

ORAL ABSTRACT PRESENTATIONS

Concurrent Session 1A: HFpEF: Unraveling the Gordian Knot

Monday, July 29, 2019, 1:00 pm - 2:35 pm

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Dual Activation of PKA and PKG by PDE1 Inhibition Facilitates Proteasomal Degradation of Misfolded Proteins and Protects Against Proteinopathy-Based HFpEF

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No current treatment is intended to target cardiac proteotoxicity or can reduce mortality of heart failure with preserved ejection fraction (HFpEF), a prevalent form of heart failure (HF). Selective degradation of misfolded proteins by the ubiquitin-proteasome system (UPS) is vital to the cell. Proteasome impairment is recently implicated in HF genesis. Activation of the cGMP-protein kinase G (PKG) or the cAMP-protein kinase A (PKA) pathways facilitates proteasome functioning. Phosphodiesterase 1 (PDE1) hydrolyzes both cyclic nucleotides and accounts for the majority of PDE activities in human myocardium. Here we report the preclinical therapeutic efficacy and a new mechanism of action of PDE1 inhibition (IC86430) for cardiac proteinopathy caused by Arg120Gly missense mutant α B-crystallin (CryAB^{R120G}). In mice expressing GFPdgn, an inverse reporter of UPS proteolytic activity, IC86430 treatment increased myocardial 26S proteasome activities and substantially decreased GFPdgn protein levels. Myocardial PDE1A expression was highly upregulated in CryAB^{R120G} mice. HFpEF was detected in CryAB^{R120G} mice at 4 months; IC86430 treatment initiated at this stage markedly attenuated HFpEF, substantially delayed mouse premature death, increased myocardial levels of Ser14-phosphorylated Rpn6 (the primary proteasome target of PKA), and reduced the steady state level of the misfolded CryAB species in these mice. In cultured cardiomyocytes, IC86430 treatment increased proteasome activities and accelerated proteasomal degradation of GFPu and CryAB^{R120G} in a PKA- and PKG- dependent manner. We conclude that PDE1 inhibition induces PKA- and PKG-mediated promotion of proteasomal degradation of misfolded proteins in cardiomyocytes and effectively treats HFpEF caused by CryAB^{R120G}; hence, PDE1 inhibition represents a potentially new therapeutic strategy for HFpEF and heart disease with increased proteotoxic stress.

H. Zhang: None. **B. Pan:** None. **P. Wu:** None. **M.D. Rekhter:** 1. Employment; Significant; Lilly Corporate Center. **A.L. Goldberg:** None. **X. Wang:** None.

Concurrent Session 1B: Mechanisms of Mitochondrial Quality Control

Monday, July 29, 2019, 1:00 pm - 2:35 pm

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Identification of Novel MICU1 Interactors Independent of the mtCU Complex

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MICU1 is an EF-hand containing mitochondrial protein that gates the mitochondrial Ca²⁺ uniporter complex (mtCU) and directly interacts with the pore-forming subunit MCU. Previous studies have shown perinatal lethality and altered mitochondrial architecture in MICU1 knockout (*Micu1*^{-/-}) mice, phenotypes that are distinct from other knockout models of mtCU components, such as MCU, and thus are likely not explained solely by changes in matrix [Ca²⁺] uptake. Further, our proteomic studies suggest that MICU1 exists in mitochondrial complexes void of MCU. This suggests that MICU1 may have cellular functions independent of mtCU regulation. To discern novel MICU1 molecular interactors we employed a biotinylation-based proteomic approach in *Mcu*^{-/-} and *Micu1*^{-/-} cells to detect proteins interacting with MICU1 using fusion protein containing BioID2, a small biotin ligase for proximity-dependent labeling. Expression of MICU1-BioID2 in *Mcu*^{-/-} cells allowed the identification of mtCU-independent interactors. Fast protein liquid chromatography (FPLC), blue native-PAGE, co-immunoprecipitation, live-cell Ca²⁺ imaging, confocal and super-resolution imaging methods were used to confirm novel roles for MICU1 in mitochondrial biology. LC-MS analysis of biotinylated proteins after avidin-based purification identified the Mitochondrial Contact Site and Cristae Organizing System (MICOS) components IMMT, CHCHD2, and CHCHD3 as interacting with MICU1 (MICU1-BioID2 expressed in *Micu1*^{-/-} cells to avoid aberrant expression). These same MICOS components were identified in *MCU*^{-/-} cells, suggesting that MICU1 could be involved in the regulation of MICOS independent of the mtCU. Further, the deletion of CHCHD2 resulted in the loss of MICOS and cristae disorganization without any observable effect on mCa²⁺ uptake. RNA sequencing revealed correlative expression changes in MICU1 and MICOS

components in response to heart failure progression during transverse aortic constriction and myocardial infarction. These results suggest MICU1 likely serves cellular functions independent of the mtCU and may serve as a key regulator of Ca²⁺-dependent signaling (EF-hands) in other cellular processes that are dysregulated during heart failure.

D. Tomar: 2. Research Grant; Significant; AHA Postdoctoral Fellowship. **J.F. Garbincius:** None. **D. Kolmetzky:** None. **M. Thomas:** None. **P. Jadiya:** None. **J.W. Elrod:** 2. Research Grant; Significant; NIH.

Concurrent Session 2A: Early Triggers of Heart Failure

Monday, July 29, 2019, 3:05 pm - 4:40 pm

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Molecular, Cellular and Systemic Mechanism of Nonlinear Dynamic Patterns of Ventricular Repolarization and Spontaneous Arrhythmic Sudden Death in Non-ischemic Heart Failure

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INTRODUCTION: Sudden cardiac death (SCD) is the leading cause of death in USA. Heart failure (HF) confers high SCD risk, but most SCD victims do not have HF or well-defined genetic abnormalities. The underlying mechanisms are poorly understood, precluding the design of more effective strategies for risk stratification and therapy. We previously showed that distinct non-linear patterns of cardiac repolarization strongly and independently predict SCD in HF patients [PMID: 27044982]. We also showed in a unique guinea pig HF model that increased levels of mitochondrial reactive oxygen species (mROS) drive spontaneous SCD, even before HF onset [PMID: 29898892]. Herein, we further dissect the underlying mechanisms. **HYPOTHESIS:** Increased mROS levels in pressure overloaded hearts reduce K⁺ currents, destabilize the resting membrane potential, increase repolarization lability and cause SCD. **METHODS:** Guinea pigs were randomized to Sham, aortic banding (AC), or AC with daily brief low-dose β -adrenergic stress (ACi) with and without *in vivo* mROS scavenger MitoTEMPO (ACi+MT). We performed time series analyses on recordings of: (1) 24-hour ECG QT interval in freely ambulating animals before sacrifice at 4 weeks; (2) pressure-volume and electrophysiology (EP) in excised perfused hearts; and (3) duration of action potential (APd), calcium transient (CaTd) and sarcomere shortening (SSd) in isolated LV myocytes. **RESULTS:** About 50% of ACi animals had SCD by 4 weeks. Increased QT entropy in ACi (at 1 week) predicted SCD over followup, similar to HF patients. Compared to Sham and ACi+MT, LV myocytes isolated from AC and ACi had reduced inward (IK1) and delayed slow (IKs) and rapid (IKr) rectifier currents; and increased beat-to-beat lability in APd, CaTd and SSd. The late Na and L-type Ca currents were similar between models. Acute exposure to isoproterenol (Iso) and carbachol (CCh) increased lability in LV myocytes compared to Iso or CCh alone. **CONCLUSIONS:** High mROS levels reduce repolarization reserve in LV myocytes and contribute, at least in part, to the non-linear dynamics of ventricular repolarization (reflected by high QT entropy), leading to spontaneous arrhythmic SCD. These findings provide important new mechanistic insight with direct clinical implications.

D.C. Vieira: None. **J.S. Crocker:** None. **K. Desai:** None. **N. Sanagala:** 2. Research Grant; Significant; 18AMTG34280046. **D.R. Wendelken:** None. **K.G. Parks:** None. **T. Liu:** None. **B. O'Rourke:** None. **S. Dey:** None. **D. DeMazumder:** 2. Research Grant; Significant; NIH NHLBI 1K99HL130662-01, NIH NHLBI 4R00HK130662, NIH NHLBI 1UH54HL119810, AHA 17UNPG33860002, AHA 18IFUNP33990024, AHA 18AMTG34280046.

Concurrent Session 2B: Beyond Myocytes and Fibroblasts: Forgotten Cells of the Heart

Monday, July 29, 2019, 3:05 pm - 4:40 pm

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Single Cell Transcriptome Analyses Reveal Novel Targets for Therapeutic Neovascularisation by Resident Endothelial Cells in the Heart

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Aims A better understanding of the pathways that regulate regeneration of the coronary vasculature is important for future strategies to treat patients with heart disease. We investigated (i) the clonal dynamics of endothelial cells (EC) associated with neovascularization in the ischemic border region (ii) transcriptional signatures of regenerative EC in the ischemic heart using single cell RNA-sequencing (iii) the functional relevance of selected targets.

Methods MI was induced in 'EC-Confetti' mice by coronary artery ligation. EC clonal proliferation was quantified or hearts dissociated for scRNAseq. Immunofluorescence staining for targets identified by scRNAseq was performed on cardiac tissue from patients with ischemic heart disease. EC proliferation was assessed *in vitro* following siRNA gene silencing.

Results EC-Confetti mice express YFP, RFP, GFP, or CFP specifically in EC. Fluorophores are inherited by EC progeny following proliferation, allowing quantitative clonal analysis. Clonal proliferation was significantly increased in the infarct border at 7 days post-MI compared to healthy hearts ($P < 0.0001$). Ten transcriptionally discrete EC clusters were defined following scRNAseq with 3 clusters predominantly composed of cells from the MI group, indicating their gene expression profiles may be relevant to neovasculogenic pathways. We selected plasmalemma vesicle associated protein (Plvap) for further study and confirmed EC-specific increased Plvap expression in ischemic border regions of human ($P = 0.002$) and mouse ($P = 0.002$) hearts, compared to healthy myocardium. siRNA gene silencing of Plvap significantly inhibited EC proliferation ($P = 0.0038$), strong evidence that Plvap can directly modulate EC function.

Conclusions Generation of new blood vessels following ischemic injury in the mouse heart is predominantly mediated by clonal proliferation of resident EC. We present a gene expression atlas of resident cardiac EC, and the transcriptional hierarchy underpinning endogenous vascular repair following MI. This resource identifies novel targets, including Plvap, that may augment myocardial perfusion post-MI, and inform future design of strategies aimed at promoting vascular perfusion in ischemic heart disease.

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Concurrent Session 3A: Heart Failure in a Dish: iPSCs to Organoids

Tuesday, July 30, 2019, 8:00 am - 9:15 am

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Identification and Characterization of a *Titin* Enhancer using CRISPR/Cas9 Genome Editing and hiPSC-Derived Cardiomyocytes

Meraj Neyazi, Manuel Schmid, Arun Sharma, Christopher N. Toepfer, Yuri Kim, Lauren K. Wasson, Seong Won Kim, Daniel M. DeLaughter, Jon A. L. Willcox, Radhika Agarwal, Angela Tai, Joshua M. Gorham, Steven DePalma, Jonathan G. Seidman, Christine E. Seidman, Harvard Medical Sch, Boston, MA

Dilated cardiomyopathy (DCM) is a leading cause of heart failure and is associated with a rate of mortality of 20% within 5 years of diagnosis. The most common genetic causes for DCM are mutations of the sarcomere protein titin (encoded by *TTN*), which occurs in 10-20% of DCM cases. Dominant DCM mutations truncate titin (*TTN*tv) and result in haploinsufficiency. Thus, strategies to increase the expression of the wild type *TTN* allele could attenuate damaging effects of *TTN*tv. Utilizing bioinformatic tools, we identified a putative enhancer for *TTN* in its intron 1. We deleted a 658 bp region from intron 1 which encompasses the region of interest in human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas9 genome editing to validate its function. Utilizing RNA sequencing and qPCR of RNA harvested from hiPSC-derived cardiomyocytes (hiPSC-CMs), we demonstrated that a homozygous deletion in this region leads to a drop in *TTN* expression compared to the wild type (WT) control (0.344-fold change, $p < 0.001$). To further characterize this region, we subdivided it into three parts which we called E1 (296 bp), E2 (206 bp), and E3 (139 bp). E1 includes a highly conserved region and a region of open chromatin as identified by the Assay for Transposase-Accessible Chromatin Sequencing (ATAC-Seq) performed on hiPSC-CMs. A homozygous E1 deletion resulted in a decreased *TTN* expression of 0.63-fold compared to the WT control ($p < 0.001$) when performing RNA sequencing on hiPSC-CMs. Both homozygous E2 and E3 deletions resulted in an increased *TTN* expression (1.56-fold change, $p < 0.001$; 1.19 fold change, $p < 0.001$). Utilizing a published sarcomere tracking platform, SarcTrack, to investigate hiPSC-CM physiology, we saw a decreased contractility of 6.6% in hiPSC-CMs carrying a homozygous E1 deletion compared to 10.1% in the WT control ($p < 0.001$). Cells carrying homozygous E2 or E3 deletions were hypercontractile (13.8%, $p < 0.001$; 13.7%, $p < 0.001$). Given our results, we hypothesize that *TTN* expression depends on the E1 region. If confirmed, we expect that increasing the activity of this enhancer using small molecules may provide a novel therapeutic target for DCM caused by *TTN*tv.

M. Neyazi: None. **M. Schmid:** None. **A. Sharma:** None. **C.N. Toepfer:** None. **Y. Kim:** None. **L.K. Wasson:** None. **S. Kim:** None. **D.M. DeLaughter:** None. **J.A. Willcox:** None. **R. Agarwal:** None. **A. Tai:** None. **J.M. Gorham:** None. **S. DePalma:** None. **J.G. Seidman:** 7. Ownership Interest; Significant; CES and JGS are founders and own shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere. **C.E. Seidman:** 7. Ownership Interest; Significant; CES and JGS are founders and own shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere..

Concurrent Session 3B: Emerging Discoveries in Myocardial Metabolism

Tuesday, July 30, 2019, 8:00 am - 9:15 am

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MCL-1 Promotes Drp1-Mediated Mitochondrial Fission as an Adaptive Response to Stress

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The anti-apoptotic BCL-2 family member, Myeloid Cell Leukemia-1 (MCL-1), is highly expressed in myocardium and plays a critical role in maintaining mitochondrial homeostasis. We have previously found that cardiomyocyte-specific MCL-1 knockout mice develop rapid cardiac dysfunction and cardiomyopathy but show little activation of apoptotic cell death. Instead, loss of MCL-1 resulted in atypical mitochondrial morphology and function. This suggests that besides its anti-apoptotic role, MCL-1 may have broader functions in regulating mitochondrial dynamics and function. MCL-1 localizes to two different mitochondrial locations in myocytes. One form exists on the outer mitochondrial membrane (MCL-1_{OM}) and a shorter cleaved form resides in the mitochondrial matrix (MCL-1_{Matrix}). We found that overexpression of MCL-1_{WT} or MCL-1_{OM}, but not MCL-1_{Matrix}, induces fragmentation and perinuclear aggregation of the mitochondria in a Drp1-dependent manner. Mutating MCL-1's BH3 domain (G198E D199A), which is required for MCL-1's anti-apoptotic function, completely abrogates its ability to induce perinuclear aggregation. Interestingly, a MCL-1-BCL-2 chimera, in which MCL-1's BH domains are replaced with those of BCL-2, is still able to induce perinuclear aggregation. This suggests that the presence of a functional BH3 domain is sufficient for induction of perinuclear aggregation. We confirmed that there is increased interaction between endogenous MCL-1 and Drp1 in response to a variety of fission-promoting stimuli, including glucose deprivation, hypoxia, and treatment with rotenone or FCCP. MCL-1 overexpression also protects against cell death in response to these stimuli, but this protection is abrogated when Drp1 is knocked down using siRNA. Additionally, Drp1 levels are significantly increased at the mitochondria in the hearts of MCL-1_{OM} transgenic mice, and this increase corresponds to an increased interaction between MCL-1 and Drp1 in these transgenic mice. Consistent with these findings, many of the mitochondria in these transgenic mice appear to be smaller than those from the WT mice, indicative of enhanced mitochondrial fission. Thus, our data suggests that MCL-1 functions as a positive regulator of Drp1-mediated mitochondrial fission.

A.G. Moyzis: None. **N.S. Lally:** None. **R.A. Najor:** None. **L.J. Leon:** None. **Å.B. Gustafsson:** None.

Concurrent Session 4A: The Future of Cardiac Fibrosis

Tuesday, July 30, 2019, 9:45 am - 11:00 am

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Cardiac Fibroblast as a Critical Constituent of the Cellular Niche Driving Cardiac Maturation

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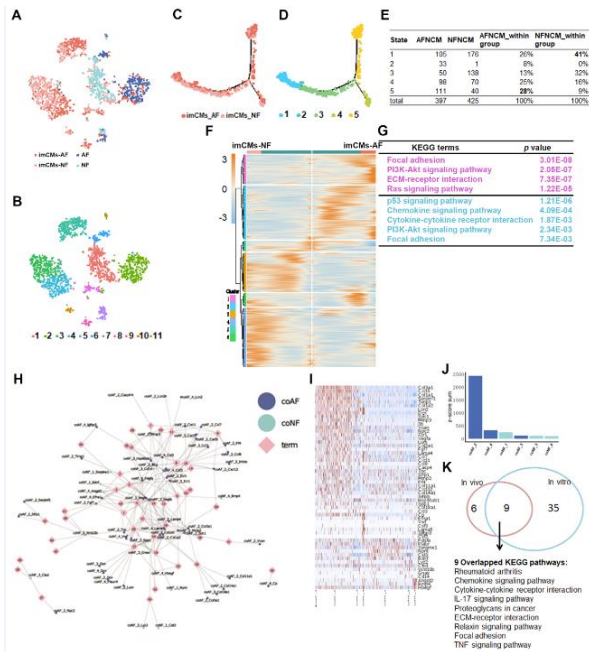
Rationale: Cardiac maturation lays the foundation of postnatal heart development and disease, yet little is known about the contributions of the microenvironment to cardiomyocyte maturation.

Objective: To identify non-cardiomyocyte cell types and important signaling pathways central to cardiomyocyte maturation.

Methods and Results: Through building cell-cell interactomes and signaling regulatory networks from single-cell RNA-sequencing data of mouse hearts at various postnatal stages, we identified switching of fibroblast subclusters state as a key driving force behind cardiac maturation. Molecular and functional maturation of immature (neonatal) mouse cardiomyocytes or human embryonic stem cell-derived cardiomyocytes were considerably enhanced when co-cultured with their corresponding adult cardiac fibroblasts *in vitro*. Further, single-cell analysis of the cardiac maturation trajectory revealed highly conserved signaling pathways and ligands in fibroblast-induced cardiomyocyte maturation *in vivo* and *in vitro*. Targeting these signaling pathways using inhibitors significantly delayed cardiac maturation in postnatal hearts, and markedly induced cardiomyocyte proliferation in infarcted hearts.

Conclusions: Our study unveiled cardiac fibroblasts as a major constituent in the microenvironment driving cardiac maturation. Targeting signaling pathways and ligand-receptor interactions involved in their crosstalk with cardiomyocytes may yield useful strategies to modulate cardiomyocyte maturation state in cardiac diseases and for regeneration purposes.

Keywords: Cardiac Maturation, Fibroblast, Single-Cell RNA-Sequencing



F. Yao: None.

Concurrent Session 4B: Diabetes and Cardiomyopathies: Where Are We Now and What is New?

Tuesday, July 30, 2019, 9:45 am - 11:00 am

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Role of p53 Acetylation in Mediating Myocardial Angiogenesis and Diabetic Cardiomyopathy

Heng zeng, **Jian Xiong Chen**, Univ Mississippi Medical Ctr, Jackson, MS

The prevalence of diabetic heart failure (HF) is increasing at an alarming rate, it is therefore urgent to identify potential therapeutic targets. Accumulating evidence demonstrates the important role of acetylation of mitochondrial proteins in HF and diabetes. In this study, we investigated the role of p53 acetylation in diabetic impairment of myocardial angiogenesis and determine whether inhibition of p53 acetylation can improve cardiac function in the setting of diabetes. The acetylation of p53 was significantly increased in hearts of diabetic db/db mice, hyperglycemic STZ mice, and high fat-diet-induced obese mice, which was associated with the reduced level of SIRT3. Knockout of SIRT3 increased p53 acetylation, which in turn led to impairment of angiogenesis and cardiac function in mice. Using cultured cardiomyocytes exposed to high glucose (HG) and diabetic db/db mice, we further explored the roles of p53 acetylation on angiogenic growth factor expression, angiogenesis and cardiac function. We found that exposure of cardiomyocytes to HG resulted in upregulation of p53 acetylation. This was accompanied by a reduction of Sirt3 expression. Transfection with Ad-Sirt3 significantly reduced hyperglycemia-induced p53 acetylation. Overexpression of Sirt3 increased the expression of HIF-1 α and Ang-1 and VEGF, together with reduction of oxygen consumption and ROS formation. Hyperglycemia-induced caspase-3 expression and apoptosis was also significantly suppressed by Sirt3. Knockdown of p53 in cardiomyocytes by using siRNA significantly increased the expression of HIF-1 α and Ang-1 and VEGF. Treatment with p53 siRNA significantly decreased oxygen consumption. *In vivo*, Ad-Sirt3 treatment significantly inhibited p53 acetylation in the heart of db/db mice. Ad-Sirt3 treatment upregulated the expression of HIF-1 α , Ang-1 and VEGF. Similarly, mutations at acetylation sites K117/161/162 and K98 of p53 (p53^{4KR}) upregulated the expression of HIF-1 α and increased angiogenesis in mouse heart. Ad-Sirt3 treatment increased angiogenesis, and reduced ROS formation and apoptosis in the diabetic heart. These led to significantly improved cardiac function. Our study suggests that p53 acetylation may be a novel target for diabetic cardiomyopathy.

H. zeng: None. J. Chen: None.

Concurrent Session 6A: Cardio Oncology: Protecting the Heart from Cancer Treatment

Tuesday, July 30, 2019, 1:30 pm - 2:45 pm

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Reactivation of Myc Transcription in the Heart Unlocks its Proliferative Capacity

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It is unclear why some tissues are refractory to the mitogenic effects of the pleiotropic transcription factor Myc, even when its expression is deregulated. We have developed an *in vivo* model permitting determination of the early transcriptional consequences of Myc activation across all tissues of an adult mouse. Myc activation induces rapid transcriptional responses, followed by cell proliferation in some, but not all, organs. Despite such disparities in proliferative response, Myc bound DNA at open promoter and enhancer elements, in representative responsive (liver) and non-responsive (heart) tissues, but failed to induce a robust transcriptional and proliferative response in the heart. Therefore, the determinants of transcriptional responsiveness are distinct from chromatin state and DNA binding by Myc. Using heart as an exemplar of a non-responsive tissue, we show that Myc-driven transcription may be re-engaged in mature cardiomyocytes by elevating levels of the P-TEFb, instating a profound proliferative response to Myc. These data indicate that the cardiac epigenomic architecture does not preclude Myc binding to E-boxes; rather, it is the inability of Myc to drive transcriptional output from Myc target genes that thwarts cardiomyocyte proliferation. Hence, P-TEFb activity is a key limiting determinant of whether or not an individual tissue is permissive for Myc transcriptional activation and mitogenesis. These data provide not only a greater understanding of how Myc transcriptional activity is determined in cellular contexts, they also indicate that modification of the expression levels of the transcriptional co-factor P-TEFb could be a means through which adult cardiomyocytes could be regenerated for the treatment of heart conditions.

C.H. Wilson: 3. Other Research Support; Modest; Receipt of research drugs from AZ. 7. Ownership Interest; Modest; patent. **M.J. Bywater:** 7. Ownership Interest; Modest; patent. **D.L. Burkhart:** None. **A. Sabò:** None. **J. Straube:** None. **V. Pendino:** None. **J.E. Hudson:** None. **G.A. Quaife-Ryan:** None. **E.R. Porrello:** None. **T.R. Kress:** None. **B. Amati:** None. **T.D. Littlewood:** None. **G.I. Evan:** 2. Research Grant; Modest; PI on Research Grant. 8. Consultant/Advisory Board; Modest; Member of AstraZeneca's Oncology External Advisory Panel.

Concurrent Session 6B: Cardiac Myofilaments: Mechanics and Regulations in Heart Failure

Tuesday, July 30, 2019, 1:30 pm - 2:45 pm

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Site-specific Acetylation of Cardiac Troponin I Regulates Myofilament Relaxation and Calcium Sensitivity

Kathleen C Woulfe, Ying H Lin, Univ of Colorado, Aurora, CO; William Schmidt, Anthony Cammarato, D Brian Foster, Johns Hopkins Univ Sch of Med, Baltimore, MD; Brandon J Biesiadecki, The Ohio State Univ, Columbus, OH; Timothy A McKinsey, Univ of Colorado, Aurora, CO

Objective: Cardiac troponin I (cTnI) is an essential regulator of cardiac contractility and relaxation. Mutations within key regions of this regulator lead to cardiomyopathies. Further, post-translational modification of cTnI through phosphorylation impacts myofibril relaxation and calcium sensitivity. Recent studies have also demonstrated that myofibril proteins are acetylated leading to faster relaxation. These studies highlight the potential significance of myofilament acetylation; however, it is not known if site-specific acetylation of cTnI can lead to mechanical changes in the myofibril. The objective of this study was to determine if acetylation at a single site of cTnI (lysine-132; K132) is sufficient to alter myofibril protein mechanics.

Methods: Adult rat ventricular cardiomyocytes infected with adenoviral constructs expressing either cTnI K132 replaced with glutamine (K132Q; to mimic acetylation) or K132 replaced with arginine (K132R; to prevent acetylation) were subjected to cell contractility and isolated myofibril mechanical measurements. Additionally, skinned myofibrils were exchanged with troponin containing wildtype (WT) or K132Q cTnI and mechanics assessed. Finally, dynamics of reconstituted thin filaments containing WT or K132Q cTnI were assessed by *in vitro* motility assay.

Results: Cardiomyocytes expressing cTnI K132Q relaxed faster and had decreased calcium sensitivity compared to WT cTnI at the whole cell and myofibril level. Relaxation or calcium sensitivity did not differ between cardiomyocytes infected with WT cTnI and cTnI K132R. Myofibrils with cTnI K132Q exchanged *ex vivo* demonstrate faster relaxation and decreased

calcium sensitivity as well as decreased motility.

Conclusions: Our results indicate for the first time that acetylation at a specific cTnI lysine can alter myofibril relaxation and calcium sensitivity. This work underscores the importance of understanding how acetylation affects specific sarcomeric proteins in the context of cardiac disease, and suggests that modulation of myofilament lysine acetylation may represent a novel therapeutic target to alter cardiac relaxation.

K.C. Woulfe: None. **Y.H. Lin:** None. **W. Schmidt:** None. **A. Cammarato:** None. **D.B. Foster:** None. **B.J. Biesiadecki:** None. **T.A. McKinsey:** None.

Concurrent Session 7A: Pathways and Mechanisms of Apoptosis

Tuesday, July 30, 2019, 3:15 pm - 4:30 pm

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Role of Beclin1 in Regulating Parkin-mediated Mitophagy

Eileen R Moreno, Mark A Lampert, Rita H Najor, Åsa B Gustafsson, Univ of California San Diego, La Jolla, CA

Autophagy is a cellular quality control mechanism involved in the selective elimination of damaged organelles and cytotoxic protein aggregates. Beclin1 is a component of the PI3 kinase complex that initiates autophagy in cells. Here, we have investigated the role of Beclin1 in autophagosome formation and Parkin-mediated mitophagy. Using Beclin1-deficient mouse embryonic fibroblasts (MEFs), we found that autophagosomes are still formed in the absence of Beclin1 in response to various treatments known to induce autophagy (FCCP, nutrient deprivation, rapamycin). Autophagic flux is also intact in the Beclin1^{-/-} MEFs, indicating an alternative mechanism of autophagy is induced. Next, we examined whether elimination of mitochondria via autophagy (mitophagy) was intact in Beclin1^{-/-} MEFs. Unexpectedly, we observed that Parkin-mediated mitophagy was significantly reduced in the absence of Beclin1. We confirmed that Parkin was recruited to depolarized mitochondria in both WT and Beclin1^{-/-} MEFs which correlated with increased ubiquitination of mitochondrial proteins. However, high resolution imaging revealed that autophagosomes failed to sequester mitochondria that had been labeled by Parkin. We observed that Parkin was degraded in Beclin1^{-/-} MEFs, but not WT, after mitochondrial stress. We also found that Beclin1 selectively localized as discrete puncta on Parkin-positive mitochondria suggesting a potential role for Beclin1 in linking mitochondria to autophagosomes. In contrast to our findings in MEFs, characterization of autophagy in mice with cardiac specific deletion of Beclin1 revealed that these hearts had a reduced number of autophagosomes which correlated with a reduction in autophagic flux as indicated by accumulation of cytosolic LC3I and p62. Beclin1-deficient hearts also had significantly reduced Parkin protein levels without changes in mRNA levels, suggesting an impairment in mitophagy. Overall, these findings suggest Beclin1 is required for the proper targeting of mitochondria into autophagosomes and that the adult heart is dependent on Beclin1 to induce formation of autophagosomes.

E.R. Moreno: None. **M.A. Lampert:** None. **R.H. Najor:** None. **Å.B. Gustafsson:** None.

Concurrent Session 8A: Cardiac Arrhythmias: From Basic Mechanisms to Precision Medicine

Wednesday, July 31, 2019, 8:00 am - 9:15 am

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Wnt Signaling Inhibition Rescues Voltage-Gated Na⁺ Current in Brugada Syndrome Patient Cardiomyocytes

Wenbin Liang, Aizhu Lu, Cencen Chu, Jerry Wang, Univ. of Ottawa Heart Inst, Ottawa, ON, Canada

Aims: Both inherited arrhythmogenic diseases (such as Brugada Syndrome) and heart failure are associated with reduced voltage-gated Na⁺ current (I_{Na}) which promotes lethal arrhythmias and sudden deaths. We and others have shown that Wnt/β-catenin signaling (Wnt signaling), which is active in heart disease, inhibits I_{Na} in rat and mouse cardiomyocytes. But whether Wnt signaling regulates I_{Na} in human cardiomyocytes and represents a novel therapeutic target is unknown. This study aims to investigate if Wnt signaling inhibits I_{Na} in human cardiomyocytes, to elucidate the underlying mechanisms, and to test if blocking Wnt signaling can rescue I_{Na} in Brugada Syndrome patient cardiomyocytes. **Methods and**

Results: Cardiomyocytes were differentiated from human induced pluripotent stem cells (hiPSC-CMs) that were derived from healthy volunteers or a Brugada Syndrome patient. Whole-cell patch-clamp technique was used for I_{Na} measurement. Activation of Wnt signaling in healthy hiPSC-CMs led to a 69% reduction in I_{Na} amplitude (peak current at -20 mV: -10.5±2.8 pA/pF, n=9 cells vs. control -33.6±4.1 pA/pF, n=6 cells, p<0.01) by reducing *SCN5A* mRNA (encoding the pore-forming α subunit of I_{Na}, Na_v1.5). In addition, Wnt signaling also reduced Na_v1.5 glycosylation causing a depolarizing shift of I_{Na} activation curve (a mechanism found in human, but not in rodent, cardiomyocytes). Blocking Wnt signaling in Brugada hiPSC-CMs with shRNA-mediated β-catenin knockdown led to a 13-fold increase in I_{Na} amplitude (p<0.01, n=9 cells),

offsetting the fundamental genetic defect. Consistent with increased I_{Na} , blocking Wnt signaling also upregulated *SCN5A* mRNA and Nav1.5 protein, without affecting expression of other cardiac ion channels in Brugada hiPSC-CMs. **Conclusions:** This study demonstrated Wnt-inhibition of human cardiac I_{Na} and, using Brugada Syndrome as an example, demonstrated that blocking Wnt signaling is a novel therapeutic strategy to rescue I_{Na} in heart disease.

W. Liang: None. **A. Lu:** None. **C. Chu:** None. **J. Wang:** None.

Concurrent Session 8B: Cardiac Inflammasome in Heart Failure

Wednesday, July 31, 2019, 8:00 am - 9:15 am

112

Endogenous-Antigen-Specific T Cell Receptor Activation of CD4+ T Cells in the Heart is Required for Maladaptive Cardiac Remodeling Due to Pressure Overload

Njabulo Ngwenyama, Francisco Carrillo-Salinas, Tufts Univ, Boston, MA; Mark Aronovitz, Tufts Medical Ctr, Boston, MA; Annet Kirabo, David G Harrison, Vanderbilt Univ, Nashville, TN; Pilar Alcaide, Tufts Univ, Boston, MA

Cardiac pressure overload is associated with an adaptive immune response that drives maladaptive cardiac remodeling. CD4+ T cells are activated and expanded specifically in the heart-draining mediastinal lymph nodes (mLNs) and infiltrate the left ventricle (LV) in response to transverse aortic constriction (TAC). However, the specific mechanisms triggering T cell activation during the progression of TAC-induced HF remain unknown. We **hypothesized** that T cell receptor (TCR)-dependent activation of CD4+ T cells by endogenous antigens initiates and sustains maladaptive cardiac remodeling during the progression of TAC induced HF. We evaluated TCR mediated CD4+ T cell activation in the LV of Nur77^{GFP} mice, which transiently express GFP exclusively upon TCR stimulation. Strikingly, we found LV-infiltrated GFP+ CD4+ T cells that increased in number as maladaptive remodeling and cardiac dysfunction progressed in response to TAC. Next generation sequencing of LV-sorted GFP+ CD4+ T cells after 8 weeks of TAC revealed a limited repertoire of TCR clones relative to the periphery, demonstrating a restricted CD4+ T cell response to endogenous antigens. We further assessed the requirement for endogenous antigens by immunizing OT-II transgenic mice, which exclusively express a TCR specific for exogenous chicken ovalbumin (OVA), with OVA in the onset of TAC. While OVA induced CD4+ T cell activation and infiltration into the LV, this was not sufficient to induce cardiac dysfunction, demonstrating the importance of an endogenous antigen-specific response. Reactive isolevuglandins (IsoLGs) are formed during oxidative stress, and rapidly adduct to self-proteins forming endogenous neoantigens that elicit CD4+ T cell activation. We treated mice with the IsoLG scavenger 2-hydroxybenzylamine (2-HOBA) in the onset of TAC, which significantly reduced accumulation of IsoLG-protein adducts in dendritic cells, prevented CD4+ T cell activation in the mLNs, and prevented cardiac dysfunction. Thus, our results demonstrate that CD4+ T cell recognition of endogenous antigens in the LV, including IsoLG-protein adducts, via the TCR is required for maladaptive cardiac remodeling and cardiac dysfunction in pressure overload-induced HF.

N. Ngwenyama: None. **F. Carrillo-Salinas:** None. **M. Aronovitz:** None. **A. Kirabo:** None. **D.G. Harrison:** None. **P. Alcaide:** None.

Concurrent Session 9A: Rare Cardiac Genetic Disorders - New Mechanistic Insights

Wednesday, July 31, 2019, 9:45 am - 11:00 am

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Recombinant Tafazzin Enzyme Replacement Therapy Rescues Metabolic and Functional Defects in a Mouse Model of Barth Syndrome

Corinne J Thomas, Junya Awata, Tufts Medical Ctr, Boston, MA; Ana A Dinca, Wei-Ming Chien, Univ of Washington, Seattle, WA; Robert Blanton, Mark Aronovitz, Gregory L Martin, Lauren Richey, Kelly Tam, Tufts Medical Ctr, Boston, MA; Douglas Strathdee, Beatson Inst of Cancer Res, Glasgow, United Kingdom; Michael T Chin, Tufts Medical Ctr, Boston, MA

Barth syndrome (BTHS) is an X-linked recessive disease where patients most commonly die from cardiomyopathy-induced heart failure before middle age. BTHS is caused by mutations in the tafazzin (TAZ) gene, resulting in defective TAZ protein. TAZ is an enzyme that generates mature cardiolipin (CL) from monolysocardiolipin (MLCL) in the mitochondrial membrane, a reaction essential for normal mitochondrial function. Current therapies can only treat the symptoms of BTHS. In this study, we propose an enzyme replacement therapy for BTHS which utilizes recombinant human TAZ fused to a cell penetrating peptide (hTAZ-CTP) to facilitate tissue uptake. The efficacy of this protein was tested *in vitro* on C2C12 TAZ-knockout (TAZ-KO) skeletal myoblasts and *in vivo* on a myocardial-specific TAZ conditional knockout mouse, modelling the cardiomyopathy associated with BTHS.

In vitro tests of TAZ-KO cells, using oxygen consumption rate as a measure of mitochondrial activity, showed treatment of the cells with hTAZ-CTP effected a partial rescue of the fatty acid oxidation capabilities of the TAZ-KO cells. *In vivo* tests showed that BTHS mice display increasing septal wall thickness over time, an effect halted upon treatment with hTAZ-CTP. Pressure-volume (PV) loop analysis indicated that heart function, impaired in the vehicle-treated BTHS mouse, was similar between treated mice and normal mice. The ratio of MLCL/CL, a direct measure of TAZ enzymatic activity, was measured in heart mitochondria isolated from BTHS and control mice after treatment. The vehicle treated BTHS mouse showed the high MLCL/CL ratio typical of BTHS patients, whereas the MLCL/CL ratios in protein-treated mice matched the much lower ratio of the control mice. Similarly, oxygen consumption rate measurements of these isolated heart mitochondria demonstrated partial rescue by hTAZ-CTP treatment. Coupled with the lack of toxicity observed in the liver, spleen, kidney, and heart due to hTAZ-CTP injection, these results indicate that TAZ enzyme replacement therapy has great potential as a future treatment for BTHS.

C.J. Thomas: None. **J. Awata:** None. **A.A. Dinca:** None. **W. Chien:** None. **R. Blanton:** None. **M. Aronovitz:** None. **G.L. Martin:** None. **L. Richey:** None. **K. Tam:** None. **D. Strathdee:** None. **M.T. Chin:** None.

General Session 10: Outstanding Early Career Investigator Award Competition

Wednesday, July 31, 2019, 11:00 am - 11:45 am

114

Myocardial B Cells Regulate the Composition of Leukocytes in the Heart as Well as Myocardial Growth

Luigi Adamo, Cibele Rocha-Resende, Sarah Evans, Jesse Williams, Hao Dun, Wenjun Li, Cedric Mpoy, Buck Rogers, Daniel Kreisel, Kory Lavine, Gwendalyn Randolph, Douglas L Mann, Washington Univ in St Louis, Saint Louis, MO

Background: B cells are the second most prevalent leukocyte in the murine naïve heart. However, since current models state that B cells continually recirculate between primary and secondary lymphoid organs without accumulating in peripheral tissues, the origin and function of this sizeable myocardial leukocyte pool remains unknown.

Goal: To investigate the origin and function of myocardial B cells in the naïve heart.

Results: Bone marrow transplant experiments showed that the murine myocardium harbors a large population of bone marrow derived B cells and a small population of embryo derived B cells. Parabiosis, heart transplant, and adoptive transfer experiments showed that all myocardial B cells recirculate between the heart, the spleen, and likely other organs. Immunohistochemistry showed that the vast majority of myocardial B cells resides in the myocardial microcapillaries and make intimate contact with the endothelium. 10X single cell RNA sequencing showed that myocardial B cells are heterogeneous and have a specific gene expression pattern different from that of peripheral blood B cells. *Ex vivo* perfusion of hearts from B cell deficient mice with wild type peripheral blood suggested that myocardial B cells “adhere” to the microvascular endothelium. *In vivo* microscopy confirmed that a subgroup of circulating B cells pauses on microvascular endothelial cells. Finally, analysis of B cell deficient and B cell depleted mice showed that B cells modulate the composition of the myocardial leukocyte pool and myocardial growth.

Conclusions: Myocardial B cells are an accumulation of a heterogeneous subgroup of circulating B lymphocytes that, upon arrival in the heart, makes intimate contact with the microvascular endothelium. Myocardial B cells regulate the composition of the myocardial leukocyte pool and myocardial mass. These findings highlight a novel unexpected relationship between B cells and myocardial physiology and suggest a paradigm shift in our current understanding of B cell biology in the naïve state.

L. Adamo: 7. Ownership Interest; Modest; Co-Founder of startup focused on developing B cell modulating therapies for the treatment of cardiac dysfunction. **C. Rocha-Resende:** None. **S. Evans:** None. **J. Williams:** None. **H. Dun:** None. **W. Li:** None. **C. Mpoy:** None. **B. Rogers:** None. **D. Kreisel:** None. **K. Lavine:** None. **G. Randolph:** None. **D.L. Mann:** 7. Ownership Interest; Modest; Co-Founder of startup focused on developing B cell modulating therapies for the treatment of cardiac dysfunction.

General Session 10: Outstanding Early Career Investigator Award Competition

Wednesday, July 31, 2019, 11:00 am - 11:45 am

115

Surgical Bilateral Stellate Ganglionectomy Reduces Mitochondrial Reactive Oxygen Species (mROS) and Prevents Sudden Cardiac Death (SCD) in Non-ischemic Heart Failure (HF)

Swati Dey, Brian O'Rourke, Johns Hopkins Univ, Baltimore, MD; Deepankar DeMazumder, Div of Cardiology and of Pharmacology and Systems Physiology, Univ of Cincinnati Coll of Med, Cincinnati, OH

INTRODUCTION: Current therapies for SCD are limited. Bilateral sympathetic stellate ganglionectomy (**BSG**) is a promising new adjunctive therapy in patients with ischemic HF who have incessant ventricular tachyarrhythmias (**VT/VF**) despite optimal medical management. The underlying mechanisms are unknown. The effect of BSG in non-ischemic HF is unclear. **HYPOTHESIS:** BSG prevents VT/VF and SCD in non-ischemic HF by restoring sympathovagal balance and reducing mROS. **METHODS:** We used a unique pressure-overload guinea pig model that closely mimics human non-ischemic HF, including a high incidence of spontaneous VT/VF/SCD. We randomized the animals to non-failing controls, HF+Sham, or HF+BSG surgery. We used intention to treat analysis to evaluate VT/VF-free survival. We analyzed continuous ECG (VT/VF burden, heart rate variability, QT variability) and echo (cardiac function). In isolated left ventricular (LV) myocytes, we measured Ca^{2+} transients and mitochondrial reactive oxygen species (mROS) using targeted ratiometric probes. **RESULTS:** Half of the HF+Sham animals experienced SCD in 4 weeks. In contrast, BSG abolished SCD, reduced VT/VF, and increased parasympathetic tone (beyond corresponding reductions in sympathetic tone). Fractional shortening of the LV was markedly reduced in HF+Sham (0.28 ± 0.02) vs. control (0.50 ± 0.01), but preserved by BSG (0.45 ± 0.02). Whereas HF+Sham increased mROS levels in LV myocytes (fractional oxidation, F_{ox} : 0.53 ± 0.015) compared to control (0.29 ± 0.020), BSG reduced mROS (0.38 ± 0.035). Mitochondrial antioxidant capacity, as indexed by H_2O_2 challenge of LV myocytes, was compromised in HF+Sham (F_{ox} : 0.81 ± 0.040) compared to control (0.44 ± 0.061), but preserved by BSG (0.48 ± 0.023). **CONCLUSIONS:** Surgical BSG prevents SCD in non-ischemic HF by reducing mROS, decreasing sympathetic tone, and by increasing parasympathetic tone (possibly by facilitating cholinergic transdifferentiation). This promising new minimally-invasive surgical therapy may be particularly useful in patients with non-ischemic HF on optimal medical therapy who require recurrent ICD shocks to prevent SCD. Patients in the early stages of HF who are not eligible for ICDs but remain at higher SCD risk may also benefit from BSG surgery.

S. Dey: 2. Research Grant; Significant; Department of Defense, PRMRP, W81XWH-17-1-0345. **B. O'Rourke:** 2. Research Grant; Modest; R01 HL134821. **D. DeMazumder:** 2. Research Grant; Significant; NIH NHLBI 4R00HK130662.

General Session 10: Outstanding Early Career Investigator Award Competition

Wednesday, July 31, 2019, 11:00 am - 11:45 am

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Inhibition of Autosis Attenuates Ischemia/reperfusion Injury in the Heart

Gihoon NAH, Rutgers Univ NJMS, Newark, NJ; Alvaro Fernandez, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Peiyong Zhai, Rutgers Univ NJMS, Newark, NJ; Beth Levine, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Junichi Sadoshima, Rutgers Univ NJMS, Newark, NJ

Although autophagy is generally protective, uncontrolled or excessive activation of autophagy can be detrimental. Recent studies provided evidence that excessive autophagy induces cell death with characteristic morphological and biochemical features, termed autosis. However, whether autophagy contributes to death in cardiomyocytes (CMs) is controversial. We here show that inhibition of autosis by targeting Rubicon attenuates ischemia/reperfusion (I/R) injury in the heart. Treatment with TAT-Beclin1 (TB1), a peptide that mobilizes endogenous Beclin 1, increased autophagic flux and induced cell death in CMs, which was inhibited by inhibitors of autophagy but not apoptosis or necrosis. CMs treated with TB1 at high doses showed typical morphological and biochemical features of autosis, namely an increased number of autophagic vacuoles with ballooning of perinuclear spaces and rescue of cell death by downregulation of Na^+ , K^+ -ATPase. To examine whether autosis is activated by I/R in the heart *in vivo*, mice were subjected to 30 minutes of ischemia followed by 24 hours of reperfusion, and autophagic flux and morphological features of CMs were examined at several time points. Autophagic flux was increased during ischemia and the early phase of reperfusion; however, more autophagosomes started to accumulate 6 hours after reperfusion due to suppression of autophagosome maturation, which was caused by increased Rubicon expression. CMs exhibited typical morphological features of autosis and Na^+ , K^+ -ATPase was significantly upregulated starting 6 hours after reperfusion. Injection of ouabain into cardiac glycoside-sensitive knock-in mice exhibited protection against I/R injury with decreased features of autosis. To normalize autophagic flux during I/R, we generated cardiac-specific Rubicon knockout (*rubi*-cKO) mice. *rubi*-cKO prevented the marked accumulation of autophagosomes and significantly reduced the size of infarct following I/R, with prominent suppression of autosis. These results indicate that autosis is triggered by dysregulated autophagic flux due to upregulation of Rubicon during the late phase of I/R in the heart and that inhibition of autosis protects the heart against I/R injury.

G. Nah: None. **A. Fernandez:** None. **P. Zhai:** None. **B. Levine:** None. **J. Sadoshima:** None.

Concurrent Session 11A: New Frontiers in the Regulation of Cardiac Gene Expression

Wednesday, July 31, 2019, 1:30 pm - 2:45 pm

117

A Novel Ubiquitination Dependent Pathway Regulating Myocardial Necroptosis and Ischemic Injury

Xiaoyun Guo, Haifeng Yin, Yi Chen, Siqi Hong, Hui He, Yachang Zeng, Rachel Steinmetz, **Qinghang Liu**, Univ of Washington, Seattle, WA

Objective: Necroptosis is a new caspase-independent, regulated form of necrosis, which has recently been implicated in ischemic cardiac injury and remodeling. This study aims to determine a novel ubiquitination-dependent mechanism that regulates necroptosis in the heart and the implications of necroptosis signaling in the setting of ischemic injury and remodeling. **Methods and Results:** Here we identified a novel ubiquitination-dependent mechanism mediated by the E3 ubiquitin ligase TRAF2 (TNF receptor associated factor-2) and the deubiquitinase CYLD (cylindromatosis), which critically regulates myocardial necroptosis and pathological remodeling. TRAF2 and CYLD specifically regulate K63-linked protein ubiquitination, which regulates signal transduction but not proteasomal degradation. Intriguingly, CYLD is up-regulated, but TRAF2 is down-regulated, in the heart after ischemia-reperfusion injury. We found that wild-type TRAF2 inhibits, whereas the ligase inactive mutant TRAF2- Δ RING promotes cardiomyocyte necroptosis. Importantly, acute deletion of TRAF2 in the adult heart in *Traf2^{fl/fl}*-MerCreMer mice induces severe dilated cardiomyopathy and lethal heart failure by promoting myocardial necroptosis. Conversely, AAV9-mediated TRAF2 gene transfer significantly attenuates ischemic-reperfusion injury. On the other hand, CYLD overexpression promotes, whereas CYLD deletion inhibits cardiomyocyte necroptosis. *In vivo*, genetic ablation of CYLD in *Cyld*^{-/-} mice attenuates ischemic myocardial injury and adverse remodeling by suppressing myocardial necroptosis. Mechanistically, TRAF2 and CYLD opposingly regulate K63-linked polyubiquitination of TAK1 (TGF β -activated kinase 1), TAK1-RIP1 interaction, and the formation of RIP1-RIP3 necrosome. These results thus identify a ubiquitination-dependent mechanism of necroptosis mediated by TRAF2 and CYLD, providing a new paradigm of necroptosis signaling. **Conclusion:** The E3 ligase TRAF2 and the deubiquitinase CYLD, as a suppressor and an activator of necroptosis respectively, opposingly regulate myocardial homeostasis, ischemic injury, and remodeling, thus representing promising therapeutic targets for heart disease.

X. Guo: None. **H. Yin:** None. **Y. Chen:** None. **S. Hong:** None. **H. He:** None. **Y. Zeng:** None. **R. Steinmetz:** None. **Q. Liu:** None.

Concurrent Session 11B: Molecular and Cellular Aspects of Heart Failure

Wednesday, July 31, 2019, 1:30 pm - 2:45 pm

118

Reduced Cardiac Transmembrane Protein 65 Resulted in Dilated Cardiomyopathy and Progressive Cardiac Fibrosis *in vivo*

Allen C.T. Teng, Univ of Toronto, Toronto, ON, Canada; Liyang Gu, Translational Biology and Engineering Program, TRCHR, Toronto, ON, Canada; Michelle Di Paola, Univ of Toronto, Toronto, ON, Canada; Meena Fatah, Diptendu Chatterjee, The Hosp for Sick Children & Res Inst, Toronto, ON, Canada; Thomas Kislinger, Princess Margaret Cancer Ctr, Toronto, ON, Canada; Robert Hamilton, The Hosp for Sick Children & Res Inst, Toronto, ON, Canada; Anthony O. Gramolini, Univ of Toronto, Toronto, ON, Canada

Intercalated discs (ICDs) are unique and functionally indispensable to the heart, but its structural organization remains less understood. Previously, we showed that an ICD-bound transmembrane protein 65 (Tmem65) was required for Connexin 43 (Cx43) localization in cultured mouse neonatal cardiomyocytes, and that reduced Tmem65 was associated with a decrease and internalization of Cx43, and impaired electrical conduction between neighboring cardiomyocytes. Here, we investigated the role of Tmem65 *in vivo* by injecting CD1 mice with recombinant adeno-associated virus 9 (rAAV9) harboring Tmem65 (or scrambled) shRNA. Quantitative polymerase chain reactions and immunoblots confirmed greater than 90% reduction in Tmem65 expression in mouse ventricles compared to control samples. Immunoblots and immunofluorescence showed reduced and internalized Cx43 in Tmem65 knockdown (KD) hearts compared to controls, respectively. Kaplan-Meier survival plot showed that all Tmem65 KD mice died within 7 weeks (> 50% death 3 weeks post viral injection), whereas no death was seen in control mice. Tmem65 KD mice developed eccentric hypertrophic cardiomyopathy in 3 weeks and dilated cardiomyopathy with severe cardiac fibrosis in 7 weeks as confirmed by H&E and Masson's Trichrome staining. Echocardiography also confirmed ventricular dilatation and showed a 60% reduction in cardiac output (19.27 \pm 1.46 mL/min in control vs. 6.63 \pm 0.52 mL/min in Tmem65 KD mice, p <0.01, n = 6 per group) at 7 weeks. Transmission electron microscopy showed an altered ICD pattern and disorganized myofibril structure in Tmem65 KD ventricles while control tissues had smooth ICDs and organized myofibers. Together, these findings suggest a critical role of Tmem65 in maintaining cardiac ICD and myofibers and loss of Tmem65 leads to cardiomyopathy *in vivo*.

A.C. Teng: None. **L. Gu:** None. **M. Di Paola:** None. **M. Fatah:** None. **D. Chatterjee:** None. **T. Kislinger:** None. **R. Hamilton:** None. **A.O. Gramolini:** None.

Concurrent Session 12B: Cellular Cross-talk in Heart Failure

Wednesday, July 31, 2019, 3:15 pm - 4:30 pm

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Multi-Omics Investigation of Cardiomyocyte-to-Fibroblast Crosstalk in Human iPSC Models

Edward Lau, Mark J Chandy, Damon R Williams, Rajani Shrestha, June-Wha Rhee, Joseph C Wu, Stanford Univ, Palo Alto, CA

Introduction

Cardiac cells communicate with each other in part through secreted proteins known as cardiokines. The total repertoire of proteins secreted by cardiac cells is unknown. We aim to apply human iPSC models and large-scale multi-omics to identify secreted proteins and the crosstalk signals they mediate.

Method

We optimized an experimental protocol to recover and identify cell-specific secreted proteins from human iPSC-cardiac cells. Human iPSCs were differentiated into cardiomyocyte (CM) and endothelial cells (EC) using established protocols, after which secreted proteins were extracted from the conditioned medium for analysis. We combined multiplexed aptamer-based large-scale protein quantification platform and high-resolution mass spectrometry to identify secreted proteins from iPSC-CM, iPSC-ECs, and primary ventricular fibroblasts. An in-silico filter was implemented to prioritize bona fide cardiokines over proteins externalized by passive lysis.

Result

We identified 146 candidate cardiokines at 1% false discovery rate, including cell-specific cardiokines as well as a common core secretome of three cardiac cells. We analyzed the data to identify potential signals secreted by cardiomyocytes to mediate fibroblast function, using a ligand-receptor model based on proteomics and single-cell transcriptomics data to prioritize cardiokines secreted by iPSC-CMs and which bind to fibroblast-expressed receptors. To examine their roles in fibrosis regulation, we selected three candidate cardiokines (PLAU, FGF7, and CXCL12) for verification using immunodetection, and exposed their recombinant proteins at multiple concentrations to human ventricular fibroblasts. The results nominated cardiokine-specific effects on recipient cell gene expression including evidence of modulation of fibroblast transcription and translation pathways.

Conclusion

We demonstrate a multi-omics approach in iPSC models to explore the large-scale human secretome of multiple cardiac cell types. The approach holds promise for identifying disease markers and understanding intercellular communication in cardiac development and diseases.

E. Lau: None. **M.J. Chandy:** None. **D.R. Williams:** None. **R. Shrestha:** None. **J. Rhee:** None. **J.C. Wu:** None.

Concurrent Session 13A: Aging and Cardiovascular Risk

Thursday, August 1, 2019, 8:00 am - 9:15 am

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Methamphetamine-induced Cardiomyopathy Associated With Mitochondrial Dysfunction, Cardiac Fibrosis and Hypertrophy

Chowdhury S Abdullah, Dept of Pathology and Translational Pathobiology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Richa Aishwarya, Dept of Molecular and Cellular Physiology, Louisiana State Univ Health Sciences Ctr-Shreveport, SHREVEPORT, LA; Shafiu Alam, Mahboob Morshed, Gopi K Kolluru, James Traylor, Dept of Pathology and Translational Pathobiology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Sumitra Miriyala, Manikandan Panchatcharam, Dept of Cellular Biology and Anatomy, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Matthew D Woolard, Dept of Microbiology and Immunology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Nicholas E. Goeders, Xioa-Hong Lu, Dept of Pharmacology, Toxicology and Neuroscience, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Paari S. Dominic, Div of Cardiology and Internal Med, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Christopher G. Kevil, A. Wayne Orr, Dept of Pathology and Translational Pathobiology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Norman R Harris, Felicity N. E. Gavins, Dept of Molecular and Cellular Physiology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA; Md. Shenuarun Bhuiyan, Dept of Pathology and Translational Pathobiology, Louisiana State Univ Health Sciences Ctr-Shreveport, Shreveport, LA

Introduction: Methamphetamine (METH) is one of the most commonly abused illicit drugs in the United States, exerting a range of adverse effects upon multiple organ systems. Cardiovascular complications are among the major causes of death in METH users. METH-induced cardiomyopathy is a poorly characterized disease entity as METH-induced molecular perturbations, and histopathological changes in the heart remain under-explored. **Objectives:** We studied histopathology in the hearts of human METH users. We also observed the histological alteration and changes in mitochondrial function in mice that received 'binge' administration of METH. **Methods and Results:** We obtained 32 autopsy heart samples from humans with positive toxicology for chronic METH use and performed Sirius Red and Masson's Trichrome (MT) staining on left ventricular (LV) sections. Notably, chronic METH user hearts showed intense perivascular and interstitial fibrosis in LVs. 'Binge' METH administration in mice for 4 weeks showed an increase in heart weight-to-tibia length and increase in myocyte cross-sectional area in WGA stained LVs compared to saline-treated mice. Sirius red and MT staining also showed an increase in perivascular and interstitial fibrosis in METH mice heart. Isolated mitochondria from METH-treated mice heart showed suppressed mitochondrial bioenergetics measured by Seahorse Analyzer. Immunoblotting in heart lysates and mitochondrial fractions showed altered mitochondrial dynamics regulatory proteins expression in METH mice compared to control saline group. METH-treated cultured neonatal rat ventricular cardiomyocytes also showed suppression of mitochondrial respiration and mitochondrial network disorganization indicating a direct effect of METH on cardiomyocytes. **Conclusions:** We report that maladaptive cardiac fibrotic remodeling is typical in a human and pre-clinical mouse model of METH abuse. 'Binge' METH exposure in mice induces cardiac hypertrophy, cardiac fibrosis, and suppression of mitochondrial respiration. Thus, chronic METH use induces maladaptive cardiac remodeling associated with mitochondrial dysfunction.

C.S. Abdullah: None. **R. Aishwarya:** None. **S. Alam:** None. **M. Morshed:** None. **G.K. Kolluru:** None. **J. Traylor:** None. **S. Miriyala:** None. **M. Panchatcharam:** None. **M.D. Woolard:** None. **N.E. Goeders:** None. **X. Lu:** None. **P.S. Dominic:** None. **C.G. Kevil:** None. **A. Orr:** None. **N.R. Harris:** None. **F.E. Gavins:** None. **M. Bhuiyan:** None.

Concurrent Session 13B: Novel Therapeutic Targets in Heart Failure

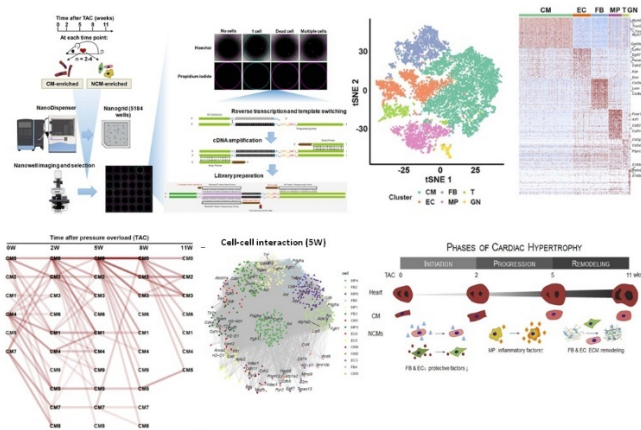
Thursday, August 1, 2019, 8:00 am - 9:15 am

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Precision Intervention of Cardiac Remodeling Based on Cellular Composition Principles Uncovered by Single-Cell Transcriptomics

Peng Yu, Zongna Ren, Li Wang, Fuwai Hosp, Beijing, China

Stress-induced cardiac remodeling forms the foundation of many cardiac diseases, yet little is known about the spatiotemporal interplay amongst cell types underlying the pathological progression of the heart from normal to a diseased state, at single-cell resolution. Here, we analyzed 11,492 single cells, including both cardiomyocytes (CMs) and non-cardiomyocytes (NCMs), at different stages in a mouse model of pressure overload-induced cardiac remodeling, and identified a full list of factors and signaling pathways important for disease progression. Through constructing cell crosstalk maps, we revealed sequential switching in NCM subtype (fibroblasts, macrophages, and endothelial cells) utilization at different stages of cardiac remodeling. Intriguingly, stage-specific pharmacological inhibition of macrophage subtype switch dramatically retarded heart transition from adaptive to a maladaptive state. Consistently, alterations of cardiac remodeling related-genes were highly conserved in human samples. Together, our study not only characterizes the molecular features of different cell types and identifies crucial factors underlying cardiac remodeling, but may also have important implications for stage- and cell type-specific precise intervention in cardiac diseases.



P. Yu: None. **Z. Ren:** None. **L. Wang:** None.

Concurrent Session 14A: Machine Learning, Big Data and AI in Heart Disease

Thursday, August 1, 2019, 9:45 am - 11:00 am

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Reliability Analysis for Image-based Non-invasive Pressure Quantification in Aortorenal Artery Systems

Hao Wu, Monsurul Khan, **Xiaoping Du**, Indiana Univ-Purdue Univ, Indianapolis, Indianapolis, IN; Alan P Sawchuk, Sch of Medicine, Indiana Univ, Indianapolis, IN; Huidan Whitney YU, Indiana Univ-Purdue Univ, Indianapolis, Indianapolis, IN

Non-invasive quantification of fractional flow reserve (FFR) based on CT angiography has great potential to replace invasive catheterization for quantifying the amount of ischemia caused by a specific arterial stenosis (AS), promising for lower medical cost and better patient care. In clinical practice, the reliability of quantifying the 4-D approximate pressure (p_a) and distal pressure (p_d) across an AS is critical since $FFR = p_d/p_a$ by definition. The objective of this work is to assess the effects of uncertainties (empiricalness) in the 3-element Windkessel model (WK3) used as exit boundary conditions (BC) on the quantification of p_d and p_a in aortorenal artery systems. A developed and validated in-house software (*InVascular*, see Fig. 1(a)), featured with revolutionarily fast speed for image-based computational hemodynamics, is used for the uncertainty quantification analysis. Fifteen total aortic and renal arteries were studied in five male patients aged 64 to 83. In each case, the effects of the uncertainty on the 4-D pressure quantification are assessed using the First Order Second Moment method. We conduct analyses where the input variables are normally distributed with their coefficients of variation being 3%. Figure 1(b) shows the effects of uncertainty on the systolic pressure (SP) with their means, standard deviations, and 95% confident intervals. The results indicate that the uncertainty (measured by standard deviation) in SP in the aorta is higher than that in the renal arteries. The uncertainty is minimal for the severe stenosis (in red), implying more reliable noninvasive pressure quantification in stenosed arteries.

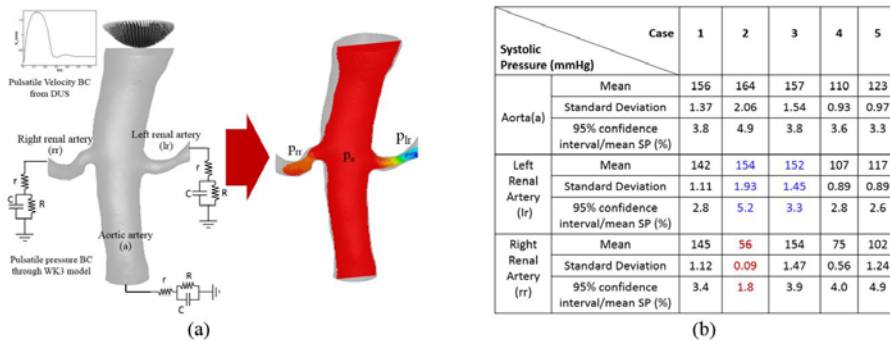


Fig. 1 Uncertainty analysis for CTA-based non-invasive pressure quantification in aortorenal artery systems: (a) schematic of image-based computational hemodynamics for 4-D pressure (*InVascular*) and (b) uncertainty quantification of systolic pressure of five medical cases. Red: with severe RAS, blue: with mild RAS, and black with no reported RAS.

H. Wu: None. M. Khan: None. X. Du: None. A.P. Sawchuk: None. H.W. Yu: None.

Concurrent Session 14B: Development of Therapeutics for Heart Failure

Thursday, August 1, 2019, 9:45 am - 11:00 am

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Osteopontin Regulates Adult Cardiomyocyte Division in a Mouse Model of Pressure Overload Induced Heart Failure

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Background. Our previous work showed that pharmacological blockade of Osteopontin (OPN) signaling can prevent and reverse heart failure induced by pressure overload in a transverse aortic constriction (TAC) mouse model. Surprisingly, OPN Knockout (KO) mice subjected to 3 month TAC had worse cardiac function and bigger hearts than wild type (WT) TAC mice, despite lack of cardiomyocyte hypertrophy. We hypothesized that OPN KO increased adult cardiomyocyte proliferation in TAC-induced heart failure. **Methods.** Male C57Bl/6 (n=17) or OPN KO (n=11) mice were subject to TAC for 3 months. The protein levels of the mitosis marker H3P was quantified using immunofluorescence in paraffin-embedded myocardial sections. Myocytes were co-stained with WGA and MLC2 to count the number of myocytes per field. Adult primary

cardiomyocytes from WT hearts were isolated and analyzed with co-immunoprecipitation (Co-IP) to study the interaction between the regeneration factor YAP1, OPN and OPN-regulated proteins such as LDLR. For validation and mechanistic studies, more Co-IP experiments were performed in proliferative human liver HEPG2 cells. To study the effect of OPN blockade on YAP1 nuclear translocation, HEPG2 cells and human iPSC- derived cardiomyocytes (hiPS-CMs) were treated with an IgG or OPN blocking antibody for 24 hours followed by immunostaining for YAP1 and PITX2. Results. Nuclear H3P normalized to myocyte count was significantly increased in OPN KO relative to WT TAC hearts (Fold Change = 1.4; p=0,04). Co-IP results revealed a novel interaction between OPN, LDLR and YAP1. Stimulation of β 2 adrenergic receptor increased the formation of this multi-molecular complex in a time-dependent manner. Blockade of OPN by a monoclonal antibody for 24 hours caused nuclear localization of YAP1 and PITX2 in HEPG2 cells and hiPS-CMs. Conclusion. OPN regulates the mitotic program in adult cardiomyocytes. Furthermore, the interaction of OPN with LDLR and YAP1 to form a new multi-molecular protein complex is regulated by β 2-cAMP signaling pathway. Importantly, OPN regulates nuclear translocation of the regeneration factors YAP1 and PITX2, suggesting that OPN signaling may be important for adult cardiomyocyte division in TAC and potentially myocardial infarction and aging.

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POSTER ABSTRACT PRESENTATIONS

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Development of Bioinspired Synthetic Exosomes with Proangiogenic Potential

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Exosomes with variable microRNA cargos are released from different cell types and stimulate angiogenesis in animal models. Therefore, exosomes are currently considered for their potential to represent a safer and easier biological-based alternative to stem cell therapies. However, the limited amount of exosomes that can be reproducibly prepared from cultured stem cells and the complexity of the cargo of endogenous exosomes make them difficult to develop as off-the-shelf “pharmaceutical products” and limit their clinical potential. These issues could be surpassed by the use of artificial (i.e. nanoparticles mimicking the natural product) exosomes carrying a therapeutic cargo. In consideration of the possibility that not all cargo components of exosomes are required for their therapeutic functions, we reasoned that bioinspired artificial exosomes (AEs) incorporating key therapeutically relevant molecules and devoid of potentially negative or indifferent factors could further improve their therapeutic potential. Therefore, we developed and tested AEs containing exosomal proangiogenic miRNAs as a “proof-of-concept” for therapeutic angiogenesis. In order to achieve this, we: (1) characterized the common microRNA cargo of endogenous proangiogenic exosomes (from stem cells, pericytes and pericardial fluid) using bioinformatics, (2) exploited this knowledge to develop off-the-shelf artificial exosomes (AEs) with proangiogenic capacities, (3) validated the angiogenic potential of the bioinspired AEs. Bioinformatics analyses integrating data of miRNA arrays and panels of proangiogenic exosomes confirmed the enrichment of let-7 family in these exosomes. After testing the angiogenic potential of different members of let-7 family, we produced AEs containing let-7b (the most potent in the family) by microfluidic micromixing. The AEs were uptaken by human ECs cultured under hypoxic conditions, without causing toxicity. let-7b-AEs transferred functional let-7b, thus decreased the expression of validated targets of let-7b in recipient cells. let-7b-AEs improved EC survival, proliferation and angiogenesis *in vitro* and *in vivo*. These data suggest the therapeutic potential of bioinspired AEs containing let-7b.

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Cardiomyocyte Renewal and Cardiac Outcomes Following Injury in Young Swine

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Objective: Infants with severe congenital heart defects (CHD) typically require lifesaving cardiac surgery. Performing this surgery during a time when the myocardium can proliferate and repair could improve successful outcomes. The mouse heart can regenerate in the first week post-partum, but the mitotic activity of cardiomyocytes (CM) is extended to 1-2-months post-partum in the pig. Thus, the regulation of CM cell cycling ability and renewal in the hearts of young large mammals after injury was examined.

Methods & Results: Pigs at postnatal day 30 (n=6) were subjected to cardiac ischemia (1-hour) by temporary occlusion of the left anterior descending (LAD) artery followed by reperfusion (IR), or to sham operation (n=6, no LAD occlusion). LAD occlusion, below the second diagonal branch, provided an effective injury as indicated by increased circulating cardiac troponin-I 2-hours after injury. In addition, ejection fraction (EF) decreased by 46% (57% to 31%) at 2 hours post-ischemia, which was then maintained to the 4-week study end point. Pigs were sacrificed 4 weeks after surgery and histology demonstrated evidence of scar formation in the area of injury. However, no change in number of proliferating CM or cell death (via pHH3 or TUNEL immunohistochemistry respectively) was detected in IR pigs vs sham, or between regions of the left ventricle.

Conclusions: Here we report a successful ischemic injury method, at an age previously not reported in swine. Pigs did not continue to decline towards heart failure following an IR injury at 1 month of life, with preservation of EF up to 4-weeks post-injury. This is also without a change in CM cell cycling activity at 2 months of age. This study highlights that even in the presence of a scar, young, large mammals can adapt to cardiac injury to maintain cardiac function. The pathways regulating scar formation and CM cell cycling are being further investigated by RNA-seq studies. If similar mechanisms operate in humans, it may be beneficial to perform CHD surgeries at a younger age when the heart is able to better tolerate injury, to ultimately improve long term outcomes.

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Imaging-Based Assay for Screening of Cell Cycle Modifying Substances in Postnatal Cardiomyocytes

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Cardiovascular diseases are the main cause of death in the industrial world. Especially large myocardial infarctions and the potentially resulting left ventricular dysfunction are chronic diseases with a poor prognosis. Due to limited endogenous cardiac repair capacity, we are investigating the regulation of the cell cycle in cardiomyocytes (CMs) as a potential treatment strategy. We hypothesize that controlled induction of cardiomyocyte proliferation will lead to an increase in muscle mass and improvement of cardiac function. We are using CMs from double transgenic α MHC-H2B-mCherry/CAG-eGFP-anillin mice for the analysis of postnatal CM development. This double transgenic system enables us to unequivocally identify CM nuclei as well as cell cycle variations like acytokinetic mitosis (mitosis without cytokinesis), resulting in binucleated CMs, and endoreplication (mitosis without cytokinesis and karyokinesis), leading to polyploid CMs. With the goal of inducing authentic cell division, we are screening for pro-proliferative substances in P1 and P6 postnatal CMs of α MHC-H2B-mCherry/CAG-eGFP-anillin mice. Here we will present the detailed analysis of several screening hits demonstrating an increase in cell cycle activity leading mainly to binucleation but only to limited cell division in CMs.

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Exosomal Transfer of Muscle Specific Mir-499 to Endothelial and Endothelial Progenitor Cells Impairs Angiogenesis in Diabetes

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Background—miR-499, a muscle specific-miR, is enhanced in diabetic heart and has been suggested to be a therapeutic target for cardiovascular disease in diabetes. Recent studies provided the evidence that overexpressing miR-499 in ECs inhibits the capillary tube networks. Here, we tested the hypothesis that under diabetic conditions, myocyte-derived exosomes transfer miR-499 to EC/EPCs thereby impairing their angiogenic properties.

Methods and Results—miR-499 expression is abundant and enhanced in heart, skeletal muscle, cardiomyocytes and skeletal muscle cells (SKMCs) of db/db mice. We observed that miR-499 level was also increased in diabetic EC/EPCs. In vitro, high D-glucose (25 mM) increased miR-499 level in SKMCs but not in ECs, suggesting that enhanced miR-499 in diabetic EC/EPCs is not regulated by hyperglycemia. To study whether SKMC-derived exosomes transfer miR-499 from SKMCs to EC/EPCs, we first examined the miR-499 levels in diabetic SKMC-derived exosomes and plasma-derived exosomes from db/db mice. We observed that miR-499 levels were greater in diabetic SKMC- and plasma-derived exosomes. Furthermore, by co-culture, we found that diabetic SKMCs increased miR-499 levels in human microvascular endothelial cells (ECs) and impaired EC tube formation and migration which was blocked by exosome inhibitor GW4869 (GW). We also observed that GW partially rescued diabetic SKMC- and plasma-derived exosome-mediated impairment in

tube formation and migration of ECs. Overexpression of miR-499 in ECs decreased angiogenic factors and impaired tube formation/migration. Furthermore, in a hind-limb ischemia model of db/db mice, GW treatment improved ischemic hindlimb blood perfusion and angiogenesis. Our study suggests that diabetes-enhanced miR-499 in myocytes can be transferred to ECs via myocyte-derived exosomes thus impairs EC/EPC function. Mechanistically, Sex-determining region Y-box 6 (SOX6), one of the validated target of miR-499, was significantly decreased in diabetic EC/EPCs. Knockdown of SOX6 impairs tube formation and migration of ECs.

Conclusions—Our results suggest that exosomal transfer of muscle specific miR-499 to EC/EPCs impairs angiogenesis and ischemic tissue injury repair in diabetes via suppression of SOX6.

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Direct Reprogramming of Fibroblasts into a Cardiovascular Tissue

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Background - Direct reprogramming of fibroblasts into cardiomyocytes (CMs), endothelial cells (ECs), or smooth muscle cells (SMCs) has emerged as a promising strategy for cell-based therapy. Moreover, tissue engineering has become a crucial means to enhance the effects of the cell therapy. However, simultaneous reprogramming of somatic cells into all three cell types or a tissue-like structure has not been investigated despite its significance. Here, we aimed to directly reprogram fibroblasts into a cardiovascular tissue containing all three cell types and determined their regenerative effects on infarcted hearts. **Methods and results** - Adult mouse fibroblasts were transfected with a pre-selected cardiac microRNA mimic, miR-208b-3p, and treated with ascorbic acid and BMP4 one day after the transfection for 9 days. At day 10, cells were reprogrammed into a tissue-like structure referred to as reprogrammed cardiovascular tissue (rCVT), which contains reprogrammed CM ($16.9 \pm 8.0\%$ positive for TNNT2)-, EC ($28.4 \pm 3.5\%$ positive for PECAM1)-, and SMC ($12.0 \pm 2.9\%$ positive for SMTN)-like cells, and 14 identified mouse proteins of extracellular matrix. Sarcomeric striations, calcium oscillations and action potentials were shown in reprogrammed CMs. Reprogrammed ECs took up Dil-conjugated acetylated low density lipoprotein and reacted with DAF-FM diacetate, suggesting production of nitric oxide by the cells. Therapeutic effects of rCVT were determined on a mouse model of myocardial infarction with echocardiography and histological analysis over 4 months. Implantation of this rCVT onto the infarcted mouse heart reduced regional cardiac strains and improved cardiac function. Histological examination showed migration of reprogrammed cells from rCVT into the infarcted hearts. Migrated rECs and rSMCs contributed to vessel formation in collaboration with host vascular cells. Migrated rCMs initially displayed immature characteristics but became mature over time and formed gap junctions with host CMs. **Conclusions** - This study demonstrates that a combination of miR-208b-3p, AA and BMP4 can directly reprogrammed single adult somatic cells into a cardiac tissue-like structure containing CMs, ECs, and SMCs, and that rCVT is effective for cardiac repair.

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Hypoxia Induced Defects in 26S Proteasome Machinery Causes Loss Of Immunoprivilege of Allogeneic Mesenchymal Stem Cells

Ejlal Abu-El-Rub, Weiang Yan, Glen Lester Sequiera, Niketa Sareen, **Sanjiv Dhingra,** St. Boniface Gen Hosp Res Cen, Winnipeg, MB, Canada

The poor survival of transplanted allogeneic mesenchymal stem cells (MSCs) in the recipient heart has dampened the overall enthusiasm of allogeneic MSCs based clinical trials. Outcome of recent studies suggests that the phenotype of transplanted cells changes from immunoprivileged to immunogenic state that leads to rejection of implanted cells by host immune system. In this study, we present a novel mechanism of immune switch in MSCs. We found that hypoxia/or ischemic environment shifts immunological landscape of MSCs from immunoprivileged to immunogenic state. The immunoprivilege of MSCs is preserved by absence of major histocompatibility complex class II (MHC-II) molecule. Our studies demonstrate that

26S proteasome-mediated degradation of MHC-II prevents its expression on cell surface in MSCs and preserves their immunoprivilege. The exposure to hypoxia results in dissociation of 19S and 20S subunits, and inactivation of 26S proteasome. This promotes accumulation of MHC-II and increases immunogenicity of MSCs. We also found that a chaperon protein HSP90 α is responsible for 26S proteasome activity in normoxic MSCs. The level of HSP90 α decreases in hypoxic MSCs that leads to increase in immunogenicity. Maintaining HSP90 α levels in hypoxic MSCs prevents 26S inactivation and preserves immunoprivilege of MSCs. Therefore, hypoxia-induced defects in 26S proteasome assembly causes loss of immunoprivilege of allogeneic MSCs. Preserving 26S proteasome activity in MSCs maintains immunoprivilege and prevents rejection of allogeneic MSCs in the heart. (This work was supported by CIHR)

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The Role of Glucose as a Promoter for Cardiac Regeneration

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Heart failure is the leading cause of death worldwide. Our focus is on non-genetic mechanisms by which cardiac regeneration can be lengthened or enhanced. Specifically, we are interested in the cyto-protective effects of glucose in cardiomyocyte growth, differentiation and proliferation and how this knowledge can be applied to regeneration therapies. Our preliminary data showed that glucose induces cardiomyocyte proliferation and inhibits cardiomyocyte maturation in human embryonic stem cells derived cardiomyocytes (hESC-CM) via the Pentose Phosphate Pathway in a dose dependent manner. Whether this pathway can be a therapeutic target for heart regeneration is unknown. **Our hypothesis is that glucose promotes neonatal heart regeneration in a murine model.** Non-Transmural cryoinjury was performed to the apex of the left ventricle in wild-type pups and cardiac specific overexpression of Glucose Transporter 1 Transgenic pups. In the acute phase (P1-P7), the level of cardiomyocyte cell proliferation was measured via flow cytometry analysis and immunostaining with PH3 and cTnnt. Glucose uptake by cardiomyocytes was measured by 18F-FDG assay and Glut1 immunostaining. In the chronic phase (P14, P21, P40), we quantified the level of fibrosis by histology (H&E and Picrosirius Red) and neovascularization by immunostaining with PECAM. Increased cardiomyocyte proliferation was observed in the Transgenic Glut1 pups. Myocardial glucose uptake declines from the muscular layer towards the trabecular layer, corresponding with maturation of the heart. We observed that Glut1 cardiomyocyte-specific overexpression resulted in improved cardiac repair compared to wild type (WT) mice at 21 days postnatally. Compared to wild-type, Glut1 hearts showed increased angiogenesis around the site of injury secondary to an increase in cardiomyocyte proliferation. This study is the first to demonstrate the potential role of glucose as a promoter for cardiac regeneration and reveal a potential mechanism for congenital cardiomyopathy associated with diabetic pregnancy.

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The Role of TNNI3K in Adult Mammalian Heart Regeneration

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Adult mammalian heart regenerative capacity is thought to be extremely limited. All fetal and newborn mouse cardiomyocytes are mononuclear and diploid, and most become polyploid during the first postnatal week. Our lab showed that mononucleated diploid cardiomyocytes (MNDCMs) retain regenerative capacity, and that the percentage of this subpopulation in the adult mouse heart is variable across strains and correlated with the regeneration capacity of mouse heart. We reported cardiac troponin I-interacting kinase (TNNI3K), a novel cardiac specific gene, to be a key regulator of MNDCM frequency. Nonetheless, a detailed mechanism of how *Tnni3k* regulates MNDCM frequency is still lacking. TNNI3K is a MAPKKK by sequence, but no change in MAPK signaling pathways (p38, ERK1/2, JNK) occurs in *Tnni3k* knock-out mice. To distinguish whether *Tnni3k* plays a kinase role, we created a mouse line harboring the kinase-dead mutant of *Tnni3k* (*Tnni3k-k489r*), which recapitulates the high MNDCM frequency of *Tnni3k* knock-out mice.

Engineered *Tnni3k* truncations in mice yield no stable protein, and these mice also show a high MNM frequency. Interestingly, we found that naked mole-rat (*Heterocephalus glaber*) has a *Tnni3k* truncation mutation, doesn't express a stable TNNI3K protein, and has a high level of MNM frequency and cardiomyocyte polyploidy. These results indicate that *Tnni3k* may influence cardiomyocyte karyokinesis and cytokinesis across mammalian species. We also found that several human *TNNI3K* polymorphisms compromise kinase activity, which may influence variation in MNM frequency and heart regenerative capacity in the human population.

P. Gan: None.

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A Novel Cellular and Genetic Approach to Investigate the Cardioprotective Role Played by Endothelial Nitric Oxide Synthase in Myocardial Infarction

Carmine Gentile, The Univ of Sydney, St Leonards, Australia; **Scott Kesteven**, Jianxin Wu, Victor Chang Cardiac Res Ctr, Sydney, Australia; **Christina Bursill**, The Univ of Adelaide, Adelaide, Australia; **Michael J Davies**, The Univ of Copenhagen, Copenhagen, Denmark; **Gemma Figtree**, The Univ of Sydney, St Leonards, Australia

The loss of regenerative properties in adult cardiomyocytes (CMs) is directly linked to their inability to proliferate. Following an extensive ischaemic event in an aged heart, fibrotic scar formation is the only repair process and eventually heart failure develops. However, molecular and cellular cues in the neonatal heart support that cardiac regeneration is possible in presence of proliferating CMs. Based on previous studies demonstrating that endothelial nitric oxide synthase (eNOS) regulates proliferation in both endothelial cells (ECs) and CMs, we hypothesized that eNOS signaling could play a cardioprotective role. To test our hypothesis, we injected different combinations of co-cultured ECs and CMs in the LV muscle wall of MI mice (permanent LAD ligation). First, injected cells were isolated from either WT or KO eNOS neonatal mice and then co-cultured to form 3D vascularized cardiac spheroids (VCSs), which were eventually transplanted in adult MI mice on the day of the procedure. Control infarcted animals received media-only (vehicle). Other mice received a suspension of co-cultured VCSs in media as follows: *i*) WT CMs and ECs; *ii*) WT CMs and KO ECs; *iii*) KO CMs and WT ECs. Following 28 days, injection of WT cells increased the ejection fraction (EF%) by 20% compared with control animals (61%±4% and 41%±11%, respectively). When eNOS was absent in either CMs or ECs, the EF% was 40%±5% and 46%±2%, respectively, suggesting that the eNOS-mediated protection is dependent on its presence in both cells. Histological analyses confirmed the presence of WT VCSs in MI mice, contributing to a thicker wall thickness compared to vehicle MI mice. No VCSs were observed in the LV wall when KO cells were injected. Therefore, our results strongly suggest that eNOS may play a major role via both an autocrine (CMs) and paracrine (ECs) mechanism. Current studies are focusing on further evaluating the mechanism(s) for this eNOS-mediated protective role. To our knowledge, this is the first study combining cellular and genetic approaches to evaluate the cardioprotective role of eNOS in the heart. A better understanding of this mechanisms may have significant impact for the development of improved molecular and cell therapeutics (including stem cells) for heart failure patients.

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Poster Session 1 and Reception

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Systemic Analysis and Discovery of Embryonic Stem Cell-derived Exosomal Long Non-coding RNAs as Potential Therapeutic Modulators of Myocardial Repair

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Cardiovascular disease is the leading cause of death in the U.S. Exogenous stem cell therapy has emerged as a novel therapeutic approach and various stem cell types, including embryonic stem cells (ESCs), bone marrow stem cells and mesenchymal stem cells, have been studied for cardiac regenerative cell therapy. Even though there was great enthusiasm with promising initial results in pre-clinical disease models, stem cell-based therapy demonstrated modest benefits in clinical trials. Emerging evidence suggests that stem cells can secrete a large number of soluble factors to evoke a resident cell response for tissue regeneration. In this perspective, stem cells-derived exosome therapy has been recently put forward as an alternative cell-free modality for cardiac repair after myocardial infarction (MI). Our previous study indicated that ESC-

derived exosomes (ESC-exo) promote endogenous cardiac repair and enhanced cardiac function following MI. However, the mechanisms underlying ESC-exo mediated cardiac repair and regeneration still need to be fully elucidated. RNA-sequencing data on ESC-exo revealed that non-coding RNAs, including long non-coding RNAs (lncRNA), are selectively sorted into exosomes and highly enriched compared to control fibroblasts. Unlike exosomal miRNA, exosomal lncRNAs have not been well-studied in the context of tissue repair. To investigate the reparative role of lncRNA in protecting against MI, we first systematically analyzed and discovered ESC-derived exosomal lncRNAs *in silico*. We analyzed novel lncRNAs from ESC- and MEF-exo based on differential expression by microarray analysis, lncRNA classification, human homology and non-coding potential prediction. For lncRNAs enriched in the ESC-exo population, we selected 2 potential lncRNAs of interest for further study: Gm4890 and XLOC 01990, which are confirmed for expression by qRT-PCR results. Gm4890 and XLOC are mainly located in nucleus rather than cytoplasm implicating their function on transcriptional regulation. Ongoing studies are evaluating the functional roles for these ESC-exo enriched lncRNAs.

G. Huang: None.

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Efficacy Evaluation of Transplantation of Three-Dimensional Adipose-derived Stem Cell Sheet With Enhanced Angiogenesis Into Cardiovascular Disease

Hyung Joon Joo, Korea Univ Anam Hosp, Seoul, Korea, Republic of; **Jong-Ho Kim**, Chi-Yeon Park, Korea Univ Coll of Med, Seoul, Korea, Republic of; **Soon Jun Hong**, **Do-Sun Lim**, Korea Univ Anam Hosp, Seoul, Korea, Republic of

Integration of adipose-derived stem cells (ASCs) and cell sheet engineering technology has received much interest in the regenerative medicine. First of all, sphere formation of ASCs (sph-ASCs) were successfully produced by poly-2-hydroxyethyl methacrylate (poly-HEMA)-coated plates as we previously reported. Sph-ASCs showed increased expression of angiogenic growth factors and cytokine secretion compared to adherent ASCs. Significantly increased expression of HIF-1 α and decreased expression of cleaved caspase-3 were detected in sph-ASCs compared to adherent ASCs. Moreover, ASC sheets were formed using thermo-responsive plates and sph-ASCs were seeded to produce ASC sheets covered with sph-ASCs (sph-ASC sheets). After 48 hrs of incubation, the cross-section of sph-ASC sheets revealed mixed and multi-layered of sph-ASCs and ASC sheets. Sph-ASC sheets showed the positive expression of HIF-1 α and angiogenic markers such as FGF-2, CD31, PDGFb, and CD144, especially higher in sph-ASC-covered sites. In addition, cytokine arrays of secretome revealed that sph-ASCs and sph-ASC sheets showed augmented angiogenic potential compared to adherent ASCs. Furthermore, four groups of AMI induction only (sham), intramyocardial injection of sph-ASC, ASC sheet transplantation, and sph-ASC sheet transplantation were compared using acute myocardial infarction (AMI) rat models. After 6 weeks of transplantation, echocardiography revealed that significantly improved ejection fraction and fraction shortening in sph-ASC sheet group compared to sham, sph-ASC, and ASC sheet groups. In conclusion, transplantation of sph-ASC sheets showed enhanced neovascularization in AMI animal models. These results indicated that the use of ASC sheets as a novel tissue engineering approach to improve the cardiac and vascular function.

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Identification of a Key Regulator for Activating Bmp Signalling and Promoting Mesodermal Differentiation in Induced Pluripotent Stem Cells

Yoshikazu Kishino, Shinsuke Yuasa, Keiichi Fukuda, Keio Univ Hosp, Tokyo, Japan

[Background] Recently, patient's own induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) are expected to be cell sources for regenerative therapy. In stem cell differentiation into cardiomyocytes, activation of BMP and Activin signalings in early phase and suppression of Wnt signalling in late phase are important. Although small molecule chemical compounds and recombinant proteins are currently used for differentiation, however, it is better that all recombinant proteins are replaced by chemical compounds, which cost cheaper and improve the efficiency of iPSC-CM differentiation. Several chemical compounds inhibiting Wnt signalling have been reported in previous researches. On the other hand, BMP signalling activator has not been developed because there is little knowledge of factors which strongly modify BMP signalling

in various cells. To address this issue, we tried to screen factors activating BMP signalling strongly. **[Methods and Results]** To develop the screening system, we used luciferase assay including BMP-responsive element (BRE) and confirmed that our screening system promptly responds to BMP-4 stimulation. Then, we focused on the molecular target, which has a strong impact on BMP signalling. We screened the possible BMP signal regulators which were reported in previous papers by RNA interference in HEK293T cells. Among many candidate regulators, we found that knockdown of *Tripartite motif 33 (TRIM33)*, E3 type ubiquitin ligase, significantly and reproducibly enhanced BRE-luciferase assay and induced the expression of *Id-1* gene as innate reporter gene of BMP signalling. Next, we investigate whether the suppression of *TRIM33* promotes the differentiation of iPSCs into mesodermal cells. We confirmed that *TRIM33* knockdown by RNA interference promoted the expression of mesodermal marker *Brachyury T* and epithelial-mesenchymal transition marker *Slug2* in 2-dimensional cardiac differentiation. **[Conclusions]** We successfully detected the factor activating BMP signalling. *TRIM33* inhibition enhanced the expression of *Id-1* gene and promoted mesodermal differentiation. In the future, chemical compounds targeting *TRIM33* may be useful for regenerative medicine as BMP signalling activators.

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New Approach for Directly Reprogrammed Endothelial Cells

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Rationale: For therapeutic neovascularization through cell therapy to treat cardiovascular diseases, recently, we successfully generated reprogrammed ECs, directly from human dermal fibroblasts (HDF) with the ETV2 transcription factor. Although functional ECs were generated, ETV2 was delivered lentiviral vector, which is clinically unsuitable. **Objective:** In this study, we aimed to generate clinically compatible rECs, by employing an adenoviral vector to minimize genetic integration. Furthermore, to prolong the cell retention and improve cell survival, we applied synthetic biomaterial, polypeptide amphiphile (PA) nanomatrix gel and tested for experimental cell therapy in animal models. **Methods and Results:** After we transduced HDFs with Ad-ETV2 (Ad-ETV2-HDF), EC gene expression was measured by qRT-PCR and flow cytometry. We found that Ad-ETV2-HDFs displayed a cobblestone-like morphology and showed notable induction of EC genes (CDH5, KDR, PECAM1). Flow cytometry analysis showed peak expression of CDH5 and KDR at D6 and continuous increase of PECAM1 over 9 days. Also, Ad-ETV2-HDFs took up acetylated LDL and bound lectin, suggesting that these cells possess functional EC characteristics. To enrich cells showing EC characters, Ad-ETV2-HDFs were sorted for KDR by FACS at day 6. qRT-PCR showed substantial enrichment of EC-specific gene expression in KDR⁺ cells. Only KDR⁺ cells (reprogrammed ECs, rECs) formed tube-like structures on Matrigel. In addition, immunostaining confirmed high expression of EC marker proteins in rECs. Next, we tested the functionality of rECs in hindlimb ischemia mouse model. At 4 weeks after injection of 4×10^5 rECs into the ischemic hindlimb, we observed rECs incorporated into host blood vessels or resided perivascular regions. Furthermore, we found more rECs in the tissues and were directly incorporated into the vessels when they were encapsulated in PA nanomatrix gel compared to bare rEC-injected mice. **Conclusions:** Together, these data strongly support the generation of Ad-ETV2-induced rECs (Ad-rECs) and enhanced retention of rECs by PA nanomatrix gel in tissue. With this study, we can develop advanced concept and techniques for clinical application of rECs and establish a new platform for future cell therapy.

S. Lee: None. **D. Chen:** None. **Y. Yoon:** None.

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Surface Modification of Stem Cell Exosomes Myocardial Infarction Specific Peptides for Non-invasive Delivery to Ischemic Myocardium

Vandana Mallareddy, Temple Univ, Philadelphia, PA

Exosomes are under extensive investigation as a next generation therapeutic agent for clinical applications in various diseases. Stem cell-derived exosomes have been shown to provide post-MI cardiac repair in small and large animals, however, their multi-dose application is limited to single intra-myocardial delivery. In this study, we investigated the heart homing peptide, CRPPR (Cys-Arg-Pro-Arg) modified mouse Embryonic Stem Cells (mESCs) exosomes as efficient

strategy for intravenous delivery of exosomes to ischemic myocardium. In order to target the infarcted heart, we fabricated the exosomes with CRPPR peptide. These peptides are MI-specific peptides, identified by phage display in earlier studies. For this study, we constructed a plasmid expressing fusion protein consisting of Green Fluorescent Protein (GFP), CRPPR and a truncated lactadherin that gets expressed on the surface of exosomes. The plasmid was transfected into the mESCs, followed by puromycin selection. The modified CRPPR exosomes released from the cells were collected by ultracentrifugation and displayed intense GFP- fluorescence indicating efficient CRPPR tagging of exosomes. The CRPPR exosomes were characterized by Nanosight, Electron microscopy and western blotting. MI was performed by LAD ligation and GFP-CRPPR tagged exosomes were intravenously (i.v) injected at a dose of 10^{10} particles per mouse and their tissue distribution was evaluated. Interestingly, unmodified exosomes were rapidly cleared from the circulation within 1 hr after i.v injection in mice whereas exosomes modified with CRPPR were still detectable. At tissue level, there was an increased accumulation of the CRPPR exosomes in the heart compared to the unmodified exosomes. In conclusion, we report developing heart homing exosomes that may efficiently delivers the therapeutic payload of embryonic stem cells to the ischemic region of the heart.

V. Mallareddy: None.

Poster Session 1 and Reception

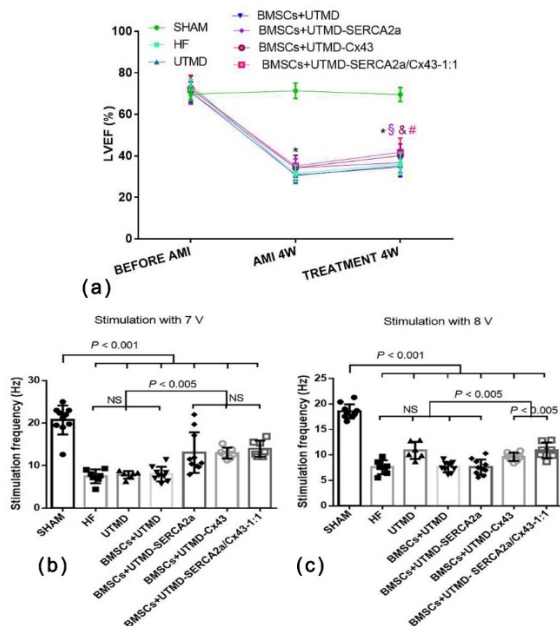
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Ultrasound Mediated Transfection of SERCA2a and Cx43 Genes Assisted Bone Marrow Stem Cells Transplantation to Improve Heart Failure and Ventricular Arrhythmia After Myocardial Infarction

Yuming Mu, Wei Wang, Baihetiya Tayier, Lina Guan, First Affiliated Hosp of Xinjiang Medical Univ, Urumqi, China

Objective We aimed to investigate the possibility of combining BMSCs transplantation (BMSCs T) with sarcoplasmic reticulum Ca²⁺ATase (SERCA2a) and connexins 43 (Cx43) genes transfection mediated by ultrasound-targeted microbubble destruction (UTMD) to treat heart failure (HF) and ventricular arrhythmia (VA) after MI. **Methods** HF rats received BMSCs T 4 weeks after AMI. Two days later, microbubbles carried SERCA2a and Cx43 genes were injected via tail vein, then ruptured by irradiation. 63 rats were divided into 7 groups : ① SHAM; ② HF; ③ UTMD; ④ BMSCs + UTMD; ⑤ BMSCs + UTMD-SERCA2a; ⑥ BMSCs + UTMD-Cx43; ⑦ BMSCs+ UTMD-SERCA2a/Cx43-1:1. Left ventricular ejection fraction (LVEF), cardiac tolerance to electrical stimulation were examined 28 days after BMSCs T. **Results** The LVEF of gene transfection groups were improved than before: BMSCs + UTMD-SERCA2a (39.6±3.6% vs. 34.3±4.3%); BMSCs + UTMD-Cx43 (40.1±5.6% vs. 35.1±5.2%); BMSCs + UTMD-SERCA2a/Cx43-1:1 (41.7±6.8% vs. 35.1±5.2%) (P < 0.05). Stimulating with 7V voltage, the frequencies of inducing VA in BMSCs + UTMD-SERCA2a, BMSCs + UTMD-Cx43 and BMSCs + UTMD-SERCA2a/Cx43-1:1 were 13.07±4.77Hz, 13.94±1.29Hz and 13.97±1.91Hz, which were higher than HF and non-gene groups (P < 0.05). With 8V, the frequencies in BMSCs + UTMD-Cx43 and BMSCs+ UTMD-SERCA2a/Cx43-1:1 were also higher than other groups but SHAM (P < 0.05). The frequency of double-gene group was higher than single-gene groups with 8V (P < 0.05). **Conclusion** Our results suggest that BMSCs T assisted by UTMD-mediated SERCA2a and Cx43 genes transfection can improve HF and VA after MI, and especially for the stability of cardiac electrical activity with double-gene.



Y. Mu: None. **W. Wang:** None. **B. Tayier:** None. **L. Guan:** None.

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Isolation & Characterization of Heart-field Specific Cardiomyocytes

Arash Pezhouman, James L Engel, Ngoc B Nguyen, Rhys JP Skelton, Peng Zhao, Blake W Gilmore, Nicholas Hornstein, Reza Ardehali, ucla, Los Angeles, CA

Cardiovascular disease (CVD) is the leading cause of death worldwide. Human embryonic stem cell (hESC)-derived cardiovascular progenitors (CVPs) or cardiomyocytes (CMs) represent a promising candidate for cell-based therapies to treat CVD. Myocardial infarction leads to extensive CM death mainly within the left ventricle, which is predominantly derived from the first heart field (FHF) during embryonic development. We postulate that the generation of chamber-specific CMs may play a key role in the development of safe and efficacious regenerative therapy. As a first step, we generated and characterized a FHF-specific TBX5-TdTomato^{+*W*} hESC reporter line. We show that TBX5⁺ cells represent an enriched population of FHF CVPs that can give rise to CMs, endothelial, and smooth muscle cells *in vitro*. Interestingly, we observed that TBX5⁻ cells can also generate contractile CMs. Bulk RNA-sequencing analysis at different stages of development suggested that TBX5⁻ cells are enriched for second heart field (SHF) CMs. To enable prospective isolation of FHF and SHF CMs, we generated a double transgenic TBX5-TdTomato^{+*W*}/NKX2-5^{eGFP^W} hESC reporter line. We performed detailed electrophysiological, functional, and transcriptional studies to characterize the heart-field specificity of these hESC-derived CMs. Electrophysiological studies revealed that, despite the presence of atrial and ventricular action potentials (APs) in both FHF and SHF, there are significant differences in their AP duration and cycle length. In addition, both FHF and SHF CMs responded appropriately to adrenergic stimuli. Single-cell RNA sequencing analysis confirmed the absence of nodal genes within these populations and provided evidence of unique molecular signatures for isolating FHF- and SHF-like CMs. Finally, we identified CORIN as a novel cell surface marker for FHF CMs. Our studies provide a platform for investigating *in vitro* cardiovascular development, drug screening, and may facilitate a safe approach for cell therapy in heart disease.

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Investigating and Inhibiting Neutrophil Extracellular Trap Formation in the Heart

Michael N Sayegh, Lanfang Wang, Eric Y. Shin, Emory Univ, Atlanta, GA; Woojin M. Han, Georgia institute of Technology, Atlanta, GA; Milton E. Brown, Michael E. Davis, Emory Univ, Atlanta, GA; Andres J. Garcia, Georgia institute of Technology, Atlanta, GA; Rebecca D. Levit, Emory Univ, Atlanta, GA

Neutrophil extracellular traps (NETs) have been observed in multiple diseases of the cardiovascular system, such as myocardial infarction and atherosclerosis. We have recently shown that adenosine (ADO) inhibits NET formation and may be an important endogenous regulator. CD73 is expressed on many cell types and catalyzes the extracellular formation of ADO from AMP. Here, we first attempt to implicate NETs in cardiac injury, and we posit that human NETs negatively impact cardiac function by influencing native leukocyte recruitment and activation. Second, we prototype a CD73-functionalized hydrogel delivery vehicle for adenosine and test its function *in vitro*. We introduced NETs obtained from stimulated neutrophils into healthy hearts by intramyocardial injection in two-month-old rats. We used echo to assess cardiac function at days 0, 1 and 3 of injection. We measured the abundance of cardiac leukocyte subpopulations by flow cytometry at days 1 and 3 after injection, with immunostaining for CD45 (all leukocytes), myeloperoxidase (MPO, neutrophils), CD68 (macrophages), CD3 (T-cells), B220 (B-cells) and citrullinated histone 3 (citH3, NETs). To deliver adenosine as a NET antagonist, we designed a polyethylene (PEG) hydrogel composed of a 4-armed PEG incorporating a VPM protease-degradable crosslinker for cargo release, and an RGD peptide to enhance gel-tissue attachment. We tested gel polymerization by measuring storage and loss moduli at 4% and 6% PEG content (w/v) and 0, 1 μ M and 1mM RGD concentrations. We functionalized the gel with CD73 to test its adenosine production capability. We found a significant decrease in cardiac function assessed by global longitudinal strain at day 1 in the NET group compared to saline. By flow cytometry, we found an increase in neutrophils, macrophages and NET formation at day 1, while B- and T-cells were increased at day 3. We found that increasing the PEG content of our hydrogel, but not RGD, increases gel stiffness. We also found that the CD73 functionalized hydrogel successfully catalyzes the formation of adenosine *in vitro*. In conclusion, we

found that NETs negatively impact cardiac function, and successfully tested an adenosine hydrogel source in vitro. Next, we will study the effect of our gel on NETs in vivo.

M.N. Sayegh: None. **L. Wang:** None. **E.Y. Shin:** None. **W.M. Han:** None. **M.E. Brown:** None. **M.E. Davis:** None. **A.J. Garcia:** None. **R.D. Levit:** None.

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Screening for Developmental and Injury-Induced Genes That Facilitate Heart Regeneration

Akansha M Shah 75235, Miao Cui, Zhaoning Wang, Wei Tan, Ning Liu, Rhonda Bassel-Duby, Eric N Olson, UT Southwestern Medical Ctr, Dallas, TX

The adult mammalian heart has a limited capacity to regenerate upon injury, such as myocardial infarction (MI). MI in adult mouse hearts results in a reduced cardiac function, due to extensive loss of cardiomyocytes and formation of scar tissue by activated fibroblasts. In contrast, the newborn postnatal day 1 (P1) mouse heart is able to regenerate and restore its cardiac function following injury. However, this regenerative ability dramatically declines after P7. The molecular properties and mechanisms that facilitate neonatal heart regeneration remain unknown. To gain further insight into the realm of cardiac regeneration, our lab performed transcriptome analysis by bulk and single cell RNA-seq on P1 and P8 hearts at various time points following MI surgery. We also performed enhancer profiling by H3K27ac ChIP-seq. Coupling of both these approaches has uncovered two different gene sets that may promote the regenerative ability of neonatal hearts: developmental genes and injury-induced genes. Amongst the neonatal-specific developmental genes, *Igf2bp3*, a RNA-binding protein, and *Hic2*, a transcriptional regulator, have been shown to promote neonatal rat ventricular cardiomyocyte (NRVM) proliferation and are currently being characterized for their involvement in neonatal heart regeneration by using gain-of-function and loss-of-function in vivo approaches. Amongst the injury-induced genes, we identified mitogens secreted from macrophages that promote NRVM proliferation. This is in line with the differential immune response elicited by P1 hearts upon MI-injury. We have also shown that an epicardial secreted factor in P1 hearts can enhance human umbilical vein endothelial cell tube formation, indicative of its role in angiogenesis. In summary, by using a multi-layered genomic analysis, we identified various genes that are involved in different cellular responses during neonatal heart regeneration. This comprehensive analysis of the neonatal regenerative response provides insights into the molecular basis of neonatal heart regeneration, and can potentially identify genes that can be modulated to facilitate adult heart regeneration.

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Neonatal Mouse Heart Regeneration is Dependent on Mononuclear Diploid Cardiomyocytes and Paracrine IGF2 Signaling

Hua Shen, Univ of Southern California, Los Angeles, CA; Michaela Patterson, Medical Coll of Wisconsin, Milwaukee, WI; Peiheng Gan, **Henry M Sucov**, Medical Univ of South Carolina, Charleston, SC

In normal mice, injury to the newborn heart is efficiently regenerated, but this capacity is lost by one week after birth when most cardiomyocytes have become post-mitotic. Concurrently during the first week, most cardiomyocytes become polyploid. Despite this temporal association, the relation of polyploidization to neonatal heart regeneration has not been adequately examined. In this study, we find that IGF2, an important mitogenic factor in heart development, is required to support neonatal mouse heart regeneration. Following injury on postnatal day 1, absence of IGF2 abolished injury-induced cell cycle entry (as indicated by phospho-histone H3 staining) during the early part of the first postnatal week, when cardiomyocytes are still mononuclear and diploid. Consequently, regeneration failed despite the later presence of additional activities that support robust cell cycle entry 7 days following injury, a time when most cardiomyocytes are polyploid and no longer able to complete cytokinesis. IGF2 originates from the endocardium/endothelium lineage and is transduced by the insulin receptor; both features distinguish the action of IGF2 in neonatal heart regeneration from that in embryonic heart development. Regeneration in injured *Igf2*-deficient neonates was rescued by three different contexts that elevate the percentage of mononuclear diploid cardiomyocytes beyond postnatal day 7. Thus, IGF2 is the primary paracrine-acting mitogen for heart regeneration during the early postnatal period, and IGF2-deficiency unmask the dependence of this process on proliferation-competent mononuclear diploid cardiomyocytes.

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Endothelial-specific Overexpression of Metallothionein Prevents Diabetes Mellitus-induced Impairment in Ischemia Angiogenesis via Preservation of Hif-1 α /sdf-1 in Endothelial Progenitor Cells

Kai Wang, Univ Louisville, Louisville, KY; Xiaozhen Dai, Chengdu Medical Coll, Chengdu, China; Junhong He, Chengkui Yang, Kupper Wintergerst, Paul Epstein, Lu Cai, **Yi Tan**, Univ Louisville, Louisville, KY

Diabetes mellitus associated dysfunction of endothelial progenitor cells (EPCs) may contribute to dysregulation of endothelial regeneration. Whether oxidative protection of EPCs by an antioxidant, such as the protein metallothionein (MT) can have positive angiogenic effects and by what mechanisms remain unclear. Endothelial-specific MT overexpression (JTMT) mice were made diabetic by high fat diet followed by streptozocin administration (HFD/STZ). Diabetic hind limb ischemia (HLI) was established by femoral artery ligation. Bone marrow mononuclear cells (MNCs) collected from JTMT mice were transplanted into db/db mice with HLI. Blood reperfusion was monitored. High glucose and hypoxia conditions in culture were adopted to mimic diabetic ischemia. Compared with wild-type (WT) littermates, JTMT mice were resistant to diabetes-induced impairment in ischemia angiogenesis and blood reperfusion in HFD/STZ diabetes. Similarly, transplantation of MT overexpressing MNCs showed better therapeutic effect on db/db mice with HLI than transplantation of WT MNCs. These changes were accompanied by increased mobilization and infiltration of EPCs in JTMT mice, and enhanced incorporation of EPCs into capillaries in JTMT MNCs transplanted db/db mice. Furthermore, EPCs from JTMT mice exhibited augmented cell survival, tube formation, and migration capacities under diabetic ischemia-like conditions. Mechanistically, the expression of hypoxia-inducible factor 1 α (HIF-1 α) and the secretion of stromal cell-derived factor (SDF-1) in blood and expression in ischemic tissues were upregulated in JTMT and JTMT-MNC transplanted db/db mice, and in cultured JTMT EPCs, which were accompanied by marked amelioration of oxidative stress. However, MT-mediated elevation of SDF-1 and improvements of function in EPCs were all abrogated by siRNA knockdown of HIF-1 α expression without effect on the anti-oxidative capacity of MT. Endothelial-specific MT elevation is sufficient to protect against diabetes mellitus-induced impairment of ischemia angiogenesis by promoting EPC function. The benefits of MT are predominantly mediated by upregulation of the HIF-1 α /SDF-1 pathway.

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Creation and Characterization of Functional, Human Pediatric-Sized Cardiac Rings With Human iPSC-derived Cardiomyocytes

Karis R Tang-Quan, Camila Hochman-Mendez, Po-Feng Lee, Texas Heart Inst, Houston, TX; Jared F Mike, Lynntech Inc, College Station, TX; Luiz C Sampaio, Doris A Taylor, Texas Heart Inst, Houston, TX

Background: Cardiovascular disease results in a growing need for heart tissue replacement therapies and more heart donors. We seek to address this need using engineered cardiac constructs made with decellularized cardiac extracellular matrix (dECM) which is known to retain bioactive cues and preserve native macro- and micro-architecture.

Objective: We aimed to create beating cardiac rings by injecting left ventricular dECM with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and to evaluate function of the recellularized cardiac tissue.

Methods and Results: Human pediatric-sized rabbit hearts (~14g) were perfusion-decellularized through the coronary vasculature, and sectioned transversely to create ~1 mm-thick rings of decellularized left ventricle. One million hiPSC-CMs were injected into decellularized cardiac rings and stimulated electrically, mechanically, both, or neither. Cell survival, attachment, distribution, and ring contractility were evaluated after 7 days with immunofluorescence and light microscopy (n=6/group). Cells in cardiac rings had higher viability than previously reported in literature (57.6% \pm 3.8 vs 50%). Cardiac rings showed 63.4% \pm 4.4% cellularity relative to human heart tissue, and staining for integrin β 1 confirmed cell-matrix attachment. Spontaneous contraction frequency was within human physiologic range (45.7 \pm 4.4 beats/min), with an average maximum contraction displacement of 34.65 μ m. The effects of electrical, mechanical, and combined stimulation will also be presented.

Conclusion: Pediatric-sized cardiac rings can be made using hiPSC-CMs and cardiac dECM. The cardiac rings showed

sufficient cell survival, attachment, and distribution for contractility, so that these tissue rings can be scaled up to create functional bioartificial hearts with dECM. Stimulation increased both conduction velocity and contractility, suggesting that optimizing biophysical stimulation increases functional output of cardiac tissue.

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Organized 3D Neo-Angiogenic and Neo-Lymphangiogenic Vascular Networks for Cardiac Regeneration

Xi Lou, **Valarmathi T. Thiruvanamalai**, Jianyi Zhang, Univ of Alabama at Birmingham, Birmingham, AL

Introduction: Cardiovascular tissue engineering has been an area of intense investigation. The major challenge to these approaches has been the inability to vascularize and perfuse the *in vitro* engineered tissue constructs. Engineering a tissue of clinically relevant magnitude requires the formation of extensive and stable microvascular networks within the tissue. **Hypothesis:** Functional vascularized cardiac graft can be generated by the interaction of multipotent human mesenchymal stem cells (hMSCs) and human induced pluripotent stem cell-derived embryonic cardiac myocytes (hiPSC-eCMs) in a 3D collagen cell carrier (CCC) scaffold. **Methods and Results:** To achieve the above aim, we have developed an *in vitro* 3D functional vascularized cardiac muscle construct using hiPSC-eCMs and hMSCs. Initially, to generate the prevascularized scaffold, human cardiac microvascular endothelial cells (hCMVECs) and hMSCs were co-cultured on CCCs for 7 days under vasculogenic culture conditions. hCMVECs/hMSCs underwent maturation and differentiation characteristic of microvessel morphogenesis and formed extensive plexuses of capillary networks. Next, the hiPSC-eCMs and hMSCs were co-cultured onto these generated prevascularized CCCs for further 7 or 14 days in myogenic culture conditions. hiPSC-eCMs/hMSCs underwent maturation and differentiation, and demonstrated spontaneous rhythmic beating. The vascular and cardiac phenotypic inductions were analyzed at the morphological, immunological, biochemical, molecular and functional levels. Expression analyses of the differentiated cells revealed neo-angiogenesis and/or neo-lymphangiogenesis as well as neo-cardiomyogenesis. **Conclusions:** Our unique 3D co-culture system provides us the apt *in vitro* functional prevascularized 3D cardiac graft that can be utilized for myocardial repair and/or regeneration.

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Tip60 Depletion Promotes Cardiomyocyte Proliferation and Attenuates Ischemic Injury in the Adult Heart

Xinrui Wang, Tina C. Wan, Carri Lupton, Mitchell Harrison, John Lough, John A. Auchampach, Medical Coll of Wisconsin, Milwaukee, WI

The pathogenesis of myocardial infarction (MI) is largely attributed to the loss of cardiomyocytes (CMs) and their insufficient regeneration. Inducing the proliferation of pre-existing CMs has emerged as a potential therapeutic strategy for cardiac repair. Results in our laboratory indicate that Tip60 (Tat-interactive protein 60 kD), a pan-acetylase protein encoded by the *Kat5* gene, inhibits CM proliferation consequent to its induction of the DNA damage response (DDR) at neonatal stages, which has recently been shown to cause CM replicative senescence. To determine whether Tip60 depletion permits re-entry of adult CMs into the cell-cycle and confers protection from MI, we are employing a line of *Kat5*^{fllox/fllox} mice wherein Tip60 is conditionally and specifically depleted in CMs via tamoxifen-induced activation of a *Myh6*-driven merCremer transgene. In uninjured hearts, Tip60 depletion results in transient thickening of the left ventricular walls, accompanied by markedly increased expression of G₂/M-phase cell cycle regulators (cyclins A2 & B1, Cdk1) and de-differentiation markers (Myh7, Osm, OsmR, Runx1), diminished CM size, decreased expression of cell-cycle inhibitors (p27, Meis1), and remarkable increases in Ki67 and pH3-positive CMs as well as non-CMs. In hearts infarcted by permanent ligation, tamoxifen administration increases fractional shortening, ejection fraction, and anterior wall thickening within 7 days, conditions that are sustained for at least 18 additional days, when reduced scarring is indicated by trichrome staining. Taken together, these results indicate that Tip60 depletion in adult heart may preserve cardiac performance after MI by inducing CM regeneration.

These findings should help advance our understanding of the molecular mechanisms that keep CMs in replicative senescence, establishing a possible therapeutic target for maintaining and restoring cardiac muscle after MI.

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Dexamethasone Inhibits Regeneration and Causes Ventricular Aneurysm in the Neonatal Porcine Heart After Myocardial Infarction

Lei Ye, Zhonghao Tao Tao, Sze Jie Loo, Liping Su, Shihua Tan, Desiree Abdurrachim, Natl Heart Ctr Singapore, Singapore, Singapore; Janise Lalic, Teck Hock Lee, Singapore Bioimaging Consortium, Singapore, Singapore; Rusan Tan, Stuart Alexander Cook, Natl Heart Ctr Singapore, Singapore, Singapore

Background: The adult mammalian heart has limited ability to repair itself following injury. Zebrafish, newts and neonatal mice can regenerate cardiac tissue, largely by cardiac myocyte (CM) proliferation. More recently, we demonstrated that the hearts of neonatal pigs (2-day old) have regenerative capacity, likely driven by cardiac myocyte division, but this potential is lost immediately after birth. The current study aims to determine whether acute inflammation is required for CM regeneration after myocardial infarction (MI). **Methods:** We examined the role of acute inflammation on regenerative capacity of the neonatal pig heart (ages: 2 days postnatal) by intramuscular injection of dexamethasone (Dex) during the first week post-MI surgery. Myocardial scar and left ventricular function were determined by cardiac magnetic resonance (CMR) imaging. Bromodeoxyuridine pulse-chase labeling, histology, and immunohistochemistry were performed to study CM cytokinesis and to quantify myocardial fibrosis. **Results:** After MI, there was early and sustained recovery of cardiac function and wall thickness in the absence of fibrosis in 2-day old pigs. In contrast, piglets received Dex injection developed ventricular aneurysm and did not recover function. Dex limited CM cytokinesis via inhibiting aurora-B protein expression and caused mature scar and thinned walls at infarct site. **Conclusions:** Dex suppresses acute inflammation, inhibits CM regeneration, and causes ventricular aneurysm in neonatal pig hearts after MI.

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Human Highly Proliferative Cells Acquire Endothelial Phenotype and Promote Healing After Experimental Myocardial Infarction

Haifeng Yin, Sergey Ryzhov, Maine Medical Ctr Res Inst, Scarborough, ME; Michael P Robich, Reed Quinn, Robert S Kramer, Maine Medical Ctr, Portland, ME; Calvin P.h Vary, Maine Medical Ctr Res Inst, Scarborough, ME; Douglas B Sawyer, Maine Medical Ctr Res Inst, Portland, ME

Introduction: The human adult heart contains a subset of mesenchymal cells characterized by high proliferative potential and capability to differentiate into different cell types. We recently demonstrated that a high level of ErbB2 expression is associated with differentiation of human highly proliferative cells (hHiPC) into endothelial cells in vitro. Based on our findings, we hypothesized that ErbB2^{high} hHiPC play a critical role in early revascularization and repair of injured heart. To test this hypothesis, we performed xenotransplantation of human ERBB2^{high} hHiPC into mouse hearts and analyzed the phenotype of transplanted cells and cardiac function on day 7 after experimental myocardial infarction (MI).

Methods: The effect of ErbB2^{high} hHiPC was tested in immunodeficient NSG mice. MI was induced by permanent ligation of the left coronary artery. Human ERBB2^{high} hHiPC (2.5×10^5 cells) were injected into the peri-infarct zone immediately after MI. Echocardiography was performed on unsedated mice before and on days 7 after MI. Examination of transplantation efficiency and hHiPC phenotype was performed using flow cytometric analysis of cell suspension obtained from left ventricles (LV).

Results: We found that up to 25% of cardiac endothelial cells were represented by cells that originated from hHiPC, as identified by specific expression of human CD31, in mouse LV, on day 7 after MI. The total number of cells expressing mouse or human endothelial cell markers, CD31 and CD105, within non-immune cardiac cell population was higher in mice that received injection of hHiPC compared to control mice (PBS injection) (4.7 vs. 2.9×10^5 cells, hHiPC vs. PBS, $p=0.026$).

Echocardiographic analysis revealed that mice which received an injection of ERBB2^{high} cells demonstrated significantly improved cardiac function compared to control mice injected with PBS (fractional shortening: 21.6% vs. 15.3% for hHiPC vs. PBS, $p=0.023$).

Conclusion: ERBB2^{high} hHiPC survive, undergo endothelial cell differentiation in vivo, and contribute to the pool of cardiac endothelial cells after experimental myocardial infarction. A population of ErbB2^{high} hHiPC obtained from adult human hearts can be used to improve the revascularization of injured myocardium or other tissues.

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Lin28 Enhances Cardiac Progenitor Cell Ability to Repair the Heart by Reprogramming Cellular Metabolism

Antonia Yuko, Justin Kurian, Nicole Kasatkin, Kelsey Busch, Fares AlHawaj, Temple Univ Sch of Med- Ctr for Metabolic Disease Res, Philadelphia, PA; Lena Ma, Temple Univ Sch of Med- Cardiovascular Res Ctr, Philadelphia, PA; Mohsin Khan, Temple Univ Sch of Med- Ctr for Metabolic Disease Res, Philadelphia, PA; Sadia Mohsin, Steven Houser, Temple Univ Sch of Med- Cardiovascular Res Ctr, Philadelphia, PA; Hong Wang, Temple Univ Sch of Med- Ctr for Metabolic Disease Res, Philadelphia, PA

Rationale: The adult Heart is largely a dormant organ supporting limited cellular turnover. In contrast, neonatal cardiac tissue proliferates and is capable of regeneration while operating under a specialized metabolic state. During transition to adulthood, cardiac metabolism undergoes a rapid shift that coincides with termination of regenerative processes. Whether altering cardiac metabolism recapitulates regenerative potential remains untested. Recently, introduction of Lin28, a metabolic regulator of pluripotency, enhances tissue repair after injury. Nevertheless, there are no studies characterizing the effect of Lin28 on cardiac repair.

Objective: Determine the effect of Lin28 on cardiac progenitor cell function and cardiac repair after injury.

Methods and Results: Lin28 expression coincides during heart development with c-kit and declines postnatal with complete abrogation in 3-week-old adult heart as measured by qRT-PCR and immunohistochemistry. CPCs were engineered with Lin28-GFP (CPCLin) lentivirus GFP expressing CPCs were used as controls (CPC-G). CPCLin demonstrated increased proliferation measured by CyQuant compared to CPC-G, concurrent with decreased apoptosis. Interestingly, CPCLin demonstrated a significant increase in lactate production, pyruvate kinase activity, glucose uptake together with enhancement of glycolysis and expression of glycolytic enzymes compared to CPC-G. Oxidative metabolism was also upregulated together with increased intracellular ATP in CPCLin compared to controls. Additionally, CPCLin demonstrated significantly reduced ROS generation as measured by CM-H₂TMROS based FACS analysis compared to CPC-G. To determine in vivo efficacy, CPCLin and CPC-G were transplanted in the heart after myocardial infarction. CPC-Lin hearts showed significant increase in cardiac structure and function 8 weeks after MI. Increased persistence and proliferation of transplanted CPC-Lin cells together with reduced apoptosis were observed in CPCLin hearts compared to control CPC-G hearts 2 days after transplantation.

Conclusions: Lin28 modification of CPCs reprograms cellular metabolism in CPCs enhancing proliferation and survival including ability to repair the heart after myocardial injury.

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Endogenous Cardiomyocyte Dedifferentiation and Cycling Revealed by Single-Cell Imaging and Single-Nucleus Transcriptomic Analysis

Yiqiang Zhang, Nuria Gago-Lopez, Ning Li, Zhenhe Zhang, Naima Alver, William R MacLellan, Univ Washington, Seattle, WA

Although the mammalian heart had been recognized as a post-mitotic non-regenerative organ, recent evidence demonstrated a measurable cardiomyocyte (CM) renewal at low rates in normal hearts and augmented in post-injury

myocardium. However, the resources and cellular processes of such a new CM formation, and the underlying molecular programs remained largely undetermined. While CMs in neonatal hearts or adult CMs in tissue culture can undergo (partial) dedifferentiation and proliferation, the roles of *in vivo* CM dedifferentiation and cycling were unclear. Here, we report a multi-reporter transgenic mouse models featuring efficient adult CM (ACM) genetic cell fate mapping and real-time CM dedifferentiation reporting. In this triple-transgenic model, upon tamoxifen-induced gene recombination, only ACMs switched from expressing red fluorescent protein (RFP) to green fluorescent protein (GFP), and non-CMs remained expressing RFP. The nuclear blue fluorescent protein (BFP) signal was expressed in normal mature ACMs but reduced in neonatal or cultured dedifferentiated ACMs. Hence, all 4 plausible modes of new CM formation can be visualized using this model. Using ImageStream for single-cell imaging analysis, we demonstrated that non-myocytes (including putative cardiac progenitor cells) contributed negligibly to new ACM formation at baseline or after myocardial infarction (MI). In contrast, there was a significant increase in dedifferentiated (e.g. GFP⁺BFP⁻) CMs in post-MI hearts compared to the sham. Cell cycling activity was enhanced within the dedifferentiated CM population compared to normal ACMs (GFP⁺BFP⁺). Furthermore, we applied massive whole-heart single-nucleus RNA-seq to dissect the transcriptomes of dedifferentiated CMs. We found that CMs in post-MI hearts identified with dedifferentiation reporter had significant down-regulations of genes in networks for hypertrophic growth, cardiac contractile functions and metabolism, but up-regulation in signaling pathways for cell survival, active cell cycle and proliferation. Therefore, cellular dedifferentiation may be an important prerequisite for endogenous CM cell cycling and proliferation and explain the measurable cardiac myogenesis seen after MI.

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Atg7-Dependent Activation of Mitochondrial Autophagy in Cardiomyocytes

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Introduction: Autophagy-related protein 7 (Atg7) has been studied for its role in cellular macroautophagy induction and regulation in different cell systems. We have previously reported that Atg7 regulates cardiac macroautophagy. However, whether Atg7 plays any role in the initiation and regulation of mitochondrial autophagy (mitophagy) in cardiomyocytes remains elusive. **Objective:** We investigated subcellular localization and molecular function of Atg7 in the regulation of mitophagy through a series of *in vitro* and *in vivo* experiments. **Methods and Results:** We isolated mitochondria from mouse hearts by subcellular fractionation and conducted biochemical experiments which revealed localization of Atg7 on the mitochondrial membrane. Next, we co-stained for Atg7 in isolated heart mitochondria stained with Mitotracker dye and found Atg7 localizes to mitochondrial membrane. To elucidate molecular function of Atg7 on mitophagy, adenovirus-mediated Atg7 overexpressed cultured neonatal rat cardiomyocytes (NRCs) were treated with mitophagy inducer carbonyl cyanide 3-chlorophenylhydrazone (CCCP) *in vitro* and stained with organelle-specific mitochondria and lysosome dyes to count mitophagy fluorescent dots by confocal microscopy. Atg7 overexpressed NRCs exhibited an increase in mitophagy dots than β -gal treated NRCs following CCCP induction. Further, we have treated cardiac specific Atg7 overexpressed (Atg7 Tg) mice and littermate non-transgenic (Ntg) mice with CCCP to assess mitophagy induction *in vivo*. Immunoblotting confirmed enhanced accumulation of mature autophagolysosomal marker LC3-II protein in mitochondrial fraction from Atg7 Tg hearts. **Conclusions:** Our *in vitro* and *in vivo* findings indicate that Atg7 localizes to the mitochondrial membrane, regulates recruitment and initiation of mitophagy machinery, and facilitates mitophagy in cardiomyocytes.

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IL4 Increases Phagocytosis of Necrotic Cells in a Cd36-dependent Manner

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Macrophages are a population of immune cells with high heterogeneity, which can acquire distinctive phenotypes under different stimuli, and each subtype has specific function in immune surveillance and tissue homeostasis. Different with traditional activated macrophages, which are mainly primed by pathogen motifs or inflammatory stimuli and are involved in pathogen clearance and immune defence, alternatively activated macrophages by Th2 cytokine IL4 have been shown to be functioning in cell debridement, promising a crucial role in tissue repair and tissue homeostasis. However, the mechanisms underlying how IL4 signaling promotes cell debridement have not been fully understood. To address this question, we aim to investigate whether and how IL4 signaling promotes necrotic cell clearance, which is the primary source of cell debris and share similar biological properties. In our study, we demonstrated that IL4 enhances phagocytosis of necrotic cells, and the downstream IL4R α -STAT6 axis signaling pathway is involved. We also found that CD36, a class B scavenger receptor, may be, at least to some extent, responsible for IL4-accelerated phagocytosis. Beside increasing engulfment of necrotic cells, IL4 treatment also enhances the processing of engulfed material, indicating multi-dimensional effects of IL4 on phagocytosis activity of macrophages. By uncovering the molecular mechanisms of IL4 promoting phagocytosis activity of macrophages, we are able to better understand the role of IL4 signaling in cell debridement, and this may help to develop new strategies in promoting tissue repair and regeneration in chronic diseases.

M. Chen: None.

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Liproxstatin-1 Treatment Protects the Myocardium Against I-R Injury By Decreasing VDAC1 Levels and Increasing GPX4 Activity

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Introduction: Ferroptosis is a non-apoptotic form of cell death characterized by iron-dependent accumulation of lethal lipid reactive oxygen species (ROS) and liproxstatin-1 is a potent inhibitor of ferroptosis. We investigated whether Liproxstatin-1 can protect the heart against ischemia/reperfusion (I/R) injury and determined the mechanism underlying cardioprotection. **Methods:** Isolated hearts from C57BL/6J mice were subjected to 35 min ischemia followed 120 min reperfusion and treated with Liproxstatin-1 (200nM). Myocardial infarct size was assessed by TTC staining at the end of reperfusion. Mitochondrial integrity was measured by analyzing cristae morphology and ROS production was assessed using Amplex red method. The calcium retention capacity (CRC) required to induce mitochondrial permeability transition pore (mPTP) opening was measured using calcium green dye. The levels of mitochondrial proteins cyclophilin D (CypD), Glutathione peroxidase 4 (GPX4), VDAC1, 2 and 3 were assessed by Western blot. And GPX4 activity was measured using a kit. **Results:** We found that liproxstatin-1 treatment decreased myocardial infarction size, ROS production, and VDAC1 expression as well as preserved mitochondrial structural integrity by protecting cristae from damage. These mitochondrial effects of liproxstatin-1 were associated with the increase in GPX4 levels and activity. But, we also found that liproxstatin-1 did not affect the CypD, VDAC2, and VDAC3 expression as well as mitochondrial CRC. **Conclusion:** Together, these results indicate that liproxstatin-1 treatment protects myocardium against I/R injury by increasing GPX4 activity resulting in the decrease in mitochondrial ROS production; preserving mitochondrial structure as well as a decrease in VDAC1 (but not VDAC 2 and 3) expression. Liproxstatin-1 cardioprotective action does not involve the regulation of the mPTP opening. These results point to the cardioprotective role of this anti-ferroptosis compound after I/R.

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NFkB Promotes Oxidative Stress-induced Necrosis and Ischemia Reperfusion Injury Through NRF2-ARE Pathway

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Objective: Despite the well-studied pro-survival function of nuclear factor- κ B (NF κ B), recent studies suggest that NF κ B may also play a pathogenic role in myocardial ischemia injury and adverse remodeling. This study aims to define a new pro-cell death role of NF κ B in response to oxidative stress and the functional implications in ischemia reperfusion (I/R) injury. **Methods and Results:** We identified an unexpected pro-cell death role of NF κ B in oxidative stress-induced necrosis, and provide new mechanistic evidence that NF κ B, in cooperation with HDAC3, negatively regulates NRF2-ARE anti-oxidative signaling through transcriptional silencing. Genetic deletion of NF κ B-p65 inhibits, whereas activation of NF κ B promotes, oxidative stress-induced cell death and HMGB1 release, a biomarker of necrosis. Moreover, simulated ischemia reperfusion (sI/R) and doxorubicin (Dox) treatment both induce NF κ B-luciferase activity in cardiomyocytes, and inhibition of NF κ B diminishes sI/R- and Dox- induced necrosis. Importantly, NF κ B negatively regulates NRF2-ARE activity and the expression of anti-oxidant proteins. Mechanistically, co-immunoprecipitation reveals that p65 is required for the association between NRF2 and HDAC3 and transcriptional silencing of NRF2-ARE activity. Further, the ability of HDAC3 to repress NRF2-ARE activity is lost in p65^{-/-} cells. The HDAC inhibitor TSA and NF κ B inhibitor BMS-345541 both increase NRF2-ARE activity and promote cell survival following sI/R. *In vivo*, NF κ B transcriptional activity in the heart is significantly elevated after I/R injury, which is abolished by cardiac-specific deletion of p65. Moreover, ablation of p65 using p65^{fl/fl}/Nkx-Cre mice reduces myocardial infarct size after acute I/R, and prevents chronic remodeling and contractile dysfunction after myocardial infarction. **Conclusions:** Our results identified NF κ B as a key regulator of oxidative stress-induced necrosis by suppressing the NRF2-ARE anti-oxidant pathway through an HDAC3-dependent mechanism. Ablation of NF κ B-p65 attenuates oxidative stress-induced necrosis and I/R injury, suggesting a new pathogenic role of the NF κ B pathway, and thus a therapeutic target, in myocardial ischemic injury and remodeling.

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Hypoxia-Induced Cardiomyocyte Mitophagy and Mitochondrial Permeability Transition are Inhibited by Bnip3 Phosphorylation

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Bnip3 is a hypoxia-inducible initiator of cardiomyocyte cell death and has also been implicated as a mitochondrial receptor for the cellular mitophagy machinery. Previous work from our group demonstrates that the prostaglandin E1 analogue, misoprostol, prevents hypoxia-induced mitochondrial dysfunction and is a potent activator of PKA. We hypothesize that misoprostol alters the phosphorylation status of Bnip3, inhibiting its ability to induce cardiomyocyte mitophagy, mitochondrial dysfunction and cell death. Using a rodent model of neonatal hypoxia, in combination with rat primary ventricular neonatal cardiomyocytes (PVNC's) and H9c2 cells, we assessed the effect of hypoxia and misoprostol drug treatments on mitochondrial function, mitophagy, and cell viability. In postnatal day 5 rats, hypoxia caused a 30% increase in the concentration of serum cardiac troponin-I, a clinically relevant marker for cardiomyocyte cell death, which was absent with the addition of misoprostol drug treatments (n=3). Using PVNC's we further demonstrated that hypoxia reduced measures of mitochondrial function including membrane potential (47%) and maximal respiration (46%), which were restored back to control levels with the addition of misoprostol (p<0.01). Furthermore, hypoxia induced a 36% increase in mitophagy, concurrent with a 210% increase in mitochondrial permeability transition, both of which were reversed with misoprostol drug treatment (p<0.01). Using a combination of mass spectrometry and mutagenesis, we also show that PKA directly phosphorylates the transmembrane domain of Bnip3 to inhibit its function. Mechanistically, when the PKA phosphorylation site on Bnip3 was neutralized, the protective effect of misoprostol on mitochondrial membrane potential, mitophagy and permeability transition was lost. Taken together, these results demonstrate a foundational role for Bnip3 phosphorylation in the molecular regulation of cardiomyocyte mitochondrial dysfunction. These findings further identify a pharmacological mechanism, through PKA, which may ultimately be able to prevent hypoxia-induced myocardial injury.

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A Genome-scale Crispr Screen Identifies Modulators Of Doxorubicin-induced Cardiotoxicity

Christopher McDermott-Roe, Univ of Pennsylvania, Philadelphia, PA

Doxorubicin is a potent chemotherapeutic used to treat various cancers. Despite efficacy, doxorubicin can cause severe and irreversible cardiotoxicity. We carried out a genome-scale CRISPR-Cas9 screen in HL1 mouse cardiomyocytes to identify genes which when disrupted, either protect against or worsen doxorubicin toxicity. Validating our approach, *Top2a* (the doxorubicin target) and *P-glycoprotein* (the doxorubicin efflux pump) were amongst the very top desensitizers and sensitizers, respectively. The screen also uncovered hundreds of other genes which significantly influenced toxicity as well as multiple over-represented gene sets, including *apoptosis*, *aromatic compound metabolism*, and *transferase complex*. Our study provides an unbiased, forward genetics-based insight into doxorubicin-induced cardiotoxicity and a springboard to define novel toxicity mechanisms and susceptibility alleles.

C. McDermott-Roe: None.

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MCL-1 Facilitates the Removal of Damaged Mitochondria via the Mitophagy Receptor BNIP3

Alexandra G Moyzis, Navraj S Lally, Rita A Najor, Leonardo J Leon, Åsa B Gustafsson, Univ of California San Diego, La Jolla, CA

Myeloid Cell Leukemia-1 (MCL-1) is an anti-apoptotic BCL-2 family protein that is necessary to maintain cardiac homeostasis in the adult heart. MCL-1 localizes to two distinct mitochondrial locations in myocytes, both on the outer mitochondrial membrane (MCL-1_{OM}) and in the mitochondrial matrix (MCL-1_{Matrix}). Our lab previously showed that cardiac-specific ablation of MCL-1 at both of these mitochondrial locations in mice led to severe contractile dysfunction and compromised mitochondrial function. Intriguingly, these defects were accompanied by signs of necrotic, rather than apoptotic, cell death. This indicates that MCL-1 has an alternate role in maintaining mitochondrial homeostasis in cardiac myocytes. Unexpectedly, we found that MCL-1 induces mitochondrial clearance in response to treatment with the chemical uncoupler FCCP in a Parkin-independent manner. Hypoxia is also known to induce mitochondrial clearance, and overexpression of MCL-1 further enhances hypoxia-mediated mitophagy. Fluorescence imaging identified MCL-1-positive mitochondria sequestered inside autophagosomes. MCL-1-mediated clearance is abrogated in autophagy deficient Atg5^{-/-} cells, confirming that clearance is occurring via the autophagy pathway. Mutation of MCL-1's BH3 domain (G198E D199A) does not affect its ability to induce clearance, suggesting that this role may be independent of its anti-apoptotic function. Also, replacing MCL-1's BH domains with those of BCL-2, does not affect its ability to induce mitophagy. Next, we investigated whether MCL-1 functions as a mitophagy receptor and promotes removal of damaged mitochondria by binding to directly to LC3 through one or more of its three putative LC3-Interacting Region (LIR) motifs. Endogenous MCL-1 and LC3 co-immunoprecipitate in response to stress induced by FCCP. However, mutating each of MCL-1's individual LIR motifs, as well as generating combined mutations in all three, does not affect MCL-1-mediated mitophagy. Instead, we found that MCL-1 interacts with the known mitophagy receptor BNIP3 both *in vitro* and *in vivo*. Thus, our data suggest that MCL-1 promotes elimination of dysfunctional mitochondria by positively regulating the mitophagy receptor BNIP3.

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Increased Mitophagy and Lysophagy in High Fat Diet and Streptozotocin Induced Diabetic Cardiomyopathy

Joy R Patel, Satoru Kobayashi, Qiangrong Liang, New York Institute of Technology Coll of Osteopathic Med, Old Westbury, NY

Heart disease and diabetes are among the leading causes of morbidity and mortality. Diabetes can result in diabetic cardiomyopathy, which further increases the risk of heart failure and mortality in diabetic patients. Diabetic cardiac injury has been associated with altered mitochondrial quality control mechanisms including mitophagy, a selective autophagy in which dysfunctional mitochondria are degraded through the lysosome. Lysophagy is the elimination of injured lysosomes by healthy lysosomes which is extremely important for all forms of autophagy. In the present study, we examined the functional status of mitophagy-lysosome system in the diabetic mouse heart. Cardiac mitophagy and lysophagy were determined by using novel dual fluorescent reporter mice, namely, mt-rosella that express mitochondria-targeted RFP-GFP fusion protein and tfgal3 that express RFP-GFP-galectin3, a galactoside-binding lectin. Mice were fed a high fat diet (60% calories by fat) for 1 month and then injected i.p. with streptozotocin (30 mg/kg/d for 3 days). Mice continued on HFD for additional 3 months. Echocardiographic data showed impaired cardiac function in the diabetic mice. Merged confocal images demonstrated that diabetes increased mitophagy flux in the heart as indicated by the red puncta from mt-Rosella and the mitochondrial LC3-II levels with and without lysosomal protease inhibitors pepstatin A and E64d. In addition, diabetes triggered lysosomal injury in the heart as shown by the increased total number of puncta (yellow plus red) from tfgal3 reporter on the merged confocal images. This was associated with accelerated lysophagy flux as shown by the red puncta, suggesting that the injured lysosomes were being actively degraded in the diabetic heart. The increased lysophagy may have contributed to the increased mitophagy flux since the enhanced elimination of injured lysosomes would be expected to help maintain a pool of healthy lysosomes which is essential for the efficient execution of all forms of autophagy. In summary, our results showed an enhanced mitophagy-lysosome system in the diabetic heart which may be an adaptive response that serves to limit diabetic cardiac injury.

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Remote Ischemic Preconditioning Rescues Cardiac Dysfunction in Atg5 Knockdown Mice

Fangfei Wang, Quan He, Andrew N Redington, Cincinnati Children Hosp Medica, Cincinnati, OH

Objective—Remote ischemic pre-conditioning (RIPC) is highly cardioprotective in models of ischemia-reperfusion (IR) injury but has not been tested in genetically modified mice. This study was design to test whether RIPC rescues cardiomyopathy induced by knockdown of autophagy-related 5 protein (Atg5). *Approach and Results*—Atg5 knockdown was induced by feeding tamoxifen diet for two weeks in Atg5^{fllox/fllox} Cre^{+/-} (KD) mice which were created by crossing Atg5^{fllox/fllox} and heterozygous cardiac-specific Cre recombinase transgenic (Cre^{+/-}) mice. Atg5^{fllox/fllox} Cre^{-/-} mice served as controls (CONT). Cardiac function was evaluated by echocardiography 28 days after tamoxifen induction. Autophagy proteins were determined by Western blot analysis. RIPC was induced by four cycles of five minutes of hind limb ischemia with tourniquet followed by five minutes reperfusion. Tamoxifen feeding knocked down Atg5 to 32% (CONT 0.91 ± 0.09 vs KD 0.29 ± 0.01, p < 0.01, n = 7), leading to 96% decrease of autophagy activity indicated by LC3-II/LC3-I ratio (CONT 0.96 ± 0.07 vs 0.06 ± 0.03, p < 0.01, n = 7). This was associated with significant cardiac dysfunction (LV ejection fraction, CONT 59.78% ± 3.26 vs KD 47.13% ± 2.50, n = 8 and 10, p < 0.01) and increased heart weight to body weight ratio (CONT 0.50 ± 0.02 vs KD 0.57 ± 0.01, n = 8 and 10, p < 0.01). RIPC increased levels of Atg5 in Atg5 KD mice by 183% (Sham 1.00 ± 0.16 vs RIPC 1.83 ± 0.14, p < 0.01, n = 11 and 13), leading to 148% increase of autophagy activity (Sham 0.99 ± 0.13 vs RIPC 1.47 ± 0.18, p < 0.05, n = 6 and 7), and resulted in rescued cardiac function in terms of heart weight (CONT 0.50 ± 0.01 vs KD 0.53 ± 0.10, n = 11 and 13) and LV ejection fraction (CONT 55.64% ± 2.45 vs 52.48% ± 1.61, n = 11 and 13). RIPC also suppressed mTOR phosphorylation by 62% compared with sham procedure in Atg5 KD mice (Sham 1.00 ± 0.18 vs RIPC 0.45 ± 0.08 p < 0.01 n = 11 and 13) which was increased by 650% in Atg5 KD compared with CONT mice (CONT 1.23 ± 0.14 vs KD 6.35 ± 0.09, p < 0.01, n = 7). *Conclusion*—We conclude that RIPC rescues Atg5 knockdown-induced cardiomyopathy. Our data adds to the emerging data suggesting benefits of RIPC beyond acute cardioprotection in IR injury.

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Exosomal AAV-mediated SERCA2a Gene Transfer Improves Cardiac Function in a Mouse Model of Heart Failure

Marta Adamiak, Divya Jha, Yaxuan Liang, Prabhu Mathiyalagan, Neha Agarwal, Erik Kohlbrenner, Elena Chepurko, Dongtak Jeong, Delaine Ceholski, Cardiovascular Res Ctr, Icahn Sch of Med, New York, NY; Nicole Dubois, Dept of Developmental and Regenerative Biology, Mindich Child Health and Development Inst, Black Family Stem Cell Inst, Icahn Sch of Med, Mount Sinai, New York, NY; Roger Hajjar, Susmita Sahoo, Cardiovascular Res Ctr, Icahn Sch of Med, New York, NY

Adeno-associated viruses (AAVs) are promising therapeutic tools for gene delivery to the heart. However, pre-existing antibodies (NAbs) to many cardiotropic AAV serotypes naturally present in humans pose a critical challenge for the translation of gene therapies to clinical applications. Here, we describe the use of exosomal AAVs (eAAV) as a robust heart gene delivery system that improves transduction efficiency while protecting from pre-existing immunity to the viral capsid. To obtain eAAV specimens from conditioned medium from AAV-producing HEK-293T cells, we have developed a state-of-the-art multi-step ultracentrifugation strategy. We demonstrated through electron microscopy-based visualization, size distribution measurements and distribution of AAV genomes in post-centrifugation iodixanol gradients, that our purification process enables isolation of eAAVs with high purity and minimal contamination with standard AAVs. Efficiency of heart targeting was then evaluated for eAAV9 or eAAV6 and standard AAV9 or AAV6 in human cardiomyocytes (hCMs) *in vitro* and in passive immunity nude mouse model *in vivo*. Regardless of the presence or absence of NAbs, we demonstrated that eAAVs are more efficient in transduction of cells in the same titer ranges as standard AAVs. To test the therapeutic efficacy, eAAV9-SERCA2a or AAV9-SERCA2a were injected intramyocardially in post-myocardial infarction (MI) mice preinjected with NAbs. Remarkably, eAAV9-SERCA2a outperformed standard AAVs 6 weeks post-MI, significantly improving cardiac function in the presence of NAbs (%EF 55.14 ± 3.50 vs. 27.31 ± 1.63 , respectively). Additionally, we demonstrated *in vivo* that eAAV9-mediated gene delivery is more specific to CMs than to other cell types present in the heart, which suggests that eAAVs preserve cardiotropic properties of AAV9 serotype. With examination of colocalization of eAAVs and markers specific for endosomes (Rab5 and Rab7) in hCMs *in vitro*, our preliminary data indicated that eAAV infectious entry potentially involves trafficking via endocytic compartments. In conclusion, these results underline the therapeutic potential of eAAVs to evade NAbs, and to facilitate the clinical translation of AAV-based gene therapies to a larger human population.

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The Effects of Adolescent Binge Alcohol Exposure to Cardiovascular Structure and Function

Lizhuo Ai, Edith Perez, Maxime Heroux, Andrei Zlobin, AnnaDorothea Asimes, Quan Cao, Loyola Univ Chicago, Chicago, IL; Charles S Chung, Wayne State Univ, Detroit, MI; Toni R Pak, Jonathan A Kirk, Loyola Univ Chicago, Chicago, IL

While the effects of alcohol consumption on the adult heart have been well studied, the impact to the adolescent heart is almost entirely unknown. Adolescents primarily consume alcohol in a binge pattern, which elevates blood alcohol content (BAC) to 0.08 g/dL within 2 hours. During adolescence the body grows rapidly, and the heart must also grow (through cellular hypertrophy) to meet this increasing demand. Our goal was to determine the impact of adolescent binge alcohol exposure on the heart, using an outbred rat model.

On postnatal day (PND) 37 (adolescence), rats were gavaged 3 g/kg EtOH for 3 days, H₂O for 2 days, and EtOH for 3 more days, then sacrificed following the last dose (Binge). The control group received only H₂O (Water). Both groups had normal food and water intake and weight gain. BAC was 0.08 g/dL in the Binge group and 0 in the Water group. Water rats experienced a ~40% increase in LV end-diastolic volume (LVEDV) due to normal growth along with a parallel increase in end-systolic volume such that ejection fraction (EF) was preserved. Conversely, Binge rats only had half that increase in LVEDV, and a decrease in systolic diameter resulting in increased EF. We hypothesize alcohol impaired normal cardiac growth, and systolic function increased to maintain cardiac output. Increased systolic function suggested beta-adrenergic activation, which was supported by increased troponin I phosphorylation at the PKA sites in the Binge group.

Doppler indicated the E/A ratio increased with age in the Water group, but in the Binge group the E/A ratio did not change with age. Further, the Binge group displayed increased single cell passive stiffness level (sarcomere length: 1.7-2.4 μ m) compared to Water, likely due to altered titin phosphorylation. Lastly, we performed RNA-seq and identified 58 down-

regulated and 10 up-regulated genes in the Binge group. Many of these genes suggest a switch in substrate utilization from fatty acid to glucose metabolism in the Binge rats.

These data reveal a previously unappreciated pathological impact of adolescent binge alcohol exposure. The young heart can compensate for these consequences at first and appear healthy. However, the long term impact of these effects may be significant and whose underlying cause was previously unknown.

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Spatially Distributed N-glycomic Regulation in Human Aortic Valve Development and Disease

Janet Saunders, Cassandra L. Clift, Medical Univ of South Carolina, Charleston, SC; Yan R. Su, David P. Bichell, Vanderbilt Univ Medical Ctr, Nashville, TN; Anand S Mehta, Richard R. Drake, **Peggi M. Angel**, Medical Univ of South Carolina, Charleston, SC

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Development of an Auto-Segmentation Technique Using a Convolutional Neural Network for the Segmentation of the Ventricular Cavity in Zebrafish

Tanveer Ashwini Teranikar, Xin Ma, Zachary Bailey, Jessica Lim, Bijan Gaire, Martin Hirsch, Won Hwa Kim, Juhyun Lee, Univ of Texas at Arlington, Arlington, TX

Light sheet fluorescence microscopy (LSFM) with a synchronization algorithm is a unique imaging technique that can be used to image the beating zebrafish heart in 4-dimensions (4-D). However, natural arrhythmic diastolic and systolic contraction and relaxation of the heart can cause aberrations in 4-D image reconstruction. These aberrations are observed as blurred lines, spikes, or holes in the myocardium of the heart, which can lead to complications when analyzing biomechanics by computational fluid dynamics (CFD). In this study, we imaged a beating zebrafish heart at 4 days post fertilization (dpf) using a 10x objective lens with our single-sided LSFM system scanning with a 2 μ m step size. The obtained images were analyzed in 3-D to ensure that there were limited aberrations in the wall of the myocardium. Then, the ventricular cavity of these 3-D images was hand segmented at various time points that were spaced within two cardiac cycles. These hand segmented images were used to train a convolutional neural network (CNN) which would have the capability of performing segmentation of the ventricular chamber automatically. This auto-segmentation tool was then used to segment the ventricular cavity of ten samples contained within one cardiac cycle that had not previously been used for training purposes. The ventricular volume of these ten images calculated after auto-segmentation was found to be comparable to the images when hand segmented. Then, a Gaussian wavelet filter was used after auto-segmentation to remove aberrations and minimize errors in the CFD simulation. Both the auto-segmented images and the hand-segmented images were analyzed using a CFD solver to simulate the hemodynamic shear stress through the cardiac cycle. The results from these simulations showed no significant difference between shear stress when the images were hand-segmented or auto-segmented, and there was no leakage observed between the two sets. The results using the auto-segmentation code are promising for future applications in cardiac research where low-quality images obtained under fast acquisition times can be enhanced without the traditional costly deconvolution techniques. This can lead to faster and more accurate analysis of the ventricular cavity for related diseases.

T. Teranikar: None. **X. Ma:** None. **Z. Bailey:** None. **J. Lim:** None. **B. Gaire:** None. **M. Hirsch:** None. **W. Kim:** None. **J. Lee:** None.

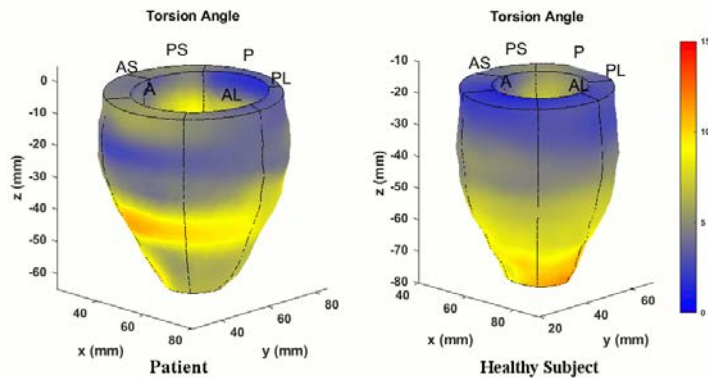
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Investigating Subclinical Cardiotoxicity in Long-term Breast Cancer Survivors Following Chemotherapy With DENSE-based 3D Strain Analysis

Bryant Baldwin, Shane Johnson, Christopher Malozzi, Michael V Cohen, Maria S Figarola, Anam Chouhdry, Eduardo Rel, Angela Y Yudice, Samuel A McQuiston, Julia Kar, Univ of South Alabama, Mobile, AL

This study investigated if left ventricular (LV) mechanical contractile parameters in addition to LV ejection fraction (LVEF) indicated cardiac remodeling and fibrosis in patients exposed to cardiotoxic chemotherapy agents (CCA). Cardiac deformation data were obtained using single-scan acquisitions with the Displacement Encoding with Stimulated Echoes (DENSE) MRI sequence. Contractile analysis consisted of automated myocardial boundary detection and unwrapping 3D DENSE phase images for intra-myocardial displacements, and followed by analyzing torsion and 3D strains with the meshfree Radial Point Interpolation Method (RPIM). Data were acquired on 13 CCA-exposed patients who were undergoing chemotherapy and/or care for cardiac complications. DENSE LVEF measurements in patients were validated against steady-state free precession (SSFP) MRI data, all contractility computations were compared to healthy subjects and Bland-Altman agreements established between strains computed by independent observers. A significant difference was not found between DENSE and SSFP LVEF computations ($52 \pm 11\%$ vs $48 \pm 15\%$, $p=0.33$). Significant differences were seen with enlarged LV diameters in patients versus healthy subjects (6.0 ± 1.1 cm vs 4.9 ± 0.7 cm, $p<0.001$) and similarly for torsion and longitudinal strain but not in LVEF results ($p > 0.05$). Bland-Altman agreements were 0.01 ± 0.06 for longitudinal strain and $0.1 \pm 1.9^\circ$ for torsion. Statistical analysis confirm changed LV size and function in patients and indicate remodeling that is otherwise not demonstrated by LVEF measurements, which may precede clinically apparent heart failure development and worsened morbidity.



B. Baldwin: None. **S. Johnson:** None. **C. Malozzi:** None. **M.V. Cohen:** None. **M.S. Figarola:** None. **A. Chouhdry:** None. **E. Rel:** None. **A.Y. Yudice:** None. **S.A. McQuiston:** None. **J. Kar:** None.

Poster Session 1 and Reception

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Evaluation of Sepsis-Related Cytokines on Endothelial Cell Permeability

Carmen E Campbell, William A Boisvert, Univ of Hawaii, Honolulu, HI

The endothelium plays a critical role in the progression from sepsis to organ failure because endothelium lines all blood vessels and organs in the body to provide a selective barrier. The endothelium is responsible for dynamically regulating the passage of small molecules, fluid, and cells between the bloodstream and tissues. In sepsis, many factors play a role in compromising the endothelium. Dysfunctional endothelium results in development of capillary leak, tissue hypoperfusion, hypoxia, edema, and shock. This study evaluates the individual contributions immunomodulatory cytokines as well as a potential synergistic effect of cytokine combinations on vascular endothelial permeability. Major components of intercellular junctions including tight junction and associated cell adhesion molecules are also assessed microscopically. Specifically, endothelial cell monolayer response to an array of sepsis-related factors including TNF-alpha, IL-1b, IL-6, IL- 8, IL-10, IL-12, IFN-g, and TGF-b by a novel approach using the in-vitro Electric Cell-substrate Impedance Sensing (ECIS) biosensor model is presented.

C.E. Campbell: None. **W.A. Boisvert:** None.

Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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The Effects of Age, Menopause, and Body Mass Index on Blood Pressure and Radial Pulse Wave in Women

Chih-Yu Chen, Renai Branch of Taipei City Hosp, Taipei, Taiwan; **Chi-Wei Chang**, Mii-Ann medical center, Taipei, Taiwan; Yi-Ting Chang, Johns Hopkins Bloomberg Sch of Public Health, Baltimore, MD; Sheng-Hung Wang, Mii-Ann medical center, Taipei, Taiwan; Gin-Chung Wang Wang, JinMu Health Technology, Taipei, Taiwan

Age, gender, and body mass index (BMI) play an important roles in cardiovascular risk. However, the independent effects of menopause on the ventricular-arterial system in female individuals have not been fully investigated. Thus, this study aimed to assess the effect of age, menopause and body mass index on different hemodynamic parameters including systolic blood pressure (SBP), diastolic blood pressure (DBP) and harmonics of radial pulse wave. This study enrolled 327 female subjects(230 pre-menopausal and 97 post-menopausal), aged 20-87 and without cardiovascular history. SBP and DBP were evaluated by an automatic blood pressure monitor. A 12-second continuous radial pulse data was recorded using medical grade pulse measuring instrument and standard protocol. Spectrum analysis of radial pressure wave was calculated and transformed into first five harmonic amplitudes(C1~C5). We further performed multivariable linear regression models to assess the independent effects of age, menopause, and body mass index on SBP, DBP, and C1 to C5 respectively. The mean age, BMI, SBP, and DBP in enrolled subjects were 43 ± 13 years, 23 ± 4 kg/m², 116 ± 16 mmHg, and 69 ± 11 mmHg. Regression analysis demonstrated that age was positively correlated with SBP($P<0.001$), whereas effect of age exerted no significant influence on DBP. BMI had positive associations with SBP and DBP($P<0.001$), while menopause had no significant effect on neither SBP nor DBP. Multivariable linear regression showed that menopause had significant effects on C1 ($P<0.05$) and C3 ($P<0.05$). C1 is associated with atherosclerosis and is an independent risk predictor for adverse cardiac events. We proposed that an increase in cardiovascular risk during menopause may be due to hormonal changes exacerbating the progression of atherosclerosis and reflected in the radial pulse spectrum. Conclusively, this study demonstrates that age and BMI have an independent effect on SBP, whereas menopause has no significant independent effect on SBP. The effects of menopause on C1 and C3, independent of age and BMI, may partially explain the reasons of dramatically increased cardiovascular risk during menopause and provide clues to the underlying mechanisms of the change in cardiovascular system.

C. Chen: None. **C. Chang:** None. **Y. Chang:** None. **S. Wang:** None. **G. Wang:** None.

Hemodynamic Status From Photoplethysmography, Signs of Successful Pregnancy

Chi-Wei Chang, Mii-Ann medical center, Taipei, Taiwan; Dan ni Xie, Shu jia Wang, Xiao ran Li, Meng jie Li, Chun Huang, An ran Wang, Li Zhou, Inst of the reproductive center of Natl Health and Family Planning Commission, Nanning, China; Xiao feng Zhu, Inst of the reproductive center of Natl Health and Family Planning Commission, Nanning, Taiwan; Yan mei Li, Inst of the reproductive center of Natl Health and Family Planning Commission, Nanning, China; Zi-Xin Huang, Chang-Zhou Pulse Health Technology, Nanning, China; Gin-Chung Wang, JinMu Health Technology, Taipei, Taiwan

The key physiological condition for successful pregnancy is a critical issue in reproductive science. Many studies have shown that the pregnancy process requires a lot of subtle cardiovascular adaptation, including a decrease in systemic vascular resistance, an increase in maternal heart rate, stroke volume, and cardiac output. Those adaptation will also lead to changes in the arterial pulse spectrum. Since pregnancy requires the mother's competency to change the hemodynamic state, an interesting question is if the pre-pregnancy hemodynamics of the cardiovascular system affect the clinical pregnancy rate of each embryo transfer. Therefore, we initiated an observational study to validate our conjecture. We recruited 116 women of childbearing age (range 22 to 41 years) who planned to carry out in vitro fertilization (IVF). Each subject underwent photoplethysmography (PPG) before embryo implantation. We calculated the first ten harmonic amplitudes (C1~C10) and phases (P1~P10) from the pulse data of PPG using the Fourier transform method, and tracked these women to 42 days after implantation to confirm the successful pregnancy. The results showed that the subjects with successful IVF had higher C4 and C7 ($P=0.006$; $P=0.018$), compared with those who failed the IVF. We then classified the enrolled patients into quartile groups of C4 and C7 respectively. Log rank test further proved that the cumulative rates of successful pregnancy were significant higher for those with the higher C4 and C7, taking groups with smaller values as reference (Figure 1). In conclusion, maternal hemodynamic state before implantation have an impact on the successful pregnancy rate during IVF.

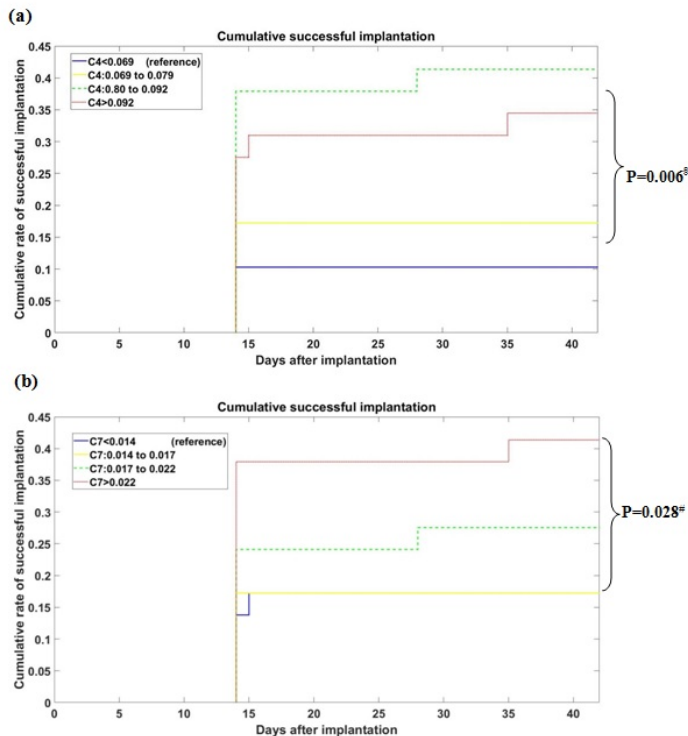


Figure 1. Cumulative rates of successful pregnancy through in vitro fertilization categorized by four quartile levels of (a) C4 and (b) C7 of pulse data from photoplethysmography (N=116). P values were the result of the log-rank test.

[§] Log-rank test between $C4 < 0.079$ and $C4 > 0.080$

[#] Log-rank test between $C7 < 0.017$ and $C7 > 0.022$

Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Development of a New Structural Family of Microbial Choline Trimethylamine Lyase Inhibitors for the Treatment and Prevention of Cardiovascular Disease

Ashraf Sabri Duzan, Cleveland State Univ / Cleveland Clinic, Cleveland, OH

Ashraf Duzan, BPharm., MS^{1,2}; Xiaodong Gu, Ph.D.^{1,3}; Adam Roberts, Ph.D.^{1,3}; Jennifer Buffa, MS^{1,3}; James Anderson^{1,3}, Valentine Gogonea, Ph.D.^{1,2,3} Stanley L. Hazen, MD, Ph.D.^{1,2,3}.

¹Department of Cellular & Molecular Medicine, ³Center for Microbiome and Human Health, ⁴Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio 44195; ²Department of Chemistry, Cleveland State University, Cleveland, Ohio 44115

Recent clinical research points to trimethylamine N-oxide (TMAO), a gut microbiota generated metabolite, as a biomarker associated with acute thrombotic event and cardiovascular disease risks, and a direct causative contributor to these adverse phenotypes. Our goal in this project is to develop novel microbial enzyme inhibitors that alter the biosynthetic pathway of TMAO in vivo through the selective potent inhibition of gut microbial choline trimethylamine (TMA) lyase activity, the rate limiting step in TMA and TMAO generation in vivo. TMA generation by gut microbiota is predominantly catalyzed by the gut microbial enzyme pair CutC/D, members of the microbial choline utilization (*cut*) gene cluster. We are using structure activity relationships (SAR) to predict new classes of chemical structures as potential efficient inhibitors. Thus, we are exploring the synthesis of new chemical compounds, non-lethal to the gut microbial community, with high inhibitory potency in multiple *in vitro* assays employing evolutionarily diverse microbial *cutC/D* (primary and secondary screens), polymicrobial communities (tertiary screens), and for inhibitors that pass the above screening assays, progression to *in vivo* studies. We are preparing and assessing inhibitors that can work either through irreversible non-competitive or competitive mechanisms, possess appropriate physico-chemical pharmaceutical properties and have minimal systemic exposure to the host in effort to minimize possibility of side effects. Our leading candidates have excellent enzyme blocking efficiency and display good pharmacokinetic/pharmacodynamics properties.

A.S. Duzan: None.

Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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Resistin Regulates ATP-citrate lyase Expression a Key Metabolic Enzyme of Lipogenesis and Histone acetylation in Human Macrophage

Nezam Haider, Pricila Moly, Weifeng Lu, Wei Zhou, Univ of Arizona, Tucson, AZ

BACKGROUND: Human resistin synthesized by macrophages is associated with insulin resistance, type 2 diabetes, atherosclerosis, and chronic inflammation. We used a comparative global protein expression profiling of human macrophage treated with resistin to identify downstream targets and intracellular signaling pathways involving hyper-resistinemia-associated metabolic disorders by HPLC-ESI-MS/MS. ATP-citrate lyase (ACLY), is an essential cytosolic enzyme for generating acetyl-CoA, a key metabolite for glycolysis, de novo lipogenesis, cholesterol synthesis, and histone acetylation. Its dysregulation is associated with diabetes, hypercholesterolemia, and oncogenesis and is a potent therapeutic target. In this study, we explored the regulation of ACLY and its phosphorylation by resistin.

METHODS: M ϕ derived from THP1 cells were treated with human resistin for 24-72 hrs. Samples of total protein tryptic digest of untreated control and resistin-treated M ϕ were analyzed by HPLC-ESI-MS/MS. Total protein were also analyzed by western blot for ACLY and its phosphorylation. Gene expression was measured by Quantitative Real-time PCR.

RESULTS: ACLY showed a significant ($p < 0.05$) differences in expression between control and resistin treated groups by quantitative mass spectrometry. ACLY formed protein-protein interaction (PPI) with 23 out of 162 significantly differentially regulated proteins. Level of ACLY protein decreased 2 fold after 48 hours of resistin treatment. However, after 72 hours of resistin treatment ACLY proteins increase with a 4-5 fold increase in its serine 455 phosphorylation (Figure 1). In human macrophage, ACLY mRNA also increased ~2 fold after 24 hours of resistin treatment (Figure 2).

CONCLUSION: Resistin significantly changed the gene expression of ACLY in humanM ϕ . Although there was an initial decrease in ACLY protein with no change in its phosphorylation. However, a longer 72 hours treatment of resistin induce ACLY protein in human macrophages and a very significant increase in its phosphorylation. ACLY plays a pivotal role in many metabolic and epigenetic processes, this late ACLY activation may mediate the effect of resistin on chronic diseases

like diabetes, atherosclerosis, and chronic inflammation.

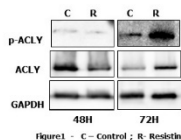


Figure 1 - C - Control ; R- Resistin

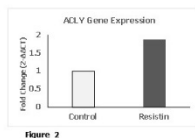


Figure 2

N. Haider: None. **P. Moly:** None. **W. Lu:** None. **W. Zhou:** None.

Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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Blood Outgrowth Endothelial Cell-derived Exosomes Mediate Therapeutic Neovascularization

Stefan P Janssens, Arief Wibowo, Hilde Gillijns, Ellen Caluwe, Denise Veltman, Univ Hosp Gasthuisberg, Leuven, Belgium; Leen Delrue, OLV Hosp, Aalst, Belgium; Jozef Bartunek, Univ Hosp Gasthuisberg and OLV Aalst, Leuven, Belgium

Background: Blood outgrowth endothelial cells (BOECs) mediate therapeutic neovascularization in experimental models. We hypothesized that BOECs promote angiogenesis via secretion of exosomes, extracellular vesicles with an important role in cell-to-cell communication.

Methods: We derived exosomes from BOECs of patients with ischemic heart failure (HF with LVEF <35%) and age-matched controls (CON) in normoxia and 1% O₂ hypoxia by differential ultracentrifugation and used nano tracking (NTA) and immunoblot analysis to identify size, number, and surface markers. We characterized protein and miRNA content by proteomic LC-MS-Orbitrap analysis of trypsin-digested exosomes and validated selected peptides using ELISA. We measured expression of pro-angiogenic miRNAs and tested in vitro angiogenic potential of HUVECs in the presence or absence of exosomes using matrigel 2D-tube formation and 3D-spheroid sprouting assays.

We compared in vivo angiogenesis using a wound healing model in nude mice.

Results: NTA showed higher exosome concentrations in hypoxia compared to normoxia ($14.38 \pm 0.23 \times 10^8$ vs $12.05 \pm 0.23 \times 10^8$ particles/ml, $n=7$, $P<0.001$) with robust expression of exosome markers TSG101 and Flotilin-1. Compared to serum stimulation (positive control), BOEC-derived exosomes similarly enhanced 2D vascular network growth and 3D spheroid sprouting angiogenesis whereas absence of serum or exosomes did not ($P<0.001$). Bioinformatic proteome analysis identified 249 shared proteins in HF and CON, and 72 proteins exclusively present in HF with abundant expression of matricellular proteins and angiogenic growth factors. Exosomes derived in hypoxia contained significantly higher levels of pro-angiogenic miR-210-3p, miR-210-5p and miR-21-3p ($P<0.05$ vs normoxia, $n=6$). Closure of the skin wound after 4 and 5 days was significantly better in animals treated with BOEC-derived exosomes and accompanied with greater density of CD31-positive neovessels.

Conclusion: BOEC-derived exosomes effectively induce vascular network formation and sprouting angiogenesis and stimulate wound healing in vivo. The identified protein and miRNA content in BOEC-derived exosomes may constitute a promising allogenic approach for therapeutic neovascularization.

S.P. Janssens: None. **A. Wibowo:** None. **H. Gillijns:** None. **E. Caluwe:** None. **D. Veltman:** None. **L. Delrue:** None. **J. Bartunek:** None.

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Anti-inflammatory Effects of Macitentan on Monocrotaline-induced Pulmonary Hypertension in a Rat Model

Kyung-hee Kim, Sejong General Hosp, Bucheon, Korea, Republic of; **Seung-Woon Rha**, **Yoonjee Park**, **Jae Kyeong Byun**, Korea Univ Guro Hosp, Seoul, Korea, Republic of

Background: Inflammation is closely linked to pulmonary arterial hypertension (PAH). Macitentan (MAC) reduces morbidity and mortality among advanced-stage pulmonary arterial hypertension (PAH) patients. However, data regarding anti-inflammatory benefits of MAC treatment at an early stage of PAH is lacking. **Methods:** One week after monocrotaline (MCT) injection, rats were randomly assigned to MAC (n=30), normal saline (MCT, n=30). Fourteen sham rats (Sham) were included for comparison. Right ventricular (RV) systolic function was assessed via echocardiography as the RV fractional area change (RV-FAC). An invasive pressure-volume analysis using a Millar conductance catheter was performed 8 weeks after MCT injection. Rats were subsequently euthanized for histopathologic analysis. Levels of pro-inflammatory cytokines (tumor necrosis factor- α , interleukin-1,6) and nuclear factor-kappa B (NF- κ B) activity in serum were determined with enzyme-linked immunosorbent assay. **Results:** RV-right atrial pressure gradient on echocardiography was significantly increased 3 weeks after MCT injection, but was maintained in the Sham. On invasive hemodynamic analyses, RV end-systolic (203 \pm 79 μ L) and end-diastolic volumes (315 \pm 83 μ L), pulmonary artery systolic pressure (90 \pm 6 mmHg), and end-systolic pressure-volume relationship (-264 \pm 23) were significantly worse in the MCT vs. in the MAC (98 \pm 45 μ L, 225 \pm 35 μ L, 39 \pm 12 mmHg, and -147 \pm 42, respectively, all p<0.05). On histopathology, both RV and lung fibrosis were significantly reduced in the MAC. MAC significantly ameliorated PAH by acting against pulmonary vascular remodeling, decreasing macrophage infiltration, and reducing pro-inflammatory cytokine expression and nuclear factor-kappa B (NF- κ B) activity in the lungs of the MCT-treated rats. **Conclusions:** MAC treatment improves haemodynamic parameters in established pulmonary hypertension. These results suggest that MAC saline ameliorates the progression of pulmonary hypertension induced by monocrotaline in rats, which may be associated with anti-inflammatory effects.

K. Kim: None. **S. Rha:** None. **Y. Park:** None. **J. Byun:** None.

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Left Ventricular Diastolic Dysfunction in HIV-infected ART-naive and HIV-negative Tanzanian Adults: A Cross-sectional Study

Justin Kingery, Weill Cornell Med, New York, NY; **Edmund Damas**, **Samuel Kalluvya**, Weill Bugando Medical Ctr, Mwanza, Tanzania, United Republic of; **Altaf Pirmohamed**, **Parag Goyal**, **Myung Hee Lee**, Weill Cornell Med, New York, NY; **Fredrick Kalokola**, **Bernard Desdarius**, Weill Bugando Medical Ctr, Mwanza, Tanzania, United Republic of; **Robert Peck**, Weill Cornell Med, New York, NY

Objective: To compare the prevalence and associated factors of left ventricular diastolic dysfunction in HIV-infected, ART naïve and HIV-negative Tanzanian adults.

Methods: A cross-sectional analysis of a longitudinal study including: 257 HIV-infected, ART naïve adults and 265 HIV-negative controls. Echocardiography and traditional risk factors were performed and determined by standard investigations. The primary outcome was prevalence of left ventricular diastolic dysfunction. Secondary outcomes were factors associated with and grade of dysfunction.

Results: Compared to HIV-negative controls, HIV-infected, ART naïve adults had a 2-fold higher prevalence of diastolic dysfunction (OR=2.06 [1.16-3.66], p=0.01). Additionally, age, female sex, obesity and hypertension were significantly associated with dysfunction in HIV-negative adults; whereas, only age and hypertension were associated with dysfunction in HIV-infected adults. As compared to HIV-negative controls, significantly more HIV-infected, ART naïve adults had higher grade dysfunction (p=0.02) and more ventricular hypertrophy. HIV-infected adults with diastolic dysfunction also expressed higher levels of inflammatory cytokines including TNF α , IL6, IL8, IL33 and sST2 as compared to both HIV-negative and HIV-infected adults without dysfunction.

Conclusions: HIV-infected Tanzanian adults have a 2-fold higher prevalence of left ventricular diastolic dysfunction in the period immediately following diagnosis and before ART initiation as compared to HIV-negative adults, and this dysfunction is of higher grade and is associated with myocardial hypertrophy. These data identify diastolic dysfunction in an international cohort in the immediate post-diagnosis, pre-ART period similar to that previously reported in the post-ART period in high-income countries. Additionally, traditional cardiovascular risk factors are not significantly associated with dysfunction in HIV-infected adults; however, cytokine patterns are present and may be important for risk stratification in this group. Therefore, studies delineating appropriate screening for this population are needed.

J. Kingery: None. **E. Damas:** None. **S. Kalluvya:** None. **A. Pirmohamed:** None. **P. Goyal:** None. **M. Hee Lee:** None. **F. Kalokola:** None. **B. Desdarius:** None. **R. Peck:** None.

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Phosphodiesterase 9 Inhibition Improves Cardiometabolic Profile in Female Mice Independent of Estrogen Status

Sumita Mishra, Guangshuo Zhu, Susana Rodriguez, William Wong, David Kass, Johns Hopkins Med, Baltimore, MD

Approximately 6.5 million Americans are living with heart failure (HF) currently and the number of persons is projected to increase 46% by 2030. One particularly notable syndrome associated with increasingly severe obesity is called - Heart failure with a preserved ejection fraction (HFpEF); which represents half of all HF patients world-wide. Epidemiologic insights have provided incremental evidence that female sex is a stronger risk factor for HFpEF, particularly post-menopausal women. Loss of estrogen after menopause reduces estrogen receptor induced-NO-cGMP-PKG activation and consequently less benefit from Phosphodiesterase 5(PDE5) inhibition which principally hydrolyze NO-derived cGMP that appears compromised in HFpEF patients. PDE9 targets cGMP generated by Natriuretic peptide (NP) signaling and is significantly increased in hearts from HFpEF patients. This supports a role of PDE9 in the heart and its value as a therapeutic target for abnormalities relevant to HFpEF. We created a murine model of postmenopausal obesity and mild cardiac stress induced HFpEF. Genetic ablation or pharmacological inhibition of PDE9 conferred higher contractile performance with increased %EF, %FS and improved diastolic relaxation and filling. Echo-MRI analysis showed an improvement in whole body metabolism with significant decrease in percent body fat associated with enhanced O₂ consumption and increased energy expenditure. The mechanisms underlying the protective effects are increased mitochondrial function and enhanced expression of transcriptional regulators *pgc1α* and *pparα*, driving mitochondrial fatty acid oxidation and increased energy dissipation. Our work identifies for the first-time reversal of changes in substrate metabolism, and mitochondrial dynamics as a direct consequence of PDE9 inhibition in heart and fat. Proteomic analysis also revealed a dynamic cross-talk of PDE9 protein with biological networks of mitochondrial membrane proteins and proteins regulating cellular metabolism. This study greatly enhances our understanding of how the cGMP/PKG activation system can be modulated in females with reduced sex hormone- paving the way for gender-selective targeting in patients with obesity and HFpEF.

S. Mishra: None. **G. Zhu:** None. **S. Rodriguez:** None. **W. Wong:** None. **D. Kass:** None.

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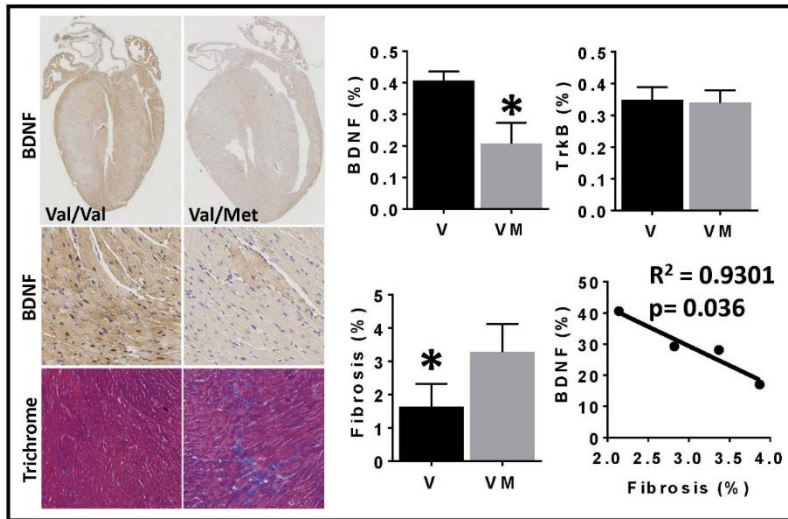
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Cardioprotective Effects of Brain-derived Neurotrophic Factor rs6265 Polymorphism in Duchenne Cardiomyopathy

Frank J Raucci Jr., Anand P. Singh, Jonathan H. Soslow, Lin Zhong, Wejdan Aljafar, Natasja Lessiohadi, Cassandra P. Awgulewitsch, Prachi Umbarkar, Qinkun Zhang, Holly M. Smith, Presley L. Cannon, Erica J. Carrier, Antonis K. Hatzopoulos, Hind Lal, Cristi L. Galindo, Vanderbilt Univ Medical Ctr, Nashville, TN

We previously identified brain-derived neurotrophic factor (BDNF) as a putative cardiac-specific biomarker for preserved cardiac function in Duchenne Muscular Dystrophy (DMD). BDNF has cardioprotective qualities that may attenuate dilated cardiomyopathy, and a common, functional BDNF single nucleotide polymorphism rs6265 (Val66-Met) may be correlated with increased risk of cardiovascular events. We hypothesized that BDNF is protective in the setting of DMD and that BDNF's beneficial effects are diminished in rs6265 allelic carriers. We compared circulating BDNF levels with cardiovascular and skeletal muscle functional parameters in DMD patients stratified by carrier status. Positive association of circulating BDNF with left ventricular ejection fraction in DMD patients was restricted to non-carriers of the rs6265 allele, whereas carriers exhibited worse cardiac function with increased BDNF, more fibrosis and worse skeletal muscle function. To more directly assess association of rs6265 with cardiac function, we used mice with knock-in of BDNF polymorphic Val66Met allele. RNASeq showed higher expression of extracellular matrix genes in Val66Met hearts, and there was a negative correlation of cardiac BDNF protein levels with fibrosis. Additionally, there were baseline echocardiographic and ECG differences, and isolated cardiomyocytes had decreased contractility. This overall cardiac phenotype was substantially enhanced when Val66Met mice were crossed with muscular dystrophy mice. Considered together, our results indicate that BDNF plays a protective role in the dystrophic heart and may represent a novel therapeutic candidate for DMD cardiomyopathy.



F.J. Raucci: None. A.P. Singh: None. J.H. Soslow: None. L. Zhong: None. W. Aljafar: None. N. Lessiohadi: None. C.P. Awgulewitsch: None. P. Umbarkar: None. Q. Zhang: None. H.M. Smith: None. P.L. Cannon: None. E.J. Carrier: None. A.K. Hatzopoulos: None. H. Lal: None. C.L. Galindo: None.

Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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Role of Substance P in Pathogenesis of Chemotherapy Associated Cardiotoxicity.

Ashiq Legi, **Prema Robinson**, Univ of Texas MD Anderson CAN, Houston, TX

Doxorubicin (DOX), is broadly considered the most active single agent available for many cancers. Doxorubicin has been known to induce, cardiotoxicity and life-threatening heart failure or acute coronary syndromes in some patients. There is an urgent need to develop new non-cardiotoxic therapies in cancer. Substance P (SP) is a neuropeptide involved in pain transmission. We had previously determined that aprepitant, an antagonist of SP receptor (neurokinin1 receptor (NK-1R)), that is routinely used to treat chemotherapy associated nausea, prevents DOX induced cardiomyocyte death in vitro. In the current studies, we determined if SP receptor antagonism could prevent chemotherapy induced cardiotoxicity in vivo. Six week old, male C57BL/6 mice were administered DOX (5 mg/kg of body weight, intraperitoneally (IP) once a week for 5 wks. In the aprepitant treated group, five days before the first injection of DOX, aprepitant (0.5 mg/kg) was administered in the drinking water, and then consequently every day until the end of the experiment. The control groups were treated with vehicle alone (1X PBS) instead of DOX without or with aprepitant pretreatment. Mice were euthanized 6 weeks after the first dose of DOX. We determined heart functions by echocardiogram in all mice. The mean ejection fraction (EF) and fractional shortening (FS), percentage, following treatment with DOX respectively decreased significantly by 25.85% and 30.73% compared to that in the control vehicle group (EF: 40.428 ± 6.68 (DOX; $n = 4$) vs $54.526 \pm 11.15\%$ (Control Vehicle group; $n = 4$) (FS: $19.51 \pm 3.66\%$ (DOX; $n = 4$) vs $28.16 \pm 7.38\%$ (Control Vehicle group; $n = 4$) (both, $P < 0.05$, ANOVA). Importantly, treatment with aprepitant in DOX treated mice normalized the EF and FS values to that present in the control vehicle group (EF; 56.80 ± 6.81 (DOX+aprepitant; $n = 4$) vs $54.526 \pm 11.15\%$ (Control Vehicle group; $n = 4$) (FS; $29.64 \pm 4.46\%$ (DOX+aprepitant; $n = 4$) v $28.16 \pm 7.38\%$ (Control Vehicle group; $n = 4$) (both, $P < 0.05$, ANOVA). These findings indicate that SP contributes to chemotherapy associated cardiac-dysfunction via interaction with its high affinity receptor, NK1R. These studies suggest that SP-receptor antagonism may be a novel option to prevent chemotherapy associated cardiotoxicity in cancer.

A. Legi: None. P. Robinson: None.

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Superiority of Sacubitril/Valsartan Over Valsartan Alone in Attenuating Pressure Overload-induced Cardiac Fibrosis and Oxidative Stress

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Background: Sacubitril/valsartan, a first-in-class Angiotensin Receptor-Nepilysin Inhibitor (ARNi), is superior to valsartan in reducing blood pressure in hypertensive patients. However, cardiac effects of sacubitril/valsartan in hypertension treatment and its potential superiority over valsartan are unclear. This study was designed to investigate effects of sacubitril/valsartan on cardiac structural remodeling and oxidative stress using a pressure overload rat model induced by ascending aortic constriction (AAC) and to address whether direct regulation of cardiac myocytes and/or fibroblasts is involved in the cardiac effects independent of afterload changes.

Methods and Results: 1 week after AAC surgery, adult male Sprague-Dawley rats were randomized for 10 weeks treatment with vehicle, valsartan, or sacubitril/valsartan. Sacubitril/valsartan was superior to valsartan in inhibiting ventricular fibrosis, while both equally inhibited cardiomyocyte hypertrophy at the cellular level. These findings were supported by *in vitro* experiments showing that combined LBQ657 (sacubitrilat, active form of sacubitril) and valsartan was superior to valsartan alone in inhibiting angiotensin II (Ang II)-induced adult ventricular fibroblast activation, but not adult cardiomyocyte hypertrophy. In adult cardiomyocytes LBQ657 effectively inhibited Ang II-induced mitochondrial superoxide, and combined LBQ657 and valsartan was superior to valsartan or LBQ657 alone. Importantly, sacubitril/valsartan was also superior to valsartan in reducing mitochondrial superoxide in cardiomyocytes from pressure-overloaded hearts and improved cardiomyocyte mitochondrial maximal and spare respiration capacity.

Conclusions: These findings provide direct evidence and novel mechanistic insights on the superior cardioprotective effects of sacubitril/valsartan in the setting of pressure overload: independent of afterload reduction, sacubitril/valsartan is superior to valsartan in attenuating cardiac fibrosis and myocyte oxidative stress and improves myocyte mitochondrial function in rats. Our study thus suggests a greater therapeutic effect of sacubitril/valsartan in the early stage of hypertensive heart disease before heart failure.

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Polycystin-1 Assembles with Kv Channels to Govern Cardiomyocyte Repolarization and Contractility

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Mutations in the gene encoding polycystin-1 (PC1) underlie autosomal dominant polycystic kidney disease (ADPKD). ADPKD patients present with multiple cardiovascular co-morbidities believed to be caused by renal dysfunction. LV hypertrophy and diastolic dysfunction can manifest during childhood or in young adults prior to a formal diagnosis of hypertension, and evidence suggests that LV function is impaired in ADPKD patients with normal or moderately reduced kidney function. These facts suggest that cardiomyocyte-autonomous effects may contribute to the cardiovascular abnormalities seen in ADPKD. Contractile function (systolic and diastolic) measured by echo was significantly reduced in PC1 cKO (*Pkd1^{F/F};αMHC-Cre*) mice compared with controls (*Pkd1^{F/F}*). PC1 cKO cardiomyocytes manifest impaired contractility and smaller and slower Ca²⁺transients. Using a multidimensional approach, we discovered that cardiomyocytes lacking PC1 have shorter action potentials (APD_{50/90}) and decreased SERCA activity. These alterations impair EC-coupling and decrease SR Ca²⁺ loading during pacing. Remarkably, square pulses under voltage clamp (-80 to +10 mV) produced Ca²⁺ transients with similar amplitude between genotypes, which highlights that alterations in action potential (AP) duration drive most of the EC-coupling changes. PC1-deficient cardiomyocytes manifested an increase in outward K⁺ currents (I_{to}, I_{Kslow1/2} and I_{ss}) but not in inward currents (I_{K1}). PC1 over-expression in HEK293T cells reduced the currents of heterologously expressed Kv4.3/2.1/1.5 channels. The inhibitory effects of PC1 on Kv4.3 currents were mediated by PC1-CT (C-terminus) through its coiled-coil domain (CCD). Interestingly, a naturally occurring human mutant PC1^{R4228X}, located in

the CCD, manifested no suppressive effects on Kv4.3 channel. Finally, to begin to test for relevance to human pathology, we found that PC1 ablation reduces AP duration, and PC1-CT over-expression had the opposite effect in human stem cell-derived cardiomyocytes. Our findings uncover a novel role for PC1 controlling action potential duration and SERCA. PC1-deficient cardiomyocytes manifest impaired contractility, likely contributing to contractile dysfunction in ADPKD patients.

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Difference in Calcium Sensitivity Between Right and Left Ventricles With Lower Expression of Calcium Binding Proteins in Right Ventricular Myocytes

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Left ventricle (LV) and right ventricle (RV) have distinctive structural and functional characteristics as well as heterogeneous physiological properties. The RV is exposed to a relatively low pulmonary vasculature, which results in less mechanical afterload. Consistent with these physiological differences, the RV has a thinner free wall than the LV, and the movement of its contraction is geometrically different. Despite these definite differences, the studies of basic excitation-contraction coupling and calcium homeostasis of RV has been less studied than in LV. To establish the interventricular difference, we evaluated the basic electrophysiological and calcium-contraction properties of myocyte with or without β -adrenergic stimulation. Analyses of contraction and Ca^{2+} -signaling and action potential duration (APD) in isolated RV myocytes showed more prominent APD prolongation with less significant changes in sarcomere shortening and calcium transient, implying less efficient E-C coupling RV myocytes. To investigate the prolonged APD of RV, we measured transient outward potassium (Ito) current and L-type calcium channel (LTCC) current of two ventricles. Although the peak current of two ion channel was not different significantly in two ventricle, the half inactivation voltage of RV Ito was smaller than LV. Comparing with LV, RV myocytes showed round peak, slower early relaxation, and faster late relaxation, suggesting the difference of calcium sensitivity between two ventricles. To investigate the difference, we examined the expression level of calcium-binding proteins that regulate myofilament activities. Using immunoblotting from enriched protein of myofilament fraction, we found that the calcium binding proteins such as troponin I were lower in RV. Taken together, our results suggest that calcium binding proteins of RV was differ from that of LV, which induce the modified calcium sensitivity. We confirmed that reduced calcium binding proteins induced the decrease of calcium sensitivity and modified E-C coupling using Bers' mathematical rat cardiomyocyte model. The calcium sensitivity of RV is a clue to explain the different physiological properties of the RV.

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Sex Dependent Differences in Cardiac Myocyte Excitation-Contraction Coupling After Chronic Stimulation of the Delta or Kappa Opioid Receptors During Normoxic and Hypoxic Conditions

Matthew Klos 44106, UH Rainbow Babies and Children's Hosp, Cleveland, OH; Ramiz Ahmad, Isabella Pua, Kayla Hicks, Shreyas Suresh, Case Western Reserve Univ, Cleveland, OH; Sherry Morgenstern, Eric Devaney, UH Rainbow Babies and Children's Hosp, Cleveland, OH

Introduction: In 2016 the US government declared opioid misuse a public emergency. While most opioid related deaths can be attributed to overdose, recent data suggest a link between opioid use and cardiovascular disease. Further, epidemiological data suggest it may be sex dependent, with females (♀) being at a somewhat higher risk than males (♂). This is paradoxical because until menopause, ♀ have a lower risk of heart disease when compared to ♂ . Healthy adult hearts express the δ and κ ORs, but not the μ OR. Therefore, the overall goals of this study using a reductionist model were to: 1). To determine if there are sex specific differences in cardiac myocyte excitation-contraction coupling between ♂ and ♀

myocytes exposed to δ or κ OR agonists during normoxia/hypoxia. 2). Examine if 17 β -Estradiol enhances or augments the effects of δ or κ OR stimulation. **Methods:** Myocytes were isolated from adult δ and κ Sprague-Dawley rats and cultured overnight in M199 HEPES media, or M199 HEPES media containing either 1 μ M DADLE (δ agonist), 20 μ M U50-488 (κ agonist), 100 nM 17 β -Estradiol, 1 μ M DADLE + 100 nM 17 β -Estradiol, or 20 μ M U50-488 + 100 nM 17 β -Estradiol. To simulate an infarct, a subset of the myocytes was incubated at 1%O₂, 5% CO₂ for 4 hours + 10 nM isoproterenol followed by 60 min normoxia (21%O₂, 5% CO₂) before recordings. Excitation-Contraction was measured using an IonOptix rig (IonOptix LLC, Milton MA) at 36 \pm 1 $^\circ$ at 1 Hz pacing. **Results:** During normoxia, δ myocytes compared to κ myocytes had an increased peak contraction and peak Ca²⁺ transient. The addition of 100 nM 17 β -Estradiol did not affect peak contraction in either sex but decreased the Ca²⁺ transient in δ myocytes while increasing it in κ myocytes. DADLE and U50-488 had no effect on κ myocyte contractility but decreased δ myocyte contractility by a minimum of 13% and a maximum of 26% with/without 100 nM 17 β -Estradiol. κ myocytes were immune to the effects of hypoxia, even exhibiting a slight increase in their peak calcium compared to control conditions, arguably because of the addition of isoproterenol. δ myocytes on the other hand, had an overall decrease in excitation-contraction coupling during hypoxia which was made worse by OR stimulation. **Conclusion:** κ myocytes are immune to the detrimental effects of δ and κ OR stimulation.

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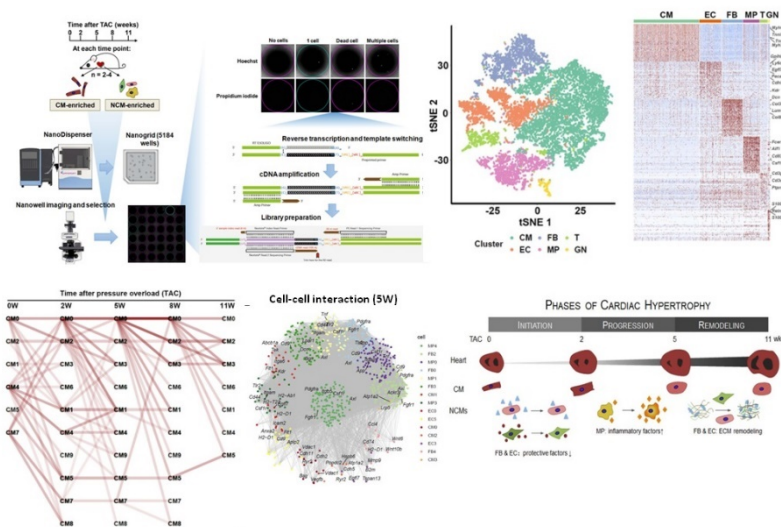
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Precision Intervention of Cardiac Remodeling Based on Cellular Composition Principles Uncovered by Single-Cell Transcriptomics

Peng Yu, Zongna Ren, Li Wang, Fuwai Hosp, Beijing, China

Stress-induced cardiac remodeling forms the foundation of many cardiac diseases, yet little is known about the spatiotemporal interplay amongst cell types underlying the pathological progression of the heart from normal to a diseased state, at single-cell resolution. Here, we analyzed 11,492 single cells, including both cardiomyocytes (CMs) and non-cardiomyocytes (NCMs), at different stages in a mouse model of pressure overload-induced cardiac remodeling, and identified a full list of factors and signaling pathways important for disease progression. Through constructing cell crosstalk maps, we revealed sequential switching in NCM subtype (fibroblasts, macrophages, and endothelial cells) utilization at different stages of cardiac remodeling. Intriguingly, stage-specific pharmacological inhibition of macrophage subtype switch dramatically retarded heart transition from adaptive to a maladaptive state. Consistently, alterations of cardiac remodeling related-genes were highly conserved in human samples. Together, our study not only characterizes the molecular features of different cell types and identifies crucial factors underlying cardiac remodeling, but may also have important implications for stage- and cell type-specific precise intervention in cardiac diseases.



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Epigenetic Mechanisms Underlying Anthracycline-Induced Cardiotoxicity

Ching Kit Chen, Yee Phong Lim, Wilson Lek Wen Tan, George C Anene-Nzelu, Shi Ling Ng, Yiqing Li, Tuan L Danh, Roger Sik Yin Foo, Natl Univ of Singapore, Singapore, Singapore

Anthracycline-induced cardiotoxicity (AC) is a major cause of morbidity in childhood cancer survivors. Doxorubicin cardiotoxicity however, is consistently observed to develop chronically over time. We sought to develop a mouse model of pediatric AC to test our hypothesis for an epigenetic mechanism, which may be operating to explain delayed-onset AC. Three week-old male and female C57Bl/6J mice were administered 5 weekly injections with doxorubicin (1mg/kg) (n=24) or saline (n=8). The dosage used was intended to closely mimic the clinical scenario. Half of the doxorubicin-treated (DOX) mice (n=12) and all controls (n=8) were sacrificed 1 week after the last injection; the rest of DOX mice (n=12) were given 4 weeks of drug-free holiday before sacrifice. Echocardiographic measurements were obtained before the first injection, 1 week after the final injection, and before sacrifice. Gene expression profiles were assessed by RNA-sequencing, reduced representation bisulphite sequencing (RRBS), and by ATAC-seq. These analyses were performed on purified pools of isolated cardiac myocytes, procured using a published Langendorff-free method. There was no death in the cohort. LV chamber dimension and wall thickness as well as fractional shortening did not differ between DOX and controls, implying the lack of obvious functional insufficiency from this dose. Similarly, DOX mice did not differ in body weight, heart weights (HW), and HW-to-tibia length ratios compared with controls. Cardiomyocyte dimensions and myocardial interstitial fibrosis were the same between groups. Despite the lack of echo and histological changes in DOX mice, RNA-seq analysis revealed profound and significant differentially expressed genes between the DOX mice and controls. Importantly, a significant proportion of downregulated and upregulated genes persisted, despite the period of drug-free holiday. Moreover, despite being taken off DOX, cardiomyocytes appeared to display new gene expression changes taking place even 4 weeks after stopping DOX. Detailed results of RNA-sequencing, RRBS and ATAC-Seq will be presented during the meeting. These results suggest that cardiomyocytes display DOX-related epigenetic alterations, which may implicate the basis for delayed-onset AC.

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CELA2A is a Pluripotent Insulinotropic Peptide

Sahar Esteghamat, James Samuel Broughton, Emily Smith, Rebecca Cardone, Tarun Tyagi, Mateus Guerra, András Szabó, Nelson Ugwu, Mitra Mani, Bani Azari, Gerald Kayingo, Sunny Chung, Mohsen Fathzadeh, Ephraim Weiss, Jeffrey Bender, Shrikant Mane, Richard Lifton, Adebowale Adeniran, Michael Nathanson, Fred Gorelick, John Hwa, Miklós Sahin-Tóth, Renata Belfort-DeAguiar, Richard Kibbey, Arya Mani, Yale Sch of Med, New Haven, CT

Coronary artery disease (CAD) is the leading cause of death worldwide. It is more prevalent in individuals with metabolic syndrome. Genetic factors that underlie the clustering of metabolic syndrome traits are not fully known. In this study, we present a cohort of 30 index cases with early onset CAD (age of onset for CAD \leq 30yr in male and \leq 35yr in female), and metabolic clustering of hypertension, hypertriglyceridemia and obesity. Whole exome sequencing in kindreds with extreme phenotypes of early onset CAD identified novel nonconservative loss of function mutations in the gene that encodes for the pancreatic elastase Chymotrypsin-like elastase family member 2A (CELA2A). we discovered that CELA2A is a circulating enzyme that reduces platelet hyperactivation, triggers both insulin secretion and degradation. CELA2A plasma levels rise postprandially and parallel insulin levels in humans. Our analyses show that the major consequence of CELA2A mutations is impaired insulin degradation with potential long-term deleterious effects of hyperinsulinemia on beta cell function and the vasculature.

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Transcription Factor Interactome in Human iPSC-derived Cardiac Progenitors is Enriched for Proteins Associated With Congenital Heart Disease

Barbara Gonzalez Teran, Maureen Pittman, Desmond Richmond-Buccola, Kaitlen Samse, Bonnie Cole, Ruth Huttenhain, Michael McGregor, Nevan Krogan, Katherine Pollard, Deepak Srivastava, Gladstone Insts, San Francisco, CA

Congenital heart disease (CHD) affects ~1% of live births and remains the leading cause of mortality in infants. While large-scale genetic studies have uncovered genes associated with CHD, distinguishing variants that confer risk from the background noise of inconsequential variants remains a challenge. Causative mutations in transcription factors (TF) essential for cardiovascular development, such as NKX2-5, GATA4 and TBX5, have been identified in familial cases of CHD, however they are rare. To expand our understanding of their molecular function and to test whether their interacting proteins may be enriched for variants associated with CHD. We defined the protein-protein interaction (PPI) network of NKX2-5, GATA4 and TBX5 using unbiased mass spectrometry in human iPSC-derived cardiac progenitors (iPS-CPs). This approach yielded a network of 172 proteins. An interdependent gene-regulatory role has been reported at the DNA-binding level for these 3 TF during cardiac development, and we also found interdependent protein interactomes where loss of one TF affected the interactome of the others. Interactomes for each were enriched in proteins involved in similar biological processes, such as chromatin remodeling and gene regulation, or previously unrelated processes such as splicing and mRNA transport. Integration of the iPS-CP-PPI network with the CHD-associated damaging variants found in the *Pediatric Cardiac Genomics Consortium* whole-exome sequencing cohort revealed statistically significant enrichment in the GATA4 interactome for de novo missense variants. In contrast, neither the TBX5 or NKX2-5 PPIs were enriched for either de novo missense or rare damaging variants. Finally, we developed a framework to rank PPIs with reported damaging variants for functional validation studies. Overall, this work identified novel protein interactors of TFs essential for cardiac development, offering new insights regarding their regulatory roles and the mechanisms through which they may cause CHD.

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Single-cell Reconstruction of Differentiation Trajectory Reveals a Critical Role of Ets1 in Human Cardiac Lineage Commitment

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) is an essential model system, but the dynamic process of this *in vitro* differentiation, especially the pivotal stages of cardiac cell lineage specification is not fully understood. Here, we generated a rich resource of Single-Cell RNA-sequencing of more than 7,500 single cells to capture the differentiation process of *in vitro* hESC to CMs across six key time points. We characterized the inter-subpopulation relationship demonstrating that co-existing side populations could play an essential role in early cardiac development by bestowing a supporting cellular microenvironment. Through analyzing the dynamic regulatory networks, loss-of-function screen, and systematically validation of binding signals through ChIP-sequencing, we pinpointed the essential regulatory role of *ETS1* in mediating early cardiomyocyte development.

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The Role of miR-987 in Homeostasis of the Aging Heart in *Drosophila*

Alyssa M Hohman, Elizabeth M McNeill, Iowa State Univ, Ames, IA

Background- microRNAs (miRNAs) have emerged as ideal candidates to regulate the genes involved in tissue homeostasis of the aging heart. Our work suggests a novel role for miR-987 in heart tissue maintenance during aging. *Drosophila* miR-987 has a highly conserved seed sequence with human miR-95 and miR-545. Both miR-545 and miR-95 have been identified as possible biomarkers in cardiovascular disease and are known to be dysregulated following cardiac events. The physiological consequences of their dysregulation, their molecular mechanism of action and specific gene targets are unknown.

Methods and Results- We have characterized the impact of miR-987 loss on homeostasis of heart morphology and function during aging using a combination of null mutants and heart specific knock down of miR-987 expression. Using SOHA (semi-automated optical heartbeat analysis), we have identified an increase in heart area and diameter around middle age (20 days post eclosion), but no significant change in arrhythmicity index, diastolic interval, systolic interval, and fractional shortening in miR-987 null mutants. We have examined the effect of miR-987 loss on lifespan and found a significant impact on male survival starting at 20 days. Using *in silico* analysis we have identified multiple (130) human conserved putative miRNA-target gene interactions to further characterize the molecular mechanisms underlying the observed phenotypes.

Conclusions- Our results suggest that miR-987 plays an important role in heart tissue homeostasis during aging and may impact the lifespan of mutant animals. The high conservation of *Drosophila* miR-987 with human miR-95 and miR-545 and their conserved predicted gene targets provides us with an excellent platform to facilitate the discovery of the molecular mechanisms underlying miRNA dysregulation in cardiovascular disease.

A.M. Hohman: None. **E.M. McNeill:** None.

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Targeting the Highly Abundant Circular RNA, circHeart, in Cardiomyocytes Attenuates Pressure Overload Induced Hypertrophy

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Objectives: Circular RNAs (circRNAs) are single-stranded non-coding RNAs that form a covalent closed looped structure and are generated during gene transcription. In our genome-wide analysis of cardiac circRNA expression in both human and mouse hearts we identified a highly abundant circRNA, named here as *circHeart* and hypothesize an important role for *circHeart* in cardiomyocyte physiology and homeostasis. **Materials and method:** We undertook FISH to visualize the localization of *circHeart*. Online tools have predicted multiple potential miRNA binding sites on *circHeart*, including the cardiac-enriched microRNA-133a (miR-133a). We assessed the interactions of *circHeart* with these miRNAs by luciferase and pull-down assays and performed AAV9-mediated RNAi knockdown and overexpression of *circHeart* in mice *in vivo*. *In vivo* phenotype was tracked by echocardiography, electrocardiogram and histological studies. Transverse aortic constriction (TAC) was also performed. **Results:** FISH imaging showed *circHeart* localized largely to the cardiomyocyte cytosol. Luciferase and reciprocal pull-down assays suggest *circHeart* can interact with miR-133a. MiR-133a is a well-known regulator of cardiac hypertrophy and antagomir-133a treatment has been shown to induce cardiac hypertrophy *in vivo*. In our studies, while AAV9 RNAi-mediated knockdown of *circHeart* has no apparent baseline effect in the heart it suppresses pathological cardiac hypertrophy post-TAC. More strikingly, AAV9-mediated overexpression of *circHeart* *in vivo* resulted in mouse mortality between 3-4 days post-transduction and surviving mice showed evidence of dilated cardiomyopathy. Known targets of miR-133a were concordantly modulated in the knockdown and overexpression mouse model. **Conclusion:** CircRNAs have huge potential role as therapeutic targets and/or disease biomarkers and represent a new avenue for cardiovascular research. Taken together, our data are consistent with a mechanistic model of *circHeart* sequestering miR-133a. Inhibiting *circHeart* abundance presents a potential target for treating cardiac hypertrophy.

T.B. Lim: None.

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Roles of PIKfyve in Cardiac Fibroblast Migration

Guangming Luo, Lois S Weisman, Univ of Michigan, Ann Arbor, MI

Cell migration is fundamental to proper development of the heart as well as other organs. Our characterization of a hypomorphic PIKfyve ^{β -geo/ β -geo} mouse mutant, revealed that has an abnormally small heart, and raised the possibility that this could be due in part to a defect in cell migration. Moreover, a few studies suggested that PIKfyve, the sole lipid kinase that generates phosphatidylinositol 3,5-bisphosphate (PI3,5P2) and provides most of the PI5P, may have a role in migration of cultured cells. However, the localization of PIKfyve and the molecular mechanisms whereby PIKfyve regulates cell movement remain elusive. Here I show that inhibition of PIKfyve significantly impairs cell migration in cultured cell lines, as well as in primary cardiac fibroblasts, which are essential for proper heart development. Moreover, expression of a hyperactive PIKfyve mutant promotes cell migration. In order to detect the localization of endogenous PIKfyve, we used CRISPR-Cas9 genome editing to tag endogenous PIKfyve with an HA peptide at its N-terminus. Interestingly, in addition to PIKfyve localization on endosomes, a pool of PIKfyve was located at the cell leading edge. We observed PIKfyve in both lamellipodia and filopodia. Notably, we found that acute addition of PI3,5P2 or PI5P for 5 min altered filopodia dynamics. Our preliminary results suggest that PI3,5P2 and PI5P alter different properties of filopodia. These findings suggest that cell migration may be controlled in part by PI3,5P2 and PI5P which arise due to PIKfyve localization to the cell leading edge. Together, these studies suggest that mechanistic insights into how PIKfyve regulates cell migration may reveal new tools or concepts that can be utilized to study heart development in mammals.

G. Luo: None. **L.S. Weisman:** None.

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The R21C Mutation in Troponin I Has a Founder Effect in South Lebanon and Causes Malignant Hypertrophic Cardiomyopathy

Akl Fahed, Harvard Medical Sch, Boston, MA; **Georges Nemer**, Fadi Bitar, Samir Arnaout, Antoine Abche, American Univ of Beirut, Beirut, Lebanon; James Ware, Imperial Coll London, London, United Kingdom; Manal Batrawi, Athar Khalil, American Univ of Beirut, Beirut, Lebanon; Steven DePalma, Barbara McDonough, Harvard Medical Sch, Boston, MA; Mariam Arabi, American Univ of Beirut, Beirut, Lebanon; Jonathan Seidman, Christine Seidman, Harvard Medical Sch, Boston, MA

Hypertrophic Cardiomyopathy (HCM) occurs in 1 of every 500 people and has a wide phenotypic variability. In the majority of cases, HCM is caused by known mutations in genes that code for sarcomere proteins. Although gene testing is widely available for HCM, knowing the phenotype caused by different gene mutations remains a challenging task. We recruited 28 families with HCM, of which 19 (67.8%) have at least one patient with pediatric onset. Index patients from 20 families received targeted sequencing for a panel of genes including *TNNI3*, and 7 families received Sanger sequencing for the *TNNI3*. We identified a missense mutation p.R21C in *TNNI3* segregating with HCM in four families from South Lebanon. Through cascade screening, we identified 30 patients from the four families; twenty of them (67%) had a clinical diagnosis of HCM with a median age of 37 years, while 9 (30%), with a median age 21 years, had no evidence of HCM on echocardiography. An additional 27 members of the families had evidence of HCM, including 22 with SCD in the setting of no past medical history, and their carrier status for p.R21C was implied from the pedigrees. Survival analysis for 57 HCM patients with the mutation revealed a markedly decreased age at first adverse event as compared to 47 HCM patients with the *MYBPC3* p.R502W mutation. Founder mutations in HCM that cause a severe phenotype are uncommon. The p.R21C mutation in *TNNI3* is the first HCM mutation described in the Lebanese population and has a founder effect in South Lebanon. Early and more frequent screening with different imaging modalities as well as tailored management might be warranted for carriers of this mutation.

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Reliable Biomimetic Culture System for Pig and Human Heart Slices

Riham Abouleisa, Qinghui Ou, Univ of Louisville, Louisville, KY; Zoë Jacobson, Tenaya Therapeutics, South San Francisco, CA; Xian-Liang Tang, Sajedah M. Hindi, Ashok Kumar, Univ of Louisville, Louisville, KY; Kathryn N. Ivey, Tenaya Therapeutics, South San Francisco, CA; Guruprasad Giridharan, Ayman Al-Baz, Kenneth Brittan, Benjamin Rood, Bradford G. Hill, Steven P. Jones, Roberto Bolli, Tamer M A Mohamed, Univ of Louisville, Louisville, KY

Rationale: Heart failure is the number one killer and drug induced cardiotoxicity is a major cause of market withdrawal. The lack of availability of culture systems for human heart tissue that is functionally and structurally viable for more than 24 hours is a limiting factor in validation of novel heart failure therapies as well as reliable cardiotoxicity testing. Therefore, there is an urgent need to develop a reliable system for culturing human heart tissue for testing drug efficacy and toxicity. **Objective:** To develop a reliable method to culture pig and human heart slices under full physiological conditions for a period of time sufficient to test therapeutic efficacy and acute drug toxicity. **Methods and Results:** Here we describe a novel biomimetic culture system that maintains full viability and functionality of human and pig heart slices (300 µm thickness) for 6 days in culture through optimization of the medium and culture conditions with continuous electrical stimulation at 1.2 Hz and oxygenation of the medium. Functional viability of these slices over 6 days was confirmed by assessing their calcium homeostasis, twitch force generation, and response to β -adrenergic stimulation. Temporal transcriptome analysis using RNAseq at day 2, 6, and 10 in culture confirmed overall maintenance of normal gene expression for up to 6 days, while over 500 transcripts were differentially regulated after 10 days. Electron microscopy demonstrated intact mitochondria and Z-disc ultra-structures after 6 days in culture under our optimized conditions. This biomimetic culture system was successful in keeping human heart slices completely viable and functionally and structurally intact for 6 days in culture. We also used this system to demonstrate the effects of a novel gene therapy approach in human heart slices. **Conclusions:** We have developed and optimized a reliable and easily reproducible culture system for pig and human heart slices as a platform for testing the efficacy of novel heart failure therapeutics as well as reliable testing of cardiotoxicity in a 3D heart model.

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iPSC Derived Cardiomyocytes Reproduce Divergent Phenotypes Caused by a LQTS Type-1 Likely Pathogenic Mutation

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The purpose of this work was to investigate if iPSC derived cardiomyocytes would reproduce the divergent phenotypes observed in a proband and mother that carried the same likely-pathogenic variant in KCNQ1 gene. A 15 years old proband had syncope and cardiac arrest while swimming being diagnosed with LQTS based on a 12 lead ECG. The mother's ECG showed no abnormalities. Genomic DNA from blood of daughter and mother was extracted using standard procedures. All coding exons and flanking intronic sequences of KCNQ1 (NM_000218) genes were amplified by polymerase chain reaction. Direct sequencing was performed on a 3500XL Genetic Analyzer. Data was analyzed with Geneious software for identification of mutations. For iPSC generation CD71+ CD36+ erythroblasts were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit on irradiated mouse embryonic fibroblast feeder layers. Differentiation into cardiomyocytes was accomplished using the protocol described by Lian et al. (2012). Electrophysiological recordings were obtained from cardiomyocytes after 30–45 days of differentiation. Cells were transferred to acrylic plates pretreated with Matrigel and kept in culture for 3 days before microelectrode action potential (AP) were registered. The ECG recordings showed a corrected QT interval (QTc) of 516 ms for the daughter and 436 ms for the mother. Sequencing showed that both daughter and mother had a c.1763C>T mutation resulting in p.Ile588Thr substitution. iPSC generated from daughter, mother and an unrelated control displayed normal karyotype and expressed pluripotent genes,

besides spontaneously differentiating into cells of the three germ layers. Differentiation into cardiomyocytes was ascertained by immunofluorescence and flow cytometry for troponin T. In all differentiations more than 70% of cells were cardiomyocytes. Action potentials recordings showed that ventricular cardiomyocytes from the daughter exhibited significantly longer action potential durations at 90% repolarization than the mother's or the unrelated control's cardiomyocytes.

Cardiomyocytes derived from iPSC cells reproduced the clinical phenotypes exhibited by daughter and mother.

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Transcriptomic Changes During Induced Pluripotent Stem Cell-derived Neural Crest Cell Differentiation Highlight Genes Involved in Endocardial Cushion and Cardiac Outflow Tract Development

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Rationale: Neural crest cells (NCCs) play a critical role in normal cardiac development, and defects in NCCs likely cause congenital heart disease (CHD). NCCs are transient and multipotent migratory stem cells that give rise to diverse tissues, including cardiac structures such as the smooth muscles of the great arteries and semilunar valves. Induced pluripotent stem cells (iPSC) can be differentiated into NCCs, as demonstrated by expression of several marker proteins including NGFR and HNK1. To better define iPSC-NCCs and to better understand the progression of iPSCs to NCCs, we have compared the transcriptomes of iPSC and iPSC-NCCs. We have also begun to investigate the consequences of loss-of-function mutations in genes implicated in CHD on the differentiation of iPSC-NCCs.

Methods and Results: PGP1 iPSCs were differentiated to NCCs and RNA was collected at 0, 5, 10, and 15 days of differentiation. RNAseq analysis showed that by day 15, 6483 genes were upregulated in NCC vs iPSCs, 6406 downregulated, and 6715 unchanged (FDR 5%). Enrichment analysis for the top 500 upregulated genes showed 4 cardiac gene ontology (GO) terms in the top 10, including 'endocardial cushion morphogenesis' (fold enrichment 11.85, $p = 0.012$). Notably, of 45 genes under GO term 'endocardial cushion development', 38 (84%) differentially expressed in NCCs by day 15 (FDR 5%). Next, we compared this RNAseq data with that of iPSC-derived cardiomyocyte (CM) differentiation in a subset of 253 genes previously implicated in CHD. Of these, 143 genes were differentially expressed in both iPSC-CMs and iPSC-NCCs. However, 27 genes (10.6%) including *MYH6*, *PITX2*, and *TBX5* were uniquely upregulated during CM differentiation, while 65 genes (25.7%) including *CHD4* and *NOTCH1* were uniquely upregulated during NCC differentiation.

Conclusion: Transcriptomic changes during iPSC-NCC differentiation, assessed by both bulk and single cell RNAseq, reveal an upregulation of genes involved in cardiac development, particularly endocardial cushions and the outflow tract.

Importantly, a subset of genes implicated in CHD are altered during iPSC-NCC differentiation but not in iPSC-CM differentiation. Thus, iPSC-NCCs offer a new model with which to investigate the pathogenesis and mechanisms of CHD.

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Substrate Elasticity Impacts Duchenne Muscular Dystrophy Cardiomyopathy Progression

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Duchenne Muscular Dystrophy (DMD) is a X-link disease affecting ~1:3500 boys per year and culminating with heart failure in early adulthood. DMD results from >200 possible genetic mutations on dystrophin. The lack of dystrophin disrupts the anchoring of the cell sarcomere to the extracellular matrix (ECM), affecting the cardiac contraction. With disease progression, in an attempt to mitigate the subcellular defects, the tissue stiffness and ECM composition remodel in association with a dilated cardiomyopathy phenotype and fibrosis. Our hypothesis is that disease progression is accelerating because of this remodelling, through a positive feedback loop involving multiple mechanosensing pathways. Here, we use a

single-cell assay platform to model the effect of fibrotic remodelling in DMD. This platform allows measuring the force production of single human-derived pluripotent stem cell (hiPSC) cardiomyocytes (CMs). The substrate stiffness can be controlled to match that of healthy (~10kPa) or fibrotic (~35kPa) tissue. In addition, single iPSC-CMs are patterned in an elongated 1:7 aspect ratio using microcontact printing of ECM protein. This enhances their intracellular structural maturity towards a more mature adult phenotype. This renders our in-vitro model more representative of the human pathology and greatly improves our measurements standardization. Our results show that single iPSC-CMs with DMD mutations have a dramatically reduced ability to produce force on stiffer substrates compared to their isogenic control. This loss of contractile function correlates with an increase in reactive oxygen species (ROS) and mitochondria dysfunction, as well as with other markers of stress response and cellular senescence. We are asking how the remodeling of the ECM stiffness and composition is signaled from the outside-in and affects the progression of the disease phenotype. The further development of our platform and approach will allow for more accurate in-vitro modeling of cardiac diseases and greatly increase our understanding of the underlying biophysics of mechanosensing.

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Hypertrophic Cardiomyopathy Mutations With Opposite Effects on β -myosin Biomechanics Show Similar Structural and Biomechanical Phenotypes in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes (hipsc-cms)

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Introduction: Hypertrophic cardiomyopathy (HCM) is the most prevalent heritable cardiovascular disease, commonly caused by mutations in beta cardiac myosin heavy chain (β MYH). We have measured the kinetics of isolated myosin proteins with different HCM mutations in β MYH and found an intriguing heterogeneity in kinetics and force production: some mutations increase whereas others decrease force and velocity; others result in no change. How these divergent molecular alterations converge to the final HCM phenotype: hypercontractility and cellular hypertrophy is still unknown. hiPSC-CMs provide a powerful tool for studying human cardiomyocyte biology including contractility, hypertrophic growth, and intracellular organization, but are limited by immature phenotypes and population heterogeneity in traditional culture environments.

Methods and Results: We developed a micropatterned hydrogel platform that enhances force generation and promotes both structural and molecular hiPSC-CM maturation. Using CRISPR/Cas-9 gene editing in an isogenic hiPSC line, we created hiPSCs with two different β MYH mutations (P710R and D239N) that result in opposite effects on force at the molecular level, using in vitro motility and laser trap methods. Cell morphology was quantified by both fluorescence and transmission electron microscopy (EM) and force generation measured by traction force microscopy. Despite having opposite effects at the single molecule level, both HCM lines showed increased contractile force and cellular hypertrophy compared to isogenic controls. Immunostaining for β MYH and EM revealed organizational and microstructural changes including sarcomere and myofibrillar disarray, and thickened z-discs in cells containing these mutations. Both mutations had alterations in AMPK, MAPK, and calcineurin signaling shown to regulate hypertrophy.

Discussion: Divergent biomechanical alterations due to HCM mutations at the single molecule level lead to common cellular-level structural and biomechanical phenotypes through activation of common signaling pathways.

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Single-Cell RNA Sequencing Reveals Pathways Dysregulation by a NFATc1 Mutation in Patient-Specific Cardiomyocytes Derived From Inducible Pluripotent Stem Cells

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Using a family-based approach with whole exome sequencing (WES) and custom bioinformatics tools we identified a novel mutation (M527L) in the Nuclear Factor of Activated T-Cells 1 gene (NFATc1). This nonsynonymous heterozygous substitution segregates in an autosomal dominant pattern within a family with a young onset Atrial Fibrillation (AF) phenotype. Patient specific inducible pluripotent stem cell derived-cardiomyocytes (iPSC-CM) from a patient carrying this variant showed abbreviated repolarization resulting in a shorter action potential duration compared to iPSC-CM from a healthy sibling control. Unlike familial monogenic AF where the mutation occurs in an ion channel gene, NFATc1 is a transcription factor that influences expression of multiple genes. Using single-cell RNA sequencing on 30 day post-differentiation iPSC-CM, we identified four subpopulations by K-mean clustering (k=4) of single cell transcriptomes and subsequent marker expression. We then performed gene-annotation enrichment analysis of differentially expressed genes specifically in the mutant (MT) and wild-type (WT) cardiomyocyte (CM) populations, detecting several key functional and pathological cardiac pathways affected in response to the NFATc1 mutation. These included Wnt, NF-kappaB and MAPK signaling, Ca homeostasis and gene categories that have been associated with atrial fibrillation. These results suggest that NFATc1 mutation may play an important role in the pathophysiology of familial atrial fibrillation by its broad impact on gene expression and subsequent pathway regulation.

Category	Term	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0038061	NIK/NF-kappaB signalin	5.59	3.60E-08	4.00E-09	1.71E-08
GO:0060071	Wnt signaling pathway, planar cell polarity pathway	5.18	1.91E-10	6.36E-11	9.07E-11
UP_KEYWORDS	Calcium	1.41	2.51E-06	4.18E-07	6.09E-06
GO:0005509	Calcium ion binding	1.42	2.23E-05	1.11E-05	1.69E-05
GO:0005887	Integral component of plasma membrane	1.23	0.005	8.75E-04	0.008
GO:0001666	Response to hypoxia	1.84	0.001	4.53E-04	3.74E-04
KEGG_PATHWAY	Gap Junction	2.03	0.001	3.66E-04	0.007

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Effect of Matrix Stiffness on Adult Cardiomyocytes Using Dynamic, Tunable, and Reversible Magnetorheological PDMS Substrates

Alexia Vite, Matthew A. Caporizzo, Unniversity of Pennsylvania, Philadelphia, PA; Elise Corbin, Delaware Univ, Newark, DE; Kenneth Bedi, Christina Yingxian Chen, Benjamin L. Prosser, Kenneth B. Margulies, Unniversity of Pennsylvania, Philadelphia, PA

The physical properties of the extracellular matrix (ECM), including stiffness and viscoelasticity, play a key role in the development of hypertensive and hypertrophic cardiac diseases at the cellular level, affecting the morphology and function of heart tissue. The ECM is a dynamic structure that is continuously changing in both normal and diseased tissue. In an extension of prior studies examining how substrates of fixed stiffness affect cardiac cells, we introduce a novel experimental model that allows exploration of cardiomyocyte functional alterations in response to rapid and reversible matrix stiffness modulation. We fabricated a biocompatible magneto-rheological elastomer (MRE) culture substrate by adding iron particles to a PDMS elastomer. In the presence of an applied magnetic field, this substrate stiffens reversibly and nearly instantaneously from 10 kPa to 50 kPa: the range observed in normal and diseased myocardium, respectively. Adult cardiomyocytes isolated from non-failing human hearts (unused organ donors) or adult rats were cultured for 24 - 48 hours on the tunable MREs. Cardiomyocytes cultured on a stiffened MRE (~50 kPa) exhibited time-dependent reductions in cell shortening and peak Ca^{2+} transient amplitude compared with cardiomyocytes cultured on a soft MRE (~10 kPa). Rates of cardiomyocyte shortening and re-lengthening were also slowed by culture on 50 kPa substrates. Using super-resolution imaging, we found that cardiomyocytes cultured on a stiffened MRE exhibited an increased microtubule network density and demonstrated increased cellular stiffness and viscoelasticity, as measured by nanoindentation. These studies indicate that adult human and rat cardiomyocytes are acutely sensitive to changes in extracellular stiffness within the pathophysiological range and tend to adapt their own stiffness to match that of their surroundings via dynamic changes in microtubule architecture. These mechanisms may contribute to the regulation of mechanical homogeneity within the myocardium. Our method provides a unique *in vitro* platform for studying mechanosensing, mechanotransduction and mechanical memory in isolated adult cardiomyocytes.

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Assessing the Efficacy of Novel RYR2 Inhibitor, EL20, in Induced Pluripotent Stem Cell Derived Cardiomyocytes from a Catecholaminergic Polymorphic Ventricular Tachycardia Patient

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Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac arrhythmia syndrome that leads to sudden cardiac death in children and young adults. The most common form of CPVT is caused by autosomal-dominant mutations in the gene encoding cardiac ryanodine receptor type-2 (*RYR2*). Mutations in *RYR2* promote excessive calcium (Ca^{2+}) leak from the sarcoplasmic reticulum (SR), triggering potentially lethal arrhythmias. Recently, we demonstrated that the tetracaine derivative EL20 specifically inhibits RYR2, normalizes Ca^{2+} handling, and suppresses arrhythmias in a CPVT mouse model. We hypothesized that EL20 will also normalize Ca^{2+} handling and arrhythmias in human induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs).

Materials/Methods: Blood samples from a child carrying RYR2 mutation R176Q/+ (RQ) and a mutation-negative relative (WT) were collected, processed into peripheral blood mononuclear cells (pBMCs), and reprogrammed into iPSCs using Sendai virus system. Functional iPSC-CMs were derived using Stemdiff™ kit. Differentiation of CMs was validated using qPCR and immunofluorescent staining. Confocal Ca^{2+} imaging was used to monitor RyR2 activity. AxioM Biosystem™ microelectrode arrays (MEA) was used to assess arrhythmogenic action potential (AP) and rates.

Results: Successfully differentiated CMs showed increased levels of Ca^{2+} -handling markers and decreased levels of stem cell markers. Baseline Ca^{2+} imaging revealed a 4-fold increase in calcium spark frequency (CaSpF) in RQ iPSC-CMs (3.08 ± 0.58 a.u., $n=12$) vs. WT (0.69 ± 0.22 a.u., $n=13$, $p < 0.01$). Strikingly, EL20 normalized CaSpF in RQ iPSC-CMs (0.60 ± 0.16 a.u., $n=9$, $p < 0.05$ vs. baseline). Furthermore, EL20 decreased the erratic beat period rate of spontaneous APs occurring in RQ iPSC-CMs to a rate similar to WT. Importantly, EL20 had no significant effect on the beat period rate or field potential duration of WT iPSC-CMs.

Conclusion: Our results demonstrate that EL20 normalizes Ca^{2+} handling and AP beat rate frequency in patient-derived RYR2-R176Q/+ iPSC-CMs with no observed off-target effects, positioning this novel RyR2-inhibitor as a promising therapeutic candidate for treatment of CPVT.

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Pathophysiological Significance of Browning of Perivascular Adipose Tissue in the Development of Atherosclerosis

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Background: Perivascular adipose tissue (PVAT) possibly plays a pivotal role in the development of atherosclerosis through its direct local action to the vessels. However, its physiological and molecular mechanisms are less understood. Visceral and subcutaneous white adipose tissues exert a brown adipose tissue-like phenotype under specific conditions such as cold exposure, i.e. browning. We here investigated the pathophysiological role of browning of PVAT against the development of atherosclerosis after endovascular injury.

Methods and Results: Endovascular injury was generated by wire insertion into the femoral artery of C57BL/6 female mice. Transcriptome analysis revealed robust upregulation of brown adipose tissue markers such as *Ucp1*, *Elovl3*, *Cox8b* and *Cidea* in injured arteries. Notably, administration of an atheroprotective agent, 17-beta estradiol, significantly inhibited these changes; *Ucp1* was the most down-regulated gene by 17-beta estradiol administration in the entire gene set (log2-fold change = -6.58, false discovery rate = 0.003). Consistently, the upregulation of browning markers after endovascular injury and these inhibitions by 17-beta estradiol were confirmed in PVAT by quantitative real-time PCR, western blot and immunohistochemical staining. The present study also demonstrated that vascular injury promotes macrophage infiltration in PVAT accompanied with upregulation of inflammatory cytokine production. Furthermore, we confirmed spatiotemporal synchronicity between browning and inflammation in PVAT by immunohistochemical staining.

Conclusions: We observed that endovascular injury elicits browning in PVAT accompanied with exacerbated inflammation, and that atheroprotective 17-beta estradiol strongly inhibits this phenomenon. These findings may provide novel insights into pathogenesis of the atherosclerosis.

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Gut Microbiota Alterations Associate With T Cell Activation and Adverse Cardiac Remodeling in Response to Cardiac Pressure Overload

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Background: The complex syndrome of heart failure (HF), a leading cause of morbidity and mortality affecting more than 24 million people worldwide, is recently becoming associated with alterations in gut microbiota, also known as gut dysbiosis, as well as with T cell mediated systemic inflammation and T cell infiltration in the heart. Specifically, certain bacteria promote T cell activation, and T cell activation contributes to adverse cardiac remodeling and cardiac dysfunction in HF.

We **hypothesize** that gut dysbiosis modulates cardiac remodeling and function in a T cell-dependent manner.

Results: C57/BL6 mice were orally treated with a well-established wide spectrum antibiotic cocktail (ABX) and subjected to transverse aortic constriction (TAC), a model of non-ischemic HF. ABX was present during the duration of TAC (4 weeks), thus sterilizing the gut completely, or during 3 weeks before TAC followed by removal during the 4 weeks of TAC duration for spontaneous recovery of the gut microbiota (TAC-REC). The lack of gut microbiota showed a reduced T cell activation in the mediastinal lymph nodes and heart T cell infiltration, as well as cardiac fibrosis and cardiac hypertrophy were prevented in response to TAC. Nevertheless, gut dysbiosis induced by bacterial recolonization only partially recovered this phenotype. In addition, we sequenced 16S RNA to study the changes in bacterial populations in Sham, TAC, and TAC-REC. TAC induced changes in the gut microbiota compared to Sham surgery, which resulted in a higher relative abundance of several genera, such as Bacteroides, and lower relative abundance of Coprococcus, Lactobacillus and Bifidobacterium, these last two normally associated to an anti-inflammatory response. At the end of the experiment, bacterial recolonization in TAC-REC mice showed different relative bacterial abundance between TAC and TAC-REC, although there were no significant changes in diversity at 4 weeks post-TAC.

Conclusion: Collectively, our findings suggest that changes in gut microbiota constitution modify T cell activation and migration to the heart in the TAC model. Exploring the role of gut microbiota in modulating adverse cardiac remodeling and cardiac function can become an optimal therapeutic target to treat HF patients.

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IKKε Deficiency Aggravates Cardiac Inflammation with Dysregulation of p52 and p38 in Myocardial Infarction

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Inhibitor of NF-κB kinase (IKK), an upstream of nuclear factor-kappa B (NF-κB), is a critical modulator for pathophysiological inflammation. IKKε is a non-classical IKK and has been studied in infectious diseases and cancers. However, the role of IKKε in a myocardial infarction (MI) has not been addressed. In this study, we used IKKε knockout mice to induce MI by coronary artery ligation. Cardiac function was analyzed by echocardiograph after 2 weeks, and fractional shortening (FS) was 16.36±4.46% in the wild type group and 13.47±1.21% in the knockout group. Cardiac fibrosis and macrophage infiltration deteriorated in the knockout group. Next, we investigated the inflammatory responses and found that the expression of inducible nitric oxide synthase (iNOS), an inflammatory marker, was much higher in both infarcted heart tissues and bone marrow-derived macrophages (BMDM) isolated from knockout mice than from wild type mice. Besides, cardiac macrophages displayed more inflammatory phenotype in the IKKε knockout group than in the wild type group. To explore the responsible mediator, we performed phosphorylated protein array and found phosphorylated p38 was

significantly downregulated in the IKK ϵ knockout BMDM. Conversely, both knockdown of p38 by siRNA and inhibition of p38 by SB203580 treatment in RAW264.7 cells upregulated iNOS induction. In the infarcted heart tissue, non-canonical NF- κ B2 (p52) protein was dramatically upregulated in the IKK ϵ knockout group than in the wild type group, while mRNA level was not different in the both groups. Immunohistochemical analysis showed nuclear accumulation of p52 in cardiomyocytes and fibroblasts in the peri-infarct lesion. Our data showed excessive inflammation in IKK ϵ knockout mice was associated with inactivation of p38 in macrophages and upregulated p52 in the infarcted myocardium. Collectively, IKK ϵ is involved in the control of inflammation resolution through modulating p38 activity and p52 post-translational modification in the infarcted myocardium.

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Single Cell Analysis of the Emergency Hematopoietic Response to Myocardial Infarction

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Myocardial infarction (MI) is the initiating event in ischemic heart disease, the most common cause of death in the world. Although MI-induced injury is sterile, it nevertheless elicits a vigorous emergency hematopoietic response in the bone marrow that supplies abundant myeloid cells to the heart, which remove dying cell debris and orchestrate healing, repair, and fibrosis. Ensemble methods such as flow sorting of immunostained cells and qPCR have provided some insights into infarct leukocyte phenotypes using candidate markers/genes, but the full diversity remains unknown. Here, we used single cell RNA-Seq to perform genome-wide transcriptomic profiling of >50,000 single cells from the hearts, blood, and bone marrow of infarcted and non-infarcted mice on days 1-4 after MI. We defined the diversification of myeloid cells, from their granulocytic and monocytic origins in the bone marrow, through the blood, and into the infarcted heart. This allowed construction of an atlas of MI-induced myeloid diversification and trafficking. Among the many observations enabled by this data were the origins of the type I interferon response. We recently discovered that MI induces a type I interferon response that can be targeted genetically or pharmacologically for therapeutic benefit. Our single cell data now reveal that interferon induced cells (IFNICs) derive from both neutrophilic and monocytic origins as early as 24 hours after MI. These cells are not only found within the heart, but can also be identified in the post-MI blood and bone marrow. Ongoing studies are investigating whether IFNICs can be detected in human blood after MI. Our results have clinical importance for understanding and modulating the immune response to myocardial injury. We show, using single cell transcriptomics, that post-MI treatment with anti-interferon alpha receptor antibody (IFNAR Ab) (the murine equivalent of a therapeutic antibody currently in Phase 3 clinical trials for lupus), completely abolishes MI-induced interferon signaling and shifts the intracardiac macrophage program towards a reparative posture. Our comprehensive atlas of the emergency hematopoietic response to MI can serve as a resource for others studying the inflammation in ischemic heart disease.

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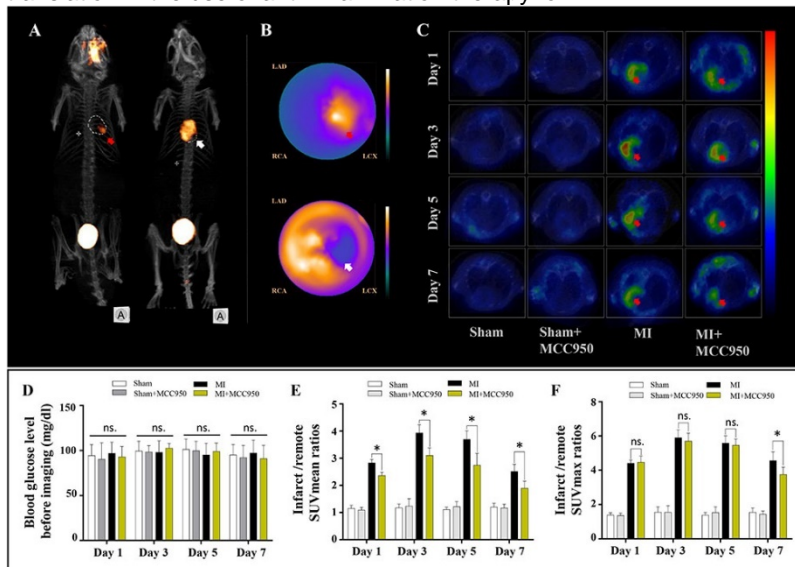
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Dynamic Multimodality Imaging Monitored Anti-inflammation Therapy for Myocardial Infarction: Exploring the Role of MCC950 in Murine Model

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Objectives: The aim of this study is applying non-invasive multimodality imaging to investigate the potential therapeutic effects of MCC950, a novel selective NLRP3 inflammasome inhibitor, in myocardial infarction (MI) model. **Methods:** C57BL/6 mice were subjected to the MI or Sham model. ^{18}F -FDG PET/CT was undertaken for monitoring any post-MI inflammation changes on days 1, 3, 5 and 7. ^{18}F -FDG PET/CT and echocardiography were both used to quantitate any alterations of infarct size and cardiac function on days 7 and 28 post-MI. **Results:** Dynamic ^{18}F -FDG PET/CT revealed that MI+MCC950 group had a lower accumulation of ^{18}F -FDG at infarct region as early as day one, and remained

lower until day seven post-MI when compared to the MI group (Infarct/remote SUV_{mean} ratios, from day 1 to 7 respectively: 2.37±0.11, 3.10±0.28, 2.74±0.43, 1.89±0.26, P < 0.05.). What's more, MCC950 inhibited infarct size expanding on day 7 (31.90±1.60% vs 36.30±3.31%, P < 0.05 vs MI group). The use of echocardiography suggested that MCC950 treatment increased ejection fraction (EF) and fraction shortening (FS), (EF: 48.72±3.86% vs 39.64±2.85%, P < 0.05; FS: 22.93±2.58% vs 17.54±1.91%, P < 0.05), meanwhile decreased end-diastolic volume (EDV) and end-systolic volume (ESV) when compared with that in the MI group on day 28 (EDV: 70.91±3.31 vs 82.40±5.30 ul, P < 0.05; ESV: 38.95±4.38 vs 50.29±3.76 ul, P < 0.05). **Conclusions:** MCC950 reduced inflammatory response, infarct size and preserved cardiac function post-MI. Dynamic multimodality imaging may be potential approaches for basic research and future clinical translation in the use of anti-inflammation therapy for MI.



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Sub-cytotoxic Levels of Heavy Metals Induce Pro-inflammatory Signaling in the Aortic Endothelium without Impairing Flow-Mediated Dilation in Rats

Pooneh Nabavizadeh, Sharina Ibrahim, Adam Fries, Jiangtao Liu, Ronak Derakhshandeh, Matthew L Springer, Univ of California San Francisco, San Francisco, CA

Introduction: Acute exposure to tobacco or marijuana secondhand smoke (SHS) causes endothelial dysfunction. The identity of specific SHS constituents that cause vascular toxicity is unclear. Heavy metals are present in SHS and at elevated levels in the blood of smokers, and may mediate acute endothelial dysfunction through reactive oxygen species formation and decreasing NO bioavailability. We assessed the effects of exposure to cadmium, lead, mercury, and arsenic at levels present in the blood of human smokers on flow-mediated dilation (FMD) as a measure of endothelial function in rats. We also evaluated the effects of heavy metal exposure on intimal structure, protein localization pattern, and inflammatory gene expression in the aortic endothelium. Hypothesis: Sub-cytotoxic levels of heavy metals impair FMD, alter intimal structure, and induce pro-inflammatory signaling in endothelium. Methods: We injected rats (n=8/group) with heavy metal cocktail or vehicle intravenously and quantitated pre- and post-exposure FMDs measures defined as percent vasodilation of femoral artery after transient ischemia. We performed en face aorta immunostaining and assessed endothelial cell axis alignment, cell length ratio, and localization pattern of PECAM-1, VE-cadherin, and vimentin. We also quantified gene expression of key endothelial proteins in aorta homogenates. Results: FMD was not impaired in the heavy metal group (8.8±3.6(SD)% vs. 12.9±8.0%, p=.31 or controls (7.5±2.7% vs. 8.8±5.8%, p=.63). No significant difference in cell length ratio and endothelial x and y axes of alignment were detected between groups (p>.8) and localization of PECAM-1, VE-cadherin, and vimentin in the aorta endothelium remained unaltered following heavy metal injection. However, expression of PECAM-1 and VE-cadherin was significantly lower in the heavy metal-treated rats, while VCAM-1 gene expression was significantly higher (p<.05). Conclusion: Acute exposure to sub-cytotoxic levels of heavy metals can induce pro-inflammatory signaling in endothelium, which could potentially lead to vascular injury. However, FMD and endothelial structure remain unchanged by heavy metal exposure.

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Evaluating Pro-Inflammatory and Pro-Resolving Lipid Modulations in an Oral 15-HETE Model of Pulmonary Hypertension

Ellen I O'Connor, Gregoire Ruffenach, Victor Grijalva, David Meriwether, Jeremy Papesh, Nasrin Dorreh, Anna Dorfman, Mansoureh Eghbali, Srinivasa Reddy, UCLA, Los Angeles, CA

Pulmonary arterial hypertension (PH) is a chronic lung disease with a mortality rate of 50% at 5 years after diagnosis. PH is characterized by progressive increase in pulmonary arterial pressure leading to right ventricular (RV) increase in afterload, hypertrophy, and failure. Despite the availability of multiple animal models of PH, finding relief or cure for PH patients has been elusive to date. Recent studies in our laboratory have shown that levels of hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs) are elevated in the lungs and plasma of patients with PH as well as in animal models of PH. In testing for causality, we showed that administration of 5 µg of 15-HETE/day to wild type male mice for 3 weeks was sufficient to cause increase in pulmonary pressure, vascular resistance, plasma levels of HETEs and HODEs, right ventricle hypertrophy, and right ventricle systolic pressure (RVSP), consistent with PH. We set out to utilize this new mouse model of PH to understand the mechanisms in PH pathology by first examining the role of lipid mediators of inflammation in the development of PH. Our lab has developed a mass spectrometry method for measuring the levels of 39 inflammatory pathway lipid mediators from the cyclooxygenase (COX) and lipoxygenase (LOX) pathways that has been validated for use in tissue, plasma, and cell supernatant. Utilizing this panel with samples from mice fed 15-HETE and supernatant from an intestinal epithelial cell line; IEC-6 cells treated with 15-HETE, we have observed significant changes in multiple lipid mediators of inflammation. In addition to seeing increases in HETEs and HODEs with 15-HETE treatment, we observed significant increases in leukotriene C4 (LTC4) and 14(S)-hydroxy docosahexaenoic acid (14S-HDHA) and trending increases in thromboxane B2 (TXB2), and leukotriene E4 (LTE4) in the plasma, significant increases in 20 different lipids in lung tissue, including LTC4, TXB2, and LTE4, and significant increases in LTC4 and 5-oxo-eicosatetraenoic acid (5-oxoETE) in the supernatant of IEC-6 cells incubated with 15-HETE for 12 hours. These results indicate that novel inflammatory lipids and pathways may play an important in the development of hypertension and help identify new targets for therapies.

E.I. O'Connor: None. **G. Ruffenach:** None. **V. Grijalva:** None. **D. Meriwether:** None. **J. Papesh:** None. **N. Dorreh:** None. **A. Dorfman:** None. **M. Eghbali:** None. **S. Reddy:** None.

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Apoai Mimetic Peptide 6f Prevent Pulmonary Hypertension Induced by Oxidized Lipids

Gregoire Ruffenach, Ellen O'Connor, Mylene Vaillancourt, Shervin Sarji, Nancy Cao, Laila Aryan, Christine Cunningham, Victor Grijalva, Soban Umar, Srinivasa Reddy, Mansoureh Eghbali, Donya Moazeni, Univ of California, Los Angeles, CA

Introduction: Pulmonary arterial hypertension (PAH) is characterized by pulmonary arterial occlusion leading to increased pulmonary arterial pressure. Recently, a growing body of evidence demonstrated a robust increase in oxidized lipids, including 15-hydroxyeicosatetraenoic acids (15HETE), in the lungs and plasma of PAH patients and animal models of pulmonary hypertension (PH). Nonetheless, the the causal role of 15HETE in promoting PH development remains elusive. In order to investigate this mechanism, we fed wild type mice with a diet rich in 15HETE and examine whether ApoA-I mimetic peptide can rescue PH induced by 15HETE.

Methods: Wild type male mice (C57BL/6) were fed for 3 weeks with regular chow (n=16-21 mice/group), 15HETE (5µg/day), 15HETE diet supplemented with either empty vector or 6F for the last week of 15HETE diet. PH development was assessed every week via serial echocardiography. Right ventricular systolic pressure (RVSP) was measured via heart catheterization. RV hypertrophy index (RV/[IVS+LV]) was measured. Lung morphology and lipid accumulation were assessed using H&E and Oil red O staining.

Results: Echocardiography revealed the first sign of PH as early as one week after starting 15HETE diet and a significant decrease in the pulmonary arterial acceleration time (PAAT) after 2 weeks of treatment (16.6±1.9 vs. 20.6±1.4 msec, p<0.05). At the end of three weeks, mice on 15HETE diet had significantly higher RVSP (31.3±1.1 vs. 38.4±2.3 mmHg, p<0.05) and RV hypertrophy index (0.26 ± 0.02 vs. 0.33 ±0.02, p<0.05). This increased pressure was concomitant with a significant increase in pulmonary arteriolar thickness in mice on 15-HETE diet compared to regular diet (35.1±0.8 vs 53.4±1, p<0.05). At the end of three weeks mice treated with 6F showed a PAAT similar to control value(19±0.5 msec) concomitant with a significantly lower RVSP than mice fed with 15HETE+empty vector (33.6 ±5.7 vs 40.3±4.6, p<0.05).

Conclusion: Our data demonstrates that APOA-I mimetic peptide 6F is able to rescue pre-existing PH induced by 15-HETE diet in wild type mice.

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Intravital Multiphoton Microscopy Reveals Increased Capillary Patrolling by Leukocytes and Cardiomyocyte Dysfunction in High Fat Diet Induced Hypertrophy

David M Small, Nathaniel H Allan-Rahill, Michael RE Lamont, Salomon Djakpa, Marvarakumari G Jhala, Yvette Zhu, Nozomi Nishimura, Cornell Univ, Ithaca, NY

Background: The study of functional cardiomyocyte adaptation and inflammatory cell behavior at the micro-scale in vivo has been challenging due to limited imaging tools. We recently developed intravital multiphoton microscopy (MPM) methods that enable visualization and quantification of cardiac dynamics at a cell-scale throughout the cardiac cycle. We aimed to determine the dynamic cellular changes that occur due to high fat diet (HFD) induced hypertrophy using intravital cardiac MPM.

Methods: ApoE^{-/-} C57Bl6 mice started a HFD at 6 weeks of age (ApoE^{-/-}-HFD, n=11), while age-matched wild-type mice (WT-ND, n=10) were fed a normal chow diet. At 26-weeks, mice were assessed by cardiac echocardiography and intravital MPM in the intact beating heart. Intravenous injections of rhodamine-6G (R6g) labeled cardiomyocytes and leukocytes, and Texas-Red dextran labeled vasculature. 3D volumes were reconstructed throughout the cardiac cycle to quantify cell motion using automated algorithms.

Results: ApoE^{-/-}-HFD hearts underwent hypertrophy compared to WT-ND with increased heart weight-to-tibial length ratio (10±0.8 vs 13±1.1) and left ventricle wall thickness (1.07±0.03 mm vs 1.13±0.06 mm, respectively, p<0.05 for both) while ejection fraction remained similar (66±3 % vs 59±3 %). In vivo MPM demonstrated that cells move a greater total distance in each cardiac cycle in ApoE^{-/-}-HFD vs WT-ND. Maximum displacement in the apex-base and anterior-posterior directions increased by 46 % in ApoE^{-/-}-HFD compared to WT-ND (30 µm vs 14 µm). R6g+ leukocytes were visible moving in capillaries. The incidence of patrolling behavior (defined as slowing moving cells, visible for longer than one heart beat) increased in capillaries of ApoE^{-/-}-HFD compared to WT-ND (3.4±0.5/min vs 0.12±0.1/min, p<0.01).

Conclusion: These results suggest that hypertrophied cardiomyocytes increase myocardial displacement, and increased leukocyte patrolling behavior is associated with HFD induced cardiac hypertrophy. Intravital cardiac MPM provides a novel perspective to study HFD induced cardiac hypertrophy by capturing the simultaneous contributions of inflammatory cells and myocyte function in the beating heart.

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Spatiotemporal Dynamics of Macrophages in vitro and in vivo

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Introduction: Macrophages play diverse roles in the cardiovascular system during health and disease. Monocyte-derived macrophages expand this repertoire of function following acute injury. Our current understanding of macrophage dynamics is limited to time scales of minutes to days, largely due to the use of low bandwidth destructive measurement techniques. We hypothesized that high bandwidth sensors that could be interrogated nondestructively, may expand our understanding of macrophage dynamics. **Methods:** We analyzed single cell RNA Sequencing to select an optimal Cre-driving promoter for monocytes and macrophages. Then, we created a macrophage calcium reporter mouse by crossing a Csf1r-Cre mouse with GCamp5 calcium reporter mouse. The monocytes and macrophages within the offspring were then nondestructively studied across space and time in vitro and in vivo using confocal microscopy. **Results:** Using flow cytometry and ex vivo confocal microscopy, the distribution of Cre-activated cells based on the constitutive fluorescence of tdTomato has been defined for

each tissue. Then, we isolated and differentiated bone marrow derived macrophages and quantified their dynamic response to innate immune stimuli commonly associated with sterile injury. We developed image analysis routines and parameterization strategies for classifying calcium responses. These studies revealed that calcium reporter BMDMs display minimal fluctuations at baseline but exhibit a dynamic response to immunogenic DNA sensing that reverberates to neighboring cells suggesting the tool can be used to infer cell communication. To study macrophage dynamics in vivo, we installed a dorsal window chamber and performed serial intravital imaging. In vivo, the monocyte-macrophage calcium reporter is spontaneously dynamic suggesting exposure to a rich but undefined collection of microenvironmental cues. **Conclusions:** Our results provide a window into the spatiotemporal dynamics of macrophages as they respond to specified innate immune stimuli in vitro or complex microenvironmental cues in vivo. In addition, the tool provides an opportunity to discover undefined pathways for intercellular communication.

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TWEAK-Fn14 Axis: A Potential Therapeutic Target for Treating Heart Failure

Sathyadev Unudurthi, Evelyn Thomas, Nehal Patel, Alexander Winkle, Daniel Gratz, Thomas Hund, The Ohio State Univ, Columbus, OH

Heart failure (HF) is a complex disease characterized by compromised cardiac structure and function. Previous work has identified a link between upregulation of pro-inflammatory cytokines, and HF. There is a critical need to gain better understanding of the inflammatory pathways in heart and identify novel therapeutic targets to regulate cardiac inflammation and HF

Recent studies have shown that under stress macrophages are recruited to heart. These macrophages are mainly implicated in the release of inflammatory cytokines that promote cardiac fibrosis, eventually resulting in heart failure. Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a multifunctional member of the TNF superfamily, which binds to fibroblast growth factor inducible 14 (Fn14), a ubiquitously expressed cell-surface receptor. The TWEAK-Fn14 axis has been previously implicated in promoting inflammation in multiple autoimmune disorders, such as rheumatoid arthritis, however, its role in heart is not well known. The objective of this study is to test the hypothesis that TWEAK-Fn14 pathway promotes cardiac inflammation under stress, and blocking this pathway could be a potential therapeutic strategy to reduce stress induced cardiac inflammation and HF. Wild type (WT) and Fn14 knock out (Fn14^{-/-}) mice were subjected to pressure overload induced stress (Trans aortic constriction or TAC) for 1 or 6 weeks. A subset of WT TAC animals were treated with the Fn14 inhibitor [L524-0366] (9mg/kg, IP daily) or vehicle beginning at day 3 following TAC. Macrophage infiltration and cardiac fibrosis were quantified in ventricular sections using F4/80 and Masson Trichrome staining respectively, and cardiac function was measured by echocardiography. Levels of monocyte chemoattractant protein-1 (*mcp-1*) were measured by quantitative PCR. Genetic or chemical inhibition of Fn14 reduced fibrosis [40% lower compared to WT TAC] and improved cardiac function [Δ EF of 21.7%, n=6 for WT TAC; Δ EF of 14.2%, n=6 for Fn14^{-/-}TAC; Δ EF of 48.5%, n=3 for vehicle; Δ EF of 27.9%, n=5 for drug] compared to WT. Fn14 inhibition also reduced *mcp-1* expression and macrophage infiltration [53.5% lower compared to WT TAC]. These results support TWEAK/Fn14 axis as a potential therapeutic target for treating HF

S. Unudurthi: None. **E. Thomas:** None. **N. Patel:** None. **A. Winkle:** None. **D. Gratz:** None. **T. Hund:** None.

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Disturbed Blood Flow-induced Platelet Transmigration Via C-type Lectin-like Receptor-2 Modulating Vascular Inflammation

Chaojun Tang, Yulan Sheng, Tao You, Lei Wang, Zhanli Xie, Soochow Univ, Suzhou, China; Michael McDaniel, Lijun Xia, Oklahoma Medical Res Fndn, Oklahoma City, OK; **Li Zhu,** Soochow Univ, Suzhou, China

Atherosclerosis preferentially develops at sites under disturbed blood flow (d-flow) that leads to steep multidirectional gradients of wall shear stress at vascular predilection sites, promoting an oxidative, proapoptotic, and proinflammatory gene expression profile in endothelial cells. Sustained d-flow causes endothelial injury characterized by leukocyte infiltration, endothelial barrier dysfunction, and eventually the formation of atherosclerotic plaques. While previous studies suggest a proinflammatory role of platelets via promoting leukocyte activation in atherogenesis, whether and how platelets are

regulated by d-flow remains largely unclear. In this study, we showed that d-flow induced by partial carotid ligation (PCL) causes platelet adhesion, endothelial damage and leukocyte recruitment in mice. Unexpectedly, PCL induces platelet transmigration into the subendothelial space. Transmigrated platelets at 2 days of PCL appeared inside the intercellular crevices beneath the damaged endothelium. After sustained d-flow (5-7 days after PCL), more adherent leukocytes and platelets with pseudopodia were observed in the seriously damaged endothelium. In investigating the mechanism of this unexpected observation, we found that mice with platelet-specific deficiency of C-type lectin-like receptor-2 (CLEC-2) showed ~80% reduction in platelet endothelial accumulation and no subendothelial localization of platelets under d-flow, indicating an essential role of CLEC-2 in mediating platelet transmigration. Enhanced platelet transmigration exacerbated monocyte accumulation, while leukocyte depletion or PSGL-1 deletion did not affect platelet transmigration under d-flow. Our results revealed unprecedented platelet transmigration mediated by CLEC-2 in the rheological regulation of vascular homeostasis, controlling vascular inflammation.

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NCLX Expression Attenuates Pathological Remodeling in Experimental Cardiac Hypertrophy and Non-ischemic Heart Failure

Joanne F. Garbincius, Timothy S. Luongo, Devin W. Kolmetzky, Alycia N. Hildebrand, John W. Elrod, Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA

Mitochondrial calcium (mCa^{2+}) uptake couples acute changes in cardiomyocyte bioenergetic demand to ATP production, but in excess triggers mitochondrial permeability transition and cardiomyocyte necrosis, as occurs during cardiac injury. Despite established roles for mCa^{2+} flux in response to acute stress, the role of mCa^{2+} signaling in chronic stress is poorly defined. As mCa^{2+} regulates the TCA cycle with the potential to affect mitochondrial signaling and/or metabolite pools required for biosynthesis, we reasoned that altered mCa^{2+} homeostasis may be an essential mechanism underlying hypertrophic growth and remodeling of the myocardium. Here, we used mice with cardiac-specific overexpression (OE) of the mitochondrial Na^+/Ca^{2+} exchanger (NCLX), the primary mediator of mCa^{2+} efflux in the heart, to test the hypothesis that mCa^{2+} signaling contributes to cardiac remodeling during sustained hemodynamic stress. We subjected NCLX OE (TRE-NCLX x α -MHC-tTA) and control (α MHC-tTA) mice to 12-wk transverse aortic constriction (TAC) or 4-wk infusion with angiotensin II + phenylephrine (AngII/PE) as experimental models of cardiac hypertrophy and non-ischemic heart failure. Cardiac function of NCLX OE and control mice was monitored throughout these studies via echocardiography and remodeling was assessed via tissue gravimetrics, histology, and qPCR gene expression analysis. Cardiac NCLX OE preserved ejection fraction, prevented afterload-induced hypertrophy and fibrosis, and attenuated the induction of both hypertrophic (*Nppa*, *Nppb*, *Acta1*) and fibrotic (*Postn1*, *Spp1*) gene programs in mice subjected to 12-wk TAC. Examination at 2-wk post-TAC revealed attenuated hypertrophy and blunted hypertrophic and fibrotic gene expression in NCLX OE mice. These data indicate that increased capacity for mCa^{2+} efflux mitigates TAC-induced remodeling, prior to the development of contractile dysfunction. NCLX OE similarly attenuated atrial hypertrophy and the induction of hypertrophic and fibrotic gene programs in mice infused with AngII/PE. Together, these findings support a critical role for mCa^{2+} in driving pathological remodeling in non-ischemic heart disease and point to NCLX as a potent therapeutic target for cardiovascular disease.

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A Micropeptide Regulator of Voltage-gated Potassium Channels Controls Cardiac Rhythm

Kelly M Anderson, Xiaorong Parks, Pornthida Poosala, Sean Lindley, Coeli Lopes, **Douglas M Anderson,** Univ of Rochester, Rochester, NY

Voltage-gated potassium (K^+) channels (VGKCs) are essential regulators of the cardiac action potential and are required to maintain normal heart rhythm and contractility. Voltage-dependent activation, conductance and membrane trafficking of K^+ channels are tightly controlled by a family of single transmembrane regulatory cofactors (KCNE1-5), which co-assemble

with pore forming K⁺ channels in cell membranes. Human mutations in voltage-gated K⁺ channels and their regulatory subunits are associated with Long QT syndrome (LQTS), a condition in which the repolarization of the heart is delayed and can lead to arrhythmias, syncope and sudden death. Here we identify a cardiac-enriched transmembrane micropeptide encoded by a small open reading frame, which we named KCNEmini, due to its sequence and structural homology with members of the KCNE family of VGKC regulators. We show that KCNEmini directly binds to and co-localizes with VGKCs in the plasma membrane and functions as a novel regulator of K⁺ channel kinetics. Disruption of KCNEmini in mice resulted in QT interval prolongation and cardiac dysfunction. These studies shed light on a previously unrecognized regulator of K⁺ handling in the heart, which may be an important future therapeutic target for the early diagnosis and treatment of human lethal cardiac arrhythmias.

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Biphasic Reprogramming of Ventricular Cardiomyocytes to Pacemaker Cells by Tbx18

Jinqi Fan, Natasha Fernandez, Hee Cheol Cho, Emory Univ, Atlanta, GA

Background: We have previously demonstrated that an embryonic transcription factor, TBX18, suffices to reprogram postnatal ventricular cardiomyocytes to induced pacemaker cells. Here, we sought to gain finer understanding of the reprogramming process by investigating genotype/phenotype of the de novo pacemaker cells in multiple temporal domains. **Methods:** TBX18-induced pacemaker cells (TBX18-iPMs) were created in vitro by somatic gene transfer of Adeno-TBX18 into neonatal rat ventricular myocytes (NRVMs). Control ventricular myocytes were transduced with Adeno- GFP. To investigate in vivo reprogramming, we delivered AAV9-TBX18 via tail vein of Hcn4(+eGFP) heterozygous transgenic mice, which expresses eGFP under the Hcn4 promoter, so as to lineage-trace de novo pacemaker cells in the ventricular myocardium. **Results:** The automaticity of TBX18-iPMs was higher than that of GFP-NRVMs during D1-D3, decreased after D3 and rebound by D14. Monolayers of the iPMs revealed multiple foci of spontaneous field potentials with lower spike amplitude, spike slope, and conduction velocity, demonstrating phenotypic reprogramming to the iPMs by D14. RNAseq and qPCR revealed two distinct phases of reprogramming; an early phase that showed loss of expression of ventricular myocyte-related gene program and a late phase that showed gain of expression of nodal gene program which plateaued by D14. For example, Hcn4 gene expression began increasing during week 1 and continued the pattern during week 2. By week 3, Hcn4-positive TBX18-iPMs appeared in clusters of NRVM monolayers while GFP-NRVMs were spread evenly throughout the monolayers. We lineage-traced de novo, TBX18-reprogrammed pacemaker cells in vivo by delivering AAV9-TBX18 via tail-vein of Hcn4(+eGFP) mice. In line with our in vitro data, in vivo pacemaker cell lineage tracing experiments indicated a progressive loss of ventricular gene program followed by gain of nodal gene program. **Conclusions:** Our data indicate that TBX18-induced reprogramming of ventricular myocytes to nodal pacemaker cells undergoes progressive changes in gene expression shedding ventricular gene/phenotype first, and then gaining nodal pacemaker genotype/phenotype, establishing fully functional nodal pacemaker by D14.

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Identifying the Transcriptome Signature of Calcium Channel Blocker in Human iPSC-Derived Cardiomyocytes

Chi Keung Lam, Lei Tian, Nadjet Belbachir, Alexa Wnorowski, Rajani Shrestha, Ning Ma, Tomoya Kitani, June Wha Rhee, Joseph C Wu, Stanford Univ, Stanford, CA

Calcium channel blockers (CCBs) are important in treating cardiovascular diseases and the acute pharmacological actions of CCBs in the hearts have been extensively studied. However, we lack the knowledge of the drug-specific effect on human cardiomyocyte transcriptome and potential physiological change after long-term exposure as patients are usually prescribed with these medications for their lifetime after diagnosis. Thus, we aimed to simulate chronic CCB treatment in human cardiomyocytes and subsequently examine both the functional and transcriptomic alterations. We differentiated cardiomyocytes from three human induced pluripotent stem cell (iPSC) lines and exposed them to four different CCBs—nifedipine, amlodipine, diltiazem, and verapamil—at their physiological serum concentrations for two weeks. Without

inducing cell death and damage to myofilament structure, CCBs elicited line specific inhibition on calcium kinetics and contractility. While all four CCBs exerted comparable inhibition on calcium kinetics, verapamil applied the strongest inhibition on cardiomyocyte contractile function. By examining cardiomyocyte transcriptome after treatment, we identified little overlap in their transcriptome signatures. Verapamil is the only inhibitor that reduced the expression of contraction related genes, such as myosin heavy chain and troponin I, consistent with its depressive effects on contractile function. Moreover, the alterations in these gene may help explain how HCM patients respond to verapamil in relieving outflow tract obstruction. In conclusion, we identified the distinct transcriptome signatures of different CCBs in human cardiomyocytes, suggesting that although the four inhibitors act on the same target, they may have distinct effects on normal cardiac cell physiology. The application of iPSC platform and transcriptomic findings may allow us to identify responders to verapamil treatment.

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Critical Role of Ryanodine Receptor Bound Calmodulin to Prevent Catecholaminergic Polymorphic Ventricular Tachycardia

Yoshihide Nakamura, Takeshi Yamamoto, Shigeki Kobayashi, Masaki Tamitani, Yamaguchi Univ, Ube, Japan; Yoriomi Hamada, Yamaguchi Univ, ube, Japan; Go Fukui, Xiaojuan Xu, Shigehiko Nishimura, Takayoshi Kato, Hitoshi Uchinoumi, Yamaguchi Univ, Ube, Japan; Tetsuro Oda, Yamaguchi Univ, ube, Japan; Shinichi Okuda, Masafumi Yano, Yamaguchi Univ, Ube, Japan

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by a single point mutation in the cardiac type 2 ryanodine receptor (RyR2). Using knock-in mouse (KI) model (R2474S/+), we previously reported that a single point mutation within the RyR2 sensitizes the channel to agonists, primarily mediated by defective inter-domain interaction within the RyR2 molecule and subsequent dissociation of calmodulin (CaM) from the RyR2. Here, we examined whether CPVT can be genetically rescued by enhancing the binding affinity of CaM to the RyR2. We first determined whether there is a possible amino-acid substitution at the CaM-binding domain in the RyR2 (3584-3603) that can enhance the binding affinity of CaM, and found that V3599K substitution showed the highest binding affinity of CaM to CaM-binding domain. Hence, we generated a heterozygous KI mouse model (V3599K/+) with a single amino acid substitution in the CaM-binding domain of the RyR2, and crossbred it with the heterozygous CPVT-associated R2474S/+ KI mouse to obtain a double heterozygous R2474S/V3599K KI mouse. The CPVT phenotypes—bidirectional or polymorphic ventricular tachycardia, spontaneous Ca²⁺ transients, and Ca²⁺ sparks—were all inhibited in the R2474S/V3599K mice. Thus, enhancement of the CaM binding affinity of the RyR2 is essential to prevent CPVT-associated arrhythmogenesis.

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Selective Right-sided Electrical Remodeling In A Pure Model Of Pulmonary Hypertension Promotes Micro-reentrant Arrhythmias

Benjamin Strauss, Emerson Obus, Nestor Bedoya, Yassine Sassi, Fadi G Akar, MOUNT SINAI SCHOOL OF MEDICINE, New York, NY

Background: Pulmonary arterial hypertension (PAH) is characterized by elevated mean PA pressures that increase right ventricular (RV) afterload, leading to RV failure. Whereas structural and mechanical decompensation in PAH are well established, the extent of electrophysiological (EP) remodeling and arrhythmia propensity are far less clear. This is, in large part, because PAH-related EP properties have been exclusively studied in the monocrotaline (MCT) model, which is confounded by major extra-pulmonary toxicities, including liver failure and direct myocardial damage. To address this knowledge gap, we performed a comprehensive evaluation of the EP substrate, including arrhythmia dynamics, in a new pure model of PAH.

Methods: Sprague Dawley rats underwent surgical left pneumonectomy followed by injection of the VEGF inhibitor Sugen

5416 (S/P), or underwent no intervention (CTRL). 5 wks later, rats were euthanized for high-resolution optical action potential (AP) mapping studies in ex vivo perfused hearts.

Results: 6/6 S/P vs 0/5 CTRL hearts were prone to pacing-induced VT ($P < .05$). Underlying this differential susceptibility was disproportionate RV sided prolongation of AP duration in S/P. This, in turn, promoted the formation of right-sided AP alternans at physiological rates in S/P but not CTRL. Correspondingly, KCND2 mRNA transcripts were downregulated by 80X in the RV of S/P relative to CTRL. While propagation was impaired at all rates in S/P, the most profound decrease in conduction velocity (53%) was always observed immediately prior to VT initiation. Nav1.5 and Cx43 mRNA expression were not altered in S/P. In contrast Col1a1, Col3a1, and fibronectin transcripts were upregulated implicating fibrosis in impaired conduction. The average wavelength was significantly decreased in S/P relative to CTRL ($P < .05$). Once generated, VT was sustained by multiple microreentrant circuits likely facilitated by the short wavelengths.

Conclusion: In this pure model of PAH, we document highly selective RV-sided EP remodeling that facilitates the initiation of multi-wavelet micro-reentry underlying VT. The S/P model represents a severe form of PAH that allows the study of EP properties without the confounding influence of extra-pulmonary toxicity.

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Mistrafficking of Integrin $\beta 1D$ Contributes to Ca^{2+} -handling Disorder and Catecholaminergic Polymorphic Ventricular Tachycardia Under Cardiac Stress

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Abstract:Background: RyR2 hyper-phosphorylation has been identified as a contributing mechanism to arrhythmogenesis. The integrin $\beta 1D$ colocalized with RyR2 and stabilized phosphorylation of RyR2, which maintains the function of the RyR2 channel. Our objective is to investigate whether the alteration of $\beta 1D$ integrin is a novel mechanism to arrhythmogenesis under cardiac stress. Methods and Results: Using transgenic mouse model $\beta 1D^{-/-}$ mice, which results in more uncoupling of $\beta 1D$ and RyR2, showed significantly increased the RyR2 ser2808 phosphorylation and Ca^{2+} -handling disorder and CPVT. Translocation of the $\beta 1D$ and uncoupling with RyR2 were discovered in the cardiac disease in human and animal model, instead of decreased $\beta 1D$. KIF5B mediated $\beta 1D$ redistributed from the center to the periphery with hyper-phosphorylation of ser2808 and Ca^{2+} -handling dysfunction. Conclusions: These results discover that translocation of $\beta 1D$ and disassociation from RyR2, which leads to hyper-phosphorylation of the RyR2 ser2808, is a novel pathway to trigger CPVT under cardiac disease.

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Pharmacological Evaluation of Novel Anti-thrombospondin-1 Decoy Strategy for Prophylaxis of Atrial Fibrillation-associated Ischemic Stroke

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Hemostatic abnormalities mainly decreased blood flow and increased stasis, and platelet hyper-aggregation increase thrombotic risk subsequent atrial fibrillation (AF) episodes. Excess elevation of extracellular glycoprotein thrombospondin 1 (TSP1) in circulation has emerged to predict prognosis of ischemic stroke. Moreover, TSP1 is found strongly associated with platelet hyper-aggregability and impaired nitric oxide response, through its cognate receptor CD47, thus exacerbating ischemic progression in AF patients. TSP1 arises as a prospective target of our candidate medicine.

Our work provides "proof-of-concept" of a novel pharmacological strategy, relying on soluble recombinant human CD47 peptide (rh-CD47p) to serve as a decoy receptor to specifically bind TSP1, and consequently neutralize TSP1-impaired vasorelaxation.

Our pilot studies were initially conducted with isolated mouse thoracic aorta. A pre-treatment with rh-CD47p (added 15 min prior to TSP1 incubation) showed a significant amelioration of TSP1-impaired endothelium-dependent vasodilation

($p < 0.0001$, rh-CD47p+TSP1 vs. TSP1, $n=6$), indicating a prophylactic effect by rh-CD47p against vasoconstriction enhanced by excess TSP1. A post-treatment set-up, where TSP1 incubation was started 15 min prior to rh-CD47p addition to mimic pre-existing high level of TSP1 in ischemic stroke, exhibited a marked reversal of TSP1-inhibited vasodilation back to the same level as controls ($p < 0.0001$, rh-CD47p+TSP1 vs. TSP1, $n=4$). Dose titration of rh-CD47p in molar ratios to TSP1 in both set-ups identified the dose range of rh-CD47p for prevention/recovery from TSP1-induced vascular dysfunction. Binding of rh-CD47p and TSP1 was first verified as the primary mechanism via Western Blot, and further quantified with a modified ELISA assay. A linear correlation was found between % of rh-CD47p bound to TSP1 and dose of rh-CD47p added ($r^2=0.8$).

Current results established the ground of rh-CD47p as a unique pharmacologic moiety for drug development to limit vasoconstriction and improve vasodilation against TSP1 in excess, thus showing great potential for proposed novel decoy CD47-coated formulation to abrogate/mitigate TSP1-associated cardiovascular complications in AF patients.

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Sk Channels in the Vascular Endothelial Cells

Young Woo Nam, Taibah Aldakhil, Adam Viegas, Hannah Vu, Rachel Lee, Pammie Wong, **Miao Zhang**, Chapman Univ, Irvine, CA

Small-conductance Ca^{2+} -activated K^+ (SK) channels are a unique group of K^+ channels, that play important roles in the vascular system and central nervous system. Four subtypes in the SK channel family are encoded by *KCNN* mammalian genes: including *KCNN1* for SK1 ($K_{Ca2.1}$), *KCNN2* for SK2 ($K_{Ca2.2}$), *KCNN3* for SK3 ($K_{Ca2.3}$) and *KCNN4* for SK4 (also IK or $K_{Ca3.1}$) channels. It is well known that the activation of SK channels expressed on the endothelial cell surface can reduce vascular tone. In our study, we found that SK channels expressed on the endoplasmic reticulum (ER) membrane can protect endothelial cells from apoptosis mediated by ER stress. Among the four subtypes of SK channels, Ca^{2+} sensitivity and subcellular localization significantly varies by subtype in endothelial cells. Ca^{2+} sensitivity of the SK1 channel subtype is negatively modulated more than that of the SK2 subtype, whereas the cell surface expression of the SK2 channel subtype is negatively modulated more than that of the SK1 subtype. The functions of SK channels to reduce vascular tone and to protect against apoptosis are greatly affected by their Ca^{2+} sensitivity and subcellular localization. It is critical to understand how each SK channel subtype is modulated, which may become promising therapeutic targets for various cardiovascular conditions.

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PRMT7 Deficiency Exacerbates Cardiomyopathy Induced Doxorubicin in Ovariectomized Mice

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The risk of cardiovascular diseases (CVDs) in female increases starkly after menopause accompanied by fibrosis and inflammation, suggesting the protective role of estrogen. Although accumulating evidences suggest the protective role of estrogen against cardiomyopathy triggered by oxidative stress or immune response, the effect of estrogen replacement therapy appears to be variable due to complex features of CVDs. Thus, precise mechanisms underlying the menopause induced CVDs are still actively under investigation. In this study, we report that protein arginine methyltransferase 7 (PRMT7) plays a protective role in CVDs. Cardiac specific deletion of PRMT7 exacerbates cardiomyopathy induced by doxorubicin in ovariectomized mouse models. In accordance with animal models, the inhibition of PRMT7 activity sensitizes cardiomyocytes to oxidative stress-induced cell death while PRMT7 overexpression alleviates it. The gene expression profiling reveals that PRMT7 deficiency enhances various stress-related pathways, especially the immune response that

might contribute to cardiomyopathy. The current study suggests an important role of PRMT7 in the suppression of oxidative stress-induced cardiomyopathy.

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Applying a Quantitative, Cell Surface Glycoproteomic Approach to Understanding the Role of Human Cardiac Fibroblasts in Advanced Heart Failure

Linda Berg Luecke, Amanda R Buchberger, Matthew Waas, Medical Coll of Wisconsin, Milwaukee, WI; Claudius Mahr, Univ of Washington, Seattle, WI; Rebekah L Gundry, Medical Coll of Wisconsin, Milwaukee, WI

Heart Failure (HF) is a complex clinical syndrome defined by the heart's inability to adequately perfuse the human body. The clinical progression of HF has been linked to the pathological activation of CF, which contributes to adverse remodeling and cardiac fibrosis. Previous studies have shown that unloading the failing heart *via* mechanical circulatory support devices contributes to reverse remodeling, improving both cardiac structure and function. However, despite the importance of CF in biology and disease, little is known regarding CF-specific mechanisms and functions during cardiac remodeling and reverse remodeling. Moreover, most human studies have relied on *in vitro* culture systems to investigate CF function, despite the fact that extended culturing leads to phenotypic changes in CF. Here, we apply state-of-the-art mass spectrometry approaches to discover and quantify cell surface and extracellular matrix (ECM) proteins to 1) develop an optimized CF culture system that allows for the preservation of their *in situ* phenotype and 2) study CF isolated from failing hearts, including failing hearts treated with mechanical circulatory support to identify novel targets for tracking and studying disease. To date, we have identified >600 surface *N*-glycoproteins on primary human CF (n=12), including those with known relevance to CF (e.g. BGN, BSG, FBN1) and those not previously described within CF (e.g. HHIP, NFACS, GPNMB). In CF samples that were passaged *ex vivo*, cell surface and ECM *N*-glycoproteins that are associated with a phenotype switch (e.g. COL5A1, ECM1, FBLN5, LAMB2) were detected and changes in their abundance correlated with increased expression of α -SMA. Our studies also identified cell surface and ECM proteins that have not been previously associated with the heart and those with known relevance to HF, cardiac remodeling and fibrosis (e.g. BGN, GLUT8, ITGA11). Current efforts are underway to quantify surface proteins of interest to determine how their abundance changes during reverse remodeling in the context of HF. Overall, this work allowed for the development of an optimized CF culture system and revealed new cell-type specific molecular targets to study and track reverse remodeling in the context of advanced HF.

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Phagocytosis-mediated Activation of Smad3 in Macrophages Mediates Anti-inflammatory Transition and Protects From Adverse Post-infarction Remodeling

Bijun Chen, Shuaibo Huang, Ya Su, Albert Einstein Coll of Med, Bronx, NY; Jonathan Graff, Univ of Texas Southwestern, Dallas, TX; Nikolaos G Frangogiannis, Albert Einstein Coll of Med, Bronx, NY

Macrophages are essential regulators in tissue injury, repair and fibrosis. Although activation of TGF- β signaling cascades critically modulates macrophage phenotype *in vitro*, the *in vivo* role of macrophage TGF- β signaling in homeostasis and disease is unknown. Our study examined the role of macrophage-specific TGF- β /Smad3 signaling in a mouse model of myocardial infarction (MI). TGF- β s markedly activated Smad3 in isolated macrophages, without affecting Smad-independent pathways. Infarct macrophages, but not neutrophils exhibited Smad3 activation. Phagocytosis rapidly and directly activated macrophage Smad3, in the absence of active TGF- β 1 release. Myeloid cell-specific Smad3 knockout mice (MyS3KO) had no baseline defects, but exhibited increased late mortality and accentuated remodeling following MI. Adverse outcome in MyS3KO mice after MI was associated with perturbations in phagocytic activity, with defective transition of macrophages to an anti-inflammatory phenotype and with scar expansion. Milk-fat globule EGF factor-8 (Mfge8) partly rescued the phagocytic defect of Smad3 null macrophages, without affecting inflammatory activity. Perturbed anti-inflammatory transition of Smad3 null macrophages was associated with marked attenuation of phagocytosis-mediated PPAR induction. We

demonstrate that Smad3 is activated in phagocytic macrophages, critically regulates their function and mediates anti-inflammatory transition, and protects the infarcted heart from adverse remodeling.

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Microfibrillar-Associated Protein 4 Regulates Maladaptive Cardiac Remodeling

Lisa E Dorn, William Lawrence, Jennifer M Petrosino, Xianyao Xu, Thomas J Hund, Ohio State Univ, Columbus, OH; Anders Schlosser, Grith L Sorensen, Univ of Southern Denmark, Odense, Denmark; Federica Accornero, Ohio State Univ, Columbus, OH

Cardiac hypertrophy is a well-known risk factor for heart failure. Cardiomyocytes are often considered the ultimate culprit of cardiac remodeling following stress, despite comprising less than half of the heart's total cells. Recent work has begun to recognize the importance of non-myocyte populations in the heart, yet how signaling between these and cardiomyocytes can dictate cardiac remodeling is still largely unknown. We have found that Microfibrillar-associated protein 4 (MFAP4) is a novel mediator of intercellular communication in the heart, and is specifically required for cardiac adaptation to stress. MFAP4 is an extracellular matrix protein that has been previously shown as a necessary component of arterial remodeling following injury, but its role in cardiac hypertrophy has not been explored. Our data revealed that, in the heart, MFAP4 is selectively secreted by vascular smooth muscle cells and endothelial cells in response to transforming growth factor β (TGF β) activation. To determine the role of MFAP4 in cardiac hypertrophy, we subjected MFAP4-deficient mice to two forms of stress: chronic pressure overload or 1 week administration of the pro-fibrotic agent Angiotensin II (AngII). MFAP4-deficient mice developed accelerated cardiac hypertrophy, fibrotic remodeling, and marked cardiac functional defects with pressure overload when compared to wild-type animals. Also, AngII administration was sufficient to exacerbate cardiac hypertrophy prior to marked cardiac functional decline in MFAP4-deficient mice, suggesting a primary role for MFAP4 in regulating cardiomyocyte growth. Furthermore, aging-induced cardiac stress led to left ventricular dilation and spontaneous development of functional defects in otherwise unstressed MFAP4-deficient mice. Mechanistically, MFAP4 possess an integrin-binding domain and can affect cardiac homeostasis downstream of cardiomyocyte integrins via altered signaling through the FAK-ERK cascade, well-known to dictate cardiomyocyte geometric remodeling. Overall, our study demonstrates a critical role for MFAP4 on cardiac hypertrophy and function post-injury, underscoring the importance of non-myocyte-derived factors on cardiomyocyte pathophysiology.

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ERBB2 and beta-1-Adrenergic Receptor Cross-talk Underlies Cardiac Dysfunction

Manveen K Gupta, Kate Stenson, Cleveland Clinic, Cleveland, OH; Kathleen Gabrielson, Johns Hopkins, Baltimore, MD; Sathyamangla V Naga Prasad, Cleveland Clinic, Cleveland, OH

ERBB2 is a member of the EGFR growth factor receptor family and a primary target for anti-cancer treatment in patients diagnosed with breast cancer. ERBB2 targeting is known to cause cardiotoxicity, resulting in cardiac dilation while, less is understood about the underlying mechanisms. Studies have shown a direct co-relationship between beta2-adrenergic receptors (β 2ARs) and ERBB2 in the cardiac hypertrophy wherein increased ERBB2 expression is associated with elevated β 2ARs. Given the key role of β 1ARs in cardiac function, we assessed potential cross-talk between ERBB2 and β 1ARs. Towards this end, we have used a transgenic mouse model of cardiomyocyte-specific overexpression of microRNA-7 (miR-7 Tg) that abrogates ERBB2 expression in the adult cardiomyocytes and associated with cardiac dilation. Radio-ligand binding in miR-7 Tg hearts showed selective increase in β 1AR density with no changes in the β 2ARs. In contrast, cardiomyocyte-specific overexpression of ERBB2 results cardiac hypertrophy and is associated with significant increase in β 2AR density with no changes in β 1ARs. These observations show that ERBB2 expression may differentially regulate β 1AR versus β 2AR providing a unique role for ERBB2 in cardiac physiology. Further, ERBB2 expression is elevated in an age-dependent manner in C57BL6 mice that may account for hypertrophic response. Consistently, miR-7 Tg mice have age-dependent

cardiac dilation consistent with the loss in the ERBB2 expression. To test for such a role, C57BL6 mice were administered vehicle, AG1875 (EGFR inhibitor) or AG825 (ERBB2 inhibitor) for two weeks to assess for changes in cardiac function. Administration of AG825 resulted significant cardiac dysfunction and dilation compared to AG1875 or vehicle controls, conferring the idea that inhibition of ERBB2 and not EGFR that mediates deleterious remodeling. Proteomic analysis showed dramatic differences in the associated proteins following AG825 compared to vehicle or AG1478 and our presentation will elucidate the results on how homeostatic ERBB2 signaling and expression is key for cardiac function and how chemotherapeutic inhibition of ERBB2 underlies deleterious pathways leading to cardiotoxicity.

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Smad3-Mediated Induction of Smad7 in Activated Myofibroblasts Protects the Remodeling Myocardium

Claudio D Humeres, Arti Shinde, Nikolaos G Frangogiannis, Albert Einstein Coll of Med, New York, NY

TGF β s are induced in the infarcted and pressure-overloaded myocardium and play an essential role in repair and remodeling by activating Smad-dependent and Smad-independent cascades. Fibroblasts are major targets of TGF β s. Activation of Smad3 signaling in cardiac fibroblasts is crucial in post-infarction repair, mediating formation of aligned myofibroblast arrays. TGF β responses are tightly regulated through induction of endogenous suppressive signals, such as the inhibitory Smads, that prevent excessive TGF β signaling. We hypothesized that the inhibitory Smad7 may be induced in activated myofibroblasts infiltrating infarcted and pressure-overloaded hearts, protecting the myocardium from adverse remodeling and fibrosis by restraining TGF β actions.

Smad7 is markedly induced in infarcted and pressure-overloaded mouse hearts, and is localized in cardiomyocytes, myofibroblasts and activated macrophages. Induction of Smad7 in myofibroblasts is mediated through TGF β /Smad3 signaling *in vitro* and *in vivo*. Mice lacking Smad7 in activated myofibroblasts (MFS7KO) and Smad7 fl/fl controls underwent non-reperused myocardial infarction and transverse aortic constriction protocols. Following infarction, MFS7KO mice had higher mortality, worse systolic dysfunction and accentuated diastolic dysfunction when compared to Smad7 fl/fl. Following pressure overload, MFS7KO mice had accentuated interstitial and perivascular fibrosis and increased cardiomyocyte hypertrophy. *In vitro*, Smad7 overexpression attenuated myofibroblast conversion and reduced expression of profibrotic genes. Conversely, Smad7 knockdown promoted a matrix-synthetic fibroblast phenotype. Smad7 overexpression abrogated TGF- β -stimulated Smad2/3 and Erk activation without affecting T β RI and T β RII phosphorylation. Akt and p38 activation were unaffected by Smad7 overexpression. In conclusion, in remodeling hearts, Smad7 induction in activated myofibroblasts restrains fibrosis, negatively regulating Smad2/3 and Erk signaling through actions downstream of the T β Rs. The protective effects of fibroblast Smad7 in cardiac remodeling may have important therapeutic implications for heart failure patients.

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Mitochondrial Calcium Uniporter Regulates Proliferative Activity of Cardiac Fibroblasts Under Angiotensin II Stimulation

Bong Sook Jhun, Yuta Suzuki, Michael W. Cypress, Univ of Minnesota, Minneapolis, MN; Peng Zhang, Ulrike Mende, Rhode Island Hosp and Brown Univ, Providence, RI; Jin O-Uchi, Univ of Minnesota, Minneapolis, MN

Introduction: Cardiac fibrosis persists in patients with heart failure (HF) even during treatment with conventional HF therapies designed to control volume/pressure overload and restore cardiomyocyte function. This suggests a critical need to develop novel and effective anti-fibrotic therapies specifically targeting cardiac fibroblasts (CFs). CFs contribute to pathophysiological remodeling such as cardiac fibrosis after cardiac injury and/or during repair in response to various profibrotic stimuli *in vivo* such as angiotensin II (Ang II). Oxidative stress promotes pathological CF proliferation, but the detailed molecular mechanisms linking mitochondria and CF proliferation remain unclear. **Hypothesis:** Ang II stimulation promotes mitochondrial ROS (mROS) generation via mitochondrial Ca²⁺ (mCa²⁺) uptake, which subsequently activates mROS-dependent proliferative signaling in CFs. **Methods:** In CFs isolated from rat hearts, mCa²⁺ uptake, mROS levels and mitochondrial morphology were measured using confocal microscopy with a mitochondria-targeted Ca²⁺ biosensor mtRCaMP1h, a mitochondrial superoxide-sensitive dye MitoSOX, and mitochondria-targeted GFP,

respectively. **Results:** Ang II ($\geq 1 \mu\text{M}$) stimulation induces Ca^{2+} release from the endoplasmic reticulum (ER) followed by increased mCa^{2+} , mROS, and mitochondrial fragmentation. In addition, Ang II activates ERK1/2- and p38-mediated proliferative pathways, which was inhibited by pretreatment with losartan, an Ang II receptor antagonist. Overexpression of a dominant-negative mCa^{2+} uniporter (MCU) with pore domain mutations abolished Ang II-mediated mCa^{2+} uptake and mROS generation, and prevented the activation of proliferative pathways. Pretreatment with a mitochondria-targeted antioxidant, mitoTEMPO, also significantly inhibited Ang II-mediated activation of the proliferative pathways without affecting ER- Ca^{2+} release or mCa^{2+} -uptake. **Conclusion:** The mROS generation via mCa^{2+} accumulation and mitochondrial fragmentation resulting from Ang II stimulation activates mROS-dependent proliferative signaling pathways in CFs. The MCU-dependent mROS generation may serve as an important molecular switch for CF proliferation during cardiac stress.

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Activated Fibroblast-specific Deletion of RhoA Reduces Cardiac Fibrosis Through Regulation of the Non-canonical p38-MAPK Signaling Pathway

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Objective: Heart failure is a progressive disease characterized by cardiomyocyte (CM) loss, interstitial fibrosis, loss of ventricular compliance and chamber remodeling. Role of cardiac fibroblasts is implicated in the process, but specific molecular mechanisms regulating their function remain unclear. Previously, we showed that in mice, in response to stress, CM-specific deletion of RhoA, a Ras-related small G protein, accelerated cardiac dilation, with significant loss of contractile function, but it also significantly reduced cardiac fibrosis. Therefore, here, we focused on understanding the role of RhoA specifically in myofibroblast transformation and the fibrotic response. **Results:** We generated mice with activated fibroblast-specific deletion of RhoA using a tamoxifen (TMX) inducible periostin-cre promoter (RhoAfl/fl::PSTN Cre/+). 10-wk-old male RhoAfl/fl::PSTN Cre/+ or control mice were treated with angiotensin-phenylephrine (AngPE) or saline for 4 wk using osmotic minipumps and fed either with TMX or regular diet. Following TMX-AngPE treatment, the RhoAfl/fl::PSTN Cre/+ mice showed significantly decreased cardiac fibrosis, as compared to controls (0.9% vs. 3.2%, respectively). Moreover, while overall cardiac function following AngPE treatment was similar at 4 wk in all groups, hearts from the TMX-RhoAfl/fl::PSTN Cre/+ mice had significantly reduced septal thickness, as compared to controls. Expression of fetal genes, ANF, BNP, and β -MHC, as well as markers of fibrosis, including α -SMA and CTGF, were similarly induced in control and TMX-RhoAfl/fl::PSTN Cre/+ mice. However, TMX-RhoAfl/fl::PSTN Cre/+ mice had reduced expression of TGF- β 1, thrombospondin-5 and Col1a2. Finally, RhoA deletion in activated fibroblasts did not affect the canonical TGF β signaling pathway, as expected; instead, we found TMX-RhoAfl/fl::PSTN Cre/+ hearts had reduced non-canonical p38-MAPK signaling, suggestive of cellular-specific effects of RhoA regulation in the onset of cardiac disease and fibrosis. **Conclusions:** These data confirm the primacy of the RhoA pathway in the fibrotic response in vivo and identify potential novel downstream targets and possible therapeutic strategies for treatment of cardiac fibrosis and heart failure.

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Pharmacological and Genetic Inhibition of Transient Receptor Potential Canonical 6 (TRPC6) as a Novel Treatment for Heart Failure and Duchenne Muscular Dystrophy (DMD) Pharmacological and Genetic Inhibition of Transient Receptor Potential Canonical 6 (TRPC6) as a Novel Treatment for Heart Failure and Duchenne Muscular Dystrophy (DMD)

Brian Leei Lin, Sumita Mishra, Grace K Muller, Djahida Bedja, Guangshuo Zhu, Jinying Yang, Mark E Anderson, Johns Hopkins Univ, Baltimore, MD; **Steve S Pullen,** Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT; **David A Kass,** Johns Hopkins Univ, Baltimore, MD

Intro: Duchenne muscular dystrophy (DMD) patients suffer from spinal deformities (scoliosis and kyphosis) and die prematurely in their thirties, mostly due to heart disease. DMD is caused by the lack of the dystrophin protein, but calcium overload contributes to pathology as a secondary insult. Inhibiting hyperactive calcium channels in DMD, such as TRPC6, is a novel therapeutic strategy for treating DMD, and we test the efficacy of pharmacological and genetic TRPC6 inhibition *in vivo*. Therefore, my objectives are: 1) determine the efficacy of chronic pharmacological TRPC6 inhibition in heart disease 2) determine the therapeutic efficacy of genetic TRPC6 inhibition in DMD and 3) identify novel mechanisms of TRPC6 hyperactivity. **Results:** 1) Using pressure-overloaded mice as our model of heart disease, we applied the first and only-known TRPC6 inhibitor (BI 749327, 30 mg/kg/day) that is both specific and bioavailable. Chronic pharmacological TRPC6 inhibition blunted a pathological calcineurin/NFAT-TRPC6 feedforward signaling pathway *in vivo*, resulting in improved fractional shortening, normalized cardiac hemodynamics, and potently reduced fibrotic signaling and fibrosis. 2) We generated dystrophin-utrophin-deficient mice, or double-knock-out (DKO) mice, a severe model of DMD that exhibits muscle weakness, spinal deformities, and premature death at ~8 weeks of age. We also generated dystrophin-utrophin-TRPC6-deficient mice, or triple-knock-out (TKO) mice, which exhibited dramatically improved kyphosis, striated muscle function, and nearly three-fold improvement in lifespan. 3) We demonstrate CaMKII mediates TRPC6-mediated contractility combining a custom cardiomyocyte stretch assay with pharmacological and genetic inhibition of TRPC6 and CaMKII. **Conclusion:** TRPC6 inhibition potently reduces fibrosis in heart disease, and dramatically improves life expectancy in DMD. CaMKII is a novel TRPC6-activating kinase, necessary for TRPC6-mediated mechanosensitive activation and force response. The striking therapeutic effects of TRPC6 inhibition in disease may be due to interruption of a kinase imbalance in which CaMKII hyperactivates TRPC6. Future studies will determine the efficacy of pharmacological TRPC6 inhibition in DMD.

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Kinase-dead PI3K γ Expression in the Cardiac Myocytes Regulates Fibroblast Signaling and Myofibroblast Differentiation

Maradumane L Mohan, Lisa M Grove, Anita Sahu, Robert S Papay, Mitchell A Olman, Cleveland Clinic Fndn, Cleveland, OH; Sathyamangla V Naga Prasad, Sathyamangla V. Naga Prasad, Cleveland, OH

Phosphoinositide 3 Kinase γ (PI3K γ) is an anti-apoptotic molecule acting through Akt pathway. Even though, role of PI3K γ in cardiac fibrosis has been established, the mechanistic details by which PI3K γ regulates cardiac myofibroblast differentiation are not clear. Myofibroblasts are hallmark of tissue fibrosis, characterized by smooth muscle α -actin (α SMA) over-expression. We have previously shown that α SMA abundance in cardiac lysates from PI3K γ null mice (PI3K γ ^{-/-}) showed significant baseline and pressure overload [Transverse Aortic Constriction (TAC)] induced upregulation compared to wildtype (WT), indicating that loss of PI3K γ predisposes the hearts towards fibrosis. Furthermore, isolated cardiac fibroblasts (CF) from PI3K γ ^{-/-} exhibited a myofibroblast phenotype with α SMA in stress fibers. Moreover, cardiomyocyte-specific over-expression of kinase-dead PI3K γ (PI3K γ _{inact}) in the global PI3K γ ^{-/-} (PI3K γ _{inact}/PI3K γ ^{-/-}) reduced α SMA abundance and myofibroblast differentiation suggesting unique kinase-independent function of PI3K γ in myocyte-initiated pathway that drives CF to become myofibroblasts. Conditioned media experiments showed that PI3K γ ^{-/-} myocytes release pro-fibrotic factors and PI3K γ _{inact}/PI3K γ ^{-/-} myocytes release fibrosis protective factors. We have previously observed that PI3K γ regulated MAPK signaling in fibroblasts in a kinase-independent manner by sequestering PP2A association and activity. Previous studies have shown that fibroblast growth factor mediated activation of the signaling pathway downregulates α SMA and that this inhibition of α SMA expression is through negative regulation by extracellular regulated kinase (ERK). Consistent with these previous observations, PI3K γ possibly mediates α SMA and myofibroblast differentiation through regulation of ERK signaling in the fibroblasts. Intriguingly, we observed presence of PI3K γ when lysates of isolated CF from PI3K γ _{inact}/PI3K γ ^{-/-} were immunoblotted for PI3K γ . These data indicate that a unique communication between myocytes and fibroblasts regulated by PI3K γ , leads to a compensatory mechanism that results in expression of PI3K γ in the fibroblasts, thereby regulating fibroblast signaling in myofibroblast differentiation.

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Novel Role for Free Fatty Acid Receptor 4 in Response to Pathologic Pressure Overload-Induced Heart Failure in Mice

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Free fatty acid receptor 4 (Ffar4, GPR120) is a GPCR for long chain fatty acids, including omega-3 polyunsaturated fatty acids (ω 3-PUFAs). Previously, we demonstrated that eicosapentaenoic acid (EPA), an ω 3-PUFA, prevents interstitial fibrosis and contractile dysfunction in pressure overload heart failure (transverse aortic constriction, TAC), but EPA was not incorporated into cardiac myocytes or fibroblasts, the traditional mechanism of action. Therefore, we hypothesized that Ffar4 is necessary for the anti-fibrotic and cardioprotective effects of EPA. In fact, we previously found that in primary cardiac fibroblasts, Ffar4 was sufficient and necessary to prevent TGF β 1-induced fibrosis. Here, we were surprised to find that Ffar4 also has important ω 3-independent effects in cardiac myocytes *in vivo*. In mice with systemic deletion of Ffar4 (Ffar4KO mice) on a standard, corn oil diet (no EPA supplementation), TAC induced significantly more cardiac hypertrophy and more systolic (ejection fraction) and diastolic dysfunction (E/A ratio), but without excess fibrosis after 4 weeks. Mechanistically, transcriptome analysis in cardiac myocytes 3 days post-TAC indicated a deficiency of transcription in Ffar4KO myocytes. Sorting based on gene ontology indicated that genes functionally categorized as cell death and inflammation, including *ilrn1*, *hmox1*, *il33*, and *ptgs2* (cox2) were not induced in the Ffar4KO myocytes. Ffar4 also activates cytoplasmic phospholipase A2 (cPLA2) inducing the production of oxylipins, and systemic deletion of cPLA2 induces exaggerated hypertrophy during development and following TAC. TAC increased cPLA2 expression in WT, but not Ffar4KO cardiac myocytes and oxylipin production was reduced in Ffar4KO mice. Finally, we confirmed that Ffar4 is expressed in human heart and down-regulated in heart failure. In summary, our data identify a novel, ω 3-independent cardioprotective role for Ffar4 in cardiac myocytes.

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Cardiac Fibroblast-derived Exosomes Mediate Endothelial Dysfunction and Heart Failure

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Objective: Endothelial cells (ECs) play critical role to maintain the normal heart function. Others and we have previously shown that stress-induced chronic inflammation mediates ECs dysfunction and restoration of IL10 (an anti-inflammatory cytokine) preserve ECs function, but, its mechanism is not clearly known. Here we hypothesized that activated cardiac fibroblasts-derived exosomes (FB-Exo) mediates cardiac ECs dysfunction and leads for cardiac pathology and IL10 treatment negatively regulates the FB-Exo contents and thus improve ECs function and biology. **Methods:** Human aortic endothelial cells (HAECs), human umbilical endothelial cells (HUVEC) and cardiac endothelial cells were cultured in EC growth media. Cells were treated with TGF β /IL10/FB-exo. Exosomes were isolated from fibroblast conditioned media by ultracentrifugation and characterized by nanosight & electron microscopy. **Results:** TGF β treatment significantly induced ECs dysfunction as shown by Matrigel assay, real-time Q-PCR and immunostaining data. IL10 treatment significantly restored TGF β -induced endothelial dysfunction as evident by immunostaining and western blot (CD31, Smad2/3 and α SMA) data. Furthermore, exosomes were isolated from saline/AngII treated neonatal heart fibroblast conditioned media by ultracentrifugation and characterized. We found that exosomes-derived from Ang II/TGF treated fibroblast were enriched with TGF β and collagen-1 α . Matrigel tube formation and quantitative PCR results suggest that activated fibroblasts secrete factors which triggered endothelial dysfunction (Matrigel/qPCR). Intriguingly, Ang II treated fibroblast-derived exosomes strongly promote endothelial cell differentiation to fibroblasts like cells. **Conclusions:** Taken together, this study demonstrates that Ang II/TGF treated fibroblasts-derived exosomes are enriched in pro-fibrotic factors and can lead to endothelial dysfunction and promotes cardiac fibrosis in PO myocardium. Ongoing investigations using molecular approaches will provide a better understanding on the mechanistic and therapeutic aspects of IL10 and modification of fibroblast-derived exosomal content on endothelial function and cardiac fibrosis failing heart.

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Cortical Bone Stem Cell-Derived Exosomes Alter Wound Healing Response in Cardiac Fibroblasts and Cardiac Endothelial Cells

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Rationale: Post-myocardial infarction (MI) results in remodeling that leads to the development of heart failure. We have previously seen improvements in post-MI wound healing and scar formation as a result of administration of cortical bone stem cell derived exosomes (CBSC exosomes).

Objectives: We sought to further elucidate the mechanism through which CBSC exosomes improved scar formation and altered wound healing through *in vitro* experimentation. We continued to explore the unique characteristics of CBSC exosomes in order to understand why they have anti-fibrotic effects.

Methods and Results: We performed migration assays on cardiac endothelial cells and cardiac fibroblasts, and saw alterations in cell migration in both cell types upon treatment with mCBSC CM and mCBSC exosomes. We discovered that migration of CECs was reduced by 20% under the influence of mCBSC CM and mCBSC exosomes, but rescued when exosomes were removed from mCBSC CM. We discovered that the removal of exosomes from mCBSC CM slowed cardiac fibroblast migration by 40%. We saw a decrease in the quantity of pro-fibrotic mRNA in cardiac endothelial cells and cardiac fibroblasts after treatment with mCBSC exosomes. Adult cardiac endothelial cell mRNA levels of VEGFA, Col4A1, and VCAM were reduced nearly two fold from control untreated cell levels following mCBSC exosome treatment. Adult cardiac fibroblast Col1A1, Col3A1, Col4A1, and MMP2 mRNA levels were reduced one-fold from control untreated cell levels following mCBSC exosome treatment.

Conclusions: Our findings show that the wound healing induced by CBSC treatment post-MI involves a mechanism altering the migration of endothelial cells, sustaining the migratory capabilities of fibroblasts, and decreasing the production of pro-fibrotic mRNA in cardiac fibroblasts and cardiac endothelial cells.

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Elucidating Sex Differences in Stress-related Atrial Remodeling and AF Risk in a Mouse Model of Atrial Dysfunction

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Atrial fibrillation (AF) is the most common arrhythmia and a major risk factor for cardiovascular mortality and stroke. Despite significant advances in AF diagnosis and management, major gender discrepancies in treatment and clinical outcomes remain. It is unclear if these are related to treatment disparities or to underlying differences in the structure and biochemical makeup of the female versus male heart. How lifestyle, diet, comorbidities and risk factors contribute to this clinical dimorphism are not well understood. My laboratory has recently developed a double-transgenic mouse line that recapitulates this gender dimorphism. These mice were generated by crossing lines with cardiac expression of an amino and carboxyl terminal fragment, β ARKnt (residues 50-145) and β ARKct (residues 495-689), of the prototypical G protein-coupled receptor (GPCR) kinase GRK2 (originally β ARK1). This line was created to investigate the therapeutic potential of dual regulation of β -adrenergic receptor signaling independent of canonical GRK2 activity. Since preliminary data demonstrate atrial dysfunction in both genders, but structural remodeling in females only, this model presents a unique tool to investigate sex differences in the molecular and cellular mechanisms of atrial remodeling. Masson's trichrome staining of 4-chamber paraffin sections demonstrated an increase in wall thickness in the β ARKnt and β ARKnt/ β ARKct mice in agreement with the increased heart weight. Interestingly, these data revealed a significant increase in left and right atrial size in the double transgenic female hearts only, with no apparent alteration in the male atria. Preliminary echo recordings demonstrated significant alterations in E/A ratio (ventricular relaxation/atrial contraction) in the β ARKnt and even more pronounced in the β ARKnt/ β ARKct male and female mice. These data suggest that although no remodeling is evident at baseline, the male β ARKnt/ β ARKct mice have underlying atrial dysfunction that may be more susceptible to additional cardiovascular stress. A better understanding of gender differences in the underlying mechanisms of atrial susceptibility may facilitate the development of safer and more effective approaches for AF management and prevention.

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Resistin Accelerates Fibroblast-Myofibroblast Differentiation and Induces Myocardial Fibrosis

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Background: Cardiac fibrosis (CF) is a major cause of heart failure in patients with ischemic insult, obesity, and diabetes. CF, characterized by excessive deposition of extracellular matrix proteins and myofibroblast differentiation, leads to profound changes in ventricular architecture and geometry, and thereby reduces cardiac function. Resistin, an adipokine has been linked to obesity, insulin resistance and diabetes. Our previous findings have implicated resistin in CF, however the molecular mechanisms underlying this process are still unclear. In this study, we investigated whether resistin regulates the transdifferentiation of fibroblasts into myofibroblasts as well as the molecular pathways leading to resistin-induced fibrotic response.

Methods: Fibroblast-to-myofibroblast differentiation was induced with resistin, TGF β 1 or FBS for 48hours in isolated cardiac fibroblasts from adult mouse and mouse embryonic fibroblasts (NIH3T3 cells). Resistin knockout mice were challenged with high fat diet for 16 weeks to stimulate cardiac lipotoxicity. Fibrotic response and markers were determined in harvested hearts.

Results: Adult cardiac fibroblasts stimulated with resistin displayed increased fibroblast-to-myofibroblast differentiation, with significantly increased levels of collagen, fibronectin, and connective tissue growth factor. Mechanistically, we demonstrated that resistin promotes fibrogenesis via Jak-Stat and c-Jun signaling pathways, independently of TGF β 1. In-vivo study confirmed the role of resistin in cardiac fibrosis. Resistin knockout mice, when challenged with high fat diet to stimulate cardiac lipotoxicity, showed a decrease in fibrosis paralleled with reduced mRNA levels of various markers of fibrosis such as collagen, fibronectin and connective tissue growth factor compared to wild type hearts. A significant improvement in heart function was also observed in resistin knockout mice.

Conclusions: To our knowledge, these findings are the first to demonstrate a role of resistin in the process of cardiac fibroblast-to-myofibroblast conversion through Jak-Stat and c-Jun signaling. These results bring new insights into the mechanisms by which resistin may contribute to cardiac fibrosis.

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The ER Unfolded Protein Response Effector, ATF6, Reduces Fibrosis and Moderates Activation of Cardiac Fibroblasts

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Fibroblasts in the heart respond to myocardial injury by infiltrating the affected area and differentiating into new cell types called myofibroblasts. These cells are characterized both by the induction of contractile proteins and the secretion of extracellular matrix proteins which form fibrotic scar tissue. Investigating the factors governing fibroblast activation is key to understanding how these cells function in the heart and may be key to future therapeutic strategies. Activating transcription factor 6 (ATF6), an effector of the endoplasmic reticulum unfolded protein response, plays critical roles in development, as well as in the differentiation of certain cell types, though it has not been studied in this regard in the heart. Our lab has demonstrated that ATF6 in cardiac myocytes is cardioprotective *in vivo* during heart disease. However, ATF6 has not been studied in cardiac fibroblasts and its effect on fibrosis in the heart is unknown. We hypothesized that ATF6 in fibroblasts is an important regulator of their function. Fibroblast activation markers including α SMA were increased in infarcted hearts with global ATF6 deletion. Additionally, hearts with pressure overload showed increased fibrosis staining in global ATF6-null mice relative to WT hearts. In isolated adult murine ventricular fibroblasts (AMVF), loss of ATF6 induced myofibroblast markers with and without the activation stimulus TGF β . ATF6 loss of function also enhanced the effect of TGF β on fibroblast contraction. These effects were associated with an increase in Smad phosphorylation, a crucial step in the TGF β pathway. Interestingly, the effect of ATF6 loss of function in AMVF was erased when treated with a TGF β receptor inhibitor. Additionally, when ATF6 was overexpressed or when endogenous ATF6 was chemically activated, myofibroblast markers were reduced and activation by TGF β was blunted. ATF6 activation was associated with induction of several TGF β /Smad pathway negative regulators including SMURF1, SMURF2, and PMEPA1, though none of these are known to be ATF6

target genes. These data suggest that ATF6 plays an important role in moderating fibroblast activation and this may contribute to previously reported roles for ATF6 in preserving cardiac function post-injury.

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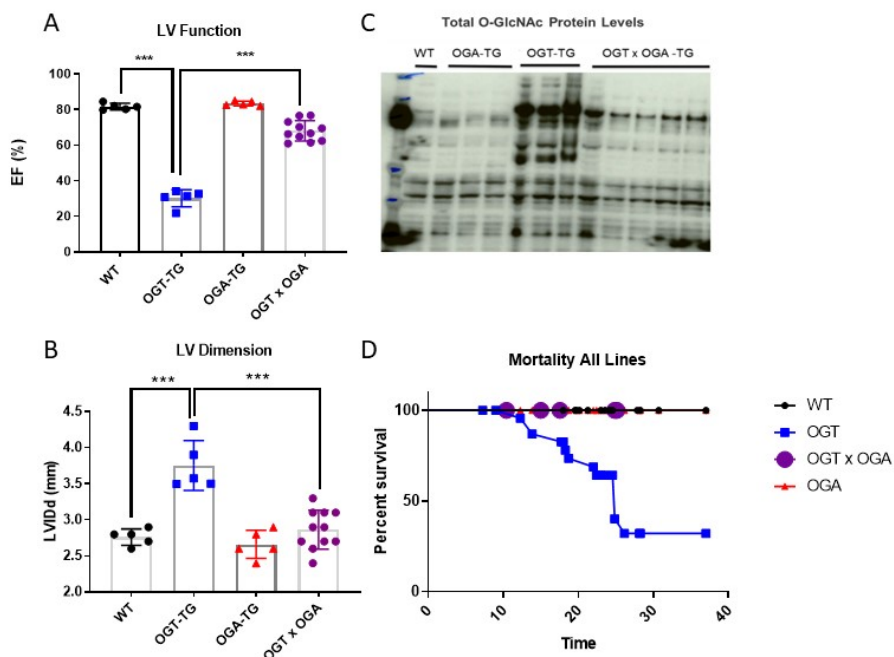
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Excessive O-GlcNAcylation Causes Heart Failure

Mahaa Umaphathi, Olurotimi Mesubi, Jon Granger, Qinchuan Wang, Yuejin Wu, Elizabeth Luczak, The Johns Hopkins Hosp, Baltimore, MD; Gerald Hart, Complex Carbohydrate Res Ctr, Athens, GA; Mark Anderson, The Johns Hopkins Hosp, Baltimore, MD

Increased protein O-GlcNAcylation (OGN) is a common feature of failing heart muscle. However, it is unknown if excessive OGN contributes to cardiomyopathy and heart failure. OGN levels are determined by the net activity of two enzymes: OGT (O-GlcNAc transferase, adds OGN) and OGA (O-GlcNAcase, removes OGN). We hypothesized that excessive myocardial OGN is a cause of cardiomyopathy. To test for a role of OGN in cardiomyopathy we developed new transgenic (TG) mouse models with myocardial overexpression of OGT or OGA. The OGT-TG hearts showed progressive decline in left ventricular (LV) systolic function, dilation, increased mass (Figure A, B) (Statistical Analysis ANOVA - *** = $p < 0.001$) and increased OGN (Figure C). OGT-TG mice showed premature mortality compared to WT littermates (Figure D). In contrast, OGA-TG mice exhibit normal contractility, do not have significantly different OGN and have normal lifespan compared to WT littermates. Hearts from OGT-TG and OGA-TG interbred mice have marked improvement of LV systolic function, lower OGN and normal lifespan. We next tested if attenuation of myocardial OGN was beneficial in acquired cardiomyopathy by performing transverse aortic constriction surgery (TAC) on OGA-TG and WT littermates. The OGA-TG hearts had lower OGN, improved LV systolic function, less hypertrophy, and lower expression of heart failure genes compared to WT littermates after TAC. Our data identify excessive OGN as an independent mechanism for cardiomyopathy, and suggest attenuation of OGN may be an effective therapy for heart failure.



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Gene Expression Profiling of Hypertrophic and Failing Cardiomyocytes Identifies New Players Involved in Heart Failure

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Pathological remodeling during cardiac disease is a detrimental response characterized by cardiomyocyte hypertrophy and fibroblast activation, which can ultimately lead to heart failure. Genome wide expression analysis on full heart tissue have been instrumental for the identification of molecular mechanisms at play. However, these data so far were based on signals derived from all cardiac cell types and a more specific view on molecular changes driving cardiomyocyte hypertrophy and failure could aid in the development of therapies aimed at improving maladaptive remodeling. Here, we generated a cardiomyocyte-specific reporter mouse (Myh6-Cre-tdTomato) that we exposed to pressure overload by transverse aortic banding (TAB). Using Fluorescence-activated Cell Sorting (FACS) we collected both hypertrophic (one week after TAB) and failing (eight weeks after TAB) cardiomyocytes. Using these cells, we performed RNA-seq and obtained subsets of genes and pathways differentially regulated and specific for either the hypertrophic or failing stages. Among these upregulated genes we identified known marker genes for cardiomyocyte failure, such as Nppb and Myh7, but also identified genes that so far have not been linked to a failing state. RNA-seq on failing and healthy human heart samples confirmed the increased expression for several of these genes regulated during cardiomyocyte failure and allowed us to show an expressional correlation to NPPB/MYH7. Moreover, we could recapitulate the upregulation of these novel genes in stressed human induced-pluripotent stem cell (iPSC)-derived cardiomyocytes. We discovered that phosphofruktokinase-platelet (PFKP) protein, a glycolytic enzyme, is strongly induced in human failing cardiomyocytes. Currently, ongoing studies are focused on defining the functional relevance of the novel failure related genes in stressed iPS-derived cardiomyocytes. Our findings suggest that cardiomyocyte-specific transcriptomic analysis allows for the identification of hypertrophic and failing gene expression profiles and helps to unveil novel genes relevant for heart disease.

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MYH14 Protects Against of Isoproterenol-induced Left Ventricular Hypertrophy and Fibrosis

Jessica J Wang, Sunny C Chang, Alex Tran, Jessica Sanchez, Max Yang, Kazuhiro Omi, UCLA, Los Angeles, CA

Objectives: We had previously identified *Myh14* as a genetic modifier of isoproterenol-induced left ventricular hypertrophy through a genome-wide association study performed among Hybrid Mouse Diversity Panel strains. Whether left ventricular hypertrophy due to *Myh14* deficiency is compensatory or maladaptive is not well established. Moreover, though MYH14 had been previously reported in the intercalated disc of cardiomyocytes, the mechanisms by which *Myh14* deficiency leads to left ventricular hypertrophy is unknown. **Methods:** We performed RNA-seq on cardiac tissues from 12-week old male *Myh14* $+/+$ and $-/-$ mice that had been treated under control versus isoproterenol conditions for 3 weeks. We examined the differentially expressed genes to identify enriched gene ontology (GO) terms altered by *Myh14* deficiency at the transcriptional level. **Findings:** Differential expression analysis showed an enrichment of transcripts coding for extracellular matrix proteins in isoproterenol treated versus control mice, many of which were further enriched in *Myh14* $-/-$ versus $+/+$ mice. Transcripts uniquely differentially expressed due to *Myh14* deficiency were enriched for GO terms such as inorganic cation import across plasma membrane, inner ear receptor cell / neuron development, response to purine-containing compound, and sensory perception of mechanical stimulus, supporting the roles these pathways play in mediating the impact of *Myh14* deficiency on cardiac remodeling. **Conclusions:** Our RNA-seq results suggest that MYH14 plays a protective role against mechanical stress. Future directions include elucidation of specific roles that the affected pathways play in mediating mechanical stress as a result by *Myh14* deficiency as well as impact on the observed phenotype of left ventricular hypertrophy and fibrosis.

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EPRS is a Critical Translational Control Factor in Cardiac Fibrosis

Jiangbin Wu, Kadium C Venkata Subbaiah, Feng Jiang, Omar Hedaya, Peng Yao, Aab Cardiovascular Res Inst, Univ of Rochester Sch of Med and Dentistry, Rochester, NY

Increased protein synthesis of pro-fibrotic genes is a common feature of cardiac fibrosis, a major manifestation of heart failure (HF). Despite this observation, critical factors and molecular mechanisms for translational control of cardiac fibrosis remain underexplored. Here, we identify a critical house-keeping translational regulator, glutamyl-prolyl-tRNA synthetase (EPRS), which preferentially regulates the translation of proline-rich (PRR) pro-fibrotic genes. In mammals, EPRS catalyzes the attachment of two amino acids, glutamic acid and proline, to their cognate tRNAs for protein synthesis. By overlapping of aminoacyl-tRNA synthetases (ARSs) induced in TGF- β treated human cardiac fibroblasts, human ARSs with genetic mutations in the congenital heart disease, mouse ARSs associated with isoproterenol (ISO)-induced cardiomyopathy by GWAS, and ISO-induced ARSs in mouse failing hearts, we identified EPRS as the only ARS involved in various cardiac pathogenesis. EPRS is induced in failing human heart compared to non-failing donor heart and functions as an integrated node downstream of multiple hypertrophic and fibrotic stimuli in murine hearts, including ISO infusion and transverse aortic constriction (TAC) surgery. Low-dose halofuginone (Halo), a prolyl-tRNA synthetase (PRS)-specific inhibitor, as well as genetic knockout of one allele of EPRS in mouse genome, reduces cardiac hypertrophy and fibrosis in ISO- and TAC-induced HF mouse models. Using RNA-Seq and polysome profiling-Seq in Halo-treated fibroblasts, we identified several novel PRR genes, including *Ltbp2*, *Furin* and *Sulf1*, in addition to collagens, which are translationally regulated by EPRS. Inhibition of *Furin* by various inhibitors attenuates cardiac fibroblast activation and collagen deposition *in vitro*. Finally, we found that inactivation of EPRS reduced translational efficiency and enhanced mRNA decay of PRR genes. Taken together, our results indicate that EPRS controls the translational activation of PRR genes in cardiac fibroblasts, and these data provide novel insights into the translational control mechanisms of cardiac fibrosis, which may promote development of novel therapeutics by inhibiting pro-fibrotic translation factors.

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Thyrotoxic Pericarditis in a Mouse Model of Graves' Disease

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Objectives: To investigate the cardiac pathological changes of hyperthyroid heart disease in mice. **Methods:** A transient model for human Graves' disease was successfully established in mice using three immunizations with recombinant adenovirus expressing the extracellular A subunit of the human thyrotropin receptor (Ad-TSHR). We induced the hyperthyroid heart mouse model by prolonging the regular injections of Graves' disease mouse model to nine months. Heart tissues were weighed and stained with hematoxylin-eosin (HE) and MASSON Trichrome staining to check and define the pathological changes. **Results:** We observed that mice in the Graves' disease hyperthyroid heart group (GH) showed enhanced cardiac hypertrophy and fibrosis. The HW/BW (ratio of heart weight to body weight) of GH group (5.528 ± 0.4014 , $n=6$) was significantly higher than control group (4.343 ± 0.03913 , $n=6$) ($P < 0.05$). In GH group, 2 of 6 hearts showed typical *cor villosum* (Image 1). The MASSON Trichrome staining exhibited aggravated cardiac fibrosis on the surface of the heart in GH group. **Conclusions:** Pericarditis as a complication of thyrotoxicosis has been reported several times in clinical work. This is the first reported case of pericarditis in the mouse model of Graves' disease, suggesting a possible link between pericarditis and thyrotoxicosis.



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Sex Differences in Anthracycline-Induced Cardiotoxicity in Young Mice

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Introduction: The role of sex as a risk factor for Anthracycline-Induced Cardiotoxicity (AIC) is not well defined. In pediatric cancer patients, most studies report that female sex is a significant risk factor for AIC. Conversely, in adult preclinical rodent studies, there is clear evidence that female sex is protective against AIC, with a possible protective role of female sex hormones and/or a detrimental role of male sex hormones. However, sex differences in AIC have not been reported in young experimental animals. Therefore, the current work aims to characterize sex differences in AIC in young male and female mice.

Methods: Five-week old male and female C57Bl/6 mice were injected with doxorubicin (DOX) 4 mg/kg/week IP for 3 to 6 weeks. After a 5-week recovery period, a cohort of control and DOX-treated mice were administered daily IP injections of isoproterenol (10 mg/kg) for 14 days to determine the effect of catecholamine stress on cardiac function and morphometry in DOX-exposed mice. Another cohort of gonadectomized and sham-operated male and female mice were injected with DOX 4 mg/kg/week for 5 weeks. Cardiac function and morphometry were measured by trans-thoracic echocardiography 5 weeks after the last DOX injection and after 14 days of isoproterenol injections.

Results: In male mice only, DOX 4 mg/kg/week for 3 to 5 weeks caused cardiac atrophy without decline in cardiac function, whereas DOX administration for 6 weeks caused cardiac atrophy and dysfunction. Female mice were protected from both cardiac atrophy and dysfunction at the 3-6 weeks regimens. Isoproterenol caused cardiac dysfunction in male and female control and DOX-treated mice. Male, but not female, DOX-exposed mice failed to develop cardiac hypertrophy when challenged by isoproterenol. Intriguingly, castration of male mice exacerbated DOX-induced cardiac atrophy, while ovariectomy had no significant effect.

Conclusion: In contrast to most clinical studies, young female mice are protected against DOX-induced cardiac atrophy and dysfunction. Juvenile exposure to DOX modulated the cardiac response to isoproterenol in a sex-dependent manner. Deprivation of sex hormones by gonadectomy did not reverse this sexual dimorphism, suggesting that other mechanisms are implicated.

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Identification of Novel MICU1 Interactors Independent of the mtCU Complex

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MICU1 is an EF-hand containing mitochondrial protein that gates the mitochondrial Ca^{2+} uniporter complex (mtCU) and directly interacts with the pore-forming subunit MCU. Previous studies have shown perinatal lethality and altered mitochondrial architecture in MICU1 knockout (*Micu1^{-/-}*) mice, phenotypes that are distinct from other knockout models of mtCU components, such as MCU, and thus are likely not explained solely by changes in matrix $[\text{Ca}^{2+}]$ uptake. Further, our proteomic studies suggest that MICU1 exists in mitochondrial complexes void of MCU. This suggests that MICU1 may have cellular functions independent of mtCU regulation. To discern novel MICU1 molecular interactors we employed a biotinylation-based proteomic approach in *Mcu^{-/-}* and *Micu1^{-/-}* cells to detect proteins interacting with MICU1 using fusion protein containing BioID2, a small biotin ligase for proximity-dependent labeling. Expression of MICU1-BioID2 in *Mcu^{-/-}* cells allowed the identification of mtCU-independent interactors. Fast protein liquid chromatography (FPLC), blue native-PAGE, co-immunoprecipitation, live-cell Ca^{2+} imaging, confocal and super-resolution imaging methods were used to confirm novel roles for MICU1 in mitochondrial biology. LC-MS analysis of biotinylated proteins after avidin-based purification identified the Mitochondrial Contact Site and Cristae Organizing System (MICOS) components IMMT, CHCHD2, and CHCHD3 as interacting with MICU1 (MICU1-BioID2 expressed in *Micu1^{-/-}* cells to avoid aberrant expression). These same MICOS components were identified in *MCU^{-/-}* cells, suggesting that MICU1 could be involved in the regulation of MICOS independent of the mtCU. Further, the deletion of CHCHD2 resulted in the loss of MICOS and cristae disorganization without any observable effect on mCa^{2+} uptake. RNA sequencing revealed correlative expression changes in MICU1 and MICOS components in response to heart failure progression during transverse aortic constriction and myocardial infarction. These results suggest MICU1 likely serves cellular functions independent of the mtCU and may serve as a key regulator of Ca^{2+} -dependent signaling (EF-hands) in other cellular processes that are dysregulated during heart failure.

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Loss of S100A1 Protein Negatively Impacts Glucose Metabolism and Energy Homeostasis in Cardiomyocytes

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Introduction: As a true metabolic omnivore, the heart is able to utilize different substrates for energy production. In the hypertrophied and early failing state, cardiac muscle leans towards glucose as a major carbon substrate to meet its energetic demand. Given that S100A1 gene therapy reverses the compromised energetic balance of the failing heart, we were interested in determining the specific role of the EF-hand calcium sensor S100A1 in cardiomyocyte energy metabolism.

Methods and results: To study metabolic changes in response to different carbon substrates, we used neonatal rat ventricular cardiomyocytes (NRVMs), which were subjected to electrical stimulation (2Hz) in culture and adenoviral-mediated anti-S100A1 shRNA silencing or scr-shRNA, and either fed with glucose, oleate or lactate only for 24hrs. Of note, lack of S100A1 expression only impaired energy metabolism of cardiomyocytes in response to glucose as reflected by a significantly decreased ATP content and subsequently enhanced AMP/ATP and p-AMPK/AMPK ratios with consecutive elevated p-ACC/ACC and Glut4 protein levels. In contrast, energy metabolism appeared to remain normal in response to oleate in S100A1-deprived cells. Targeted metabolomics yielded a tremendous decrease in glucose-6-phosphate levels as the first key step in glucose metabolism despite enhanced AMPK activation in glucose-fed S100A1-deficient cardiomyocytes.

Conclusion: Our novel in vitro data indicate that S100A1 protein may be crucial for cardiomyocytes to meet their energetic demand when glucose is the major substrate. Since the latter becomes the preferred substrate for functionally compromised heart over fatty acids, lack of S100A1 expression - as a result of fetal gene reprogramming - most likely impairs the ability of the heart to sufficiently utilize glucose to meet its energetic demand. These data shed new mechanistic light on the sustained therapeutic efficacy of S100A1 gene therapy of heart failure where S100A1 may be a key factor enabling the heart to

sufficiently use glucose to reverse its compromised energetic state. Further studies answering these pressing questions are underway for an informed design of a best-case patient enrollment scenario for a first-in-men clinical study.

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Reduced Expression of the Cardiac Sodium Channel Nav1.5 Triggers Enhanced Fatty Acid Metabolism and Oxidative Stress

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SCN5A encodes the voltage-gated Na⁺ channel Nav1.5 that is best known for its role in cardiac conduction. We recently identified a microRNA (miR-24) binding site within the *SCN5A* coding region and found that its activity is modulated by an adjacent synonymous SNP (rs1805126). In humans, we linked the rs1805126 minor allele with decreased cardiac *SCN5A* expression and increased non-arrhythmic death in HF patients. To understand how lower *SCN5A* expression may lead to worse HF, we evaluated *SCN5A*^{+/-} haploinsufficient mice, which develop cardiac fibrosis after 12 months of age. Given that oxidative stress often precedes fibrosis and is increased in HF, we assessed ROS levels in *SCN5A*^{+/-} mouse hearts and found a 2.5-fold increase relative to wildtype (WT) hearts. We also performed co-expression analyses using human cardiac mRNA profiling data and unexpectedly found that *SCN5A* is linked to *PPARA*, a key driver of fatty acid oxidation (FAO, a predominant source of cardiac ROS), and to gene networks related to glucose metabolism and oxidative phosphorylation (OXPHOS). Along these lines, we found that *SCN5A*^{+/-} mouse hearts show mRNA changes indicative of increased FAO/OXPHOS and decreased glycolysis, with a coincident broad up-regulation of PPAR target genes. Metabolomics data further indicated that glycolytic flux is perturbed in *SCN5A*^{+/-} mouse hearts, and cardiac myofiber respiration assays showed that these hearts exhibit enhanced FAO. To test if *SCN5A*^{+/-} mice suffer worse HF outcomes, we subjected young adult male mice to thoracic aortic constriction (TAC), a model of cardiac hypertrophy progressing to HF. While WT mice showed typical hypertrophic responses and signs of HF, *SCN5A*^{+/-} hearts were resistant to TAC-induced hypertrophy, which based on prior reports, may be the result of elevated FAO. Overall, our data support the notion that lower cardiac *SCN5A* expression leads to an overreliance on FAO and accumulation of ROS in heart, which may exacerbate HF in patients. Together, our studies point to unforeseen roles for Nav1.5 in cardiac metabolism, opening several new paths of investigation. Future studies will interrogate 1) if human hearts with low *SCN5A* expression show signs of oxidative stress, and 2) if *SCN5A*^{+/-} mice suffer worse HF after myocardial infarction.

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Cardioprotective Effects of Plant-derived Fat in Low Carbohydrate Diet on the Progression of Heart Failure

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Background The association between low carbohydrate diet (LCD) and cardiovascular disease and mortality remains unknown. Results from meta-analyses have reported that cardiovascular mortality increased when the carbohydrate in LCD was replaced for animal-derived fat, whereas mortality decreased when the substitution was plant-derived fat. However, the molecular mechanism is yet to be elucidated. We aimed to assess the effects of the LCD under two conditions, LCD with animal-derived fat (LCD-A) and LCD with plant-derived fat (LCD-P), on mouse cardiac function. **Methods** Using 10-week old male C57B/6J mice, we performed transverse aortic constriction surgery to generate a pressure overload model of heart failure and subjected them to either normal diet, LCD-A or LCD-P for 4 weeks. Cardiac function was measured by echocardiography at 4 weeks after surgery, and the extent of inflammation was assessed by immunohistological analysis. Expression levels of genes were assessed with RNA sequence. **Results** LCD-A accelerated left ventricular dilatation and systolic dysfunction (P<0.01 vs. normal diet), whereas LCD-P ameliorated cardiac hypertrophy (P<0.01 vs. normal diet) as revealed by echocardiography. Immunohistological analysis demonstrated that LCD-A resulted in a significant increase in the infiltration of F4/80-positive macrophages, reflecting exacerbated inflammation (P<0.01 vs. LCD-P, P<0.01 vs. normal diet).

Consistent with histological findings, inflammation-related and cell cycle-related gene expressions were upregulated only in LCD-A. On the other hand, mitochondrial fatty acid oxidation gene expression including PPAR α targets such as *Cpt1a*, *Slc25a20*, *Acadvl*, *Hadha* and *Acaa2* were upregulated only in LCD-P ($P < 0.05$, vs. normal diet), which may indicate the preserved energy metabolism during the pathogenic process of heart failure. **Conclusions** These results suggest that the effects of the LCD on cardiac function vary between the sources of fat alternatives to carbohydrate intake. LCD-P induces PPAR α activation, an important regulator of mitochondrial lipid metabolism, that may be therapeutically relevant for heart failure treatment. Further studies are required to unveil the precise molecular mechanism.

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The Role of Estrogen in Protection of Skeletal Muscle Function in Diastolic Dysfunction

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Diastolic dysfunction (DD) is prevalent in elderly post-menopausal women. In addition to impaired cardiac relaxation and output, DD is associated with loss of skeletal muscle mass, muscle strength and function, and exercise capacity. Understanding the mechanisms of estrogen (E2)-mediated regulation of skeletal muscle structure and function in DD can have broader implications in understanding the progression of heart disease in post-menopausal women. In muscle, E2 regulates autophagy and mitophagy, processes that orchestrate degradation and recycling of damaged cellular components to maintain healthy tissue and conserve energy. However, this role of E2 under a physiological stress such as DD, is largely unknown. Our study aimed to understand the relationship between E2, autophagy/mitophagy and muscle function in DD. We hypothesized that estrogen receptor (ER) signaling plays a direct role in regulation of autophagy and mitophagy-mediated maintenance of muscle mass and muscle mitochondrial function in DD. Sham or ovariectomy (OVX) surgeries were performed to induce E2-deficiency, followed by induction of DD by administering hypertension agents L-NAME and angiotensin II. NMR analysis revealed significantly reduced total lean mass in OVX mice with DD, further confirmed by direct measurement of gastrocnemius muscle mass. Expression of ER α target-genes PDK4 and STAT3 were reduced in muscle of OVX mice with DD. DD significantly reduced LC3B II/I ratio in muscle, indicative of reduced autophagy. Consistent with this, expression of transcription factor FoxO3, a master-regulator of multiple autophagy and mitophagy genes, was reduced in OVX mice with DD compared to sham control mice. Expression of SDHA gene coding for mitochondrial respiratory chain subunit II, was reduced in sham and OVX mice with DD compared to sham control mice. Mitochondrial respiratory function measured from isolated muscle mitochondria, was reduced in sham and OVX mice with DD compared to sham control mice, and in OVX mice with DD compared to OVX control mice, indicating compromised skeletal muscle mitochondrial function. Our findings indicate that OVX-induced E2-deficiency and DD leads to muscle impairments, including defects in mitochondrial function.

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Mitochondrial Translation Machinery Defects Causes Cardiomyopathy and *in vivo* Functional Screening of Therapeutic Targets

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More than 95% of ATP consumed in the heart is derived from oxidative phosphorylation (OxPhos) in the mitochondria. Besides over a thousand proteins encoded by nuclear DNA, mitochondrial gene expression machinery generates 13 proteins for the OxPhos system that employs mitochondrial ribosomal proteins for translation. Mitochondrial ribosome protein S5 (MRPS5) encoded by MRPs gene family, is located at the mammalian mitochondrial ribosome 28S subunit and its importance has not been explored in the heart. Western blotting showed that MRPS5 expression was decreased in TAC-

induced hypertrophic mouse hearts (*in vivo*), and in phenylephrine or isoproterenol-stimulated cardiomyocytes (*in vitro*). In the failing hearts of patients, Mrps5-mediated mt-CO1 was markedly decreased as well. To determine the functional role of MRPS5 in the heart, we generated an inducible cardiac-specific knockout mouse and showed that Mrps5-deficient mice developed time-dependent cardiac hypertrophy, fibrosis and heart failure. Mitochondria in the Mrps5-deficient adult cardiomyocytes exhibited membrane swelling and cristae collapsed together with decreased oxygen consumption and ATP production. Mitochondrial calcium homeostasis was also disrupted. Combined analysis of transcriptomics and metabolomics spatiotemporally identified 20 potential target genes associated with Mrps5 deficiency-mediated cardiac pathological processes. Through *in vivo* functional screening via adeno-associated virus (AAV) mediated gene delivery, we further narrowed down and confirmed that Kruppel-like factor 15 (Klf15) remarkably rescued cardiac phenotype in the Mrps5-deficient mice with maintained mitochondrial homeostasis, suppressed cardiac hypertrophy and fibrosis, as well as preserved cardiac function. Further RNA-sequencing and biochemical analysis mechanistically revealed that genes involved in glycolysis/ gluconeogenesis, PPAR signaling pathway and biosynthesis of amino acids, markedly altered when exploited AAV-Klf15 gene therapy in Mrps5-deficient mice heart. Our results demonstrates Mrps5 is essential for mitochondrial homeostasis and cardiac function and uncovers Klf15 as a potential therapeutic target for treating cardiac mitochondrial diseases.

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Modulation of Energy Metabolism by Metformin Prevents Diet Induced Cardiac Dysfunction in a Mouse Model of Adult Congenital Heart Disease

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Objective: Congenital heart disease (CHD) is the most frequent birth defect worldwide. Improved surgical and treatment interventions have led to a significant increase in the number of adult patients with CHD, now referred to as ACHD. However the mechanisms whereby ACHD predisposes patients to heart dysfunction are still unclear. ACHD is strongly associated with metabolic syndrome, but how ACHD interacts with poor modern lifestyle choices and other comorbidities, such as hypertension, obesity, and diabetes, is mostly unknown. **Methods:** We used a newly characterized mouse genetic model of ACHD to investigate the consequences and the mechanisms associated with combined obesity and ACHD predisposition and metabolic intervention studies by metformin. **Results:** ACHD mice placed under metabolic stress (high fat diet) displayed decreased left ventricular ejection fraction. Comprehensive physiological, biochemical, and molecular analysis showed that ACHD hearts exhibited early changes in energy metabolism with increased glucose dependence. These changes preceded cardiac dysfunction mediated by exposure to high fat diet and were associated with increased disease severity. Restoration of metabolic balance by metformin lead to improved liver function in both control and ACHD mice and prevented the development of heart dysfunction in ACHD predisposed mice. Metabolomic analysis of these animals revealed that metformin leads to an ACHD specific increase in metabolites associated with fat acid oxidation, likely reflecting upregulation of FAO. **Conclusions:** This study reveals that early metabolic impairment reinforces heart dysfunction in ACHD predisposed individuals and diet or pharmacological interventions can be used to modulate heart function and attenuate heart failure. Our current hypothesis is that metformin treatment leads to normalization of energy use by ACHD heart by enhancing FAO and we are currently performing CRISPR/Cas9 mediated deletion of key metabolic genes to characterize their role in ACHD. This data indicates that early manipulation of energy metabolism may be an important avenue for intervention in ACHD patients to prevent or delay onset of heart failure and secondary comorbidities.

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Maternal High Fat, High Sucrose Diet-induced Cardiac Mitochondrial Abnormalities in the Offspring are Transmitted via the Oocyte Nucleus

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Introduction: Maternal obesity correlates with higher cardiovascular disease risk in human offspring. We found high fat, high sucrose (HF/HS) diet results in accretion of abnormal mitochondria in mouse oocytes, and chow-fed offspring of HF/HS-fed dams exhibit transgenerational transmission of abnormal cardiac mitochondria through male and female germlines. Nuclear or mitochondrial inheritance can occur via the female germline, whereas male germline inheritance implicates nuclear epigenetic changes. However, recent reports indicate sperm mitochondria transmission is possible. Whether the mechanism is via effects on the nucleus or germ cell mitochondria is unknown. **Hypothesis:** Maternal HF/HS-feeding results in cardiac mitochondrial dysfunction in offspring due to effects on the oocyte nucleus. **Methods:** C57BL/6 female mice were chow- or HF/HS-fed, and mated to chow-fed C57BL/6 males. Female pronuclei were exchanged between embryos obtained from chow- and HF/HS-fed dams. Embryos without pronuclear transfer were additional controls. All embryos were transferred into chow-fed ICR dams. Cardiac mitochondrial structure (transmission electron microscopy) and function (high-resolution respirometry) were evaluated in 12-week-old offspring. **Results:** Adult offspring born of embryos having maternal pronuclei from chow-fed dams and ooplasm from chow- or HF/HS-fed dams had mitochondrial structure or oxygen consumption levels comparable to offspring born of control chow-fed dam embryos. In contrast, offspring born from embryos with maternal pronuclei from HF/HS-fed dams and ooplasm from chow-fed dams had abnormal cardiac mitochondrial structure (round shape, cristal rarefaction) and reduced oxygen consumption (~40%), comparable to embryo transfer offspring of HF/HS-fed dams. **Conclusion:** Shown for the first time, abnormal cardiac mitochondria in obese dam offspring result from HF/HS diet effects on the oocyte nucleus (likely epigenetic changes during oogenesis). Elucidating diet/obesity-induced oocyte epigenetic changes, and the mechanisms for induction and transgenerational transmission, could enable therapies to target inheritable heart disease risk in humans.

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Neonatal Cardiomyocyte-Released Signaling Protein Reduces Adipocyte Differentiation

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The heart continuously cycles through periods of muscle contraction and relaxation to pump blood throughout the body, which requires a constant supply of metabolic substrates to the heart to power these contractile cycles. To ensure adequate energy availability, the heart must communicate with other organ systems to maintain global metabolic energy homeostasis. In addition to the role of the sympathetic nervous system in global metabolic regulation, mounting evidence suggests cardiomyocytes can release signaling factors that alter global energy balance through communication with other organ systems, including the liver and adipose tissue. To identify potential cardiomyocyte-released circulating factors, we collected media conditioned by neonatal rat ventricular myocytes (NRVM-CM) and screened for released-factor activity using an *in vitro* adipocyte differentiation assay. Treatment of differentiating 3T3-L1 adipocytes reduced lipid accumulation and decreased expression of adipocyte proteins, demonstrating NRVM-released factor activity. Released factor activity was also under the control of regulators of GPCR signaling as overexpression of G-protein coupled receptor kinase 2 (GRK2) further enhanced the NRVM-released factor activity. We further determined that NRVM released-factor activity was abolished by trypsin treatment and could be segregated into >30kDa CM fractions using centrifugal filter units. To identify proteins responsible for the NRVM-released factor activity, NRVM-CM retained in the 30kDa centrifugal filter units (containing all proteins >30kDa) was analyzed by mass spectrometry. This revealed a number of signaling proteins with known adipocyte regulatory roles that could potentially regulate adipocyte physiology. We confirmed these results by examining NRVM cell lysate and concentrated NRVM-CM by Western blot to test for the presence of decorin, one of the proteins identified by mass spectrometry known to have adipocyte regulatory roles. These results provide a framework to identify potential *in vivo* cardiomyocyte-released factors that regulate global energy balance.

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Reduced HtrA2 Protein Levels in Mitochondria but Elevated Levels in Cytosol of Left Ventricular Myocardium of Dogs with Chronic Heart Failure

Ramesh C Gupta, Vinita Singh-Gupta, Hani N Sabbah, Henry Ford Hosp, Detroit, MI

Background: The high-temperature requirement serine peptidase 2 (HtrA2) is a pro-apoptotic mitochondrial serine protease involved in caspase-dependent as well as caspase-independent programmed cell death. When HtrA2 protein is tightly associated with mitochondria (MITO), its protease activity is masked. In contrast, once the protein is released from MITO into the cytosol (CS), its protease activity is exposed. Once active HtrA2 activates caspase-3, a pro-apoptotic enzyme, by degrading the caspase-3 inhibitor XIAP (x-linked inhibitor of apoptosis protein). Recent studies have shown that translocation of HtrA2 protein from the MITO to the CS occurs in mice hearts during experimental myocardial ischemic and reperfusion. **Hypothesis:** In this study we examined if translocation of HtrA2 from MITO to CS occurs in LV myocardium of dogs with chronic stable heart failure (HF). **Methods:** LV tissue from 7 dogs with chronic HF produced by multiple sequential intracoronary micro-embolizations (LV ejection fraction <35%) and 7 normal (NL) dogs was used to prepare crude homogenate (HG) and to isolate MITO and CS fractions. Using primary antibodies, HtrA2 protein was determined in HG, MITO and CS fractions by Western blotting coupled with chemiluminescence. GAPDH was used as an internal loading control for both HG and CS fractions. Porin was used as an internal loading control for MITO fractions. HtrA2 band intensity was normalized to GAPDH for HG and CS and to porin for MITO. **Results:** GAPDH and porin protein levels were unchanged between NL and HF dogs. Compared to NL, HtrA2 protein levels in dogs with HF were unchanged in HG (0.76 ± 0.04 vs. 0.73 ± 0.08), significantly reduced in MITO (0.17 ± 0.02 vs. 0.40 ± 0.05 , $p < 0.05$) and significantly increased in CS (0.90 ± 0.07 vs. 0.36 ± 0.05 , $p < 0.05$). **Conclusions:** Compared to NL dogs, HtrA2 protein levels are significantly reduced in MITO but increased in cytosol fractions from LV myocardium of dogs with chronic stable HF; a maladaptations that promotes cardiomyocyte apoptosis and progressive LV dysfunction. Inhibiting the translocation of HtrA2 from MITO to CS may have therapeutic potential for the preventing progressive dysfunction of the failing heart.

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Increased Drp1 Acetylation Mediates Lipid Overload-induced Cardiomyocyte Death & Heart Dysfunction

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Rationale: Lipid overload can cause diabetic cardiomyopathy (DCM), which is characterized by cardiomyocyte death, myocardial remodeling, and eventually contractile dysfunction. How excessive lipid supply impacts cardiac metabolism leading to DCM remains poorly understood.

Objective: To investigate the role and mechanism of a mitochondrial fission protein, dynamin-related protein 1 (Drp1), in lipid overload-induced cardiomyocyte death and DCM.

Methods and Results: Mice fed with high fat diet (HFD) for 18 weeks exhibited DCM phenotype including myocardial insulin resistance, fibrosis, hypertrophy, cardiomyocyte death and mild contractile dysfunction. Cultured adult cardiomyocytes incubated with high levels of palmitate also exhibited increased cell death and openings of the mitochondrial permeability transition pore. Mitochondrial fission was stimulated by HFD in the heart or by palmitate incubation in cultured adult cardiomyocytes. Mechanistically, lipid overload increased acetyl-CoA level and decreased NAD⁺ level in the heart and in adult cardiomyocytes, leading to upregulation of Drp1 acetylation at lysine 642 (K642). Elevated Drp1 acetylation increased Drp1 protein level, its oligomerization and its phosphorylation at the activating serine 616 (S616) site. The excessively activated Drp1 translocated to mitochondria, induced fission, and bound with VDAC1 to promote cardiomyocyte death. Preventing Drp1 acetylation (K642R mutation), inhibiting Drp1 activity, or supplementing NAD⁺ ameliorated palmitate-induced fission and cardiomyocyte death. In the hearts of HFD-fed monkeys, we observed increased Drp1 acetylation, oligomerization, and phosphorylation, which were accompanied with markers of DCM.

Conclusions: These findings uncover a novel mechanism mediating lipid overload-induced heart hypertrophy and

dysfunction. Excessive lipid supply created an intracellular environment that facilitated Drp1 acetylation, which increased its activity and mitochondrial translocation causing cardiomyocyte death. Thus, Drp1 could be a critical mediator for metabolic stress-induced heart dysfunction as well as a potential target for therapy.

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The Effect of YAP/TAZ on Glycolysis and Mitochondrial Oxidative Phosphorylation in Cardiomyocytes

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The heart requires a high rate of adenosine triphosphate (ATP) production to maintain healthy cardiac function and viability. Anaerobic glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) are the main metabolic pathways by which ATP is generated in mammalian cells, including cardiomyocytes. Yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), which are major downstream effectors of the Hippo signaling pathway, play an important role in the regulation of heart size and cellular homeostasis of cardiomyocytes. However, interplay between YAP/TAZ and cardiac energy metabolism has been poorly understood. Here we examined the effect of YAP/TAZ on glycolysis and OXPHOS in isolated neonatal rat ventricular cardiomyocytes (NRVMs). Extracellular acidification rate (ECAR; an index of glycolysis) and oxygen consumption rate (OCR; an index of OXPHOS) of NRVMs transduced with adenoviruses harboring YAP, TAZ, or LacZ (control) for 6 days were assessed by Seahorse XFe96 analyzer. Both YAP and TAZ significantly increased not only the ECAR of basal glycolysis (1.5-fold and 1.3-fold, respectively, $p < 0.05$), glycolytic maximal capacity (1.4-fold and 1.3-fold, respectively, $p < 0.05$), and glycolytic reserve capacity (1.3-fold and 1.2-fold, respectively, $P < 0.05$) ($n = 12-15$) but also basal OCR (1.3-fold and 1.3-fold, respectively, $p < 0.05$) ($n = 6-10$) compared to control LacZ. Quantitative PCR analysis showed that mRNA levels of Pfkfb3, a key glycolytic enzyme, and subunits of protein complexes I through V in the electron transport chain are significantly increased (1.3-1.9-fold, $n = 5-6$, $p < 0.05$) by both YAP and TAZ compared with control. These results suggest that YAP/TAZ increase ATP production by accelerating glycolysis and OXPHOS in cardiomyocytes.

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Single Cell Analysis of Postnatal Heart Development and Disease

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A fundamental challenge in understanding cardiac biology and disease is that the remarkable heterogeneity in cell-type composition and functional states have not been well characterized at single-cell resolution in maturing and diseased mammalian hearts. Massively parallel single-nucleus RNA sequencing (snRNA-Seq) has emerged as a powerful tool to address these questions by interrogating the transcriptome of tens of thousands of nuclei isolated from fresh or frozen tissues. snRNA-Seq overcomes the technical challenge of isolating intact single cell from complex tissues including the maturing mammalian hearts, reduces biased recovery of easily dissociated cell types and minimizes aberrant gene expression during the whole-cell dissociation. We have recently applied sNucDrop-Seq, a droplet microfluidics-based massively parallel snRNA-Seq method, to investigate the transcriptional landscape of postnatal mouse hearts in both healthy and mitochondrial disease states. By profiling the transcriptome of nearly 20,000 nuclei, we identified major and rare cardiac cell types and revealed significant cellular heterogeneity in the postnatal developing heart. When applied to a mouse model of mitochondrial cardiomyopathy, we uncovered profound cell type-specific modifications of the cardiac transcriptional landscape at single-nucleus resolution. Here, we expanded these earlier studies and used our dataset to further decipher the cardiac cell type-specific gene regulatory networks. Our analysis reveals novel insights into the key nodes of gene networks that control the postnatal development and disease-associated changes of different cardiac cell types. Our ongoing work is using genetic mouse models to validate these findings from single cell analysis.

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Mitochondrial Complex I Induced Myocardial Stunning Following Cardiopulmonary Resuscitation

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Background - Cardiogenic shock following cardiopulmonary resuscitation (CPR) for sudden cardiac arrest is common, occurring even in the absence of acute coronary artery occlusion, and contributes to high rates of post-CPR mortality. The pathophysiology of this shock is unclear and effective therapies for improving clinical outcomes are lacking. **Methods and Results** - Using a murine model of asystolic cardiac arrest, we investigated the pathophysiology of post-CPR cardiogenic shock and discovered that duration of cardiac arrest (4, 8, 12 or 16-minute) prior to CPR determined post-resuscitation success rates, degree of neurological injury, and severity of myocardial dysfunction. Post-CPR cardiac dysfunction was not associated with myocardial necrosis, apoptosis, inflammation, or mitochondrial permeability transition pore opening and recovered within several days, indicative of myocardial stunning. Post-CPR myocardial stunning was associated with increases in ventricular and mitochondrial reactive oxygen species (ROS, $P < 0.001$ vs Sham, respectively). Seahorse micropolarimetry of isolated post-CPR cardiac mitochondria revealed decreased rates of maximal oxygen consumption rates (OCR) for both Complex I and Complex II vs controls ($P < 0.01$ vs Sham, respectively), indicating inhibition of mitochondrial oxidative phosphorylation. Paradoxically, in the presence of ADP stimulated coupled respiration, post-CPR mitochondria demonstrated increased OCR ($P < 0.05$ vs Sham) and increased rates of proton leak ($P < 0.05$ vs Sham), suggesting Complex I as the site of ROS generation. These findings were not observed at complex II. S1QEL, a complex I-specific superoxide inhibitor, administered during CPR, decreased myocardial ROS generation while improving post-CPR myocardial function ($P < 0.01$ vs CPR control), neurological injury ($P < 0.01$ vs CPR control), and survival ($P < 0.01$ vs CPR control). **Conclusions** - Our results demonstrate that cardiogenic shock following resuscitation from cardiac arrest is consistent with myocardial stunning mediated by mitochondrial complex I injury and ROS generation. Targeting this mechanism represents a novel and practical therapy for improving sudden cardiac arrest resuscitation outcomes.

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Drp1 Protects the Heart Against High Fat Diet-Induced Diabetic Cardiomyopathy

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Type II diabetes induces cardiomyopathy characterized by hypertrophy, diastolic dysfunction and mitochondrial dysfunction. We have shown recently that clearance of damaged mitochondria by mitophagy mediated through an Atg7-dependent mechanism serves as an essential mechanism to maintain the quality of mitochondria during the initial development of diabetic cardiomyopathy. Our previous study showed that disruption of Drp1 inhibits mitophagy and causes mitochondrial dysfunction at baseline and in response to pressure overload. We therefore investigated whether Drp1 is essential for mitophagy during high fat diet (HFD)-induced diabetic cardiomyopathy. Mice were fed either a normal diet (ND) or a HFD (60 kcal % fat). Mitophagy, evaluated with Mito-Keima, was increased after 3 weeks of HFD ($p < 0.05$). However, this increase in mitophagy induced by HFD consumption was abolished in tamoxifen-inducible cardiac-specific Drp1 knockout (Drp1 cKO) mouse hearts ($p < 0.05$), in which both diastolic dysfunction ($p < 0.05$) and systolic dysfunction ($p < 0.05$) were exacerbated. Furthermore, the increase in LC3-dependent general autophagy in response to HFD consumption was also abolished in Drp1 cKO mice ($p < 0.05$). Electron microscopic analyses showed elongated mitochondria and accumulation of lipid droplets in Drp1 cKO mice ($p < 0.05$) in the presence of HFD consumption. Fibrosis and cell death were increased in Drp1 cKO mice during HFD consumption ($p < 0.05$). Thus, Drp1 is essential for mitophagy during HFD consumption and serves as an essential mitochondrial quality control mechanism, thereby protecting the heart against diabetic cardiomyopathy.

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Oxidized Low Density Lipoprotein and Angiotensin II Causes Vascular Senescence Via AT1R Signal-mediate Mitochondrial Fission

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Introduction: Metabolic stress including oxidized low density lipoprotein (ox-LDL) and angiotensin II (AngII) cause mitochondrial dysfunction and vascular senescence. Mitochondria are highly dynamic organelles that constantly change their morphology to fusion or fission. This study aims to clarify how mitochondrial dynamics are involved in the etiology of vascular senescence. **Methods:** VSMC and HUVEC were stimulated by either ox-LDL or AngII. We also conducted in vivo experiment using C57BL6 (WT), apolipoprotein E (ApoE) deficient and the double knockout of ApoE and AT1R (DKO) mice. **Results:** Either ox-LDL or AngII induced excessive mitochondrial fission though phosphorylation of Drp1 at Ser616, mitochondrial dysfunction assessed by JC1, reactive oxygen species production measured with Mito-Sox Red and Amplex assay and cellular senescence analyzed by senescence associated beta galactosidase staining and immunoblot of p53 and p21 in cells. Administration of mdivi-1, a specific inhibitor of Drp1, restored imbalance of mitochondrial dynamics and these detrimental alterations. Treatment with angiotensin II type1 receptor (AT1R) inhibitor also inactivated Drp1 and improved imbalance of mitochondrial dynamics, mitochondrial dysfunction and cellular senescence. These results suggest that ox-LDL- or AngII-induced mitochondrial dysfunction and cellular senescence were derived from Drp1-dependent mitochondrial fission. Administration of AT1R inhibitor to HUVEC or SMC with ox-LDL prevented cells against excessive mitochondrial fission, its dysfunction and cellular senescence. The degree of vascular senescence was higher, the number of fused mitochondria and mitochondrial function were lower and mitochondrial oxidative stress were higher in either C57BL6 with AngII infusion or ApoE KO mice than those of control or WT mice, respectively. Treatment with mdivi-1 to these mice reduced mitochondrial fission, oxidative stress and attenuated vascular senescence. The number of fused mitochondria and its function were higher and the degree of vascular senescence were lower in DKO than those in ApoE KO mice. **Conclusion:** Either ox-LDL or AngII causes cellular and vascular senescence through AT1R signal-mediated mitochondrial fission.

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Smyd1 is Required for Cell Survival During Glucose Deprivation

Amira Sabry, Keiko Cawley, Katie Sciuto, Amanda Horiuchi, David Nix, Chris Stubborn, Yukio Saijoh, Alexey V Zaitsev, **Junco S Warren**, Univ of Utah, Salt Lake Cty, UT

Our recent study demonstrated that the histone methyltransferase Smyd1 positively regulates cardiac metabolism through transcriptional control of PGC-1 α , a key regulator of mitochondrial energetics. However, it is largely unknown whether Smyd1 plays a role in adaptive responses to metabolic stress in cardiomyocytes. Here, we hypothesized that Smyd1 is required for cell survival during metabolic stress through maintaining energetic balance. To address this hypothesis, neonatal rat ventricular myocytes (NRVMs) were cultured in glucose-free media for 24 hours and real-time quantitative PCR was performed. We found that Smyd1 and its downstream target PGC-1 α were upregulated during glucose starvation, concurrent with the significant increase of mRNA levels of *PPAR α* and *CPT1b*, the key regulators of fatty acid β -oxidation (all $p < 0.05$), indicating enhanced energetics. Control NRVMs were able to withstand 24-hr glucose starvation, maintaining mitochondrial inner membrane potential (MIMP) and the cell membrane integrity. However, siRNA-mediated *Smyd1* knockdown (siSmyd1) prior to glucose starvation led to massive cell death, evidenced by the dissipation of MIMP and the uptake of the normally cell-impermeable indicator YO-PRO. To better understand the mechanism of siSmyd1-NRVMs vulnerability to glucose starvation, we examined the expression of genes involved in metabolism and apoptosis at the early stage of glucose starvation (3 hr), when autophagy was activated as evidenced by upregulation of LC3 and FoxO1. We found that the upregulation of PGC-1 α , *PPAR α* , *CPT1b*, as well as LC3 and FoxO1, adaptively induced by glucose starvation in control NRVMs, were all abolished by *Smyd1* knockdown, concomitant with a significant increase in gene expression of tumor necrosis factor receptor type 1-associated DEATH domain protein (Tradd). Moreover, RNA-seq analysis revealed that *Smyd1*-knockdown *per se* led to upregulation of the genes involved in regulation of apoptosis and cell death (i.e. Tradd, Casp8, Ripk3, Smad1). These data suggest that upregulation of Smyd1 in response to metabolic stress is a critical adaptive

mechanism for cell survival, operating via enhancement of fatty acid metabolism, activation of autophagy, and suppression of cell death signaling.

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Altered Mitochondrial Flash Activity and mPTP Opening in the Aged Heart Are Reversed by Elamipretide Treatment

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Rationale: The mitochondrial theory of aging pinpoints mitochondria as the major reactive oxygen species (ROS) production site and target of oxidative stress during aging. Mitochondrial flash (mitoflash) is recently discovered excitable event that is a manifestation of ROS production combined with pH change and coupled with mitochondrial permeability transition pore (mPTP) opening. Our preliminary data shown that 8-week SS-31 peptide (elamipretide) protects the functional performance of the aged mouse heart. This study aims to investigate mitoflash activity and mPTP opening in the aged heart, especially whether and how SS-31 protects the heart function involving modulation of these activities.

Methods and Results: By using confocal microscopy imaging on the isolated rat cardiomyocytes with overexpression of mitochondrial targeted cpYFP, we found mitoflash activity in the cells from old (26 mo) was higher than that in young (5 mo) rat cells (2.2 ± 0.2 in old vs 1.3 ± 0.2 in young, /1000 μm^2 /100s, n=27-64, p<0.05). SS-31 10 μM treatment normalized the mitoflash activity to the young level. Similarly, by photon-excitation induced mPTP opening, we found that aging increased the mPTP opening and 1 μM SS-31 stabilized the mPTP. Furthermore, by using Seahorse Assay on intact primary isolated cardiomyocytes, we found mitochondrial basal respiration in cells from old mice was higher than that in young mouse cells, however, the maximal respiration rate was without significant difference. The increased basal respiration was attributed to a higher proton leak in old cardiomyocytes (131 ± 14 in 24 month vs 94 ± 11 in young, pmol/min/800cells, n=17-35, p<0.05). Interestingly, acute (2 hrs) *in vitro* treatment of the old cardiomyocytes with SS-31 100 nm decreased proton leak. Moreover, SS-31 (100 nM) decreased the superoxide production as measured by ratio of MitoSOX Red to MitoTrackerGreen.

Conclusion: These results indicate that SS-31 directly protects cardiac aging through rapid rejuvenation of mitochondrial respiration in cardiomyocytes, and in particular, by reducing proton leak, mitochondrial flash activity, and decreasing the mPTP opening. This study helps to uncover the mechanism of the cardiac aging and the protective effect from SS-31 treatment.

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Mitoq Regulates Redox-related Non-coding Rnas to Improve Mitochondrial Network in Pressure Overload Heart Failure

Jiajia Song, Seulhee Kim, Mary N Latimer, Kah Yong Goh, Sumanth D Prabhu, Gangjian Qin, Victor Darley-Usmar, Xiaoguang Liu, Adam R Wende, Martin E Young, **Lufang Zhou**, Univ of Alabama Birmingham, Birmingham, AL

Background. Previous studies show that mitochondrial network excitability, or the propagation of ROS signals, is impaired in cardiomyocytes from failing hearts. While oxidative stress has been implicated in heart failure (HF)-associated mitochondrial network abnormality, the effect of mitochondrial-targeted antioxidant, such as mitoquinone (MitoQ), on mitochondrial network in pressure overload hearts has not been demonstrated. We hypothesize that MitoQ improves mitochondrial networks in HF via regulation of redox-related cardiac remodeling-associated non-coding RNAs.

Methods and results. To test the hypothesis, C57BL/6J mice were subjected to ascending aortic constriction (AAC) to induce left ventricular (LV) pressure overload, followed by 7 days of MitoQ treatment (2 μmol). Doppler echocardiography revealed severe LV dilation and decreased ejection fraction following AAC, which were attenuated by MitoQ. Electron microscopy and immunostaining showed that inter-mitochondrial and mitochondria-sarcoplasmic reticulum (SR) network structure were altered in HF myocardium, in parallel with reduced expression of mitofusin proteins (e.g., MFN1 and MFN2) compared to sham-operated animals. MitoQ blunted mitofusin protein downregulation and improved mitochondrial networks. Our data also identified a MitoQ-mediated mechanism of mitofusin expression in HF by ameliorating the dysregulation of

redox-related cardiac remodeling-associated long non-coding RNAs and microRNAs (i.e. Plscr4-miR-214 axis).

Conclusion. The present study indicates that MitoQ improves inter-mitochondrial and mitochondrial-SR structural organization in pressure overload hearts by attenuating the dysregulation of cardiac remodeling-associated non-coding RNAs.

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Neddylation is Essential for Cardiac Metabolic Maturation in the Developing Heart

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Changes in metabolic milieu during development trigger a metabolic shift from fetal-type glycolysis to adult-type fatty acid oxidation in the developing heart. Disruption of this metabolic shift has been linked to congenital heart disease, a major cause of birth-related mortality and morbidity worldwide. Neddylation is a post-translational modification that covalently attaches a small ubiquitin-like protein, NEDD8, to target proteins. Previously we reported that neddylation is essential for cardiomyocyte proliferation through sustaining the YAP signaling. However, a link between neddylation and cardiac metabolic maturation has not yet been established. Here, we abrogated neddylation by cardiac-specific deletion of NAE1, an essential subunit of the sole NEDD8 E1 enzyme, via α MHC^{Cre} in mice, which led to significant reduction of NAE1 proteins and neddylated proteins in the heart. Temporal histological and functional analyses revealed that mice lacking neddylation displayed cardiac hypoplasia and ventricular non-compaction at E16.5, which became more pronounced by P1, eventually leading to heart failure and perinatal lethality by P7. Transcriptome analysis identified that the defects in cardiac chamber maturation are associated with upregulation of glycolytic genes and downregulation of oxidative metabolic genes. Transmissive electron microscopy revealed accumulation of lipid droplets and degenerative/immature mitochondria in hearts deficient of neddylation. In vitro, pharmacological inhibition of neddylation impairs mitochondrial membrane potential and respiration, and suppresses fatty acid utilization in oleic acid-primed cultured cardiomyocytes. Mechanistically, HIF1 α , a potent regulator of cardiac metabolic reprogramming, is a novel NEDD8 target. Inhibition of neddylation causes accumulation of HIF1 α proteins and consequently disrupts the expression of HIF1 α downstream targets in vitro and in vivo. Silencing of HIF1 α attenuates inhibition of neddylation-induced glycolytic rates in cardiomyocytes. Taken together, our findings highlight the importance of neddylation in the developing heart and identify neddylation as a novel regulator of HIF1 α signaling to promote developmental metabolic switch.

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Lpp3 Deficiency Impairs Mitochondrial Function And Enhances Myocardial Lpa Mediated Signaling

Sumitra Miriyala, Manikandan Panchatcharam, Md. Shenuarin Bhuiyan, Diana Escalante Alcalde, Kevin McCarthy, Christopher Kevil, LSUHSC-SHREVEPORT, Shreveport, LA

The bioactive lysophosphatidic acid (LPA) plays a well-known role in atherosclerotic disease, whereas, its role in myocardial function remains virtually unexplored. Following acute myocardial infarction, serum LPA concentration rises by six-fold over control human subjects, suggesting LPA may contribute to the pathogenesis of myocardial infarction. LPA production involves hydrolysis of lysophosphatidylcholine by the secreted enzyme autotaxin, whereas lipid phosphate phosphatase-3 (LPP3) catalyzes LPA dephosphorylation to generate lipid products that are not receptor active. We present the first evidence that cardiac ischemia/reperfusion (I/R) injury enhances myocardial autotaxin levels and decreases myocardial LPP3 expression, and this is associated with increased serum LPA levels. Upon reperfusion, reactive oxygen species production arises as a burst of superoxide from mitochondria following I/R injury. The redox-sensitive transcription factor NFAT has been shown to bind to the autotaxin promoter and induce its expression. Therefore, we looked at the autotaxin and LPP3 regulation in mice following I/R injury in the myocardium. After 1h ligation followed by 3h reperfusion in the

myocardium, we observed a 3-fold increase in the autotaxin protein levels, whereas LPP3 protein levels were significantly downregulated as observed through Western blot analysis in these myocardial ischemic tissues. Autotaxin and miR-92a mRNA expression levels were significantly upregulated, whereas KLF2 and LPP3 mRNA expressions were significantly downregulated following I/R injury at 24 hours. Western blot analysis showed a 3-fold increase autotaxin protein levels and immunohistochemistry of human infarct tissues at 24 hours showed disruption of the sarcomere with decreased LPP3 staining. We found that I/R injury transactivates miR-92a, and inhibit KLF2, an upstream activator of LPP3. Taken together, *in vivo* data, from the myocardial I/R injury and human infarct tissues, suggest that regulation of autotaxin and LPP3 activity might cause the rise in serum LPA levels as reported with acute myocardial infarct patients

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Cysteine 202 of Cyclophilin D is a Site of Multiple Post-translational Modifications and Plays a Role in Cardioprotection

Georgios Amanakis, Junhui Sun, Natl Insts of Health, Rockville, MD; Matthew Brody, Cincinnati Children's Hosp, Cincinnati, OH; Tyler Bauer, Shane McGinty, Jennifer Boylston, Chengyu Liu, Natl Insts of Health, Rockville, MD; Jeffery Molkentin, Cincinnati Children's Hosp, Cincinnati, OH; Elizabeth Murphy, Natl Insts of Health, Rockville, MD

Cyclophilin-D (CypD) is a well-known regulator of the mitochondrial permeability transition pore (PTP), the main effector of cardiac ischemia/reperfusion (I/R) injury characterized by oxidative stress and calcium overload. However, the binding of CypD to the PTP is poorly understood. Cysteine 202 of CypD (C202) is highly conserved across species and can undergo redox-sensitive post-translational modifications. To study the importance of C202, we developed a knock-in mouse model using CRISPR where CypD-C202 was mutated to a serine (C202S). In a Langendorff perfused heart model, infarct size is reduced by 51% in CypD-C202S hearts compared to WT (n=4, p=0.003). Isolated cardiac mitochondria from CypD-C202S mice also have a 49% higher calcium retention capacity compared to WT (n=5, p=0.011). We hypothesized that oxidation of C202 might target CypD to the PTP and that the cardioprotection in CypD-C202S mice is due to a post-translational modification on C202. Indeed, isolated cardiac mitochondria subjected to oxidative stress exhibit less binding of CypD-C202S to the proposed PTP component ATP synthase by 48% compared to WT (p=0.012, n=4). We previously found C202 to be S-nitrosylated in ischemic preconditioning. Cysteines can also undergo S-acylation (attachment of a fatty acid), and C202 matched a S-acylation motif. To assess S-acylation of CypD-C202 we employed a resin-assisted capture. Lysates were treated with N-ethylmaleimide to block free cysteines, and then with hydroxylamine to free cysteines from fatty acids. Subsequently, free cysteines were captured by a sepharose resin, followed by western-blot of the eluate. CypD-C202 is abundantly S-acylated, but CypD-C202S hearts exhibit less S-acylation than WT by 63% (n=6, p<0.001). We investigated the importance of S-acylation of CypD-C202 in cell death in a mouse embryonic fibroblast (MEFs) model. CypD-C202S MEFs exhibit 20% less oxidative cell death than WT (n=5, p=0.003). Inhibition of S-acylation with 2-bromopalmitate attenuates oxidative cell death in the WT by 20% (n=5, p=0.018), while CypD-C202S MEFs are unaffected. Thus, S-acylation and oxidation of CypD-C202 possibly target CypD to the PTP, sensitizing it under oxidative stress and making them potent regulators of cardiac I/R injury.

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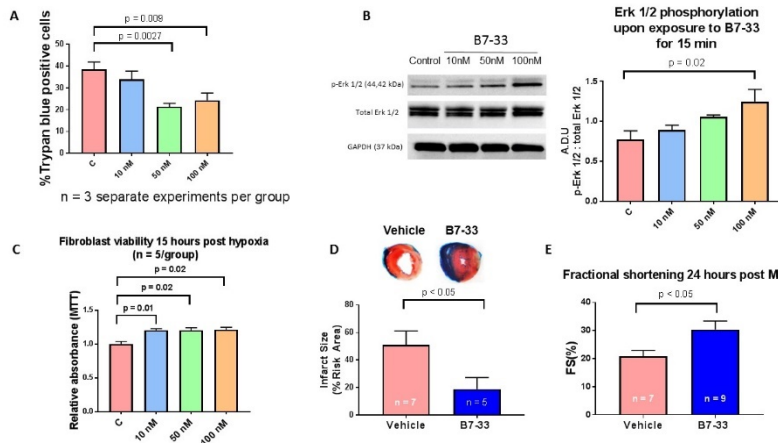
B7-33, a Functionally Selective Relaxin Receptor 1 Agonist, Exerts Protective Effects Against Myocardial ischemia-Reperfusion Injury in Mice

Teja Devarakonda, Adolfo G Mauro, Geronimo Guzman, Chad Cain, Anindita Das, Virginia Commonwealth Univ, Richmond, VA; Praveen Praveen, M A Hossain, Univ of Melbourne, Melbourne, Australia; Fadi N Salloum, Virginia Commonwealth Univ, Richmond, VA

Background: B7-33 is a peptide derived from the B-chain of human relaxin, and is shown to elicit biased signaling at relaxin receptor 1 (RXFP1) in human cell lines. The influence of B7-33 on myocardial ischemia-reperfusion (IR) injury and associated cardiac adverse remodeling is unknown.

Methods and Results: Primary cardiomyocytes and cardiac fibroblasts were isolated from adult CD1 mice and subjected to simulated ischemia-reoxygenation (SIRO). Myocytes were placed in 1% O₂ chamber for 40 min, followed by 1 h of reoxygenation with control (myocyte media) or B7-33 (10, 50 or 100 nM)-infused media. Trypan blue staining showed a significant decrease in cell death with B7-33 concentrations of 50 nM and 100 nM (**Fig. A**). Treating myocytes with B7-33 for 15 min exhibited a dose-dependent increase in Erk1/2 phosphorylation, which reached statistical significance at 100 nM (**Fig. B**). Fibroblasts subjected to 4 h of hypoxia and 15 h of reoxygenation had increased viability (MTT assay) with B7-33 at all concentrations (**Fig. C**). *In vivo*, CD1 mice were subjected to IR injury via coronary artery ligation for 30 min, followed by 24 h of reperfusion. Vehicle (saline) or B7-33 (10 µg/kg) was injected i.p. at the onset of reperfusion. After 24 h, B7-33-treated mice demonstrated decreased infarct size (TTC stain) (**Fig. D**) and preserved fractional shortening (M-mode echo, **Fig. E**) compared to vehicle-treated mice.

Conclusion: Reperfusion therapy with B7-33 reduces infarct size post-MI, preserves cardiac function and protects cardiomyocytes and fibroblasts against SIRO. We propose that B7-33 might be effective against acute MI and a possible alternative to recombinant relaxin for clinical utility.



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Therapeutic Effects of SK Channel Activator on Cardioprotection in the Setting of Cardioplegic Ischemia-Reperfusion and Cardiopulmonary Bypass

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Background: Inactivation of small-conductance calcium-activated potassium (SK) channels contributes to coronary microvascular dysfunction in patients after cardioplegic arrest, cardiopulmonary bypass (CP/CPB) and cardiac surgery. SK-channel activation is cardioprotective in rodent isolated heart model of myocardial infarction and ischemia/reperfusion. In the current study, we investigated the impact of SK activation on myocardial protection in a clinically relevant large animal model of pigs subjected to cardioplegic ischemia/reperfusion and CPB.

Methods and Results: Ten Yorkshire pigs were subjected to 1 hour of CP/CPB followed by 1 hour of reperfusion. Pigs received either cold blood hyperkalemic CP alone (CP control) or CP containing the selective SK channel activator NS309 (10⁻⁶M, n = 5/group). Left ventricular (LV) function was assessed by using pressure-volume loop analysis. Coronary arteriolar relaxation response (in-vitro) to the endothelium-dependent vasodilators adenosine diphosphate (ADP, 10⁻⁹-10⁻⁴M) and substance P (10⁻¹²-10⁻⁷M) and independent vasodilator sodium nitroprusside (SNP) was evaluated with video-microscopy. Inclusion of NS309 in cold blood CP tends to improve the recovery of LV function compared with the CP controls. CP/CPB and reperfusion significantly reduced endothelium-dependent relaxation response to ADP, 10⁻⁵M) and substance P (10⁻⁸M) as compared to control (p<0.05). In contrast, CP-containing NS309 significantly improved the recovery of endothelium-dependent relaxation response to ADP and substance P as compared with the control group (p<0.05, respectively).

Conclusion: In pig model of CP/CPB, inclusion of the selective SK channel activator in the cold blood cardioplegia improves recovery of coronary arteriolar endothelial function and microvascular relaxation, which may contribute to increased myocardial perfusion and improved LV function.

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Downregulation of Delta and Kappa Opioid Receptors in Post-heart Transplantation in Human and Rats

Mebratu A Gebrie, Alessio Rungatscher, Daniele Linardi, Anna Andrioli, Sajeela Ahmed, Naseer Ahmed, Mahmoud Ismail, Alberto Forni, Maddalena Tessari, Livio San Biagio, Alessandro Soave, Elizabetta Milani, Giulio Innamorati, Giovanni B Luciani, Giuseppe Faggian, Univ of Verona, Verona, Italy

Over the past decade, multiple data regarding local opioid regulation of heart function have been demonstrated. However, opioid receptors (ORs) have not yet characterized in post-heart transplantation. Since a transplanted heart is devoid of innervations and autonomic innervations modulate ORs, we assessed the hypothesis that a possible down-regulation of κ -OR and δ -OR in heart transplants.

Endomyocardial biopsies were collected in 15 male patients at 30 days of orthotopic heart transplantation and in 15 control male patients during aortic valve surgery in human. Another study performed in 20 heterotopic heart transplants (HHT) and 20 naïve hearts of Sprague-Dawley male rats. The mRNA transcript encoding the Oprd1 and Oprk1, KOR and DOR proteins, distribution, and cellular localization were identified using RT-qPCR, Western blot, IHC, and IF, respectively. Transcripts of mRNA encoding the Oprd1 and Oprk1 were detected in hearts of human and rats. Expression of Oprd1 ($p=0.036$) and Oprk1 ($p=0.004$) was significantly reduced in transplanted hearts. In Western blot analysis, KOR and DOR levels of expression were significantly lowered ($p=0.03$, KOR; $p=0.01$, DOR) in HHT compared to naïve hearts in rats. Immunohistochemistry analysis demonstrated a lighter labeling on the distribution of DOR and KOR in heart transplants' tissue in both rats and human. In both species, dual labeling myocardial isoforms (KOR and DOR) co-localized in cardiomyocytes of transplant and control hearts.

These findings suggest that the down-regulation of DOR and KOR receptors in hearts transplant of both human and rats might have a possible increased susceptibility to ischemia-reperfusion injury.

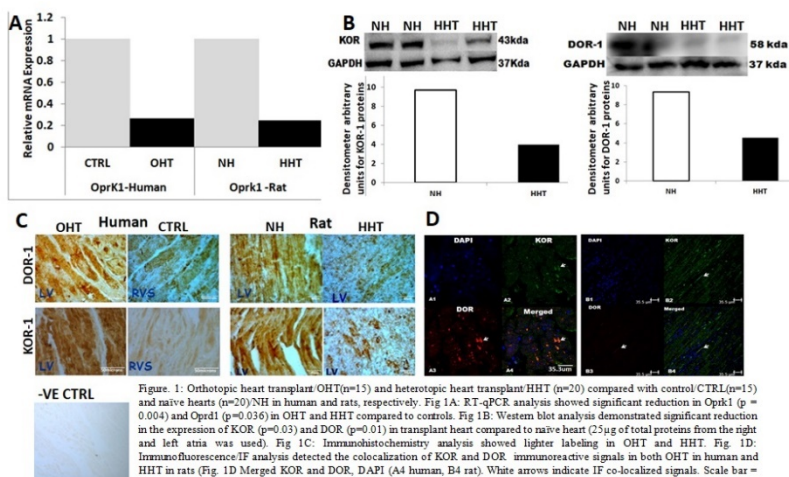


Figure 1: Orthotopic heart transplant OHT($n=15$) and heterotopic heart transplant HHT ($n=20$) compared with control CTRL($n=15$) and naïve hearts ($n=20$)NH in human and rats, respectively. Fig 1A: RT-qPCR analysis showed significant reduction in Oprk1 ($p=0.004$) and Oprd1 ($p=0.036$) in OHT and HHT compared to controls. Fig 1B: Western blot analysis demonstrated significant reduction in the expression of KOR ($p=0.03$) and DOR ($p=0.01$) in transplant heart compared to naïve heart (25 μ g of total proteins from the right and left atria was used). Fig 1C: Immunohistochemistry analysis showed lighter labeling in OHT and HHT. Fig 1D: Immunofluorescence-IF analysis detected the colocalization of KOR and DOR immunoreactive signals in both OHT in human and HHT in rat (Fig 1D Merged KOR and DOR, DAPI (A4 human, B4 rat). White arrows indicate IF co-localized signals. Scale bar = 35.5 μ m. LV, Left Ventricle, RVS, Right Ventricular Septum. $P<0.05$ is considered statistically significant.

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Engineering Rodent TRPV1 to Mimic Chicken TRPV1 Reduces Capsaicin-induced Calcium Influx in H9C2 Cells

Background and Aim: Birds, unlike mammals, are resistant to the spicy hot sensation when consuming chili peppers. The inability for birds to sense the hot sensation is accredited to genetic divergence in the transient receptor potential vanilloid 1 (TRPV1) when compared to mammals. Recently, we described how TRPV1 is important for regulating myocardial ischemia-reperfusion injury in rodents. Here, we tested our hypothesis that K710 is a crucial mediator of capsaicin-induced calcium influx in myocardial H9C2 cells. **Methods and Results:** Sequence alignment of the C-terminus TRP domain of TRPV1 among species was performed. Unlike mammals having K710 TRPV1, birds instead have N710 TRPV1 (Fig 1A). Site-directed mutagenesis of K710N was performed on wild type (WT) rTRPV1 with a pCMV6-entry vector. The empty vector, WT and K710N plasmids were transfected into H9C2 cells and the cellular localization of TRPV1 channel were detected by immunofluorescent staining and channel activity by capsaicin challenge with live cell calcium imaging with Fura-2AM. Interestingly, the K710N mutation caused the TRPV1 channel to be retained intracellularly and co-localized with PDI, an endoplasmic reticulum marker (Fig 1B, Bar=25µM). K701N cells treated with 1µM capsaicin showed a reduced response to capsaicin compared to wild type TRPV1 transfected cells (Fig 1C-E, 0.11 ± 0.01 in WT vs. 0.03 ± 0.01 in K710N, $n=10$, $P<0.01$). **Conclusions** Site-directed mutagenesis of K710N caused rTRPV1 to be insensitive to capsaicin and suggests a potential target for limiting calcium influx from myocardial ischemia-reperfusion injury which could be a novel protective strategy to prevent damage from a heart attack.

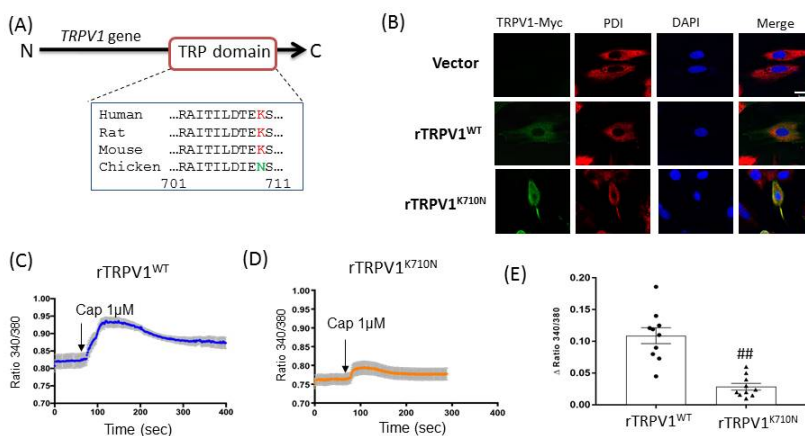


Fig.1 (A) Sequence alignment of TRPV1 among species; (B) Cellular localization of TRPV1 and PDI in H9C2 cells; (C, D) Calcium influx induced by capsaicin in rTRPV1^{WT} or rTRPV1^{K710N} transfected H9C2 cells; (E) Average peak increases (Δ) in fluorescence ratio 340/380, $n=10$, ## $P<0.01$, compared with rTRPV1^{WT}.

S. He: None. **P. Sinharoy:** None. **E. Gross:** None.

Poster Session 1 and Reception

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Ischemia and Reperfusion Injury Following Cardioplegic Arrest is Attenuated by Age and Testosterone Deficiency in Male but Not Female Mice

Anjali Ghimire, Elise S Bisset, **Susan E Howlett**, Dalhousie Univ, Halifax, NS, Canada

Cardiovascular disease increases with age in both sexes and treatment can require cardiac surgery, where the heart is pre-treated with a protective cardioplegic solution before exposure to ischemia and reperfusion (I/R). While endogenous estrogen is thought to be beneficial in I/R, the role of testosterone in cardioprotection is not well understood and whether age modifies responses to I/R is unclear. We investigated sex- and age-specific differences in I/R injury in hearts pre-treated with a clinically relevant cardioplegic solution. Hearts were isolated from adult (6-9 mos) and old (22-28 mos) C57BL/6 mice of both sexes ($n=27$). They were Langendorff-perfused with Krebs-Henseleit buffer (15 min; 37°C), followed by St. Thomas'2 cardioplegia (6 min; 6-7°C). This was followed by 90 min of global ischemia (room temperature) and 30 min of reperfusion (37°C). Hearts were then perfused with triphenyltetrazolium chloride to quantify infarct area. The role of testosterone was investigated in gonadectomized (GDx; 6-9 mos) male mice and serum testosterone was measured with an ELISA. Functional recovery in reperfusion was significantly better in old males than in adult males. Left ventricular developed pressure (LVDP) recovered to $67.3 \pm 7.4\%$ in old compared to $31.6 \pm 12.3\%$ in adult male hearts ($p<0.05$). Similar results were seen for rates of pressure development (+dP/dt; $p<0.05$) and decay (-dP/dt; $p<0.05$). Also, infarct areas were markedly smaller in old male hearts ($15.5 \pm 1.8\%$) than in younger hearts ($48.1 \pm 7.7\%$; $p<0.05$). By contrast, hearts from adult and old females exhibited a similar degree of post-ischemic functional recovery and showed no age-dependent difference in infarct

size. Serum testosterone levels declined with age in males but were low regardless of age in females, which suggested a potential benefit of low testosterone. In support of this, hearts from GDX males exhibited much better recovery of LVDP in reperfusion when compared to hearts from intact males (values were $64.8 \pm 7.7\%$ vs. $29.9 \pm 12.3\%$; $p < 0.05$). GDX hearts also had smaller infarcts than hearts from intact males ($p < 0.05$). These results suggest that low testosterone levels may be protective against I/R injury following cardioplegic arrest in the setting of aging, especially in old males.

A. Ghimire: None. **E.S. Bisset:** None. **S.E. Howlett:** 8. Consultant/Advisory Board; Significant; Consult for DGI Clinical.

Poster Session 1 and Reception

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Bone Marrow Niche-Mediates Cardiac Repair is Associated With Adiponectin in Retnla Deficiency

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Background: Resistin like α (Retnla) is known to be upregulated in anti-inflammatory macrophages and to modulate lipid profile in atherosclerosis. However, the role of Retnla is unknown in the cardiac pathology. **Methods:** Myocardial infarction (MI) was induced in wild type, Retnla knockout (KO), and Retnla transgenic (TG) mice. Cardiomyocytes, cardiac fibroblasts, macrophages and bone marrow cells were treated with Retnla protein for mechanistic studies. For an adoptive transfer, bone marrow cells were isolated from KO mice to transplant to the infarcted heart. **Results:** TUNEL staining showed the number of apoptotic cardiomyocytes was higher in the Retnla KO mice than in the TG mice 1 day after MI. Cardiac function and angiogenesis were significantly better in the Retnla KO group, while were worsen in the TG group. Retnla is an anti-inflammatory marker in macrophages, but the expressions of inflammation-related genes were not different between both groups. Interestingly, however, the numbers of macrophages isolated from bone marrow, peritoneum, and cardiac tissue of Retnla KO mice were higher than in the wild type mice. Moreover, phospho-histone H3-positive proliferating cells in the bone marrow were much more abundant in the Retnla KO mice than in the wild type mice. Adiponectin was highly expressed in bone marrow of KO mice, and we transplanted bone marrow cells from KO mice to recipient wild type mice. Two weeks after MI, recipient group transplanted with bone marrow cells from KO mice showed better angiogenesis, reserved cardiac function, and more anti-inflammatory macrophages. In Retnla-treated cardiomyocytes, bax, phospho-p38, and p21 were notably upregulated, while bcl2 and adiponectin were significantly reduced. To examine whether Retnla acted negatively on adiponectin, we administrated a synthetic adiponectin receptor agonist AdipoRon to Retnla-treated cardiomyocytes. AdipoRon blunted the effect of Retnla on cardiomyocytes. These data showed better cardiac recovery was associated with bone marrow-derived adiponectin in the Retnla KO group. **Conclusion:** Our study suggested that substantial cardiac recovery was strongly associated with adiponectin derived from highly proliferating bone marrow cells in Retnla KO mice.

Y. Kim: None. **H. Jeong:** None. **H. Kang:** None. **M. Kim:** None. **Y. Ahn:** None.

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Role of Acidic pH in Linking SIRT1 and Cardioprotective Metabolism

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Metabolic acidosis is the hallmark of the ischemic period of ischemia-reperfusion injury and acidic pH is cardioprotective via unclear mechanisms. We previously reported induction of neomorphic activities in several dehydrogenase enzymes under acidic pH, leading to accumulation of non-canonical metabolite L-2-hydroxyglutarate (2-HG). Lysine deacetylase SIRT1 is required for cardioprotection via ischemic preconditioning (IPC) and 2-HG levels are elevated in IPC in a SIRT1 dependent manner. We hypothesized that acidic pH may be an independent variable that partly drives the metabolic character of not only ischemia, but also IPC. This hypothesis was tested using LC-MS/MS based metabolomics analysis of perfused hearts. Exposure of hearts to ischemia, hypoxia, IPC or intracellular acidosis resulted in partially overlapping metabolomes, with a number of metabolic adaptations previously attributed as either ischemic or IPC-driven, being induced by acidic pH alone. Furthermore, we observed that SIRT1 activator β -nicotinamide adenine mononucleotide induced cellular acidification in isolated cardiomyocytes. In the same cells it was observed that SIRT1 activation resulted in inhibition of sodium proton exchanger NHE1. These results suggest (i) a novel signaling mechanism in which SIRT1 is a cellular pH regulator via NHE1, and (ii) a role for acidic pH as a link between SIRT1 and cardioprotective metabolic adaptations.

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Exercise-induced Autophagy and Nadph Oxidase 2 Downregulation Prevents Cardiotoxicity Induced by Doxorubicin

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Endurance exercise (EXE) preconditioning prior to DOX treatment confers cardioprotection; however, whether EXE postconditioning (i.e., EXE intervention after the completion of DOX treatment) is cardioprotective remains unknown. Thus, the aim of the present study was to investigate if EXE postconditioning provide cardioprotection by testing the hypothesis that EXE-autophagy upregulation and NADPH oxidase 2 (NOX2) downregulation would be linked to cardioprotection against DOX-induced cardiotoxicity. C57BL/6 male mice were assigned into three groups: control (CON, n=10), doxorubicin (DOX, n=10), and doxorubicin + endurance exercise (DOX+EXE, n=10). Animals assigned to DOX and DOX+EXE groups were intraperitoneally injected with DOX (5 mg/kg each week for four weeks). 48 hours after the last DOX treatment, the mice assigned to DOX+EXE performed EXE on a motorized treadmill at a speed of 13-15 m/min for 60 min per day for four weeks. EXE prevented DOX-induced apoptosis and mitigated tissue damages. While DOX did not modulate auto/mitophagy, EXE significantly enhanced its flux (increased LC3-II levels, reduced p62 levels, and increased autophagosomes with mitochondria) along with increased mitochondrial fission (DRP1) and reduced fusion markers (OPA1 and MFN2). Interestingly, EXE-induced autophagy against DOX occurred in the absence of alterations of autophagy inducer AMPK or autophagy inhibitor mTOR signaling. EXE prohibited DOX-induced oxidative damages by suppressing NOX2 levels but without modulating other key antioxidant enzymes including MnSOD, CuZnSOD, CATALASE, and GPX1/2. Our data provide novel findings that EXE-induced auto/mitophagy promotion and NOX2 downregulation are linked to cardioprotection against DOX-induced cardiotoxicity. Importantly, our study shows that EXE postconditioning intervention is effective and efficacious to prevent DOX-induced cardiac injuries.

Y. Lee: None. **I. Kwon:** None. **Y. Jang:** None. **L. Cosio-Lima:** None.

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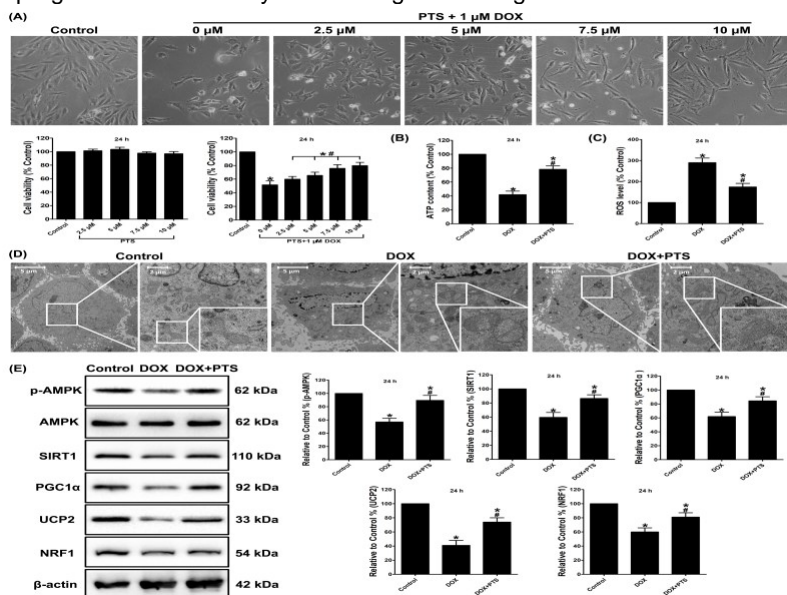
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Pgc1a Activation by Pterostilbene Ameliorates Acute Doxorubicin Cardiotoxicity via Reducing Mitochondrial Oxidative Stress Through Enhancing Ampk and Sirt1 Cascades

Dong Liu, Shubin Qiao, Jiansong Yuan, Fuwai Hosp, Beijing, China

BACKGROUND Doxorubicin (DOX) is a widely used and potent anticancer agent, but DOX dose-dependently induced cardiotoxicity greatly limits its use in clinic. Pterostilbene, a natural analog of resveratrol, is a known antioxidant and exerts myocardial protection. The present study explored the actions and detailed mechanisms of pterostilbene on DOX-treated cardiomyocytes. **EXPERIMENTAL APPROACH** We investigated the effects of pterostilbene on established acute DOX-induced cardiotoxicity models in both H9c2 cells treated with 1 μ M DOX and C57BL/6 mice with DOX (20 mg/kg cumulative dose) exposure. Cell viability, ATP content, oxidative parameters, transmission electron microscopy, western blot analyses were respective performed. AMPK and SIRT1 siRNA, Compound C and EX527 treatment were further conducted to elucidate the underlying mechanism about the involvement of AMPK, SIRT1 and PGC1 α cascades. **KEY RESULTS** Pterostilbene markedly upregulated the cell viability and ATP content in DOX-treated H9c2 cells. Both *in vitro* and *in vivo* studies revealed that pterostilbene inhibited the acute DOX exposure-caused oxidative stress and mitochondrial morphological disorder via the PGC1 α upregulation through activating AMPK and via PGC1 α deacetylation through enhancing SIRT1. However, these effects were partially reversed by knockdown of AMPK or SIRT1 *in vitro* and treatment of Compound C or EX527 *in vivo*. **CONCLUSION AND IMPLICATIONS** Our results indicate that pterostilbene protects cardiomyocytes from acute DOX exposure-induced oxidative stress and mitochondrial damage via PGC1 α

upregulation and deacetylation through activating AMPK and SIRT1 cascades.



D. Liu: None. S. Qiao: None. J. Yuan: None.

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DJ-1 Preserves Mitochondrial Function in the Ischemic Heart by Reducing the Glycation of Complex I

Yvanna Pantner, Emory Univ, Atlanta, GA; Yuuki Shimizu, Nagoya Univ Graduate Sch of Med, Nagoya, Japan; Rohini Polavarapu, Lian Li, Lih-Shen Chin, John Calvert, Emory Univ, Atlanta, GA

Background: DJ-1 is a cytoprotective protein implicated in many cellular processes, including the oxidative stress response and the glycation stress pathway. DJ-1 has proteolytic activity when cleaved under conditions of oxidative stress. We recently found that the cleaved form of DJ-1 attenuates ischemia-reperfusion (I/R)-induced heart failure by reducing glycation stress. Mitochondrial electron transport proteins are susceptible to glycation stress. Here, we sought to determine if DJ-1 regulates the glycation of Complex I after I/R injury. **Methods and Results:** Initial studies found that compared to wild-type control mice, mitochondria from DJ-1 deficient (DJ-1 KO) hearts showed increased levels of advanced glycation end products and carboxymethyl-lysine (CML) following I/R. Additionally, CML was shown to be bound to complex I at higher levels in DJ-1 KO hearts. This corresponded with reduced Complex I activity and reduced mitochondrial oxygen consumption in the presence of Complex I substrates. To confirm an association between DJ-1 and Complex I, wildtype animals were subjected to I/R injury and co-immunoprecipitation revealed higher levels of DJ-1 bound to Complex I after I/R. To further determine if DJ-1 influences the glycation of Complex I, an adenoviral approach (AAV9-CMV-DJΔc) was used to over-express the cleaved form of DJ-1. Under I/R conditions, mitochondrial CML levels were attenuated in hearts treated with AAV9-CMV-DJΔc compared to hearts treated with a control virus. Moreover, mitochondria from AAV9-CMV-DJΔc treated hearts showed improved Complex I activity and oxygen consumption. **Conclusion:** These data demonstrate that DJ-1 plays a role in maintaining Complex I efficiency when under ischemic conditions. In elucidating a specific mechanism for DJ-1's role in attenuating ischemia-reperfusion-induced heart failure, these data break new ground for potential therapeutic strategies using DJ-1 as a target.

Y. Pantner: None. Y. Shimizu: None. R. Polavarapu: None. L. Li: None. L. Chin: None. J. Calvert: None.

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Chemogenetic Activation of Paraventricular Oxytocin Neurons Reduces Cardiac Dysfunction During Heart Failure

Jeannette Rodriguez Gonzalez, Jhansi Dyavanapalli, Carla Rocha, Mary Kate Dwyer, John Schloen, Kathryn J Schunke, David M Mendelowitz, Matthew W Kay, The George Washington Univ, Washington, DC

There is a distinctive cardiac autonomic imbalance in HF consisting of increased sympathetic activity and decreased parasympathetic tone. Recent work has indicated that activation of paraventricular nucleus (PVN) oxytocin neurons increases co-release of oxytocin with glutamate to elevate cardiac vagal tone yet little is known regarding the long-term (16 week) benefit. We hypothesize that activation of oxytocin neurons in the PVN will blunt the progression of HF by reducing inflammation and fibrosis at 16 weeks post-HF initiation than in untreated counterparts. Rats underwent trans-ascending aortic constriction (TAC) to induce HF. In a subset of HF rats (TAC+OXT), oxytocin neurons were activated by excitatory designer receptors exclusively activated by designer drugs (DREADDs). Rats were implanted with ECG telemetry devices and underwent a treadmill protocol to measure peak effort capacity and heart rate recovery (HRR). Cardiac structural measurements were obtained using echocardiography. After 16 weeks, hearts were rapidly excised, Langendorff perfused to measure mechanical function, and prepared for histology and western blot analysis. Indices of cardiac function (stroke volume, ejection fraction, cardiac output, and fractional shortening) indicated preserved function in TAC+OXT compared to TAC. HRR after peak effort capacity values were lowest in TAC and were significantly greater in TAC+OXT ($p \leq 0.05$), indicating preservation of cardiac parasympathetic tone. LV developed pressure was significantly impaired in TAC compared to sham as well as significantly improved in TAC+OXT compared to TAC ($p \leq 0.05$). Contractility and relaxation were significantly impaired in TAC compared to sham ($p \leq 0.05$), while TAC+OXT animals had values not different from sham. TAC animals had collagen III levels 5X higher than TAC+OXT. Histological analysis indicated increased fibrosis in disease groups compared to sham. TAC+OXT had IL-1 β levels significantly lower than TAC ($p \leq 0.05$). PVN oxytocin neuron activation slows the decline of cardiac function in 16 week post-TAC rats, likely by reinstating cardioprotective parasympathetic activity. This novel method has translational potential since intranasal oxytocin administration has been used in the clinic.

J. Rodriguez Gonzalez: None. **J. Dyavanapalli:** None. **C. Rocha:** None. **M. Dwyer:** None. **J. Schloen:** None. **K.J. Schunke:** None. **D.M. Mendelowitz:** None. **M.W. Kay:** None.

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Role of Snf1-related Kinase as a Regulator of Chromatin Modifications and Dna-damage Response in Heart Injury

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Introduction: Altered DNA damage response (DDR) can result in phenotypic changes that share features with age-associated pathological conditions, including metabolic and cardiovascular abnormalities. Snf1-related kinase (SNRK) is a serine/threonine kinase that belongs to the AMPK family. Our recent data demonstrates that SNRK expression is upregulated in heart failure (HF) and low energy states and that SNRK improves cardiac mitochondrial efficiency and cell survival. Here, we assessed the hypothesis that in response to stress, SNRK translocates to the nucleus where it modulates nuclear processes to enhance DNA repair and ultimately reduces cardiomyocyte death.

Results: We first showed that the nuclear distribution of endogenous SNRK in cardiomyoblast is enhanced under low nutrient and oxidative stress. Next, we performed global phosphoproteomics analysis on cells with overexpression of wild type SNRK and mutant kinase-dead SNRK (D158A). We found significant increase of several phosphopeptides involved in nuclear processes, in particular in the regulation of chromatin remodeling and DDR. SNRK downregulation resulted in an increase in nucleus volume and heterochromatin content, with increased nuclear dysmorphia when subjected to oxidative stress.

Despite having lower expression of DDR signal effectors p53 and Mdm2, these cells accumulated higher levels of pH2AX (DDR marker) and displayed higher cellular death after oxidative stress, consistent with increased DNA damage. Etoposide treatment in SNRK knockdown cells failed to induce p53 levels despite pH2AX accumulation. Additionally, pharmacological stabilization of p53 protein through nutlin-3A treatment did not increase p53 in SNRK knockdown cells. These findings suggest that SNRK regulates p53 expression, thereby acting as a new modulator of DDR.

Conclusions: Our results demonstrate increased nuclear localization of SNRK under stress conditions, and that SNRK regulates phosphorylation of nuclear proteins involved in chromatin remodeling and DDR. Thus, SNRK may induce its pro-survival cardiac functions through regulation of chromatin architecture and enhancing DDR. Those novel findings can contribute to development of new therapies against HF.

P.J. Stanczyk: None. **Z. Zilber:** None. **A. de Jesus:** None. **H. Chang:** None. **H. Ardehali:** None.

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Cardioprotective Effect of Canstatin Against Myocardial Infarction in Rats

Akira Sugiyama, Rumi Ito, Muneyoshi Okada, Hideyuki Yamawaki, Sch of Veterinary Med, Kitasato Univ, Towada, Aomori, Japan

Background: We recently reported that canstatin, an endogenous substance derived from C-terminus of type IV collagen $\alpha 2$ chain (COL4A2), is immediately degraded by cathepsin S in infarcted area after myocardial infarction (MI). It also protects H9c2 cardiomyoblasts from hypoxic injury. In this study, we aimed to examine the cardioprotective effect of canstatin against MI.

Methods and Results: Left anterior descending artery (LAD) in male Wistar rats was ligated to make MI model. Recombinant canstatin (20 $\mu\text{g}/\text{kg}$) or vehicle was intraperitoneally administered for 28 days. Canstatin improved survival rate of MI rats from 47.1% (8 of 17) to 72.7% (8 of 11). Canstatin suppressed dilation (left ventricular internal dimension at end-systole; canstatin: 0.5 cm vs. vehicle: 0.6 cm, $P<0.01$) and dysfunction (ejection fraction; canstatin: $72.8\pm 3.6\%$ vs. vehicle: $62.8\pm 2.7\%$, $P<0.01$) of left ventricle in MI rats as determined by echocardiography. Hematoxylin and eosin and picosirius red stainings showed that canstatin inhibited size of cardiomyocytes (canstatin: $17.6\pm 0.6\ \mu\text{m}$ vs. vehicle: $23.2\pm 0.3\ \mu\text{m}$, $P<0.01$) and fibrotic area (canstatin: $4.6\pm 0.7\%$ vs. vehicle: $10.6\pm 1.6\%$, $P<0.01$) in non-infarcted area, respectively. Western blot analysis showed that canstatin inhibited increase of type I collagen expression in non-infarcted area (canstatin: $137.2\pm 24.4\%$ vs. vehicle: $348.7\pm 74.3\%$, $P<0.05$). Langendorff perfused heart was used after canstatin protein was deleted by injection of small interference (si)RNA against COL4A2 gene for 48 h. Then LAD of perfused heart was ligated for 2 h. Triphenyl tetrazolium chloride and TdT-mediated dUTP nick end labeling stainings showed that canstatin deletion enhanced formation of infarcted area (COL4A2 si: $37.6\pm 6.4\%$ vs. Control si: $17.0\pm 3.5\%$, $P<0.05$) and apoptosis of cardiomyocytes (COL4A2 si: $49.0\pm 3.7\%$ vs. Control si: $27.3\pm 2.3\%$, $P<0.01$), respectively.

Conclusions: We for the first time demonstrated that canstatin exerts cardioprotective effects against MI through the inhibition of hypertrophy and fibrosis in non-infarcted area and also the inhibition of apoptosis of cardiomyocytes in infarcted area. Our data indicate canstatin-treatment as a novel therapeutic strategy for MI.

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Time-Dependent Changes in Myocardial Edema Overestimate the Ischemic Area-at-Risk Up to One Week After Reperfused Myocardial Infarction in Swine

Brian R Weil, George Techiryian, John M Canty Jr., Univ at Buffalo, Buffalo, NY

Objective: Assessment of tissue salvage after myocardial infarction (MI) requires accurate quantification of the ischemic area at risk (AAR). Although the AAR is typically measured retrospectively within one week after reperfusion, dynamic changes in tissue composition may affect the precision of this approach. Given recent cardiac MRI data demonstrating a transient reduction in myocardial edema 24 hours after reperfused MI, we tested the hypothesis that this is an optimal timepoint for accurate retrospective AAR assessment.

Methods: Swine ($n=27$) were subjected to a 1 hour LAD occlusion, during which the AAR was assessed by contrast enhanced CT-derived quantification of the myocardial perfusion defect. Phthalocyanine blue was administered at the end of occlusion ($n=7$) or during re-occlusion of the LAD 3 hours ($n=5$), 24 hours ($n=5$) or 7 days ($n=10$) after reperfusion for pathologic determination of the AAR. Serial echocardiography was performed in the 24 hour reperfusion group to assess end diastolic wall thickness (EDWT) as a surrogate index of post-MI edema.

Results: When assessed before reperfusion, the AAR was similar by CT and postmortem pathology (**Table**). However, AAR measurements 3 hours, 24 hours, and 7 days after reperfusion overestimated CT AAR mass by $38\pm 7\%$, $31\pm 10\%$, and $37\pm 5\%$, respectively. Echocardiography showed a marked increase in EDWT 1 and 3 hours after reperfusion, followed by a decrease at 24 hours that remained significantly higher than pre-ischemia values.

Pre-Reperfusion vs. Post-Reperfusion AAR								
	No Reperfusion (n=7)		3 Hours Reperfusion (n=5)		24 Hours Reperfusion (n=5)		7 Days Reperfusion (n=10)	
	CT	Pathology	CT	Pathology	CT	Pathology	CT	Pathology
AAR (grams)	23.2 ± 3.2	23.4 ± 2.9	23.7 ± 1.2	32.9 ± 2.8*	16.9 ± 0.5	22.2 ± 1.7*	17.2 ± 1.2	23.3 ± 1.5*
AAR (% of LV)	27.5 ± 3.2	27.0 ± 3.4	20.6 ± 1.8	25.0 ± 2.5*	21.1 ± 1.0	25.6 ± 2.0*	20.5 ± 1.1	22.4 ± 1.1 [#]

Values are mean ± SEM. *p<0.05 vs. CT; [#]p=0.09 vs. CT. AAR indicates ischemic area-at-risk.

Serial Echocardiography (n=5)				
	Pre-Ischemia	1 Hour Reperfusion	3 Hours Reperfusion	24 Hours Reperfusion
Anterior EDWT (mm)	8.4 ± 0.6	13.3 ± 0.8*	13.9 ± 0.7*	10.6 ± 0.6 ^{#%}
Δ EDWT vs. Pre-Ischemia (%)	---	59.6 ± 11.1	66.5 ± 10.2	26.7 ± 8.3 ^{#%}

Values are mean ± SEM. *p<0.05 vs. Pre-Ischemia; [#]p<0.05 vs. 1 Hour Reperfusion; [%]p<0.05 vs. 3 Hours Reperfusion. EDWT indicates end-diastolic wall thickness.

Conclusion: Despite partial resolution of edema 24 hours after reperfusion, AAR measurement at this time does not provide an accurate assessment of the AAR obtained during the initial coronary occlusion.

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Signal Transducer and Activator of Transcription 3 Inhibits the Opening of Mitochondrial Calcium Uniporter Against Cardiac Ischemia/reperfusion in Hydrogen Peroxide Postconditioning

Lan Wu, Shanghai Univ of Med & Health Sciences, Shanghai, China; **Ji-liang Tan,** Zhong-yan Chen, Shanghai Insts for Biological Sciences, Chinese Acad of Sciences, Shanghai, China; **Gang Huang,** Shanghai Univ of Med & Health Sciences, Shanghai, China

Reactive oxygen species (ROS) generated during early reperfusion activated signal transducer and activator of transcription 3 (STAT3) contribute to intermittent hypobaric hypoxia (IHH)-afforded cardioprotection against ischemia/reperfusion (I/R)-induced mitochondrial calcium overload, but its mechanisms are not fully understood. This study investigated whether ROS signaling activated STAT3 is involved in moderate hydrogen peroxide postconditioning (H₂O₂PoC) mimicked IHH against cardiac I/R injury and the possible downstream target of STAT3 in mitochondria that mediates H₂O₂PoC-afforded cardioprotection. Moderate H₂O₂PoC not only improved the post-ischemic recovery of myocardial contractile performance and reduce the infarct size in isolated rat I/R hearts, but also alleviated cytosolic Ca²⁺ and mitochondrial Ca²⁺ overload and improved Ca²⁺ transients and cell contraction in rat I/R cardiomyocytes. However, the cardioprotective effects of moderate H₂O₂PoC was abrogated by janus kinase 2 (JAK2)/STAT3 inhibitor AG490 in rat hearts as well as adenovirus-delivered shRNAs specific for STAT3 and the opener of mitochondrial calcium uniporter (MCU) spermine, respectively, in rat adult cardiomyocytes with simulated I/R. Besides, the moderate H₂O₂PoC-afforded cardioprotection abrogated by spermine could be rescued by STAT3 overexpression in rat cardiomyocytes with simulated I/R. Moreover, H₂O₂PoC enhanced the expression of serine 727 phosphorylation of STAT3 and STAT3 in mitochondria of rat cardiomyocytes with simulated I/R. Furthermore, immunofluorescence and co-immunoprecipitation assay revealed a co-localization/interaction of STAT3 and MCU in rat cardiomyocytes with moderate H₂O₂PoC at reperfusion 5 min and reperfusion 30 min but not in I/R cells. Co-immunoprecipitation further confirmed that STAT3 interacted with the N-terminal domain (NTD) of MCU in rat cardiomyocytes with moderate H₂O₂PoC. These findings indicate that STAT3 mediates the cardioprotection of moderate H₂O₂PoC against I/R injury by alleviating mitochondrial calcium overload through inhibiting MCU opening via the interaction with the NTD of MCU. Our finds provide new insight into the mechanisms of STAT3 in the cardioprotection.

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SGLT1 is Essential for Cardioprotection During Ischemia-Reperfusion Injury via Enhanced Glucose Utilization in Diet-induced Obese Mice

Akira Yoshii, Nagoshi Tomohisa, Yusuke Kashiwagi, Haruka Kimura, Yoshiro Tanaka, Yuhei Oi, Keiichi Ito, Takuya Yoshino, Toshikazu D Tanaka, Michihiro Yoshimura, Jikei Univ Sch of Med, Tokyo, Japan

Recent large clinical trials have shown that sodium-glucose cotransporter 2 (SGLT2) inhibitors reduced cardiovascular events in diabetic patients. However, the regulation and functional role of cardiac SGLTs (SGLT1 is the dominant isoform) compared with those of other glucose transporters (insulin-dependent GLUT4 is the major isoform) remain incompletely understood. Given that glucose is a preferential substrate for myocardial metabolism during ischemia-reperfusion injury (IRI), we hypothesized that SGLT1 contributes to cardioprotection during IRI via enhanced glucose transport, particularly in insulin-resistant phenotypes. The hearts from mice fed a high-fat diet (HFD) for 12 weeks or a normal-fat diet (NFD) were perfused with either non-selective SGLT-inhibitor phlorizin (to inhibit SGLT1) or selective SGLT2-inhibitors (tofogliflozin, ipragliflozin, canagliflozin) during IRI using Langendorff model. After ischemia-reperfusion, HFD impaired left ventricular developed pressure (LVDP) recovery compared with NFD. Although phlorizin-perfusion impaired LVDP recovery in NFD, a further impaired LVDP recovery and dramatically increased infarct size (indicated by CPK release into perfusate and TTC staining) were observed in HFD with phlorizin-perfusion. Meanwhile, none of the SGLT2-inhibitors significantly affected cardiac function or myocardial injury after ischemia-reperfusion under either diet condition. The plasma membrane expression of GLUT4 was significantly increased after IRI in NFD but was substantially attenuated in HFD, the latter of which was associated with a significant reduction in both myocardial glucose uptake and cardiac tissue ATP content. In contrast, regardless of the diet condition, SGLT1 expression remained constant during IRI, whereas SGLT2 was not detected. Of note, phlorizin considerably reduced myocardial glucose uptake and decreased cardiac tissue ATP content after IRI, particularly in HFD. In conclusion, cardiac SGLT1 but not SGLT2 plays a compensatory protective role and contributes to cardiac energy metabolism during acute phase of IRI via enhanced glucose utilization, particularly under insulin-resistant conditions, in which IRI-induced GLUT4 upregulation is compromised.

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Inhibition of Resistin Protects against Heart Failure Injury by Targeting Gadd45 α

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BACKGROUND: Resistin is an adipocyte-secreted hormone, increased Resistin levels are associated with the development of insulin resistance, diabetes, and cardiovascular disease. However, the role of Resistin in heart failure is still unclear. The aim of the present study is to investigate the molecular mechanisms underlying Resistin's action in heart failure. **METHODS:** C57BL/6 (WT) and Resistin knockout (RKO) mice were used. A mice transverse aortic constriction (TAC) induced heart failure model was established, and cardiac function and hemodynamic changes were measured by echocardiography and left ventricular pressure volume loops. A recombinant adeno-associated virus 9 (AAV9) vector was used to deliver resistin gene into the myocardium. RNA sequence was used to determine differentially expressed mRNA in heart from RKO and WT mice subjected to TAC or sham surgeries. Quantitative real-time PCR (qRT-PCR) and Western blot analyses were used to verify the expression levels of various fibrosis-related factors (collagen I, Fibronectin, and CTGF) and hypertrophy-related markers (ANF, β -MHC and Acta1). **RESULTS:** Resistin inhibition attenuated while resistin overexpression (via AAV9-Resistin) exacerbated TAC-induced hypertrophy and cardiac dysfunction in WT and Resistin KO mice. Improvement in cardiac function was associated with decreased myocardial fibrosis as evidenced by histology and decreased mRNA levels of collagen I, fibronectin and CTGF in RKO-TAC hearts compared to WT or RKO-AAV9. In addition, myocardial tissue expression levels of ANF, β -MHC and Acta1 were also reduced in the RKO-TAC hearts. RNA sequencing analysis reveals that Gadd45a was significantly increased in RKO-TAC mice. The expression level of Gadd45a was verified by qRT-PCR and Western blot. Gadd45a expression was significantly downregulated in the RKO-TAC hearts following AAV9-Resistin delivery, suggesting that Resistin may control the regulation of Gadd45a. **CONCLUSIONS:** Resistin gene knockout increased Gadd45a expression in mouse model of heart failure, significantly reduced myocardial fibrosis and

hypertrophy and improved heart function. The results of this study indicate that inhibition of resistin is a potential therapeutic method for treating heart failure.

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MicroRNA-133a Attenuates the Development of Thoracic Aortic Aneurysm

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Rationale: Thoracic aortic aneurysms (TAA) result from dysregulated remodeling of the vascular extracellular matrix, and may occur as result of altered resident cellular phenotype. MicroRNA-133a is reduced in clinical TAA specimens and plays an inhibitory role in regulation of pathological phenotypic switch of vascular cells. Accordingly, this study tested the hypothesis that miR-133a replacement attenuates the development of TAA. **Methods and Results:** TAA was induced in wild type mice (0.5M CaCl₂ application, 15 minutes). Following 4 weeks, aortic diameter was increased compared to sham operated controls (1094 ± 38 vs 654 ± 8 μm; p<0.05, n=7). Copy number of miR-133a, quantitated by ddPCR, was reduced in TAA tissue (0.28 ± 0.04 vs 0.68 ± 0.11 copy per U6; p<0.05 vs control, n=7). While no change in smooth muscle cell specific Desmin nor α-smooth muscle actin were detected, an increase in copy number of the fibroblast specific discoidin domain receptor 2 (DDR2) and smooth muscle cell myosin heavy chain 11 (MYH11) were observed in TAA tissue. Combined, these results are consistent with the emergence of a population of myofibroblasts with TAA development. Accordingly, fibroblasts were isolated from TAA and sham operated control tissues (n=10). Copy number of miR-133a was reduced in TAA fibroblasts (0.41 ± 0.14 vs 1.60 ± 0.43 copy per U6; p < 0.05 vs control). TAA fibroblast phenotype was compared to controls; adhesion was reduced (-0.85±0.02 vs -0.77±0.02), migration was increased (63±4% vs 47±4%), and rate of collagen disk contraction over 7 hours was increased (-12.2±1.6 vs -8.5±1.0) (all values, p<0.05 vs control). Finally, after TAA induction in mice, a single tail vein injection of either a miR-133a overexpression or scrambled sequence (control) lentivirus was performed. Following 4 weeks, miR-133a replacement attenuated TAA development (800 ± 18 μm vs 1016 ± 16 μm; p < 0.05 vs control; n=8). **Conclusion:** Aortic fibroblast phenotype is altered during TAA progression, and miR-133a replacement attenuates the development of TAA in a murine model. These unique findings suggest stable alterations in aortic fibroblasts may be associated with pathological extracellular matrix remodeling, and regulation by miR-133a may lead to a novel therapeutic strategy.

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Nanopore and Poly(a)-sequencing Reveals a New Role for Rbfox2 in Alternative Polyadenylation and Poly(a)-sequencing Reveals a New Role for Rbfox2 in Alternative Polyadenylation Regulation

Jun Cao, Univ of Texas Medical Branch, Galveston, TX

Background: The RNA binding protein RBFOX2 is implicated in human heart diseases. However, RBFOX2-regulated RNA networks are not well defined. RBFOX2 has a well-characterized role in alternative splicing (AS) while accumulating evidence suggests that RBFOX2 may also have a role in alternative polyadenylation (APA). Recent studies showed that RBFOX2 binds to regions close to poly(A) sites in the 3'UTR of pre-mRNAs. In addition, RBFOX2 binds to the cleavage and polyadenylation specificity factors. Therefore, we aimed to determine whether RBFOX2 has a role in regulating APA.

Method: We employed poly(A)click sequencing (PAC-seq) and DPAC (Differential Poly(A) Cluster analysis) computational pipeline to identify differential poly(A) usage and mRNA abundance. We also used nanopore sequencing to identify different spliced variants and the coordinated AS and APA events.

Results:

We report that knockdown of RBFOX2 in embryonic rat heart derived cells leads to altered alternative polyadenylation (APA) of hundreds of genes. RBFOX2-mediated APA changes impacted both mRNA levels and generation of different gene isoforms. Nanopore sequencing identified full-length transcripts regulated by RBFOX2 and revealed RBFOX2-mediated isoform switches via both APA and AS in cardiac cells. Notably, RBFOX2-regulated APA networks affect genes such as *Tpm1* and *Tnnt1* involved in cardiac contractility. Identification of RBFOX2-regulated RNA networks provides novel insights into the pathogenesis of heart diseases in which RBFOX2 is involved and pave the way for designing therapeutics.

Conclusions: RBFOX2 regulates alternative polyadenylation via splicing dependent and independent mechanisms. RBFOX2-mediated APA affects mRNA levels of contractile genes.

J. Cao: None.

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Identification of Novel Long Non Coding RNAs Involved in Pitx2>Wnt>microRNA Signaling Pathways Leading to Atrial Fibrillation

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Atrial fibrillation is the most prevalent cardiac arrhythmia in humans. Genetic and genomic analyses have recently demonstrated that the homeobox transcription factor Pitx2 plays a fundamental role regulating expression of distinct growth factors, microRNAs and ion channels leading to morphological and molecular alterations that promote the onset of atrial fibrillation. We now address the plausible contribution of a novel class of non-coding RNA in this Pitx2>Wnt>miRNA signaling pathway, i.e. long non coding RNAs (lncRNAs). *In silico* analyses of annotated lncRNAs in the vicinity of Pitx2, Wnt8 and Wnt11 chromosomal loci identified five novel lncRNAs with differential expression during cardiac development. Among them, Wnt11_45188, Wnt11_44934 and Wnt8_2010Rik displayed preferential atrial-specific expression during embryogenesis. In addition, Wnt11_44653 displayed moderate expression during embryogenesis but peaked preferentially in the right atrium vs left atrium and ventricle in adulthood. Wnt11_45188, Wnt11_44934 and Wnt8_2010Rik were distinctly regulated by Pitx2, Wnt8 and Wnt11 and Wnt8_2010Rik is severely up-regulated in conditional atrial-specific Pitx2 deficient mice. We also demonstrate that arrhythmogenic microRNAs such as miR-1, miR-133 and miR-29 distinctly regulate these lncRNAs. Furthermore, angiotensin II administration to atrial cardiomyocyte cell cultures leads to significant up-regulation of these three newly identified lncRNAs, supporting a link to AF cardiovascular risk factor, i.e. hypertension. Overall, we have identified three novel lncRNAs that are distinctly regulated in the Pitx2>Wnt>miRNA signaling pathways which might be therefore implicated in the gene regulatory network leading to atrial arrhythmogenesis.

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A Novel Human Specific Long Noncoding RNA PG1 Controls Human Cardiogenesis via Regulating Beta-catenin Activity

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Long noncoding RNAs (lncRNAs) can regulate gene expression, signaling pathway, cellular and tissue functions at various stages. Recently, accumulating evidences suggest that lncRNAs play important roles in cell fate specification and organ development. We found hundreds of lncRNAs, which show lineage-specific expressions in human embryonic stem (ES) cell-derived multipotential cardiovascular progenitor cells (MCPs), cardiomyocytes (CMs), smooth muscle cells (SMs) or endothelial cells (ECs), by using whole transcriptome sequencing. Among MCP-specific lncRNAs, we identified a novel human lncRNA, PG1, which controls cardiovascular differentiation from human pluripotent stem cells. We found PG1 could regulate the early segregation of nascent mesoderm cells towards cardiac verse hematopoietic fate. Knockout of PG1 in human induced pluripotent stem (iPS) cells and H1 ES cells by CRISPR/Cas9 significantly increased hematopoietic differentiation from pluripotent stem cells when compared with wild type controls. Furthermore, our data suggest PG1 could bind beta-catenin protein to modulate Wnt signaling activity to regulate nascent mesoderm specification. In summary, we identified a novel human specific lncRNA, PG1, which plays an essential role in early stage human heart development.

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Translation Determines the Acute Cardiac Response to Ischemia/Reperfusion

Christoph Hofmann, Fereshteh S Younesi, Ole M Schwerdt, Etienne Bioleau, Hugo A Katus, Christoph Dieterich, Mirko Völkers, Univsklinikum Heidelberg, Heidelberg, Germany

Introduction: Since transcription is a relatively slow process, translation of preexisting mRNA networks might affect cardiac gene expression thereby rapidly adapting the myocardium to stress before changes in transcription take place. The mTOR kinase links nutrient and energy levels to protein synthesis by regulation of mRNA translation. Inhibition of mTOR complex 1, the responsible pathway for translational regulation, protects against reperfusion injury, however the underlying mechanisms are unknown. Through rapid restoration of myocardial energy levels upon reperfusion an mTOR-dependent translationalome could adapt early changes in gene expression inducing a maladaptive gene network, independent from transcription, which mediates reperfusion injury.

Hypothesis: Reperfusion induces an mTOR-mediated overshooting protein synthesis response that contributes to reperfusion injury by rapidly and selectively translating preexisting mRNAs.

Methods and Results: To induce reperfusion injury mice were subjected to 60 minutes of LAD ligation and increasing times of reperfusion. Puromycin incorporation showed an overshooting protein synthesis response of cardiac myocytes upon reperfusion that was especially prevalent in the peri-infarct region. Protein synthesis was induced by increased formation of the eIF4F translation initiation complex through the mTORC1-4EBP1 signaling axis. Cardiac myocyte specific ribosome profiling combined with RNA-seq of hearts 2 days after reperfusion showed that the acute cardiac response to reperfusion was mediated by translation of specific mRNAs encoding proteins which determine early events of reperfusion such as leukocyte infiltration and inflammation. Inhibition of the mTORC1-4EBP1-eIF4F axis attenuated this specific protein synthesis response. Importantly, 4EGI-1, a specific inhibitor of eIF4F complex formation, bypassed off-target effects of previously used mTOR inhibitors and protected cardiac myocytes from cell death upon reperfusion.

Conclusions: During reperfusion protein synthesis is strongly induced in the peri-infarct region by mTORC1 and contributes to reperfusion injury by rapidly translating specific mRNAs that mediate the early gene expression response to reperfusion.

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Characterization of Circular RNAs in Vascular Smooth Muscle Cells With Vascular Calcification

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Circular RNAs (circRNAs) are generally formed by back splicing, and are expressed in various cells. Vascular calcification, a common complication of chronic kidney disease, is often associated with cardiovascular disease. The relationship between circRNAs and vascular calcification has not yet been studied. Inorganic phosphate was used to treat rat vascular smooth muscle cells to induce vascular calcification. CircRNAs were identified by analyzing RNA sequencing data, and their expression change during vascular calcification was validated. The selected circRNAs, including circSamd4a, circSmoc1-1, circMettl9, and circUxs1 were resistant to RNase R digestion, and mostly localized in the cytoplasm. Silencing circSamd4a promoted vascular calcification, while overexpressing it reduced vascular calcification. In addition, microRNA (miRNA) microarray and luciferase reporter assay suggested that circSamd4a could act as miRNA suppressor. Our data shows that circSamd4a have anti-calcification roles by functioning as miRNA sponges. Since circSamd4a is conserved in humans, they can serve as novel therapeutic targets in resolving vascular calcification.

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FTO-mediated mRNA Demethylation Regulates Cardiac Contractile Protein Expression and Function

Prabhu Mathiyalagan, Marta Adamiak, Joshua Mayourian, Yassine Sassi, Yaxuan Liang, Neha Agarwal, Divya Jha, Shihong Zhang, Erik Kohlbrenner, Icahn Sch of Med at Mount Sinai, New York, NY; Xiaoke Yin, Kongs Coll London, London, United Kingdom; Jiqu Chen, Maria G Trivieri, Rihab Bouchareb, Rajvir Singh, Kenneth Fish, Kiyotake Ishikawa, Icahn Sch of Med at Mount Sinai, New York, NY; Manuel Mayr, King's Coll London, New York, United Kingdom; Djamel Lebeche, Roger J Hajjar, Susmita Sahoo, Icahn Sch of Med at Mount Sinai, New York, NY

Background: Exciting new discoveries in RNA biology underscore the importance of post-transcriptional chemical modifications to mRNAs (epitranscriptome) in regulating RNA stability, nuclear export, cellular compartmentalization, splicing, translation and degradation. The most abundant and functionally relevant modification in RNA, N⁶-methyladenosine (m⁶A) is reversibly demethylated by one of the m⁶A demethylases, fat mass and obesity-associated protein (FTO) whose function in the mammalian heart remains incompletely understood.

Materials and Methods: We used clinical human samples, preclinical pig and mouse models and primary cardiomyocytes to study m⁶A and FTO in the heart and in cardiomyocytes. We modulated FTO expression using AAV9 (in vivo), adenovirus (in vivo and in vitro) and siRNAs (in vitro). We investigated m⁶A-induced changes to contractile protein expression using m⁶A RNA immunoprecipitation sequencing (MeRIP-seq) and stable isotope labeling of amino acids in cell culture (SILAC).

Results: We discovered in human heart failure that reduced FTO expression is associated with aberrant increase in m⁶A mRNA methylation, which is conserved in swine and mouse models of myocardial ischemia (MI). AAV9-mediated FTO gene delivery in mouse MI attenuated m⁶A increase and improved cardiac function with enhanced contractility, angiogenesis and reduced fibrosis. At the molecular level, FTO-mediated mRNA demethylation serves to increase contractile protein expression in mouse hearts as well as in isolated primary cardiomyocytes. By comparing human and mouse transcriptome-wide m⁶A maps with SILAC proteomic profiling from cardiomyocytes, we identified FTO-mediated m⁶A demethylation is transcript-specific and leads to altered protein expression of several key contractile, angiogenic and regenerative proteins. **Conclusion:** Using new RNA-based investigations, we uncovered a novel regulatory layer beyond the genome working at the level of epitranscriptome governing cardiac function. Our findings on the dynamic nature of the cardiac m⁶A-epitranscriptome will lead to deeper understanding of the mechanism of cardiac remodeling on one hand and innovative therapeutic interventions on the other.

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MicroRNA-574-FAM210A Axis Maintains Mitochondrial Translational Homeostasis and Influences Pathological Cardiac Remodeling

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Rationale: Translational control is a critical regulatory step in altering protein synthesis under disease conditions. Aberrant synthesis of mitochondrial proteins impairs cardiac function and causes heart disease. However, the mechanism of translational control in mitochondria and during cardiac disease remains underexplored.

Objective: We have found that multiple pathogenic cardiac stressors induce the expression of miR-574 guide and passenger strands (miR-574-5p/3p) in humans and mice. Here, we aim to define a new miR-574-FAM210A axis that regulates cardiac mitochondrial translational homeostasis and prevents adverse cardiac remodeling.

Methods and Results: Echocardiography, histology and biochemical analyses were used to evaluate the cardiac function of miR-574 knockout mice, which exhibit severe cardiac hypertrophy, fibrosis, and cardiac dysfunction under heart failure-triggering stresses. miR-574-5p/3p mimics that were delivered systematically using nanoparticles reduced cardiac pathogenesis with disease insults. Transcriptome analysis of miR-574-null hearts has uncovered *FAM210A* as a common target mRNA for miR-574-5p/3p. Interactome and translational state analyses identify a FAM210A-bearing trimeric complex that promotes the translation of mitochondrial encoded electron transport chain genes and modulates mitochondrial activities. Moreover, the phenotypic characterization of tamoxifen-inducible CM-specific Fam210a knockout mice suggests that FAM210A plays a critical role in maintaining normal cardiac mitochondrial morphology and function as well as cardiac health and organismal viability.

Conclusions: miR-574-5p and miR-574-3p protect against pathological cardiac remodeling through regulation of FAM210A, a key translational control factor of mitochondrial encoded genes. Thus, we discovered a novel miR-574-FAM210A pathway that modulates cardiac mitochondrial translational homeostasis and influences cardiac remodeling in heart failure.

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Poster Session 1 and Reception

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A HaloTag-TEV Genetic Cassette for Mechanically Probing Native Titin

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Single-molecule methods using recombinant proteins have generated transformative hypotheses on how mechanical forces interact with titin in the sarcomere, enabling muscle contraction. However, testing these mechanical hypotheses on native titin in its natural environment has remained inaccessible to conventional genetics, biophysics and molecular biology tools. To overcome these limitations, here we demonstrate a genetically engineered knock-in mouse model carrying a HaloTag-TEV insertion in titin. Using our system, we have specifically severed the titin filament by digestion with TEV protease, and found that the response of muscle fibers to length changes requires mechanical transduction through titin's intact polypeptide chain. HaloTag-based covalent tethering has enabled directed examination of the dynamics of native titin under physiological forces using recently developed magnetic tweezers. At physiological pulling forces lower than 10 pN, titin domains are readily recruited to the unfolded state, and produce 41.5 zJ mechanical work during refolding. Our results support an active role of titin in muscle contraction in coordination with actomyosin motors.

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Loss of Myosin Binding Protein H-Like Causes Cardiac Conduction Abnormalities

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A premature truncation (R255X) in *MYBPHL* associates with human dilated cardiomyopathy (DCM) and arrhythmias. Loss of *Mybphl* in mice causes DCM and arrhythmia. *MYBPHL* encodes myosin binding protein H-like (MyBP-HL) and is expressed highly in the atria. We hypothesize that MyBP-HL is required for proper conduction system function. Immunofluorescence microscopy on normal human atria showed MyBP-HL staining in all atrial cardiomyocytes with a sarcomere A-band pattern. Atria from the heterozygous (het) *MYBPHL* R255X mutant carrier lacked MyBP-HL staining. Human induced pluripotent stem cell-derived cardiomyocytes from the het *MYBPHL* R255X carrier and control cell lines were also examined. MyBP-HL was found in a subset of control cardiomyocytes, whereas R255X cells showed no MyBP-HL, suggesting that the R255X allele exerts a dominant-negative effect on the normal *MYBPHL* allele. Immunofluorescence microscopy in wild-type (WT) mouse ventricles identified MyBP-HL-positive ventricular cardiomyocytes that co-localized with the ventricular conduction system marker contactin-2 near the atrioventricular node and in a subset of Purkinje fibers. *Mybphl* het ventricles have 10% as many MyBP-HL-positive cells compared to WT. Surface telemetry revealed atrioventricular block and atrial bigeminy and intracardiac pacing revealed a shorter atrial relative refractory period and inducible atrial tachycardia in *Mybphl*-null mice. Ca^{2+} transients measured with confocal microscopy revealed that isolated *Mybphl*-null atrial cardiomyocytes had an increased occurrence of triggered Ca^{2+} waves and more heterogeneous Ca^{2+} release than WT controls. Super-resolution microscopy revealed ryanodine receptor disorganization in *Mybphl* het and

null atrial cardiomyocytes compared to WT controls. Abnormal Ca²⁺ release, shorter atrial refractory period, and dilated atria could account for the observed atrial arrhythmias, bigeminy, and atrial tachycardia.

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Pharmacologic Characterization of the Cardiac Myosin Inhibitor, CK-3773274: A Potential Therapeutic Approach for Hypertrophic Cardiomyopathy

Darren T Hwee, James J. Hartman, Jingying Wang, Yangsong Wu, Julia Schaletzky, Preeti Paliwal, Ken Lee, Khanha D Taheri, Eddie Wehri, Chihyuan Chuang, Bradley P Morgan, Fady I Malik, Eva R. Chin, Cytokinetics, Inc., South San Francisco, CA

Hypercontractility of the cardiac sarcomere appears to underlie pathological hypertrophy and fibrosis in select genetic hypertrophic cardiomyopathies. Here, we characterize the small molecule, CK-3773274, as a novel cardiac myosin inhibitor that decreases contractility *in vitro* and *in vivo*. In bovine cardiac myofibrils, CK-3773274 decreased myosin ATPase activity in a concentration-dependent fashion (IC₅₀: 1.26 μM). CK-3773274 specifically inhibited myosin activity, as it reduced myosin ATPase activity in a concentration-dependent manner in the absence of other sarcomere proteins, including actin, troponin, and tropomyosin. CK-3773274 (10 μM) reduced fractional shortening by 84% in electrically paced, isolated adult rat cardiomyocytes relative to control without any effect on the calcium transient. The effect of CK-3773274 on cardiac contractility *in vivo* was assessed in healthy male Sprague Dawley (SD) rats using single oral doses ranging from 0.5 to 4 mg/kg. Fractional shortening (FS) and left ventricular dimensions were determined by echocardiography at select time points over a 24-hour period. One hour after dose administration, CK-3773274 significantly reduced fractional shortening in a dose-related fashion by 20-70% relative to vehicle treatment (FS %: vehicle: 47.9± 1%; 0.5 mg/kg: 39 ± 2%; 4 mg/kg: 15 ± 4%; mean ±SEM, p<0.05 vehicle vs. all doses) without any changes to heart rate. Lastly, the effect of CK-3773274 was evaluated by echocardiography in healthy beagle dogs. Left ventricular ejection fraction (LVEF) was evaluated following single oral doses ranging from 0.75-3 mg/kg over a 48 hour period. 2 hours after dosing, CK-3773274 decreased LVEF in a dose-related fashion by approximately 15-50% relative to vehicle treatment (LVEF vehicle: 74.6 ± 3 %; 0.75 mg/kg: 62.5 ± 3%; 2 mg/kg: 44.9± 3%; 3 mg/kg: 36.8 ± 2%; mean ±SEM, p<0.05 vehicle vs. all doses). In conclusion, CK-3773274 is a novel, small molecule, cardiac myosin inhibitor that reduces cardiac contractility *in vitro* and *in vivo*. Cardiac myosin inhibition may be a viable approach to treat the underlying hypercontractility of the cardiac sarcomere in hypertrophic cardiomyopathies.

D.T. Hwee: 1. Employment; Significant; \$10,000 or more during last 12 months.. 7. Ownership Interest; Modest; Equity position. **J.J. Hartman:** 1. Employment; Significant; \$10,000 or more during last 12 months.. 7. Ownership Interest; Modest; Equity position. **J. Wang:** 1. Employment; Significant; \$10,000 or more during last 12 months. **Y. Wu:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **J. Schaletzky:** None. **P. Paliwal:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **K. Lee:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **K.D. Taheri:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **E. Wehri:** 1. Employment; Significant; \$10,000 or more during last 12 months. **C. Chuang:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **B.P. Morgan:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **F.I. Malik:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **E.R. Chin:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position.

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Cardiomyocyte Hypercontractility is an Adaptational Response to Stiffness in High-Fat Diet Mice

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Diabetic heart disease typically features a restrictive/ hypertrophic cardiac phenotype characterised by diastolic dysfunction with preserved systolic performance. Little is known about the early adaptational mechanisms that underpin maintenance of systolic performance in diabetic diastolic dysfunction and how this may contribute to disease progression. This study aimed to evaluate cardiomyocyte contractility in a high-fat diet (HFD) mouse model of diastolic dysfunction. Echocardiography (GE Vivid 9) was performed in 33 wk old male C57Bl/6 mice fed a high-fat diet (HFD, 43% kcal fat, 24 wks duration). Isolated cardiomyocytes (paced 2Hz, 2.0mM Ca²⁺, 37C) were subjected to progressive axial stretch. Sarcomere length/ shortening, tension and intracellular calcium transients (Fura-2AM, 5µM) were simultaneously measured (Myostretcher, Ionoptix). HFD hearts displayed *in vivo* (22.2±1.4 vs 16.7±1.0 E/E'; p<0.05) and *in vitro* diastolic dysfunction (0.244±0.039 vs 0.075±0.017 nN/pl/ %cell stretch; p<0.05). HFD cardiomyocytes exhibited length-dependent hypercontractility with augmented developed tension amplitude at maximal stretch (4.891±0.942 vs 2.484±0.677 nN/pl; p<0.05), but not basal stretch. Hypercontractility could not be attributed to a difference in Ca²⁺ transient amplitude but was correlated with length dependence of Ca²⁺ sensitivity (n=17 cells, R²=0.4039 p<0.05). During progressive cardiomyocyte stretch, systolic tension slopes were strongly correlated with diastolic tension slopes (cardiomyocyte stiffness) (n=30 cells; R²=0.8442, p<0.05) while there was no difference in the ratio of systolic/diastolic tension slopes indicating maintained length-dependent activation. These findings provide the first evidence that cardiomyocyte hypercontractility and stiffness are strongly linked. High-fat diet cardiomyocytes may operate near maximal contractile capacity to maintain basal cardiac output, potentially limiting capacity for cardiac output increase in response to physiological stressors. An aberrant myofilament post-translational modification profile represents a potential mechanism through which cardiac dysfunction arises during the development of diabetes.

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Poster Session 1 and Reception

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Cardiac Myosin Binding Protein C Phosphorylation Regulates Calcium Homeostasis

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Rationale: Cardiac myosin binding protein-C (cMyBP-C) is heavily phosphorylated to regulate normal cardiac function under basal conditions. However, its phosphorylation level is significantly decreased in patients with heart failure and atrial fibrillation. Furthermore, decreased cMyBP-C phosphorylation causes reduced myofilament contractility and decreased calcium sensitivity. The impact of such decreases in cMyBP-C phosphorylation and myofilament calcium sensitivity on the overall calcium handling and potential induction of arrhythmogenesis is not known.

Objective: To determine the necessity and sufficiency of cMyBP-C phosphorylation to regulate calcium cycling and contractility at the isolated cardiomyocyte level. **Methods and Results:** Contractile properties and calcium kinetics were measured in intact cardiomyocytes isolated from 3-month-old, mixed sex, mice expressing phospho-ablated (S273A/S282A/S302A) cMyBP-C (AAA) or phospho-mimetic (S273D/S282D/S302D) cMyBP-C (DDD) and nontransgenic (NTG) control mice. AAA cells displayed a significant decrease in fractional shortening compared to NTG and DDD cells (9.4% vs 12.8% in NTG, and 12.3% in DDD, p<0.0001). Similarly, AAA myocytes demonstrated abnormal calcium kinetics with increased diastolic calcium levels (20%, p<0.001 vs. NTG and DDD) and prolonged decay time of the calcium transient (26%, p<0.01 vs NTG and DDD) when compared with NTG and DDD myocytes. Caffeine-induced calcium release in AAA myocytes indicated no change in SR calcium content, while sodium-calcium exchanger function, assessed as the time constant (τ) of calcium decline, was increased (89%, p<0.01 vs NTG). However, these depressive effects in AAA myocytes were relieved by isoproterenol (100 nmol/L) stimulation. Furthermore, stress conditions (2 Hz + ISO) increased after-contractions in AAA cardiomyocytes (60% in AAA vs 13% in NTG, p<0.001 and 15% in DDD, p<0.001). In addition, preliminary data indicate increased susceptibility of the AAA mice to arrhythmias under stress conditions *in vivo*.

Conclusion: Dephosphorylation of cMyBP-C is sufficient to reduce sarcomere contractility and impair calcium cycling resulting in spontaneous after-contractions and arrhythmias under stress conditions.

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Titin Truncations in Human Heart Tissue

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Truncating variants in the gene for titin (TTN_{tv}s) strongly associate with dilated cardiomyopathy (DCM) and peripartum cardiomyopathy (PPCM). How TTN_{tv}s lead to cardiomyopathy is unclear. Both haploinsufficient and dominant negative mechanisms have been proposed. To begin to address this question, we have obtained left ventricular myocardium from explanted hearts from patients with DCM or PPCM undergoing cardiac transplantation, as well as myocardium from non-failing donor hearts. Targeted exome sequencing identified 8 hearts bearing TTN_{tv}s in isoforms expressed in adult heart. Modified gel electrophoresis of extracts from these samples revealed no apparent band at the expected (truncated) molecular weight predicted for each TTN_{tv}, indicating that truncated titin protein is likely absent. In addition, the total amount of titin protein appeared preserved in TTN_{tv} extracts, arguing against haploinsufficiency in these hearts. RNAseq of TTN_{tv}-positive hearts revealed balanced representation of all identified heterozygous variants, i.e. preserved allelic balance, indicating that the alleles encoding TTN_{tv}s are appropriately transcribed, and do not undergo nonsense mediated decay. Together these data support neither haploinsufficiency nor dominant negative mechanisms, and leave open the question of how TTN_{tv}s contribute to the development of DCM and PPCM.

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The Effect Of Phosphodiesterase 9a Inhibition In 2 Mouse Models Of Diastolic Dysfunction

Mei Methawasin 85724, Josh Strom, Zaynab Hourani, John E. Smith III, Henk L. Granzier, Univ of Arizona, Tucson, AZ

Low myocardial cGMP-PKG activity is associated with increased cardiomyocyte stiffness in HFpEF. cGMP is mainly hydrolyzed by PDE (phosphodiesterases)5a and PDE9a. Importantly, PDE9a protein expression was reported to be upregulated in human HFpEF myocardium and chronic administration of PDE9a inhibitor reversed pre-established cardiac hypertrophy and systolic dysfunction in mice subjected to TAC (Transverse Aortic Constriction).

We evaluated whether PDE9a inhibition had a beneficial effect on diastolic dysfunction (DD). We investigated two mouse models of DD: 1) TAC-DOCA (TAC with deoxycorticosterone acetate (DOCA) supplementation) and 2) LepR^{db/db}, a metabolically induced DD models. Mice were subjected to subcutaneous implantation with osmotic minipumps containing either PDE9a inhibitor (PF-4449613) or vehicle for 4 wks. PDE9a transcript was not upregulated in myocardium of either TAC-DOCA or LepR^{db/db} mice. PV analysis revealed that in TAC-DOCA mice, inhibition of PDE9a resulted in reduced LV diastolic stiffness, but impairment of systolic function observed as ventricular-vascular decoupling. In LepR^{db/db} mice, cardiomyocyte stiffness was slightly but significantly reduced in PDE9a inhibitor treated animals. No differences in myocardial fibrosis or cardiac morphometry were observed.

The effect of acute PDE9a inhibition was investigated in intact cardiomyocytes isolated from LVs of TAC-DOCA mice. Cellular work loop force measurement was conducted before and after administration of PDE9a inhibitor combined with ANP (Atrial Natriuretic Peptide) for 15 mins. Cardiomyocytes from TAC-DOCA had high diastolic stiffness compared to sham hearts, however, no stiffness reduction was observed after acute inhibitor treatment.

Conclusion. Chronic inhibition of PDE9a ameliorates LV diastolic stiffness, but suppresses systolic function in TAC-DOCA induced DD mice. The LV stiffness reduction is in part due to cardiomyocyte stiffness reduction. The advantage of PDE9a inhibition on diastolic function might be more evident, with less systolic suppression, in hearts with PDE9a upregulation.

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Cell-Substrate Adhesion Regulates Early Steps of Myofibrillogenesis in Human Cardiomyocytes

Abigail C Neininger, Nilay Taneja, Dylan T Burnette, Vanderbilt Univ, Nashville, TN

Forces generated by myofibrils within cardiomyocytes must be balanced by adhesion to the extracellular matrix proteins and to other cardiomyocytes for proper heart function. Loss of this force balance results in cardiomyopathies and heart failure. Using a sarcomere assembly assay we previously developed utilizing human induced pluripotent stem cell-derived

cardiomyocytes, we show coupling of focal adhesions to myofibrils during early steps of *de novo* myofibrillogenesis is essential for myofibril maturation. Increasing the extent of adhesion by inhibition of Focal adhesion kinase (FAK), a known regulator of adhesion dynamics, or by increasing the concentration of fibronectin on which the cardiomyocytes are cultured, led to precocious myofibril formation. Decreasing the extent of adhesion by siRNA-mediated FAK knockdown or by decreasing the concentration of fibronectin attenuated myofibrillogenesis. In each case, increased or decreased adhesion extent inversely correlated with rate of retrograde flow of myofibril precursors known as muscle stress fibers (AKA, non-muscle stress fiber-like structures or pre-myofibrils). This suggests a relationship between cell-substrate adhesion and substrate coupling to muscle stress fibers, facilitating their maturation into myofibrils. Taken together, our findings implicate a role for classical mechano-transduction in the assembly of sarcomere containing myofibrils.

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Motivating Myosin: 2 deoxy-ATP Induced Structural Alterations That Increase Myosin Activity

Michael Regnier, Univ of Washington, Seattle, WA

When 2-deoxy-ATP (dATP) replaces ATP as the contractile substrate, cardiac myosin binding to actin and the rate of cross-bridge cycling is increased. To understand the structural basis of this we employed low-angle x-ray diffraction and computational modeling. Molecular Dynamics (MD) simulations of pre-powerstroke myosin suggest dADP.Pi (vs. ADP.Pi) induces changes in contact pairs for nucleotide binding that translate to structural changes on the actin binding surface, exposing positive charge in regions that make electrostatic interactions with actin. Brownian Dynamics simulations suggest M.dADP.Pi binds more rapidly to actin at distances (angstrom) associated with weak binding. X-ray diffraction analysis of resting demembrated cardiac muscle indicated a large increase in the $I_{1,1}/I_{1,0}$ intensity ratio for M.dADP.Pi, suggesting myosin moves towards thin filaments. This difference was eliminated at low ionic strength (100 vs. 170mM), where more protein surface charge is exposed, thus decreasing the electrostatic interaction advantage of M.dADP.Pi. The S_{M3} meridional reflection indicated spacing between myosin crowns was increased for M.dADP.Pi at rest, making it similar was to the activated position of M.ADP.Pi during contraction, with no change in axial ordering (I_{M3}). In intact soleus muscle of transgenic mice with 1% dATP (99% ATP), time-resolved x-ray structure indicates myosin dissociation from actin following tetanic contraction is slightly slower (vs WT) but the first-order myosin layer line (*MLL 1*) intensity recovers faster, suggesting dATP increases the rate myosin heads return to an ordered resting state. At rest the radii to the center of mass of myosin heads (R_m) and $I_{1,1}/I_{1,0}$ were larger, indicating myosin heads were closer to actin. MD simulations of post-powerstroke myosin showed greater dADP mobility in the binding pocket via altered interactions with key amino acids, resulting in altered conformation of residues on the actin binding surface in regions interacting with actin. Combined our results suggest that with dATP myosin moves closer to actin and S1 heads are more activated in resting muscle, staying primed for reactivation following relaxation, all via increased myosin-actin electrostatic interactions.

M. Regnier: None.

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Biomechanics and Calcium Handling of Thin Filament Hypertrophic Cardiomyopathy Variants

Giuliana G Repetti, Christopher N Toepfer, Amanda C Garfinkel, Harvard Medical Sch, Boston, MA; Gabriela Venturini, Univ of São Paulo, São Paulo, Brazil; Jonathan G Seidman, Christine E Seidman, Harvard Medical Sch, Boston, MA

The mechanism by which genetic variants of the thin filament cause hypertrophic cardiomyopathy (HCM) has not been fully elucidated. Induced pluripotent stem cell (iPSC)-derived cardiomyocytes can provide a rapidly generatable disease modeling tool to study HCM in these variants. While variants in the thick and thin filament both cause HCM, cardiomyocytes that harbor pathogenic variants in the thin filament genes troponin I (*TNNI3*) and troponin T (*TNNI2*) display a distinct molecular phenotype, which we hypothesize differs from the thick filament HCM phenotype. Both thin and thick filament mutant cardiomyocytes display increased measured oxygen consumption rate assessed by metabolic assays, however we hypothesize that the mechanism that drives metabolic change is not common between thick and thin filament variants. Mutations in the thick filament typically shift myosin conformations during relaxation towards a higher energy utilizing a

disordered relaxed state (DRX) that enables ATP hydrolysis with more free myosin heads, and this likely accounts for their increased oxygen consumption. However thin filament mutations cause myosin heads to preferentially adopt a conformation in which the myosin heads are sequestered and unable to bind actin. This super relaxed state (SRX) is associated with energy conservation, which would predict reduced contractility. Yet contractility data from thin filament mutants replicate the classic HCM phenotype of hypercontractility and disturbed sarcomere relaxation. To further probe the mechanism by which thin filament variants drive HCM pathophysiology we have employed methodologies to assess calcium transients in iPSC-derived cardiomyocytes harboring thin filament variants. *TNNT2* variants recapitulate this disrupted calcium handling.

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Myosin Regulatory Light Chain: A Major Player in Defining the 'OFF' State of Cardiac Myosin

Na Sa, Ivan Tomasic, Sampath Gollapudi, Suman Nag, MyoKardia, S San Fran, CA

Myosin regulatory light chain (RLC) is a regulatory subunit of the myosin molecule, which plays a major role in stabilizing the Interacting Head Motif (IHM) of myosin in non-striated muscle. However, in striated muscle, its purpose is less well defined, and it is only in the past decade that researchers have started unraveling the function of RLC and its phosphorylation in muscle contraction. In this study, we report the role of human cardiac RLC in forming the Super-Relaxed State (SRX) of myosin in reconstituted full-length cardiac myosin thick filaments and show that removal of RLC destabilizes the ability of myosin to form the SRX state. Phosphorylation of the RLC with MLCK has the same effect, which renders the myosin to the 'OFF' state as confirmed by its ATPase activity. Interestingly we found that Ca^{2+} and not Mg^{2+} binding to synthetic myosin filaments had differential effects on the population of the myosin SRX state. At systolic Ca^{2+} myosin exhibits lower SRX state than at diastolic Ca^{2+} . This effect is obliterated in RLC-lacking myosin constructs such as sS1, or RLC stripped myosin filaments suggesting that Ca^{2+} binding to RLC can activate the myosin from the 'OFF' state to a more active state. Naturally occurring cardiomyopathy-causing RLC mutants R58Q, K104E and D94A did not alter this property, but two of them, K104E and D94A abolished the phosphorylation regulation of the SRX state of myosin. Altogether, these observations demonstrate that either RLC phosphorylation or binding of systolic Ca^{2+} can reduce the number of accessible myosin heads for contraction and could form the basis of the Ca^{2+} -mediated activation of the sarcomeric thick filament and mutations in RLC can alter these abilities differentially.

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Poster Session 1 and Reception

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Efficient Large-scale Sarcomere Tracking (sarctrack) to Assess HCM Variants in iPSC-CMs

Christopher Toepfer, HMS and Oxford, Boston, MA; Arun Sharma, Amanda Garfinkel, Marcelo Cicconet, Radhika Agarwal, HMS, Boston, MA; Anant Chopra, Christopher Chen, BU, Boston, MA; Jonathan Seidman, Christine Seidman, HMS, Boston, MA

Variants that drive HCM and associated adverse patient outcomes are found in the cardiac sarcomere. These variants range from those that are known to be pathogenic to those that are likely pathogenic or even variants of unknown significance (VUS). CRISPR/Cas-9 engineering has accelerated our ability to generate variants in iPSC to probe changes in cellular function and assess cellular pathogenicity in VUSs. However, iPSC-CMs are not as functionally mature as adult cardiomyocytes. For this reason we have developed a platform to assess contractile function directly at the level of the sarcomere. We use a custom built MatLab algorithm to assess sarcomere length, contraction time, relaxation time, and beat rate of individual sarcomeres within iPSC-CMs. Sarcomeres are visualised using reporter lines that have been engineered with an N-terminal TTN-GFP. We assess the contractile function of thick filament variants in MYH7 and MYBPC3. We show the ability to detect changes in key contractile parameters. This platform allows the screening of pharmacological compounds against these reporter lines with engineered variants.

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Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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AAV1.SERCA2a Gene Therapy as a Novel Targeting Approach Against Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is characterized by a progressive accumulation of scar tissue and a fibroproliferative process, leading to respiratory failure and ultimately to death within 3-5 years after the diagnosis. To date, despite extensive research efforts in experimental and clinical studies, IPF remains an increasing cause of morbidity and mortality. Therefore, there is a critical need to identify new therapeutic target and strategy for treating patients with IPF. Previous studies have demonstrated that pharmacological disruption of Ca²⁺ signaling improves lung function and inhibit lung fibrosis in the Bleomycin (BLM) mouse model of PF. We hypothesized that SERCA2a downregulation potentiates the development of PF and SERCA2a gene transfer inhibits PF.

Here, we found that the SERCA2a expression was decreased in lung biopsy from patients with IPF and in the BLM mouse model of PF. In vivo, our results showed that intratracheal aerosolized adeno-associated virus serotype 1 (AAV1) encoding human SERCA2a (AAV1.SERCA2a) reduces lung fibrosis and associated vascular-remodeling. SERCA2a gene therapy also decreases right ventricular pressure and RV hypertrophy in both prevention and treatment protocols, in comparison with BLM-PF mice treated with a control AAV1 carrying luciferase. In vitro, our results demonstrated that SERCA2a overexpression inhibits fibroblast proliferation, migration and fibroblast-to-myofibroblast transition induced by TGF- β 1 treatment. Thus pro-fibrosis gene expression is prevented by blocking NF- κ B/IL-6-induced STAT3 activation. This effect is signaled toward inhibitory mechanism of SMAD/TGF- β signaling through the repression of OTU domain-containing ubiquitin aldehyde-binding proteins 1 (OTUB1) and forkhead transcription factor M1 (FOXO1) expression. Collectively, this study demonstrated that SERCA2a gene therapy may be a potential therapeutic target for PF.

M. Bisserier: None.

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Tuberin S1365 Phosphorylation Regulates Mechanistic Target of Rapamycin Complex 1 (mTORC1) Pathological Signaling While Sustaining Metabolic Sensor Function

Brittany L Dunkerly-Eyring, Miguel P Vera, Christian U Oeing, Mark J Ranek, David A Kass, Johns Hopkins Univ, Baltimore, MD

Rationale: The Mechanistic Target of Rapamycin complex 1 (mTORC1) integrates signaling and sensory inputs to maintain cardiomyocyte homeostasis, and itself is negatively regulated by the signaling nexus tuberin (TSC2). We identified a novel TSC2 phosphorylation site S1365 (pS1365) targeted by protein kinase G (PKG), which suppressed hormonal growth factor (PE or ET1)-stimulated mTORC1 activity to attenuate pathological cardiomyocyte hypertrophy. This was recapitulated during growth factor stimulation with expression of a phospho-null (S1365A) or a phospho-mimetic (S1365E) TSC2 that exacerbated or blunted mTORC1 activation, respectively. The nature of TSC2 pS1365 as a potential metabolic sensor is unknown and will provide mechanistic insight into the TSC2 kinase input hierarchy that regulates the homeostatic function of mTORC1.

Objective: To determine how pS1365 affects the ability of TSC2 to integrate metabolic dependent signals to regulate mTORC1.

Methods/Results: TSC2 KO MEFs were infected with TSC2 WT or S1365A adenovirus, and then stimulated with ET1 (hormonal stress that also activates mTORC1), and 2-DG (AMPK stimulation). Both groups responded with similar decreases in mTORC1 activation regardless of pS1365. Phosphorylation of the AMPK site on TSC2 (S1387) was increased in all groups despite the presence of a phospho-null S1365. Both MEFs and neonatal rat cardiomyocytes (NRCMs) infected with TSC2 WT, S1365A, or S1365E adenovirus similarly increased mTORC1 activation with insulin (PI3K-Akt-TSC2 pathway) treatment. Serum withdrawal from NRCMs reduced mTORC1 activation in all groups regardless of whether a WT, S1365A, or S1365E TSC2 was expressed. In NRCMs subject to hypoxia (a combination of Erk, Akt, AMPK signaling), there was a similar observation with only nominal changes between WT, S1365A, and S1365E TSC2.

Conclusions: The energy and nutrient sensing role of the TSC2-mTORC1 pathway remains intact regardless of the phospho-status of TSC2 S1365. These findings provide important mechanistic insight into the function of TSC2 pS1365 as a potent suppressor of pathological mTORC1 activation while not affecting the ability of TSC2 to respond to the metabolic dependent signals – AMPK (energy), PI3K-Akt (insulin), and serum starvation.

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(Pro)renin Receptor Promotes Development of Age-related Skeletal Muscle Atrophy *via* Activation of Wnt/beta-catenin and YAP Signaling Pathways

Jin Endo, Keio Univ Sch of Med, Tokyo, Japan

Prevention and treatment of age-related skeletal muscle atrophy, also known as sarcopenia, is crucial. However, the molecular mechanisms underlying sarcopenia remain unclear. (Pro)renin receptor ((P)RR) is a multifunctional protein that regulates the tissue renin-angiotensin system and activates the Wnt/ β -catenin signaling pathway and is involved in the progression of aging and sarcopenia. We developed a gain-of-function model of age-related sarcopenia *via* transgenic expression of (P)RR. (P)RR-transgenic (Tg) mice died early and exhibited muscle atrophy with the histological features of sarcopenia. Wnt/ β -catenin signaling was activated and the regenerative capacity of muscle progenitor cells was impaired in (P)RR Tg mice. Moreover, forced expression of (P)RR in C2C12 myoblasts suppressed myotube formation *via* augmentation of the Wnt/ β -catenin signaling. Dickkopf-related protein 1 significantly attenuated sarcopenia in (P)RR Tg mice. In addition, YAP/TAZ signaling contributed to the development of (P)RR-induced sarcopenia in accordance with the activation of Wnt/ β -catenin signaling. The present study demonstrated that (P)RR Tg mouse could be a novel sarcopenia-like model, and (P)RR-Wnt-YAP signaling pathway might play a major role in the pathogenesis of sarcopenia.

J. Endo: None.

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Homeobox A4 Suppresses Vascular Smooth Muscle Cell Phenotypic Switching as a Novel Regulator of YAP/TEAD Transcriptional Activity

Masahiro Kimura, Takahiro Horie, Takeshi Kimura, Koh Ono, Dept of Cardiovascular Med, Graduate Sch of Med, Kyoto Univ, Kyoto, Japan

The Hippo signaling pathway is involved in the pathophysiology of various cardiovascular diseases. Yes-associated protein (YAP) and transcriptional enhancer activator domain (TEAD) transcriptional factors, the main transcriptional complex of the Hippo pathway, were recently identified as modulators of phenotypic switching of vascular smooth muscle cells (VSMCs). However, the intrinsic regulator of YAP/TEAD-mediated gene expressions in VSMCs remains to be elucidated, then we sought to investigate a novel regulator of YAP/TEAD transactivation involved in vascular diseases. We first investigated novel YAP/TEAD regulators using lentiviral shRNA library in HEK 293T-based TEAD-responsive reporter cell line and detected Homeobox A4 (HOXA4) as a potent repressor of YAP/TEAD transcriptional activity. HOXA4 attenuated YAP/TEAD-mediated gene expression independently of YAP phosphorylation, and co-immunoprecipitation assays revealed that HOXA4 interacts with TEADs but not YAP. Mechanistically, HOXA4 attenuated YAP/TEAD-mediated transcription by competing with YAP for TEAD binding. We also clarified that the expression of HOXA4 is relatively abundant in the vasculature, especially in VSMCs. In vitro experiments in human VSMCs showed HOXA4 maintains the differentiation state of VSMCs via inhibition of YAP/TEAD-induced phenotypic switching characterized by cell morphology, cell proliferation, and gene expression patterns. We generated Hoxa4-deficient mice and confirmed the downregulation of smooth muscle-specific contractile genes and the exacerbation of vascular remodeling after carotid artery ligation *in vivo*. Our results demonstrate HOXA4 is a novel repressor of VSMC phenotypic switching by inhibiting YAP/TEAD-mediated transcription. These findings give us a better understanding of the vascular pathophysiology and a novel therapeutic approach for vascular remodeling.

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Poster Session 1 and Reception

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Beclin1 as a Critical Regulator of the Endosomal Degradation Pathway

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Effective cellular quality control is crucial for cellular homeostasis and cardiac health. Defects in the pathways that regulate the removal of damaged proteins and organelles contribute to heart failure. Autophagy is well known for its role in removing protein aggregates and organelles; however, we recently found that the small GTPase Rab5 and early endosomes also participate in Parkin-mediated mitochondrial clearance. Beclin1 is a scaffolding protein which can form distinct PI3K protein complexes to regulate different processes such as autophagy. Here, we have investigated the role of Beclin1 in regulating the endosomal degradation pathway in mice. We have generated inducible cardiac-specific Beclin1 KO mice to investigate the *in vivo* role of Beclin1 in myocytes. We found significant contractile dysfunction as early as one week after deletion of Beclin1, which led to increased mortality. Loss of Beclin1 led to rapid development of cardiac hypertrophy as characterized by increased *Myh7*, *Nppa*, *Nppb*, myocyte size, and heart weight/body weight. Furthermore, we found that loss of Beclin1 led to increased inflammatory markers *Il6* and *Tgfb1*, as well as increased DNA damage/apoptosis in the heart. Ultrastructural analysis revealed Beclin1 deficiency led to significant myocardial disarray, demonstrating the importance of Beclin1 for proper myocyte alignment. These mice also had reduced autophagic flux but increased IGFR-beta receptor protein levels, indicating defects in both the autophagy and endosomal degradation pathways. Additionally, immunofluorescence analysis revealed that Beclin1 colocalizes with Rab5 at the early endosome in cells, and coimmunoprecipitation experiments confirmed their interaction. Together, these data establish the essential role of Beclin1 in cellular homeostasis as a central regulator of endosomal activity.

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Bcl-xL-Ser14 Phosphorylation is Critical for Compensatory Cardiac Hypertrophy

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Cardiac hypertrophy is an adaptive response at least initially since it reduces wall stress. Phosphorylation of Bcl-xL at Serine (Ser) 14, which dissociates Bcl-xL from Bax and promotes apoptosis, is increased in the heart within one hour after transverse aortic constriction (TAC)-induced pressure overload (PO). Here, we investigated how the increased Ser14-phosphorylation affects hypertrophy during PO. The Bcl-xL knock-in (KI) mice, in which Ser14 was replaced with Ala (Ser14Ala), exhibited a significantly greater mortality than wild-type (WT) mice ($p=0.001$) after TAC, with elevated end diastolic pressure (LVEDP, 34.6 vs 16.5 mmHg, $p<0.05$), impaired systolic function (EF, 38.2% vs 67.5%, $p<0.001$), and increased fibrosis (1.6-fold, $p<0.001$). The level of apoptosis was similar between the KI and WT mice one week after TAC, as assessed by TUNEL staining. The KI mice showed less cardiomyocyte and cardiac hypertrophy (cardiomyocyte size, 0.71-fold; heart weight/tibia length, 0.88-fold, both $P<0.001$). Adult cardiomyocytes isolated from the KI mice two days after TAC showed significantly lower contractility compared to those isolated from WT mice (0.32-fold, $p<0.001$). Mechanistically, gene set enrichment analysis using the RNA-seq data obtained from one-day TAC hearts showed that ion channel activity-related gene sets enriched in WT mice are downregulated in the KI mice. In line with this result, angiotensin II (Ang II) increased Ser14-phosphorylation and cytosolic Ca^{2+} level in WT-MEFs *in vitro*, whereas MEFs isolated from the KI mice showed a significantly lower elevation of cytosolic Ca^{2+} against Ang II. Proteomics analysis showed that Ankyrin, an anchoring protein that targets and stabilizes ion channels on the membrane, interacts with endogenous Bcl-xL in the heart. Taken together, these data suggest that phosphorylation of Bcl-xL at Ser14 is critical for augmenting Ca^{2+} release from the sarcoplasmic reticulum by modulating the ion channel activity in part via Ankyrin against PO or AngII, thereby developing compensatory hypertrophy and maintaining contractile function. Our findings indicate that increasing the Bcl-xL-Ser14 phosphorylation during acute phase PO could be a potential therapeutic strategy for maintaining cardiac function.

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Suppression of Store Operated Ca^{2+} Entry Components, dStim and dOrai, Results in Dilated Cardiomyopathy

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Cardiac disease is a leading cause of morbidity and mortality throughout the western world with current treatment options limited to palliative pharmacological or invasive therapy. The discovery of curative treatments will rely on thorough understanding of the molecular mechanisms that govern cardiac patho-physiology. Significantly, irregularities in calcium (Ca^{2+}) homeostasis are a major contributing factor to the pathogenesis of cardiac diseases, and targeting Ca^{2+} signaling mechanisms may therefore be an important approach to novel therapeutic development. Our lab has recently identified a requirement for the store operated Ca^{2+} entry (SOCE) pathway, whereby Ca^{2+} enters the cell in response to endo/sarcoplasmic reticulum (ER/SR) store depletion, in *Drosophila* cardiac physiology. Heart-specific suppression of key SOCE pathway components, Stim and Orai, induced dilated cardiomyopathy characterized by enlarged end-diastolic and end-systolic diameters and decreased fractional shortening. Animals with SOCE suppression had a significant delay in development and ultimately died considerably earlier than controls, suggesting pathological impairment of cardiac function. Additionally, Stim and Orai suppressed hearts exhibited highly disorganized or disrupted myofibrils, suggesting either defective heart development, tissue remodeling, or degeneration resulting from disrupted Ca^{2+} homeostasis. We are currently testing these possibilities by restricting Stim and Orai suppression to specific lifecycle stages, and we are analyzing Ca^{2+} transients in intact hearts to determine whether SOCE is required for contractile Ca^{2+} cycling or may have a contraction-independent signaling role. Collectively, our results demonstrate an essential role for SOCE in normal cardiac physiology, and present how powerful genetic tools and *in vivo* analyses in *Drosophila* will allow us to define the mechanistic basis for this requirement.

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Release of 12,13-diHOME From Brown Adipose Tissue Modulates Inotropy and Lusitropy in Old Mice

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The US demographic is shifting to an elderly population. It is estimated that by 2030, 20% of the population will be comprised of people over 65 years of age. The incidences of cardiovascular and metabolic diseases are increased with aging. A contributor to the age-induced metabolic dysfunction is a decrease in Brown Adipose Tissue (BAT). In addition to being a thermogenic organ, BAT is also an endocrine organ via the release of signaling lipids known as batokines. We have previously shown that there is a significant reduction in the batokine 12,13-diHOME contributing to the metabolic dysfunction with aging. However, the specific role of 12,13-diHOME in the age-induced impairments in cardiac function remains unknown. Thus, we hypothesized that supplementing 12, 13-diHOME would improve cardiac metabolism and function. In this study, we injected 12, 13-diHOME to young (3 months) and old (18months) C57Bl/6 wild type mice either acutely or long term (via an intraperitoneal injection daily for 2 weeks). Cardiac function was measured via echocardiography and hemodynamics via intra-left ventricular catheterization. In young mice, acutely supplementing 12, 13-diHOME greatly enhanced cardiovascular function and hemodynamics (e.g., cardiac output, systolic function, diastolic function, contractility, and relaxation). Similar effects were also observed in old mice that were acutely supplemented with 12,13-diHOME. These data suggest that 12,13-diHOME is a potent inotropic and lusitropic modulator in young and old mice. However, long term (2 weeks) treatment of 12, 13-diHOME on either young or old mice had no chronic effects on cardiac function and/or structure, unlike other inotropic agents. Taken together, these data demonstrate that 12,13-diHOME is a potent regulator of heart function and may be a viable long-term treatment strategy.

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Distinct cGMP Compartmentalization by Membrane Guanylate Cyclases

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Introduction: In adult cardiomyocytes (CM), cGMP produced by two membrane guanylate cyclases (GC-A and GC-B) play distinct roles. Using Förster Resonance Energy Transfer (FRET) and Scanning Ion Conductance Microscopy (SICM)-based approaches, cGMP compartmentation by GCs and phosphodiesterases (PDEs) were assessed.

Methods: cGMP was measured in CM isolated from transgenic mice expressing membrane targeted cGMP sensor (red cGES-DE5). SICM was used to define the t-tubules across sarcolemma and its nano-pipette filled with natriuretic peptides (CNP and ANP) was used to locally stimulate GC in t-tubules and crest.

Results: CNP activating GC-B induced maximum cGMP increase, while ANP activating GC-A did not increase cGMP. Pre-inhibiting PDE2 before ANP stimulation massively increased cGMP production to functionally significant levels as measured by PLN phosphorylation in western blot. cGMP in unstimulated cells was regulated mostly by PDE3. Local ANP stimulation in t-tubules, but not in crest, induced cGMP increase and CNP induced cGMP increase in both t-tubule and crest. After methyl- β -cyclodextrin treatment, ANP induced cGMP in both t-tubules and crest suggesting a role for lipid rafts in trapping GC-A in t-tubules. In transfected HEK293 cells, Fluorescence Recovery After Photobleaching (FRAP) showed that membrane diffusion of YFP-tagged GC-A was decreased after methyl- β -cyclodextrin treatment.

Conclusions: GC-A exclusively located in T-tubules produce cGMP locally, which is tightly regulated by PDE2, while GC-B is expressed across the sarcolemma and produces global cGMP.

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Golgi Localized β_1 -adrenergic Receptors Stimulate Golgi PI4P Hydrolysis by PLC ϵ to Regulate Cardiac Hypertrophy

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Phospholipase C (PLC)-mediated PI4P hydrolysis pathway at the Golgi is important for regulating cardiac hypertrophy. cAMP stimulates this pathway via Epac-mediated activation of Rap1 which directly binds to and activates PLC ϵ . Here, we demonstrate that a membrane permeant β -adrenergic agonist, dobutamine (dob), and the endogenous β -adrenergic agonist, norepinephrine (NE), induce Golgi PI4P hydrolysis in neonatal rat ventricular myocytes (NRVMs) and adult ventricular myocytes. However, a membrane impermeant β -adrenergic agonist, isoproterenol (iso), does not. In addition, a membrane permeant β AR antagonist, metoprolol, but not a membrane impermeant antagonist, sotalol, fully reverses dob-mediated PI4P hydrolysis. Taken together, this suggests that internal β -ARs are required for inducing PI4P hydrolysis. Subsequently, we used YFP-tagged mini Gs proteins that recruit to Gs-coupled receptors upon their activation, to monitor the location of activated receptors. Dob and NE induced robust and rapid recruitment of mini Gs to the plasma membrane and the Golgi, however, iso only induces recruitment to the plasma membrane. Additionally, inhibition of PLC ϵ at the Golgi with either siRNA or the RA1 domain of PLC ϵ , or the use of an Epac inhibitor also inhibits dob-mediated PI4P hydrolysis. This suggests that PI4P hydrolysis by dob requires Golgi-localized PLC ϵ and Epac. Inhibition of the β AR with Golgi-targeted Nb80 prevented PI4P hydrolysis by both dob and NE confirming that Golgi-localized β ARs are required for PI4P hydrolysis. Also, Oct3 transporter inhibitors prevent NE-induced PI4P hydrolysis but dyngo, an inhibitor of receptor internalization, had no effect. This indicates that agonist transport and not receptor internalization is required for NE-mediated Golgi β AR activation and PI4P hydrolysis. Furthermore, dob stimulates an increase in cell size and ANF expression in NRVMs that is significantly inhibited by metoprolol but less effectively by sotalol. Taken together, these data suggest that Golgi β ARs are involved in mediating cardiomyocyte hypertrophy and may serve as a novel target for treating heart failure.

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PKA is Needed for Acute Adaptation for Cardiac Stress

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In adult mammalian hearts, the ventricles are composed of mostly binucleated myocytes (BVMs) and some mononucleated myocytes (MVMs), which have different properties. However, if the gene expression profile and pathological responses of these two populations of myocytes are different have not reported. We found that these two populations of myocytes differentially expressed 250 genes. While many RNA synthesis and processing genes, and some antiapoptotic genes were highly expressed in MVMs, BVMs had genes related to self-destruction (autophagy and ubiquitination system genes) enriched. In general, expression of genes involved in carbohydrate metabolism and cell proliferation was also more but the expression of genes involved in fatty acid metabolism was less in MVMs. The expression of some of these genes was confirmed in single-molecule pulldown (SiMPull) method in separated MVMs and BVMs. MVMs grew more in pressure overloaded hearts. MVMs were more resistant to apoptosis induced by β -adrenergic stimulation both *in vitro* and *in vivo*, which was due to cytosolic and mitochondrial Ca^{2+} and ROS could be more easily increased in BVMs leading to more damage to mitochondria. Ca^{2+} current, myocyte contraction and Ca^{2+} transients in MVMs had less responses to β -adrenergic stimulation (isoproterenol, 1 μ M). Furthermore, MVMs gene expression upon β -adrenergic stimulation showed more adaptive responses while BVMs showed more proapoptotic gene (e.g., p53 and AIF), protein post-translational modification and muscle differentiation gene expression. This study suggests that MVMs and BVMs are distinct subpopulations of VMs with different gene expression profile and pathological responses. It underscores the importance of differential pathological responses of cells having different nuclear numbers .

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Deacetylation of Lc3 Drives Autophagy and Proteome Remodeling in Skeletal Muscle During Oncometabolic Stress

Anja Karlstaedt, Heidi Vitrac, Benjamin D Gould, Rebecca Salazar, McGovern Medical Sch at UTHealth, Houston, TX; David Taylor, Weston Spivia, Daniel Soetkamp, Cedars-Sinai Medical Ctr, Los Angeles, CA; An Dinh, McGovern Medical Sch at UTHealth, Houston, TX; Koen Raedschelders, Cedars-Sinai Medical Ctr, Los Angeles, CA; Helen Burks, Walter Schiffer, Daniel McNavish, Blake Hanson, McGovern Medical Sch at UTHealth, Houston, TX; Roberta A Gottlieb, Jennifer VanEyck, Cedars-Sinai Medical Ctr, Los Angeles, CA; Heinrich Taegtmeier, McGovern Medical Sch at UTHealth, Houston, TX

Metabolic rewiring is a hallmark of cancer and muscle cells. In isocitrate dehydrogenase 1 and 2 mutant tumors, increased plasma levels of the oncometabolite D-2-hydroxyglutarate (D2-HG) are associated with systemic effects, including myopathy. Our recent *in vivo* work showed that increased D2-HG supply by IDH-mutant cells causes heart and skeletal muscle atrophy, and decreases cellular ATP and NADH. Although heart failure and cachexia in cancer are commonly associated with chemotherapy, cancer survivors have a 5-fold increased risk of heart failure independent of any cytostatic treatment. The connection between metabolic and proteomic remodeling in this context remain poorly understood. We hypothesize that D2-HG-mediated alpha-ketoglutarate dehydrogenase (AKGDH) inhibition in myocytes results in metabolomic perturbations, increases autophagy and proteomic remodeling. Here, we report that LC3, a key regulator of autophagy, is activated in the nucleus of myocytes in presence of D2-HG through deacetylation by the nuclear deacetylase Sirt1. Activation of Sirt1 is driven by increased NAD^+ levels through D2-HG-mediated AKGDH inhibition. We used LC3 mutants with arginine and glutamine replacements at lysine residues to show that deacetylation of LC3 at K49 and K51 by Sirt1 shifts LC3 distribution

from the nucleus into the cytosol, where it is able to undergo lipidation at pre-autophagic membranes. Live cell imaging with GFP-tagged LC3 in L6 myocytes indicated that the cycle of acetylation-deacetylation allows LC3 to redistribute from the nucleus to the cytosol within less than 24 h. Co-immunoprecipitation of LC3 followed by proteomics analysis revealed that LC3 binds to dynein in presence of D2-HG. Furthermore, D2-HG promoted skeletal muscle atrophy and reduced grip strength in wild-type C57BL/6 mice *in vivo*. Using LC-MS/MS-based proteomics and metabolomics combined with RNA-sequencing, we assessed the effect of D2-HG on a systems level in skeletal muscle. Pathway-enrichment analysis revealed that D2-HG induces upregulation of key metabolic enzymes involved in glycolysis and the pentose phosphate pathway. In short, autophagy activation supports proteome remodeling in muscle cells during IDH-mutant leukemia.

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Live Tracking Mouse Model for Endogenous Exosomes from Heart

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Exosomes are emerging new category of messengers communicating among cells, tissues and organs. Understanding the kinetics of exosome communication *in vivo* are a critical foundation for studying exosome functions and developing exosome based drug-delivery models. Current studies of exosome *in vivo* trafficking largely rely on the administration of labeled exogenous exosomes. These methods may not fully represent endogenous exosome kinetics due to *ex vivo* exosome manipulations. Here, we established the first inducible endogenous exosome tracking mouse model that tracks endogenous exosome released from cardiomyocytes *in vivo*. The ultrasensitive and stable Nano-luciferase (NanoLuc) was selected as a reporter due to its 150-fold stronger signal intensity compared to traditional Firefly and Renilla luciferases, and the longest luminescent half-life amongst all known luciferases. We fused NanoLuc reporter with exosome surface marker CD63 for specific labeling of exosomes. The cardiomyocyte-specific α MHC promoter followed by a loxP-STOP-loxP cassette was engineered for a temporal control of labeled exosomes originating from cardiomyocytes. We crossed this cardiomyocyte-specific transgenic mouse with a tamoxifen-inducible Cre mouse (R26CreERT2) to achieve an inducible expression of the reporter. The exosome labeling and distribution were assessed by a luciferase assay and non-invasive bioluminescent live imaging. As expected, CD63NanoLuc expression was tightly controlled and only detected in cardiomyocytes upon induction. The endogenous exosomes released from cardiomyocytes were labeled and detected *in vitro* in a cell culture supernatant, and *in vivo* in the animal plasma. A paracrine uptake of the Nanoluc-labeled exosomes by cardiac fibroblasts *in vivo* was demonstrated and quantified. A signature distribution profile of the endogenous exosomes released from cardiomyocytes was exhibited. For the first time, this exosome tracking model enables elucidating the endogenous exosome trafficking pattern, and allows future studies of exosome behavior under different conditions. It provides a useful tool for the exploration of biological functions, mechanisms and clinical applications of exosomes in a broad spectrum of researches.

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Systems Network Genomic Analysis Reveals the Role Of MURC/Cavin-4 in Cardiac Ischemia/reperfusion Injury

Masahiro Nishi, Takehiro Ogata, Naohiko Nakanishi, Yusuke Higuchi, Akira Sakamoto, Yumika Tsuji, Satoaki Matoba, Kyoto Prefectural Univ of Med, Kyoto, Japan

Background: Ischemia/reperfusion (I/R) injury is a critical issue in the development of treatment strategies for ischemic heart disease. Muscle-restricted coiled-coil protein (MURC)/Cavin-4, which is a component of caveolae, is involved in the pathophysiology of dilated cardiomyopathy and cardiac hypertrophy. However, the role of MURC in cardiac I/R injury remains unknown. **Objective:** To elucidate the role of MURC in cardiac I/R injury. **Methods and Results:** The systems network genomic analysis based on PC-corr network inference on microarray data between wild-type and MURC knockout (KO) mouse hearts described a network of discriminating genes associated with reactive oxygen species (ROS). To

demonstrate the prediction, we investigated the effect of MURC deletion in cardiac I/R injury. MURC deletion in IR-injured mouse hearts decreased infarct size and preserved heart contraction with the inhibition of ROS production and ROS-related gene expressions such as EGR1 and DDIT4 as well as EGR1 protein level. PC-corr network inference integrated with a protein-protein interaction network prediction showed that MURC is also involved in the apoptotic pathway. We confirmed the upregulated activity of STAT3, which is a transcription factor of anti-apoptotic signaling, with the increase of BCL2 mRNA expression and protein level and the decrease of cleaved Caspase 3 protein level in MURC KO compared with WT mouse hearts after I/R. TUNEL assay showed that MURC modulates the apoptosis in cardiomyocytes exposed to hydrogen peroxide. STAT3 inhibitor cancelled the cardioprotective effect of MURC deletion in I/R-injured heart and the anti-apoptotic effect of MURC knockdown in cardiomyocytes. **Conclusions:** Our findings suggest that MURC plays a pivotal role in the regulation of ROS-induced cell death and STAT3-mediated anti-apoptotic signaling in cardiac I/R injury. MURC may be a therapeutic target for cardiac I/R injury.

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Klotho Gene Deficiency Causes Heart Failure via Disruption of Phosphate Metabolism

Kai Chen, **Zhongjie Sun**, Univ of Tennessee HSC, Memphis, TN

Heart failure is the major cause of mortality for patients with chronic kidney damage (CKD). Although a decrease in plasma klotho levels has been linked to chronic kidney disease (CKD), the relationship between Klotho and heart failure is unclear. Here, using Klotho mutant homozygous (KL $-/-$) mice, we found that Klotho deficiency caused hyperphosphatemia and heart failure. Normalization of serum phosphorous by dietary phosphate restriction rescued Klotho deficiency-induced heart failure in male mice. However, low phosphate diet did not rescue hyperphosphatemia and heart failure in KL $-/-$ female mice. Therefore, hyperphosphatemia may be an important mechanism of Klotho deficiency-induced heart failure. Dietary phosphate restriction did not prevent estrogen depletion in KL $-/-$ female mice, suggesting that estrogen depletion may involve in Klotho deficiency induced hyperphosphatemia and heart failure. Normalization of serum estrogen level by *17 β -estradiol* prevented cardiac remodeling and heart dysfunction in KL $-/-$ female mice. Moreover, treatment with *17 β -estradiol* normalized phosphate metabolism via regulating renal NaPi co-transporter expression. Treatment with *17 β -estradiol* abolished mitochondrial dysfunction and cardiac apoptosis in Klotho deficient mice. This study demonstrates for the first time that hyperphosphatemia is an important mediator of heart failure which can be prevented by dietary phosphate restriction in male KL $-/-$ mice. Estrogen deficiency mediates hyperphosphatemia leading to heart failure due to klotho deficiency in female mice.

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Reducing Lumen Disorder in Cerebral Arteriovenous Malformation

Jiayi Yao, Xiuju WU, Daoqin Zhang, Li Zhang, Kristina I Bostrom, **Yucheng Yao**, UCLA, Los Angeles, CA

Lumen integrity in vascularization requires fully differentiated endothelial cells (ECs). Here, we report that endothelial-mesenchymal transitions (EndMTs) emerge in ECs of cerebral arteriovenous malformation (AVMs) and causes disruption of the lumen or lumen disorder. We show that excessive Sry-box 2 (Sox2) signaling is responsible for the EndMTs in cerebral AVMs. EC-specific suppression of Sox2 normalizes endothelial differentiation and lumen formation, and improves the cerebral AVMs. Epigenetic studies show that induction of Sox2 alters the cerebral-endothelial transcriptional landscape, and identify jumonji domain-containing protein 5 (JMJD5) as a direct target of Sox2. Sox2 interacts with JMJD5 to induce EndMTs in cerebral ECs. Furthermore, we utilize a high throughput system to identify the beta-adrenergic antagonist pronethalol as an inhibitor of Sox2 expression. Treatment with pronethalol stabilizes endothelial differentiation and lumen formation, which limits the cerebral AVMs.

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Disease and Phenotype-relevant Genetic Variants Identified From Histone Acetylomes in Human Hearts

Chukwuemeka George Anene Nzelu, Wilson Tan, Eleanor Wong, Mick Lee, Matias Ilmari Autio, Susan Tan, Pan Bangfen, Natl Univ of Singapore, Singapore, Singapore; Michael Morley, Kenneth Margulies, Thomas Cappola, Univ of Pennsylvania, Philadelphia, PA; Marie Loh, John Chambers, Nanyang Technological Univ, Singapore, Singapore; Shyam Prabhakar, Genome Inst of Singapore, Singapore, Singapore; Roger Foo, Natl Univ of Singapore, Singapore, Singapore

Identifying genetic markers for heterogeneous complex diseases such as heart failure has been challenging, and may require prohibitively large cohort sizes in genome-wide association studies (GWAS) in order to meet genome-wide statistical significance. On the other hand, chromatin quantitative trait loci (QTL), elucidated by direct epigenetic profiling of specific human tissues, may contribute towards prioritising sub-threshold variants for disease-association. Here, we captured non-coding genetic variants by performing enhancer H3K27ac ChIP-seq in 70 human control and end-stage failing hearts, mapping out a comprehensive catalogue of 47,321 putative human heart enhancers. 3,897 differential acetylation peaks (FDR 5%) pointed to pathways altered in heart failure (HF). To identify cardiac histone acetylation QTLs (haQTLs), we regressed out confounding factors including HF disease status, and employed the G-SCI test to call out 1,680 haQTLs (FDR 10%). RNA-seq performed on the same heart samples proved a subset of haQTLs to have significant association also to gene expression (expression QTLs), either in *cis* (180), or through long range interactions (81), identified by Hi-C and Hi-ChIP performed on a subset of hearts. We validated 2 of the haQTLs through base editing to show that the presence of those SNPs indeed affects gene expression in human embryonic stem cells-derived cardiomyocytes. Furthermore, a concordant relationship between the gain or disruption of transcription factor (TF) binding motifs, inferred from alternative alleles at the haQTLs, implied a surprising direct association between these specific TF and local histone acetylation in human hearts. Finally, 62 unique loci were identified by colocalisation of haQTLs with heart-related GWAS datasets. Disease-association for these new loci may indeed be mediated through modification of H3K27-acetylation enrichment and their corresponding gene expression differences.

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Epigenetic Regulation of Cardiomyocyte Proliferation and Regeneration by Swi/snf Complex Subunit Arid1a

Cornelis J Boogerd, Ilaria Perini, Phit La, Britt van der Swaan, Jari B Berkhout, Lieneke Kooijman, Hubrecht Inst, Utrecht, Netherlands; Danielle Versteeg, Univ Medical Ctr Utrecht, Utrecht, Netherlands; Eva van Rooij, Hubrecht Inst, Utrecht, Netherlands

Adult mammalian hearts do not regenerate following ischemic injury, causing permanent damage to the myocardium, often leading to heart failure. In contrast, neonatal mouse hearts can fully regenerate after injury, however this ability is lost few days after birth. Loss of regenerative capacity coincides with profound changes in the epigenetic landscape. Yet, the mechanisms controlling cardiomyocyte proliferation remain poorly understood. To identify epigenetic mechanisms that underlie cardiomyocyte regeneration in response to ischemic injury, we subjected mice to sham or ischemia-reperfusion injury (IR) and performed RNA-Seq at multiple timepoints after injury. Multiple SWI/SNF chromatin remodeling complex subunits were upregulated after IR, including the AT-rich interactive domain-containing protein 1A (*Arid1a*), which has previously been implicated in tissue regeneration. Here, we show that *Arid1a* is abundantly expressed in cardiomyocytes during development, and is reactivated in a subset of adult cardiomyocytes after IR. Moreover, *ARID1A* is highly expressed in cardiomyocytes in human failing hearts, suggesting an important function in injury response. Cardiomyocyte-specific *Arid1a* ablation around birth (*Arid1a cKO*) in mice induced cardiomyocyte hyperplasia, and severe cardiac enlargement at 2 weeks of age. *Arid1a cKO* hearts displayed increased expression of key cell cycle genes, and HIPPO target genes, suggesting *Arid1a* is required for cell cycle withdrawal in neonatal cardiomyocytes. When *Arid1a* was inducibly removed from adult cardiomyocytes (*Arid1a icKO*), hearts had normal gross morphology without visible signs of cardiac pathology. Next, we performed IR injury on *Arid1a icKO* mice and observed increased cell cycle activity in mutant border zone cardiomyocytes. To further explore the mechanisms by which *Arid1a* functions in cardiomyocytes, we are currently performing ChIP-Seq and protein interaction studies. These data suggest that *Arid1a* regulates cardiomyocyte proliferation

and function. Upregulation of *Arid1a* in cardiomyocytes after injury may suppress proliferation and regeneration. Suppression of *Arid1a* after ischemic injury may prove to be a novel target for therapeutics to enhance cardiac regeneration.

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A Novel Algorithm for the Collective Integration of Single Cell Rna-seq During Embryogenesis

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Background: Single cell RNA-seq (scRNA-seq) over specified time periods has been widely used to dissect the cell populations during mammalian embryogenesis. Integrating such scRNA-seq data from different developmental stages and from different laboratories is critical to comprehensively define and understand the molecular dynamics and systematically reconstruct the lineage trajectories. **Methods:** Here, we describe a novel algorithm to integrate heterogenous temporal scRNA-seq datasets and to preserve the global developmental trajectories. scNCA not only successfully corrected the batch effects, but also preserved the global structure of gene expression. For both balanced and imbalanced synthetic scRNA-seq data, we found that scNCA had significantly better performance of batch correction than mnnCorrect and Seurat alignment, on mixing cells from different batches, revealing the global trajectories, and bringing together the cells from the same lineage. **Results:** We applied this algorithm and approach to integrate 3,387 single cells from seven heterogenous temporal scRNA-seq datasets, and reconstructed the cell atlas of early mouse cardiovascular development from E6.5 to E9.5. Using this integrated atlas, we identified an *Etv2* downstream target, *Ebf1*, as an important transcription factor for mouse endothelial development. **Conclusions:** In summary, we presented scNCA as a novel tool to correct the batch effect of temporal scRNA-seq. We used scNCA to integrate 3,387 single cells from seven heterogenous temporal scRNA-seq datasets of mouse early cardiovascular development, and identified an *Etv2* downstream target, *Ebf1*, as an important transcription factor for mouse endothelial development. We provide the R/TensorFlow implementation of scNCA at <https://github.com/gongx030/scNCA>. The integrated mouse early cardiovascular development data can be explored at <https://heartmap.umn.edu/scNCA>.

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Poster Session 1 and Reception

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Chromatin Remodeling Mechanisms by Bromodomain PHD Finger Transcription Factor in Cardiac Hypertrophy and Heart Failure

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Alteration of chromatin conformation has a profound effect on gene expression, and can lead to heart failure, which remains a major cause of death worldwide. ATP-dependent chromatin remodelers utilize the energy of ATP to remodel chromatin in specific regions of the genome. However, the mechanisms of ATP-dependent chromatin remodeling and how they connect to the genome remain poorly understood. We have discovered that the nucleosome remodeler Bromodomain PHD-finger Transcription Factor (BPTF), the largest subunit of the Nucleosome Remodeling Factor (NURF), plays an important role in cardiac hypertrophy. Our preliminary data show that BPTF is enriched in failing human hearts, a phenomenon similarly observed in mouse hearts subjected to pressure overload hypertrophy (TAC). BPTF is up-regulated in primary neonatal rat ventricular myocytes (NRVM) and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) stimulated with the hypertrophic agonist phenylephrine (PE). Strikingly, specific silencing of BPTF in NRVM and hiPSC-CMs, inhibits the expression of the atrial natriuretic peptide (ANP), a marker of cardiac hypertrophy. Chromatin immunoprecipitation performed in NRVM show that BPTF binds to the ANP promoter, but not to GAPDH. Proteomics experiments performed in failing human hearts revealed a multi-protein complex formed between BPTF and novel chromatin regulators. At the level of the

nucleosome, BPTF in combination with one of its partners, remodels chromatin by facilitating nucleosomes “sliding” along the DNA. This mechanism is mediated by the binding of BPTF to histone H3 trimethylated at Lysine-4 (H3K4me3), an “active” mark of transcription also up-regulated in the early phase of cardiac hypertrophy in NRVM and in mouse TAC hearts. Our study suggests a novel chromatin signature controlled by BPTF, that dictates specific gene patterns implicated in pathological cardiac hypertrophy. Our study uncovers a new epigenetic mechanism controlling pathological cardiac remodeling and heart failure.

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CRISPR-based Gene Activation for Transcriptional Reprogramming of Mammalian Cardiomyocytes

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Cardiac remodelling is accompanied by silencing of genes necessary for cardiac homeostasis including Krüppel-like factor 15 (*Klf15*), an anti-hypertrophic factor. Due to a lack of tools for gene activation, we aim to adapt a CRISPR-based approach for gene expression control for mammalian cardiomyocytes. We generated a transgenic mouse model (TG) expressing an enzymatically inactive, gRNA-programmable Cas9 protein under *Myh6* promoter control fused to transcriptional activators (VPR) to drive gene expression. As a proof of concept, we systemically injected mice at postnatal day P4 with AAV9 carrying a triple gRNA expression cassette containing gRNAs targeted to the *Klf15* promoter region. Significant *Klf15* activation was observed at P10 up to 10-fold compared to controls in three independent TG lines. This *Klf15* activation was sufficient to drive target gene expression of *Aldh2* and *Adhfe1* (controls = WT + saline, WT + AAV9, TG + saline, n ≥ 3 per group, ANOVA and Bonferroni correction). Importantly, at the neonatal stage, the *Klf15* promoter is characterized by low H3K27ac presence indicating that endogenous gene activation can enhance transcription from an epigenetically inactive locus. We demonstrated that CRISPR/Cas9 mediated loss of *KLF15* expression results in impaired contractile function in a 3D model of engineering heart muscle, similar to the *Klf15*^{-/-} mouse heart phenotype. Encouraged by the evolutionary conserved *KLF15*-mediated mechanisms, we aimed to generate a human induced pluripotent stem cells, which constitutively express dCas9VPR (hiPSC-dCas9VPR) for gene activation in hiPSC-derived cardiomyocytes to complement the *in vivo* findings upon disease. We targeted the transgene integration to the AAVS1 site by CRISPR gene editing, confirmed correct integration by PCR followed by sequencing and hiPSC-dCas9VPR were successfully differentiated into beating cardiomyocytes. To this end, we have tested gRNAs targeted to the *KLF15* promoter region in HEK293 cells and observed significant *KLF15* activation of up to 5.5-fold (n = 3, ANOVA and Bonferroni correction). In summary, we report transgenic mammalian tools for endogenous gene (re-) activation purposes to identify potential therapeutic targets for preventing heart failure progression.

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Regulation of Hypoxia by Chromatin Reader Protein Kinase C Binding Protein 1 (PRKCBP1)

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PRKCBP1 (also known as RACK7 and Zmynd8) is a polyvalent chromatin reader known to cooperatively bind acetylated and methylated nucleosomes. Recently it has been shown to regulate transcription and cancer progression by coordinating histone methylation modifications affecting enhancer and promoter regions of genes. The role of PRKCBP1 in the cardiac myocardium is unexplored. Hypoxia-inducible factor 1 α (HIF-1 α) upregulation and stabilization is a common feature of both cancer and myocardial ischemia, promoting cellular functions such as proliferation, glucose metabolism and angiogenesis. Here we investigated the mechanism by which PRKCBP1 modulates the cardiac hypoxic response.

We hypothesize that PRKCBP1 inhibits the HIF-1 response in the hypoxic heart by reducing enhancer activity of HIF-1 target genes and altering availability of HIF-1 binding sites.

We have found that in transgenic mice with a mutation that increases HIF-induced expression of PRKCBP, the effect of induced oxygen-stable HIF is markedly diminished. These mice did not exhibit the typical HIF-1 over-expression phenotype of dilated vessels, increased heart size and reduced ventricular function. Semi-quantitative rtPCR analysis of mouse neonatal cardiomyocytes transfected with CMV-driven expression plasmids for PRKCBP1 and oxygen-stable HIF-1 α showed striking reduction of multiple HIF-1 target genes such as PDK1 (45% reduction relative to Ehbp1) compared to the HIF-1 α plasmid alone. RNAi mediated knockdown of PRKCBP1 removed this negative regulation (65% increase). Analysis of human PRKCBP1 and HIF-1 α ChIP-seq data indicate that PRKCBP1 binds to the enhancer of 78% of HIF-1 regulated genes. ATAC-seq data suggest that PRKCBP1 affects genome-wide chromatin accessibility, with loci-specific modifications at numerous HIF-1 target genes, such as EGLN3. These data suggest that PRKCBP1 may be acting both by modulating enhancer activity *in cis*- to HIF-1 target genes and by preventing HIF-1 binding to hypoxia response elements of target genes.

We have discovered a new regulator of HIF-1 action that modifies the hypoxic response, likely through chromatin remodeling. This new form of regulation may modify the pathophysiology of ischemia and provide new targets for therapy.

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Etv2 Transcriptionally Regulates *Yes1* and Promotes Cell Proliferation During Embryogenesis

Bhairab Singh, Wuming Gong, Satyabrata Das, Joshua Theisen, Javier Sierra-Pagan, Demetris Yannopoulos, Erik Skie, Pruthvi Shah, Mary Garry, Daniel Garry, Univ of Minnesota, Minneapolis, MN

Etv2, an Ets-transcription factor, governs the specification of the earliest hemato-endothelial progenitors during embryogenesis. While the transcriptional networks during hemato-endothelial development have been well described, the mechanistic details are incompletely defined. In the present study, we described a new role for Etv2 as a regulator of cellular proliferation via *Yes1* in mesodermal lineages. Analysis of an Etv2-ChIPseq dataset revealed significant enrichment of Etv2 peaks in the upstream regions of cell cycle regulatory genes relative to non-cell cycle genes. Our bulk-RNAseq analysis using the doxycycline-inducible Etv2 ES/EB system showed increased levels of cell cycle genes including *E2f4*, *Gadd45g* and *Ccne1* as early as 6h following Etv2 induction. Further, EdU-incorporation studies demonstrated that the induction of Etv2 resulted in a ~2.5-fold increase in cellular proliferation, supporting a proliferative role for Etv2 during differentiation. Next, we identified *Yes1* as the top-ranked candidate that was expressed in *Etv2-EYFP*⁺ cells at E7.75 and E8.25 using single cell RNA-seq analysis. Doxycycline-mediated induction of Etv2 led to an increase in *Yes1* transcripts in a dose-dependent fashion. In contrast, the level of *Yes1* was reduced in *Etv2 null* embryoid bodies. Using bioinformatics algorithms, biochemical, and molecular biology techniques, we show that Etv2 binds to the promoter region of *Yes1* and functions as a direct upstream regulator of *Yes1* during embryogenesis. These studies enhance our understanding of the mechanisms whereby Etv2 governs mesodermal fate decisions early during embryogenesis.

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Poster Session 1 and Reception

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Acetyl-CoA Production by Specific Metabolites Promotes Cardiac Repair After Myocardial Infarction via Mediating Histone Acetylation

lenglam Lei, Shuo Tian, Wenbin Gao, **Zhong Wang**, Univ of Michigan, Ann Arbor, MI

In this study, we set out to identify an interlinked metabolic and epigenetic network that produces acetyl-CoA for histone acetylation and gene regulation, and determine whether this network promotes heart repair and protection after I/R injury. Myocardial ischemic reperfusion (I/R) injury induces dramatic metabolic changes and is accompanied with extensive epigenetic changes. Recent studies reveal that energy metabolism and chromatin epigenetics are intimately linked in cellular

functions. In particular, acetyl-CoA is a building block for energy metabolism and histone acetylation. However, the acetyl-CoA mediated regulatory machinery integrating metabolic pathways and chromatin modifications has been under-explored in heart repair and protection.

We conducted a screen of energy metabolites in promoting histone acetylation and heart repair both *in vivo* and *in vitro*. Our screen identified that acetate, pyruvate, and octanoic acid (8C) but not citrate and nonanoic acid (9C) improved heart function after MI in rats. In particular, 8C administration resulted in the most significant heart functional recovery after MI. More importantly, in a more clinically relevant setting, 8C injection at the time of reperfusion 45 minutes after left anterior descending coronary (LAD) ligation showed comparable repair effect to that of 8C administration before LAD ligation, suggesting that 8C could be a very effective metabolic natural product to treat MI. Mechanistically, 8C promoted histone acetylation in CM chromatin after IR injury and inhibited CM death by activating expression of anti-oxidant genes Nrf2, HO1, and NQO1. We further established that the 8C promoted histone acetylation and heart repair was transduced by metabolic enzyme medium-chain acyl-CoA dehydrogenase (MCAD) and histone acetylase GCN5/KAT2A.

Therefore, this study has elucidated an interlinked metabolic/epigenetic network comprising 8C, acetyl-CoA, MCAD, and KAT2A in stimulating histone acetylation and anti-oxidative stress gene expression to combat heart injury. This study provides a novel strategy for treating myocardial I/R disease at the interface of metabolism and epigenetics.

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Poster Session 1 and Reception

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KLF15-TCF7L2-dependent Cardiac Transcriptional Reprogramming Induces Cardiomyocyte and Vascular Cell Remodeling in the Mammalian Heart

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Integrative biochemical and omic-based approaches have refined our understanding of how cells integrate the Wnt signal at the chromatin level to yield specific cellular responses. However, this aspect remains unexplored in the heart. Since Wnt/ β -catenin signaling activation is a hallmark in pathological cardiac remodeling, we aimed to characterize the specific Wnt cardiac transcriptional network regulation, amenable to therapeutic intervention in the adult heart.

In the adult heart, we found the transcription factor KLF15 occupying regulatory regions of tissue remodeling genes containing Wnt transcriptional activator, TCF7L2, binding sites, which are silenced in the healthy myocardium but are active during pathological remodeling. Supporting KLF15 repressive roles, its loss resulted in cardiac TCF7L2 activation, maladaptive reprogramming and failure *in vivo*. We demonstrated that KLF15 possess transcriptional age-specific repressive functions controlling Wnt signaling, cardiomyocyte de-differentiation and vascular cell (VC) remodeling. Employing different transgenic mouse models we further identified a cooperative program inducing aberrant VC remodeling, caused by a reduction of KLF15 with a concomitant TCF7L2 activation in cardiomyocytes. Furthermore, we characterized a cardiac specific Wnt transcriptional inhibitory complex consisting of KLF15 directly interacting with β -catenin and TCF7L2 and identified the amino acids critical for these interactions. Next, using a CRISPR/Cas9-mediated approach we generated *KLF15* knockout (KO) hESC lines differentiated into functional cardiomyocytes and used for engineering human myocardium (EHM) generation. *KLF15* KO EHMs showed activation of TCF7L2-dependent transcription as well as impaired function in comparison to control lines, recapitulating the *Klf15* KO mouse phenotype.

Altogether, we uncover an exquisite evolutionary conserved cardiac specific regulation mediated by KLF15 on Wnt signaling in myocardium offering a basis for designing highly specific pharmacological intervention for controlling Wnt cardiac-specific gene activation to prevent irreversible heart failure. We also underscore the significance of KLF15-Wnt dynamics in VC remodeling of the adult heart.

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Enhanced Neovascularization by ETV2 via Angiogenesis in Myocardial Infarction Models

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Attempts to regenerate cardiac vessels in order to treat ischemic myocardial disease have continued to be an old goal in the field of gene therapy. However, most studies that have applied proangiogenic factors have shown that the effects were limited. We examined the therapeutic effects of the ETV2 gene, a factor induced by postnatal injury, which is involved in the differentiation and specification of endothelial cells in the developmental stages in animal models of ischemic myocardial infarction via the adenoviral expression system. The ETV2 gene, using adenoviral vectors, was efficiently delivered in normal heart and myocardial infarction models and induced gene expression. In the myocardial infarction model of the control group, the function of the heart deteriorated after induction over time, but deterioration in the adenoviral ETV2-treated myocardial infarction group was delayed. To confirm whether this effect was due to angiogenesis, the expression of proangiogenic factors was examined, and the expression of vascular endothelial growth factor and angiopoietin were significantly increased in the group treated with ETV2. This result was reproduced via ETV2 gene expression at the cellular level. In order to confirm the effects of angiogenesis more deeply, we confirmed that the dividing vascular endothelial cells expressed Ki67. As a result, it was confirmed that a significant number of Ki67-positive cells were present in the functional blood vessel region in the group treated with ETV2. Taken together, the results suggest that angiogenesis plays a role in regenerating cardiac vessels when the ETV2 gene is introduced into the myocardial infarction model, which may be considered as a candidate for treatment of ischemic heart disease.

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Poster Session 2 and Reception

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Polyploidy Increases in Murine Cardiomyocytes Following Myocardial Infarction

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Introduction: *De novo* cardiomyogenesis versus polyploidy in myocardial homeostasis, aging, and response to injury is a controversial research area of intense investigation. Our lab recently created the Fluorescent Ubiquitin Cell Cycle Indicator transgenic (FUCCI-Tg) mouse model to study cardiomyocyte (CM) cell cycle progression. Therefore, the FUCCI-Tg model was used to track CM cell cycle correlated to ploidy state in response to myocardial infarction (MI).

Hypothesis: Adult FUCCI-Tg cardiomyocytes progress into S/G2/M phase of cell cycle by 10 days after infarction resulting in binucleation rather than *de novo* cardiomyogenesis.

Methods and Results: CMs isolated from FUCCI-Tg were analyzed 3 and 10 days following MI using confocal microscopy and flow cytometry. At 3 days post-MI, the ratio of mono- to binucleated CMs remained unchanged from non-injury CMs. At day 10 post-MI, frequency of mononuclear CMs significantly decreased compared to normal or 3-day post-MI CMs. Coincident with nucleation state, myocytes were only found to enter S/G2/M phase at day 10 post-MI. These results were verified by visualization of FUCCI in isolated CMs using Amnis ImageStream flow cytometry. Ploidy state and CM size was assessed in the infarction / border (left ventricle (LV)) and remote zone (right ventricle (RV)) at day 10 post-MI and compared to the normal and sham LV and RV. Binucleation significantly increased in the LV after MI compared to normal LV, whereas RV CM binucleation and size significantly increased in both sham and MI at 10 days after MI compared to normal RV.

Conclusion: Adult murine CMs enter cell cycle in response to MI but primarily undergo endomitosis / endoreplication rather than complete cell cycle reflecting increases in nucleation and/or myocyte size rather than *de novo* cardiomyogenesis. Future studies will assess CM ploidy in mouse strains purported to possess enhanced cardiomyogenesis following MI injury and the biological significance of ploidy for mediating myocardial repair.

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Poster Session 2 and Reception

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Adiponectin Receptor 3 is Associated With Endothelial Nitric Oxide Synthase Dysfunction and Predicts Insulin Resistance in South Asians

Mark Chandy, Nazish Sayed, Edward Lau, Chun Liu, Tzu-Tang Wei, Ian Y-L Chen, Dilip Thomas, June Rhee, Brad Oh, Lejla Pepic, Stanford Univ, Palo Alto, CA; Mansoor Husain, Univ of Toronto, Toronto, ON, Canada; Thomas Quertermous, Shriram Nallamshetty, Joseph Wu, Stanford Univ, Palo Alto, CA

Type 2 diabetes (T2DM) is a global epidemic affecting over 400 million people and causes significant morbidity and mortality. T2DM has a strong association with cardiovascular disease (CVD), the number one cause of death globally with 17.5 million deaths per year. A precursor of T2DM, insulin resistance is central to the development of T2DM and is a risk factor for CVD. Insulin resistance is difficult to diagnose and individuals are often untreated prior to the onset of T2DM or CVD. South Asians are more likely to have insulin resistance, diabetes and cardiovascular disease when compared to age matched European cohorts. The molecular mechanisms of why South Asians are predisposed to insulin resistance and consequently cardiovascular disease were investigated using induced pluripotent stem cells (iPSCs) derived endothelial cells (iPSC-EC). Endothelial cells line blood vessels of the cardiovascular system. Unlike previous models, iPSC-EC are unique because they contain the individual's genetic information and the environmental influences retained in epigenetic marks are removed via reprogramming and differentiation. iPSC-ECs from insulin resistant South Asians show evidence of impaired insulin signaling as evidenced by decreased Akt phosphorylation, and paradoxically overexpression of eNOS and adiponectin receptor 3. In the cellular milieu of prediabetes, insulin resistance iPSC-ECs show impaired tubule formation and nitric oxide release. When adiponectin receptor 3 expression is suppressed using siRNA, eNOS expression decreases and expression of components of the insulin signaling cascade are improved to levels observed in control iPSC-ECs. Multiple linear regression modeling of clinical characteristics and gene and cellular phenotype was used to develop a scoring system that predicts a patient's risk of developing insulin resistance and hence subsequently diabetes and cardiovascular complications. To our knowledge, this is the first iPSC-derived endothelial cell risk calculator with the potential to identify South Asian patients at risk for developing insulin resistance and cardiovascular disease before disease onset, which would allow for the early implementation of interventions that prevent morbidity and mortality.

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Poster Session 2 and Reception

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CX3CR1⁺ Multipotent Progenitors Generate Cardiovascular Lineage Cells Under Cardiac Microenvironments

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In regenerative medicine, investigating the ontogenetic properties of cells is necessary to develop appropriate therapeutic strategies for tissue regeneration. It was previously reported that CX3CR1⁺ cells give rise to tissue-resident macrophages in multiple organs independently of definitive hematopoiesis. However, the fate and roles of CX3CR1⁺ cells during organogenesis are still unclear. Here, we identified CX3CR1⁺ multipotent progenitors that contribute to embryonic organogenesis via genetic lineage tracing approaches in mice. *Cx3cr1* lineage cells were observed in the YS and the embryo at E8.5-9.5. It turned out that *Cx3cr1* lineage cells generated multiple cell types in various organs including the heart, intestine, liver, kidney, retina, and skeletal muscle. In the developing heart, *Cx3cr1* lineage cells incorporated into the myocardium and committed to cardiovascular lineages including cardiomyocytes (CMs) and endothelial cells (ECs). In the adult mouse heart, the percentage of *Cx3cr1* lineage CMs was approximately 20% including ~5% fusion with pre-existing CMs. Next, we developed a method to generate CX3CR1⁺ cells from differentiating mouse embryonic stem cells (mESCs) using CX3CR1 as a sorting marker. The mESC-derived CX3CR1⁺ cells exhibited a capacity to differentiate into CMs and ECs *in vitro*. When cocultured with the fetal mouse heart *ex vivo*, mESC-CX3CR1⁺ cells migrated and incorporated into the myocardium where they generated CMs and ECs. When encapsulated with PA-RGDS nanomatrix and injected into the post-MI adult mouse heart, mESC-CX3CR1⁺ cells showed robust survival and produced CMs and ECs in the host myocardium. Taken together, we identified CX3CR1⁺ cells as multipotent progenitors that retain a capacity to generate cardiovascular lineage cells under cardiac-specific microenvironments. This study offers a new insight into the mechanism of embryonic organogenesis and a potential of CX3CR1⁺ cells for cardiac regeneration.

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Poster Session 2 and Reception

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Deterministic Paracrine Repair of Injured Myocardium using Microfluidic Cocooning of Heart Explant-Derived Cells

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Previous work has shown that cocooning heart explant-derived cells (EDCs) within protective nanoporous gel capsules before intra-myocardial injection increases the retention of transplanted cells and the paracrine production of nanoparticles to improve post infarct cardiac function. In this study, we investigated the influence of cocoon size and intracapsular cell number on cell-treatment outcomes using a newly developed microfluidic-based (MF) cellular cocooning platform.

Methods/Results: Traditional vortex-based encapsulation (Vx) inherently provides cocoons of varying diameters (30-100 μm ; $68\pm 5 \mu\text{m}$). By altering the flow pressure ratios and the nozzle diameters within the MF chip, we encapsulated human EDCs within small ($51\pm 1 \mu\text{m}$, MF50) and large ($90\pm 1 \mu\text{m}$, MF90) diameter nanoporous gel cocoons for comparison with standard Vx-defined capsules ($71\pm 1 \mu\text{m}$, MF70). MF cocooning mirrored the expected Poisson distribution with smaller cocoons having a greater proportion of single cells while larger diameter cocoons contained greater proportions of multicellular aggregates. Immunodeficient mice underwent left coronary artery ligation 1 week before randomization to echocardiographic guided intra-myocardial injection of EDCs (suspended or variable diameter cocoons) or vehicle. Increasing cocoon diameter stimulated progressive salutary effects on post-infarct function (ejection fraction), scar burden and newly generated peri-infarct blood vessels (isolectin B4+) and cardiomyocytes (BrdU+/TNT+) 4 weeks after treatment. Bioluminescent imaging of luciferase tagged cells revealed increasing cocoon diameter reduced the rate of cell clearance from injured tissues. Disrupting cell-cell contact within the capsules (using a custom antibody cocktail to block E/P-selectin and N-cadherin) reduced the amount and profile of pro-healing cytokines + nanoparticles delivered to injured myocardium. **Conclusions:** Increasing cocoon diameter and cell occupancy within protective nanoporous gel cocoons boosts paracrine-mediated repair of damaged myocardium by slowing clearance of cells from injured tissues and the number of cytokines + nanoparticles secreted by micro-encapsulated cells.

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Poster Session 2 and Reception

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Functional Maturation of Human iPSC-Derived Cardiomyocytes by Prolonged 3D Culture in Engineered Cardiac Tissue Constructs

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The advent of human induced pluripotent stem cell (hiPSC) technology has revolutionized the way we study inherited diseases, as it allows us to study the effect of mutations in a human context. This is particularly true in the heart where species-specific differences has dramatic effects on heart rate, and expression of protein isoforms involved in excitation-contraction coupling. A major drawback of using hiPSCs is, however that cardiomyocytes derived from hiPSC (hiPSC-CM) are relatively immature, limiting their utility as a tool to study adult onset cardiac diseases.

We postulated that culturing hiPSC-CM in a 3D environment in engineered cardiac tissue constructs (ECT) would promote hiPSC-CM maturation.

ECTs were generated from lactate-selected day 30 hiPSCs that were mixed with isogenic hiPSC derived cardiac fibroblasts in a 10:1 ratio. Cells were mixed with fibrinogen and thrombin and seeded into molds under vacuum in FlexCell dishes to form ECT. ECTs were harvested 14-21 or 42-52 days later and subjected to functional testing, transcriptional analysis and top-down mass-spectrometry of sarcomeric proteins to assess maturation.

Functionally, we found that prolonged culture of hiPSCs in ECT resulted in increased calcium transient amplitude (0.856 vs. 0.462; $P < 0.01$) and an acceleration of calcium kinetics (calcium release time 67.7 ms vs. 109.3 ms; $P < 0.01$ and calcium decay time 148.3 ms vs. 188.0 ms; $P < 0.05$). This acceleration of calcium kinetics with time in culture was also true for twitch force kinetics (time to peak twitch force 178.6 ms vs. 208.0 ms; $P < 0.05$). Furthermore, beta adrenergic stimulation had a much greater effect on twitch kinetics in older ECTs (reduction in contraction time of 26.5% vs. 7.1%; $P < 0.01$).

Transcriptional analysis revealed an increase in expression level of the beta 1 adrenergic receptor in older ECTs, while top-down mass spectrometry showed increased expression and phosphorylation of cardiac troponin I (cTnI) and mono-phosphorylated cTnI, as well as decreased phosphorylation of alpha-tropomyosin, all markers of myocardial maturation. Taken together, these data supports our hypothesis that prolonged culture of hiPSC-CM in ECT promotes maturation of the calcium handling system and the contractile apparatus.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

Plasminogen Regulates Mesenchymal Stem Cell-mediated Tissue Repair After Ischemia Through Cry61

Hao Duan, Zhengqiang He, Alan Mitteer, Hyun-Jun Kim, Eujing Yeo, Hongyu Han, Ling Qin, Yi Fan, **Yanqing A Gong**, Univ of Pennsylvania, Philadelphia, PA

Objective Stem cell transplantation has emerged as a promising strategy for improving post-ischemic tissue repair. However, the poor survival and persistence of the transplanted cells including mesenchymal stem cells (MSCs) in the hostile ischemic microenvironments represents a major therapeutic barrier. Previous work shows that plasminogen (Plg) promotes post-injury tissue repair by mobilizing hematopoietic stem cell from bone marrow to circulation. Here our studies aim to define the role of Plg in microenvironment-dependent, MSC-mediated tissue repair after hindlimb ischemia. **Approach and Results** Our studies show that Plg stimulates MSC proliferation and migration under normoxia and promotes MSC survival under hypoxia. Hindlimb ischemia was induced in Plg-deficient (Plg^{-/-}) and wild-type (Plg^{+/+}) mice by ligation of femoral artery, followed by injection with fLuc/tomato⁺ MSCs into the ischemic limbs. Our data show that Plg deficiency abolishes MSC survival, migration, and proliferation in the ischemic limbs, and abrogates MSC-mediated blood reperfusion, neovascularization and tissue repair after ischemia, suggesting that Plg is critical for MSC-mediated tissue repair. Furthermore, multiplex cytokine array analysis identifies Cyr61, a pro-angiogenic factor, as a downstream target of Plg in MSCs. Plg cleaves and activates Cyr61, which is required for Plg-stimulated MSC survival and migration. Finally, Plg-mediated Cry61 cleavage further promotes EC migration and neovascularization in vitro and in vivo. **Conclusions** Our study suggests that Plg promotes MSC survival and persistence in ischemic limbs and improves tissue repair. We identify an underlying mechanism, by which Plg induces Cyr61 cleavage and activation in MSCs, enhancing MSC survival and migration and promoting EC migration and neovascularization. Thus, targeting Plg/Cyr61 may offer exciting therapeutic opportunities for strengthening MSC therapy in ischemic diseases.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

Modeling Atrial Fibrillation in a Dish Using Atrial iPSC Derived Cardiomyocytes

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Mutations in multiple genes have been linked with familial atrial fibrillation (AF) but the underlying pathophysiologic mechanisms and implications for therapy remain poorly understood. To characterize the electrophysiological phenotype of an AF-linked *SCN5A* mutation and assess novel mechanism-based therapies, we generated patient-specific atrial induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from a carrier with an *SCN5A*-E428K mutation and an unaffected family member using a differentiation protocol optimized for atrial differentiation generated atrial iPSC-CMs. The atrial iPSC-CMs carrying *SCN5A*-E428K displayed increased window and late Na⁺ current (I_{Na,L}), increased beating frequency and irregularity with triggered beats and prolongation of the action potential duration (APD) versus control atrial iPSC-CMs. The multi-electrode array (MEA) recordings of mutant atrial iPSC-CMs showed spontaneous arrhythmogenic activity with beat-to-beat irregularity. We further showed that targeted inhibition of the I_{Na,L} with ranolazine normalized the aberrant electrophysiologic phenotype in the *SCN5A*-E428K atrial iPSC-CMs and mexiletine reversed the sodium channel gating defects, and reduced beating frequency and irregularity. Our study illustrates the potential use of atrial iPSC-CMs for modeling AF in a dish, elucidating the underlying cellular mechanisms, and identifying novel mechanism-based therapies custom-tailored for individual patients.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Mir148a Family Regulates Cardiac Differentiation of Human Embryonic Stem Cells by Inhibiting The *Dll1*-mediated Notch Signaling Pathway

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) can differentiate into spontaneously beating cardiomyocytes in vitro, and hold great promise for cardiovascular disease modeling, therapy and drug discovery. However, these applications were hampered by low yield and purity of the in vitro differentiated cardiomyocytes. Although miRNAs represent a type of potential candidates to promote cardiac differentiation, most of cardiomyocyte-enriched miRNAs have not been functionally investigated until now. This study investigated the roles of *MIR148A* family in cardiac differentiation from hESCs. The *MIR148A* family is composed of *MIR148A*, *MIR148B* and *MIR152*, three highly conserved miRNAs sharing same seed sequences. The expression levels of all *MIR148A* family members were progressively increased during cardiac differentiation in vitro. By using CRISPR-Cas9-mediated knockout, we demonstrated that triple knockout of *MIR148A* family (*MIR148A*-TKO), rather than individual knockout of each members, could grossly inhibited TNNT2⁺ cardiomyocyte generation. Ectopic expression of *MIR152* significantly restored the cardiac differentiation efficiency of *MIR148A*-TKO hESCs. The transcriptome analysis identified a total of 1071 upregulated genes and 766 down-regulated genes in the *MIR148A*-TKO hESC-derived cells compared to wild-type hESC-derived cells at day 4 of cardiac differentiation. Gene Ontology analysis revealed that most genes down-regulated in *MIR148A*-TKO hESC-derived cells were involved in the events of lateral/cardiac mesoderm development, while the up-regulated genes were mainly involved in the events of paraxial/somitic development. Furthermore, the NOTCH ligand Delta-like1 (*DLL1*) was validated as the target gene of *MIR148A* family, and the knockdown of *DLL1* could inhibit target gene expression of Notch signaling pathway, and promote cardiac differentiation of *MIR148A*-TKO hESCs. Our findings demonstrate a new function of *MIR148A* family during cardiac differentiation. Synergistic inhibition of *DLL1*-mediated Notch signaling represents a major mechanism for the *MIR148A* family to inhibit undesired lineage formation and promote hESC differentiation into cardiomyocytes.

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Poster Session 2 and Reception

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Regulation of Cardiomyocyte Maturation by an RNA Splicing Regulator Rbfox1

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The maturation of stem cell derived cardiomyocytes (iCMs) is incomplete relative to the fully matured adult myocytes. Lack of maturation represents a major limitation to the applications of iCMs as heart disease models or heart failure therapies. Current attempts to promote iCMs maturation, such as prolonged culture, mechanical stretching and electronic pacing, are often based on empirical methods with poor reproducibility or little mechanistic basis. In order to better understand the molecular mechanisms driving cardiomyocyte maturation, we performed extensive transcriptome analyses in neonatal vs. adult hearts. In addition to metabolic and cell cycle regulatory pathways, Gene Ontology analysis revealed RNA splicing regulation is significantly enriched in the transcriptome reprogramming during postnatal maturation in heart. Specifically, we find a cardiomyocyte enriched RNA splicing factor Rbfox1 is dramatically induced in the perinatal maturing mouse hearts. Ectopic expression of Rbfox1 in neonatal cardiomyocytes markedly promotes the cellular and molecular features of adult cardiomyocyte, including contractility, calcium handling, sarcomere organization, morphology, electrophysiology and gene

expression. Most remarkably, expression of RBFox1 in human iPSC derived cardiomyocytes promotes similar maturation process as observed in the neonatal rat myocytes. At mechanistic level, RBFox1 expression in the iCMs enhances transcriptome maturation as indicated by targeted RNA splicing in genes involved in muscle contraction, gene expression, RNA processing and sarcomere organization. In summary, we have uncovered a novel molecular path towards neonatal myocyte maturation in perinatal murine hearts by targeted modulation of cardiomyocyte transcriptome via RNA-splicing. This approach has potential to be employed as a molecular approach to promote human iCMs maturation.

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Poster Session 2 and Reception

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A Novel Combination Therapy Using First Trimester Human Umbilical Cord-Derived Mesenchymal Stromal Cells and Endothelial Progenitor Cells Significantly Improves Angiogenesis and Cardiac Recovery Following Myocardial Infarction

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Introduction: Umbilical cord-derived MSC show pericyte-like characteristics and demonstrate significant angiogenic potential via paracrine and direct support to the vasculature. Endothelial progenitor cells (EPC) have the potential to initiate vascular structures while maintaining paracrine properties following ischemic injury. **Hypothesis:** A combination therapy of first trimester umbilical cord MSC (FTM HUCPVC) with rat EPC will promote significant angiogenesis, cardiomyocyte survival and lead to functional cardiac recovery in a rat model of MI. **Methods:** MI was induced by LAD ligation on 8-week-old Fox^{nu} rats. 1 week later, rats with notable decrease (<30%) in ejection fraction (EF) were randomly separated into 4 treatment groups (n=9) and received intramyocardial injection of: **G1:** Media; **G2:** FTM HUPVC (3x10⁶ cells/rat); **G3:** EPC (2.2x10⁶ cells/rat); **G4: FTM HUCPVC - EPC combination** (1:2, total cells 3x10⁶ cells/rat). Endpoint analysis was at 5-days and 4-weeks after injection. **Results:** 5-days after injection, exclusively **G4** animals showed significant cardiac recovery, improved EF and FS compared to **G1-G2-G3** (p<0.01). IHC analysis showed significantly less apoptosis (caspase-3), more protease activity and sarcomeric actin in combination groups **G4** compared to **G1-G2-G3** (p<0.001). More FTM HUCPVC and EPC colocalized adjunct to small capillaries in **G4**. Significant increase of human Angpt-2 was detected in **G4** compared to **G1-G2-G3** (p<0.0001). Analysis at 4-weeks post-injection showed significant improvements in end-systolic volume (128µl) and EF (43%) in **G4** compared to **G2** (160µl) (35%) **G3** (180µl) (30%) respectively (**B**). LV contractility (dpdt, tau) significantly improved in **G4** compared to **G1, G2, G3** (p<0.001). Significant reduction in scar tissue and increased LV myocardial mass was found in **G4 and G2**. IHC analysis showed significantly more and larger (9µm) capillaries localized in LAD injured myocardium in **G4** compared to **G1-G2-G3** (p<0.001). Significantly more sarcomeric actinin and connexin-43 was identified in **G4** compared to **G1-G2-G3**. **Conclusions:** Our results show the superior potential of FTM HUCPVC - EPC combination cell therapy for faster and greater cardiovascular repair and recovery compared to single-cell-type treatments for MI.

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Poster Session 2 and Reception

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Surface Engineering Strategies to Study Diseases of Heart and Skeletal Muscle

Aditi Jain, Venkatraman Ravi, Jafar Hasan, Manisha Behera, Kaushik Chatterjee, Nagalingam Ravi Sundaresan, Indian Inst of Science, Bangalore, India

There is an increasing need to develop simplified *in vitro* platforms that mimic the tissue environment to understand cardiovascular and musculoskeletal diseases. Towards this objective, we first explored different surface engineering strategies for culturing cardiomyocytes, which could be used for investigating disease conditions like cardiac hypertrophy. Firstly, we investigated the possibility of using human hair derived keratin as a simple, efficient and cost-effective substrate for culturing cardiomyocytes. Cardiomyocytes grown on keratin expressed cardiac specific markers and displayed

spontaneous contraction. We further evaluated the development of cardiomyocyte hypertrophy upon treatment with the agonist, phenylephrine. We observed the induction of hypertrophy at the transcriptional as well as signaling level. We also observed a marked increase in protein synthesis in these cells indicating the development of hypertrophy. Next, we employed microscale topography to confine cardiomyocytes along ridges which closely resembles mammalian heart. Cardiomyocytes grown on micro-ridges showed global alignment and elliptical nuclear morphology. Calcium currents traversed the cardiomyocytes in a directional manner and were also responsive to hypertrophic stimuli. Like cardiomyocytes, we also investigated the effect of aligned topography on primary myoblasts using nanofibers. These nanofibers retained the myotubes in culture for longer duration as compared to myotubes formed on flat surfaces. Recently, we have seen that once the myoblasts grown on flat surfaces become confluent they spontaneously differentiate to form myotubes even in the absence of differentiation cues. However, myoblasts grown on aligned fibers remain in their undifferentiated state and differentiate only upon induction with differentiation media. These results highlight the suitability of using keratin for cardiomyocyte culture and also emphasize the importance of topography in assessing cardiac and musculoskeletal function. We propose that studies which take into account the morphology of the cells offer greater potential towards clinical translation.

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Poster Session 2 and Reception

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Wnt / β -catenin Inhibitor Differentiates Human Mesenchymal Stem Cells into Myogenic Lineage *in vitro* and Improved Cardiac Function *in vivo* in Rat Model of Myocardial Infarction

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Cardiovascular disease (CVD) is one of the major causes of morbidity and mortality. At present, available therapeutic options for the treatment of cardiovascular diseases are limited and provide solution only to reduce the symptoms of CVD. The indigenous capability of cardiac tissue to meet the degeneration is limited. The feasible option is stem cells based regenerative medicine to repair or regenerate the myocardial cells and restore normal cardiac function. The aim of the present study is to assess the potential of small molecules for the differentiation of MSCs into cardiomyocytes. In this study, MSCs were cultured *in vitro* and were characterized by immunochemistry, and flow cytometry for the presence of MSCs markers, CD90, CD73, CD44, CD29, and for tri-lineage differentiation. MSCs were treated with wki-4 for 14 days to induce cardiac differentiation, and were characterized for the presence cardiac markers by gene and protein expression for GATA-4, α -actinin, cTnT, cTnI, and myosin heavy chain, and found positive for these markers. These induced cardiac progenitor cells were transplanted into the infarcted myocardium of rats, where they exhibited increased persistence, engraftment, and homing in the infarcted region, and expressed cardiac markers within the border zone. Transplanted group improved left ventricular wall thickness at 4 weeks post injury, and reduced infarct size. Functional performance of the hearts was analyzed through M-mode echocardiography, which results in significant ($p < 0.05$) improvement in the heart function including left ventricular internal diameter systolic and diastolic, ejection fraction, fractional shortening, end systolic volume and diastolic volume, and stroke volume compared to control. Histological examination of the heart sections 4 weeks post MI showed that MSCs home towards the site of injury. The transplanted cells expressed cardiac markers and contributed to the cardiac tissue recovery. The results of the present study demonstrate that myocytes derived of MSCs enhance the regeneration potential of the infarcted myocardium.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Adipose-derived Stem Cells With Thymosin Beta 4 Enhanced Neovascularization in Mouse Ischemic Hind Limb Model

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Adipose-derived stem cells (ASCs) have potential of differentiating into endothelial lineage and are easily obtainable, therefore they are promising candidates for stem cell therapy. Thymosin beta 4 (Tb4) is G-actin sequestering protein that

exerts a broad range of functions such as cell migration and angiogenesis. In this study, we investigated the effects of Tb4 on angiogenic and endothelial differentiation potential of ASCs. Exogenous treatment of Tb4 (100 ng/mL) induced a significant increase in endogenous mRNA expression of Tb4, and morphological changes with increased cell length in ASCs. In addition, Tb4-treated ASCs showed significantly higher endothelial differentiation potential than untreated ASCs after the induction of endothelial differentiation for 3 weeks. The expression of endothelial markers such as PDGFRb and CD144 were significantly enhanced by the treatment of Tb4 with the endothelial differentiation induction media compared to untreated ASCs. Indeed, Tb4-treated ASCs expressed higher expression of angiogenic genes including Ang-1, vWF, Tie1, CXCR4, uPAR, FGFR4, IGFR2, and VE-Cadherin than untreated ASCs, both cultured in the endothelial differentiation induction media. Moreover, a scratch wound healing assay revealed that the treatment of Tb4 significantly increased cell migration compared to untreated and Tb4-siRNA transfected ASCs. In the microbead sprouting assay, the treatment of Tb4 significantly augmented the number of sprouts per bead and the length of sprouts compared to untreated and Tb4-siRNA transfected ASCs. Furthermore, transplantation of ASCs with Tb4 induced neovascularization improved the blood flow in mouse ischemic hind limb models, compared to sham, ASC-transplanted, and Tb4-transplanted groups. Taken together, therapeutic application of ASCs with Tb4 could be useful to enhance endothelial differentiation and neovascularization.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Postnatal Cardiac Tissue Harbors Progenitor Cells With Unique Metabolic Profile

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Rationale: The developmental cardiac tissue is a proliferative organ capable of regeneration that extends briefly into the postnatal period. Studies show that the heart undergoes alterations in metabolism that coincide with cessation of regenerative processes during postnatal development. Whether cardiac cells in the early postnatal heart exhibit metabolic properties that favor regeneration remains unknown.

Objective: To determine whether postnatal cardiac tissue harbors CPCs with unique metabolic profile.

Methods and Results: CPCs were isolated from C57BL/6 mice aged 2-day (NCPC), 2-month (adult CPC) and 2-year old (ACPC). Morphological assessment showed NCPCs were smaller and rounded compared to CPCs and ACPCs and expressed putative stem cell markers and were negative for hematopoietic marker CD45. Interestingly, NCPCs expressed elevated levels of pluripotency markers such as LIN28 compared to ACPCs and CPCs. Increased viability, proliferation rate, metabolic activity and reduced doubling times were observed in NCPCs compared to CPCs and ACPCs measured by CyQuant, MTT, and Resazurin assays. NCPCs demonstrated a youthful phenotype and were resistant to H₂O₂ induced stress compared to ACPCs and CPCs as measured by β -Galactosidase and TUNEL labeling respectively together with a unique expression of paracrine factors as assessed by proteome profiler array. Interestingly, NCPCs demonstrated increased ATP generation, glycolytic activity and reduced oxidative phosphorylation compared to CPCs and ACPCs measured by Seahorse assay parallel with enhanced expression of glycolytic enzymes measured by qRT-PCR and western blot. NCPCs showed increased lactate production and pyruvate kinase activity and low mitochondrial membrane potential. Interestingly, mitochondrial fuel dependency assay showed increased glutamine dependency as the fuel for mitochondrial oxidative phosphorylation. RNA-sequencing analysis demonstrated increased expression of metabolic signaling in NCPCs compared to CPCs and ACPCs.

Conclusions: Postnatal cardiac tissue possesses progenitor cell population with unique metabolic profile that coincides with enhanced functional properties of NCPCs suggesting their potential therapeutic value for cardiac repair.

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Poster Session 2 and Reception

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Electrical Stimulation of Pediatric Cardiac-derived c-kit⁺ Progenitor Cells Improves Retention and Cardiac Function in Right Ventricular Heart Failure

Joshua T. Maxwell, David Trac, Ming Shen, Milton Brown, Michael E. Davis, Carly Zaladonis, Emily Baker, Martin L. Li, Daniel I. Jacobs, Emory Univ, Atlanta, GA

Nearly 1% of babies born in the US will be diagnosed with a congenital heart defect (CHD). While surgical therapy has improved survival, many of these children go on to develop right ventricular heart failure (RVHF). The emergence of cardiovascular regenerative medicine as a potential therapeutic strategy for pediatric HF has provided new avenues for treatment with a focus on repairing or regenerating the diseased myocardium to restore cardiac function. While primarily tried using adult cells and adult disease models, stem cell therapy is relatively untested in the pediatric population. Cardiac-derived c-kit⁺ progenitor cells (CPCs) have been widely studied as a cell-based therapy for cardiac pathologies; however, unless these cells are extracted at a very young age their therapeutic efficacy has been shown to be diminished. Finding novel ways to enhance the reparative potential of these cells is thus of critical significance. We previously reported pediatric CPCs (isolated from patients between 1-5 years of age) respond to electrical stimulation (ES) by initiating intracellular calcium oscillations. Here, we investigate the ability of ES to enhance the retention and therapeutic function of pediatric CPCs in an animal model of RVHF. Human CPCs isolated from pediatric patients were exposed to chronic ES and implanted into the RV myocardium of rats. Cardiac function and cellular retention analysis showed electrically stimulated CPCs (ES-CPCs) were retained in the heart at a significantly higher level and longer time than control CPCs and also significantly improved right ventricular functional parameters. ES also induced upregulation of extracellular matrix and adhesion genes, such as integrins $\beta 1$ and $\beta 5$, and significantly increased *in vitro* survival and adhesion of cells. Lastly, we show that ES induces CPCs to release higher levels of reported pro-reparative factors *in vitro*, including angiogenin, FGF, HGF, and VEGF. These findings suggest electrical stimulation can be utilized to increase the retention, survival, and therapeutic effect of human c-kit⁺ progenitor cells. Furthermore, our insights into the mechanisms of increased retention and paracrine function of ES-CPCs can have implications on a variety of cell-based therapies.

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Poster Session 2 and Reception

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Approach of mRNA Delivery by Using Nanoparticles to Recover Endocardial Notch Signaling

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Notch signaling plays a significant role in cardiac development and morphogenesis, especially for cardiac chamber development. Failure of mechanotransduction of endocardial Notch signaling in ventricular chamber causes lack of trabeculation. Advent of nanoparticle (NP) technology offers a therapeutic approach of targeted delivery. Although delivering mRNA is an effective method, it is challenging due to stability and cell defense mechanisms. To overcome the limitations and effectively deliver mRNA to endocardium, we used Poly(lactic-co-glycolic acid) (PLGA) synthesized NPs, which have been used in various drug delivery systems due to its biocompatibility, efficient encapsulation of hydrophilic and hydrophobic materials, specific targeting effects, and lower payload concentration. We hypothesized our PLGA NPs are able to deliver *NICD* mRNA to target endocardium to overexpress Notch signaling in a Notch inhibited zebrafish animal model. Using double emulsion, we synthesized PLGA NPs at 55–65 kDa and performed *in vitro* test on HUVECs. $69.8 \pm 4.3\%$ of Rhodamine B was successfully loaded into the NPs, and after incubation with HUVECs the signal lasted for 28 days after burst release. Hemolysis and whole blood clotting assays proved that NPs did not interfere with the blood clotting process or lyse blood cells. We characterized mRNA incorporated PLGA nanoparticles and incubated with HUVECs after antibody conjugation. To test *in vivo* study, we injected *gata1a* morpholino oligomer (MO) to *Tg(fli1:gfp)* transgenic line to inhibit hematopoiesis, which reduces wall shear stress (WSS) of ventricular chamber leading lack of trabeculae. At 2 days post fertilization (dpf), *NICD* mRNA incorporated nanoparticles were injected into the circulatory system of *gata1a* MO injected zebrafish to rescue trabeculation. At 5 dpf, zebrafish hearts were harvested and analyzed via qRT-PCR to compare Notch related gene expression among control, *gata1a* MO injected, and rescue group. The *NICD* mRNA injected group successfully rescued the expression level of Notch components despite a lack of WSS and mechanosensitive Notch signaling via *gata1a* MO injection. This finding will provide an initial therapeutic approach of *NICD* mRNA for trabecular related congenital heart disease.

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A 3D iPSC-derived Scaffold-Assisted Microfluidic Model of Ventricular Ejection

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Organ-on-a-chip technology fosters the potential of in vitro models of cardiac function. Nevertheless, modeling the ventricular ejection has been challenging due to the lack of 3D scaffolds of ventricular-like geometry that can accommodate the tissue while providing structural stability and contractile directionality. Using two-photon direct laser writing, we fabricate a concave, 1mm³ scaffold that is then embedded in a microfluidic, organ-on-a-chip system. We seed the system with iPSC-derived cardiac tissue and demonstrate the ability of the scaffold to preserve the 3D concave tissue structure, promote cardiomyocyte alignment and generate significant ejection fractions. The presented system sets the foundation for novel in vitro studies on ventricular function and remodeling.

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Poster Session 2 and Reception

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Ready-made Microvessels Robustly Integrate Into the Infarcted Coronary Vasculature Promoting Graft Perfusion, Enhancing Cardiac Remuscularization and Function

Sara Nunes Vasconcelos, Xuetao Sun, Jun Wu, Ren-Ke Li, Univ Health Network, Toronto, ON, Canada

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer an unprecedented opportunity to remuscularize infarcted hearts. However, the majority of hiPSC-CMs die post transplantation into the ischemic environment, limiting their regenerative potential. CM death occurs in the first few days post-transplantation due to ischemia. Attempts to promote intramyocardial vascularization (i.e. delivery of growth factors or cells) have not led to significant improvements due to poor cell retention and the long time required for new vessels to form and carry blood compared to the rapid death of transplanted CMs. Here, we used ready-made microvessels harvested from adipose tissue - that form a vasculature and carry blood within the first days post subcutaneous implantation - to re-vascularize ischemic rat hearts and improve hiPSC-CM survival. We performed left anterior descending artery ligation in immunocompromised rats to model MI. hiPSC-CMs (10×10^6) with (CM+V) or without (CM-only, control) microvessels were delivered by intra-myocardial injection 2 weeks post MI. Cardiac function was assessed by echocardiography and pressure-volume loop. Delivery of microvessels from GFP rats allowed assessment of microvessel persistence. Compared to hiPSC-CM transplantation alone, microvessels promoted a 6-fold increase in hiPSC-CM survival, with reduction in scar size and a significantly superior functional recovery. While delivery of CM-only stabilized the heart preventing further fractional shortening decline, delivery of CM+V resulted in reversal of fractional shortening loss. This was supported by PV-loop data (ejection fraction: sham, ~20%; CM-only, ~31%; CM+V, ~39%; 4 wks). Moreover, microvessels showed unprecedented persistence and integration (>60%, 4 wks), resulting in 2-fold increase in vessel area and graft perfusion (as early as day 5 post-transplantation). This was achieved despite the very low number of cells ($\sim 2 \times 10^5$) delivered in the form of microvessels. These findings provide a novel approach to cell-based therapies for MI whereby incorporation of ready-made microvessels can serve as a personalized delivery system to improve functional outcomes in cell replacement therapies.

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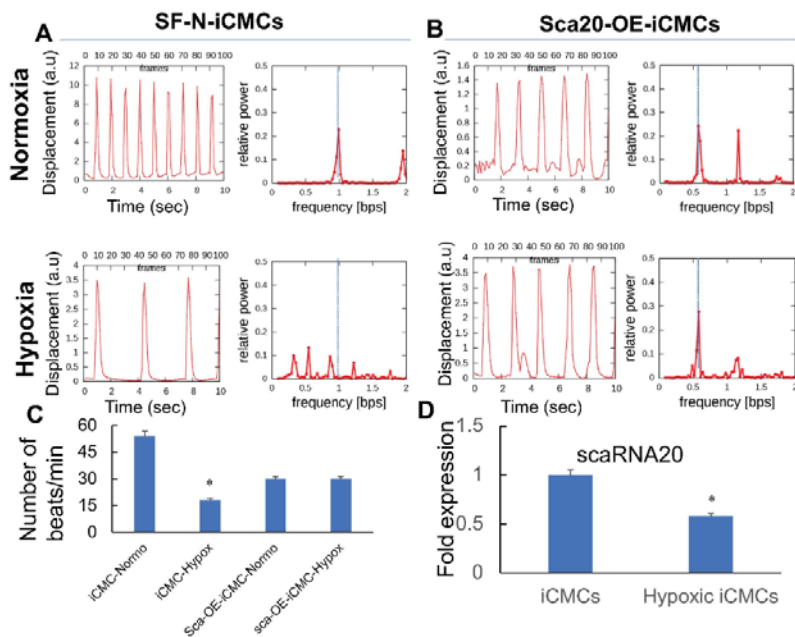
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Beneficial Effects of scaRNA Modulated Human iPSC-derived Cardiomyocytes Exposed Under Hypoxic Conditions

Sheeja Rajasingh, Vinoth Sigamani, Andras Czirok, Dona Greta Isai, Vijay Selvam, **Johnson Rajasingh**, Univ of Kansas Medical Ctr, Kansas City, KS

Introduction: Studies have shown that mRNA splicing is regulated by small cajal-body associated RNAs (scaRNAs) and plays a significant role in mammalian cardiac development and differentiation, but the potential contribution to human in vitro differentiation of iPSCs into CMCs remains unknown. In this study, the comparison of scaRNA20 modified and unmodified iPSCs derived-CMCs (iCMCs) will allow us to understand the role of scaRNAs in cardiac differentiation. **Hypothesis:** We

hypothesize that the scaRNA20 modulated iCMCs are capable of withstanding better at low-oxygen environment than the normal iCMCs which will facilitate for cardiac therapy. **Methods and Results:** To show how ischemic cardiomyopathy effects on CMC contractility, skin fibroblast derived normal iCMCs (SF-N-iCMCs) and scaRNA20-OE-iCMCs were subjected to hypoxic condition (1% O₂). Our particle image velocimetry (PIV) analysis shows that the contractility is reduced in normal iCMCs whereas the scaRNA20-OE-iCMCs maintained the contractility under hypoxic condition (**Fig. A-C**). Moreover, the qRT-PCR data show that the SF-N-iCMCs exposed to hypoxic condition decreased the expression of scaRNA20 (**Fig. D**). These data suggest that the scaRNA20-OE-iCMCs are capable of withstanding the low-oxygen environment at the infarcted heart. **Conclusions:** We report for the first time the beneficial effects of scaRNA20 modulated CMCs prior to transplantation will be an effective therapy for cardiac regeneration. <!--EndFragment-->



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Poster Session 2 and Reception

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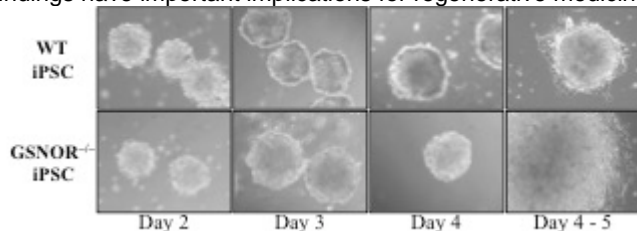
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S-nitrosylation Promotes Cell Cycle, Cell Viability and Proliferation by Activating the Snail/Slug Pathway in miPSC-derived CM

Alessandro Salerno, Amarylis Wanschel, Konstantinos Hatzistergos, Raul Dulce, Wayne Balkan, Joshua Hare, Univ of Miami, Miami, FL

INTRODUCTION: Epithelial-to-mesenchymal transition (EMT), is a crucial step in cardiac differentiation. Snail and Slug are increased and E-cadherin is decreased during EMT. Expression of *Snail* and *Slug* is reduced by GSK3 β , a key regulator of the Wnt/ β -catenin signaling pathway. S-nitrosylation (S-NO) of GSK3 β inhibits its activity. **OBJECTIVE:** We hypothesized that in the absence of S-nitrosogluthione reductase (GSNOR), the Snail/Slug pathway is activated in miPSC-derived cardiomyocytes. **METHODS:** We compared the initial growth and differentiation of iPSCs derived from GSNOR^{-/-} (iPSC^{GSNOR-/-}) and wild type (WT [control], iPSC^{WT}) mice on days (D) 0-6 in cell culture. Cell morphology was assessed and MTT, BrdU and transwell invasion assays performed to determine cell proliferation, invasion and migration. EMT-related transcription factors and members of the GSK3 β pathway were measured at D0-D6. **RESULTS:** During early differentiation, iPSC^{GSNOR-/-} cells exhibited accelerated loss of pluripotency markers and, from D4, greater proliferation/differentiation, and apparent EMT compared to iPSC^{WT}. MTT, BrdU and migration assays demonstrated that loss of GSNOR stimulated cell proliferation and migration. *Slug* and *Snail* were upregulated and *E-Cadherin* was downregulated in iPSC^{GSNOR-/-} suggesting that increased NO levels reduced GSK3 β activity in iPSC^{GSNOR-/-}. **CONCLUSIONS:** Our results suggest that the deletion of GSNOR affects early CM differentiation and promote EMT. These

findings have important implications for regenerative medicine and provide new targets for iPSC-based therapy.



A. Salerno: None. **A. Wanschel:** None. **K. Hatzistergos:** None. **R. Dulce:** None. **W. Balkan:** None. **J. Hare:** 7. Ownership Interest; Significant; Vestion Inc, Longeveron. 8. Consultant/Advisory Board; Significant; Vestion Inc, Longeveron.

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Organ-on-chip Model for Investigating Autonomic Innervation of the Cardiac Microenvironment

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Cardiac sympathovagal imbalance is a dysfunction of the parasympathetic and sympathetic nervous systems (PSNS and SNS) that, in tandem, regulate cardiac output in response to environmental stimuli. This autonomic nervous system (ANS) imbalance increases the risk of cardiac arrhythmias and sudden cardiac failure due to an inability to modulate heart rate following overexertion or excessive stress. Therefore, there is an immediate need to understand the underlying cellular mechanisms of cardiac innervation to develop new strategies to restore ANS balance. Moving away from the complexity and variability of *in vivo* models, we developed an *in vitro* microphysiological model of the cardiac ANS. Custom “cut and assembled” microfluidic organ chips were fabricated to support the 3D culture of cardiac cells and ANS neurons within a biomimetic scaffold. Using this system, on-chip innervation of the cardiac microenvironment was confirmed via immunostaining (Figure 1). Further, we demonstrated trending differences in cardiomyocyte beating between those innervated by each ANS neuron population (PSNS vs. SNS: 37.4 ± 6.7 bpm vs. 44.8 ± 7.9 bpm, $n = 8$, $p = 0.0716$) via video microscopy and custom software (MATLAB®). Investigations to alter cardiomyocyte function to promote the innervation of different neural populations within the cardiac microenvironment are ongoing. Specifically, beat rate will be increased/decreased with pharmacological agents and innervation quantified using commercial neuron tracing software (NeuroLucida®). Altogether, the development and use of this model will enable the systematic investigation of novel

therapies to restore cardiac sympathovagal balance.

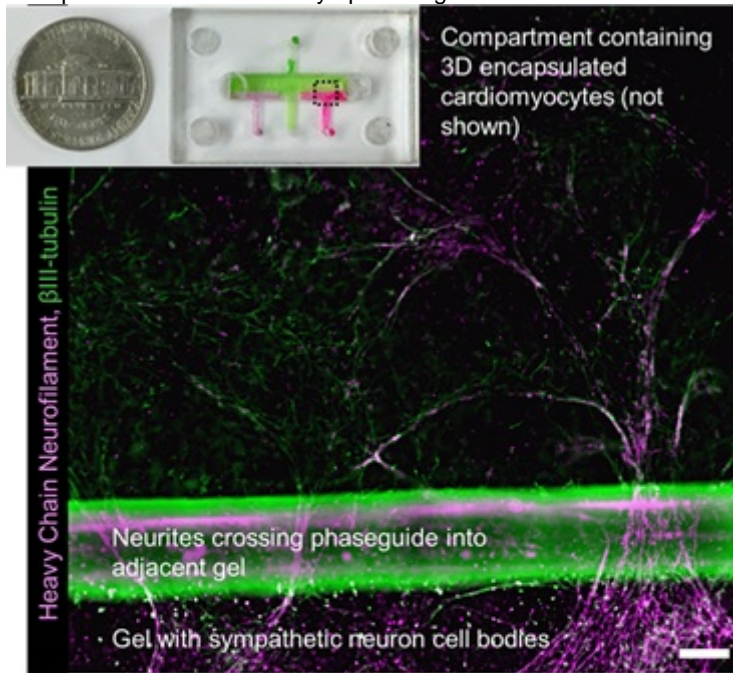


Figure 1. Custom microfluidic chip with distinct gel compartments for cardiac cells (green), parasympathetic neurons (purple), and sympathetic neurons (pink). The devices utilize a phase guide to compartmentalize cell-laden 3D hydrogels in the basal channel, and polycarbonate membrane to enable medium diffusion from the apical channel and mimic circulation. Immunofluorescent image of neurites extending towards cardiac cells. Scale: 100 μ m

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Placental Cdx2 Cells Regenerate Injured Myocardium

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The limited regeneration of adult mammalian heart has prompted the need to recognize novel strategies that can restore contractile function in heart disease. However, in cell-based therapies the lack of an appropriate cell-type that can differentiate to cardiomyocytes *in vivo* persists as an ultimate unmet need. Our prior study demonstrates that experimental myocardial injury in pregnant mice triggers the flux of fetal cells via the maternal circulation into the injured heart where they undergo differentiation into diverse cardiac cell fates. Among those fetal cells, the expression of Caudal type homeobox2 (Cdx2); a trophoblast stem cell marker was unique. To understand the intriguing role of placental Cdx2 cells in cardiomyogenesis, we utilized a lineage-tracing strategy to label fetal-derived Cdx2 cells with enhanced green fluorescent protein (Cdx2-eGFP). Cdx2-eGFP cells were characterized and assayed for cardiac differentiation *in vitro* and *in vivo* using a mouse model of myocardial infarction. Cdx2-eGFP cells clonally proliferated and differentiated into spontaneously beating cardiomyocytes and vascular cells *in vitro*, signifying a multipotent nature compared to the Cdx2 negative cell population. When administered via tail vein to infarcted wild-type male mice, Cdx2-eGFP cells selectively and robustly homed to the injured heart and differentiated to cardiomyocytes and blood vessels, significantly improving the contractility noted by magnetic resonance imaging. Proteomics and immune transcriptomics studies of Cdx2-eGFP cells compared to embryonic stem (ES) cells reveal that they appear to retain 'stem'-related functions of ES cells, but exhibit unique signatures for homing and survival in addition to being immunologically naive. Blocking CXCR4, during the migration of Cdx2-eGFP cells to SDF1 α suggested a possible role for SDF1-CXCR4 signaling in the mechanistic basis of homing. Advancing towards a translational role, we demonstrate that CDX2 expressing cells can be isolated from the chorionic region of human term placenta. Our

results herein may represent a paradigmatic shift in the way we approach early embryonic lineages and cell fate choices and will establish the translational potential of placental Cdx2 cells for cardiac repair.

S. Vadakke-madathil: None. **G. Larocca:** None. **K. Raedschelders:** None. **J. Yoon:** None. **J. Tripodi:** None. **V. Najfeld:** None. **J.E. Van-Eyk:** None. **H.W. Chaudhry:** 7. Ownership Interest; Significant; HWC is founder and equity holder of VentriNova, Inc. and inventor of pending patent 'Methods of Cardiac Repair.

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Cardiomyocyte Maturation and Multinucleation in Postnatal Swine

Nivedhitha Velayutham, Christina M Alfieri, Emma J Agnew, Kyle W Riggs, Richard S Baker, Farhan Zafar, Katherine E Yutzey, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH

Objectives: Cardiomyocyte (CM) cell cycle arrest and decline of mononucleated diploid CMs have been implicated in loss of regenerative potential in postnatal mouse hearts. Sarcomeric and extracellular matrix (ECM) maturation also occur concurrently in mice, influencing CM proliferative arrest. Recent studies show a 3-day neonatal period of cardiac regeneration in pigs similar to mice, but the dynamics of postnatal pig CM growth are unknown. Our objective is to explore cardiac cell cycling, growth and maturation in postnatal pigs to understand the events guiding loss of cardiac regenerative capacity in large mammals.

Methods & Results: Left-ventricular tissue from farm pigs (White Yorkshire-Landrace) at Postnatal day (P)0, P7, P15, P30, 2 months (2mo) and 6mo were utilized. CM dissociations revealed predominant CM mononucleation at birth in swine, with persistence of ~50% (1186/2537 cells, n=5) mononucleated CMs at P15, and ~10% (227/1785 cells, n=4) at 2mo. By 6mo, pig CMs are entirely multinucleated, exhibiting 4-16 nuclei per cell. Assessing hypertrophic growth in dissociated pig CMs revealed longitudinal CM growth relative to increased nucleation at all ages. However, onset of diametric hypertrophy only occurs beyond 2mo. When nuclear pHH3 and mRNA expression of cell cycle genes was assessed, pig hearts show robust cell-cycling up to 2mo. Also, fetal TNNI1 and MYH6 are active up to 2mo in pig hearts. Ongoing studies on collagen remodeling indicate ECM remodeling in swine occurs beyond P7. Future studies are designed to identify nuclear ploidy in postnatal pig CMs and measure cytokinesis.

Conclusions: Cardiac maturational events are staggered over a 2-6 month postnatal window in pigs, with older pig hearts exhibiting extensive CM multinucleation and differences in longitudinal versus diametric CM growth. These fundamental variations in CM growth characteristics are important to consider when designing preclinical trials for cardiac regenerative strategies in pigs. Also, despite a similar period of regenerative capacity as mice, pig hearts do not undergo loss of CM cell cycling and mononucleation until 2mo after birth. Utilizing pigs may thus offer unique opportunities to study aspects of heart regeneration unavailable in other animal models.

N. Velayutham: None. **C.M. Alfieri:** None. **E.J. Agnew:** None. **K.W. Riggs:** None. **R.S. Baker:** None. **F. Zafar:** None. **K.E. Yutzey:** None.

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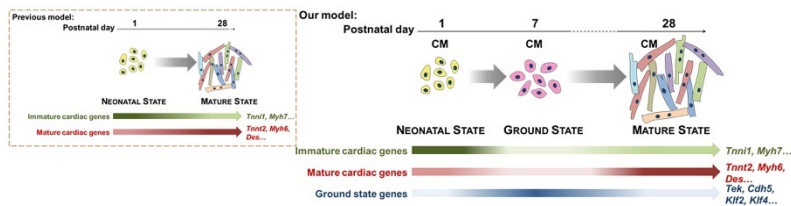
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Postnatal Resetting of Cardiomyocytes Toward Ground State as a Principal Step in Heart Maturation

Li Wang, Zheng Li, Fang Yao, Peng Yu, Dandan Li, Zongna Ren, Bingying Zhou, Fuwai Hosp, Chinese Acad of Medical Sciences, Beijing, China

The mammalian postnatal heart undergoes orchestrated and systematic changes to culminate in functional maturation, yet little is known about the cellular and molecular foundations underlying these processes. Here, we reported that cardiomyocytes (CMs) epigenetically and transcriptionally shut down cardiac gene expression immediately after birth, and reset into a ground state with potential enhancers for further programming CMs into distinct subtypes during heart maturation. Large-scale single-cell RNA-sequencing and genetic lineage tracing confirmed the presence of a postnatal ground state of CMs (pngCMs) that were in highest abundance around postnatal day 7 (P7), and recapitulated the developmental route of postnatal CM development. Importantly, this fate resetting mechanism used by CMs was conserved in neonatal heart regeneration upon surgical resection. Furthermore, P7-pngCMs exhibited remarkable regenerative capacity in infarcted hearts. Collectively, our data uncovered CM fate resetting as a pivotal mechanism in postnatal heart

development and regeneration, and supported a new model to understand how organs adapt to abrupt environmental changes to gain maturity upon birth.



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Role of MLF1 in Cardiomyocyte Cell Cycle Regulation

Feng Xiao, Jainy Savla, Alisson Campos Cardoso, Ana Macedo Periera, Shalini Muralidhar, Diana Canseco, Hesham A Sadek, UT Southwestern Medical Ctr, Dallas, TX

The neonatal mammalian heart is capable of significant regeneration after cardiac injury. However, that ability is lost in the early postnatal period, coinciding with the development of cardiomyocyte cell cycle arrest. Myeloid leukemia factor 1 (MLF1) is a protein expressed in hematopoietic cells, skeletal muscle and cardiac muscle. In hematopoietic cells, MLF1 is thought to be a negative cell cycle regulator, possibly acting through a p53-dependent mechanism. However, the role of MLF1 in the cardiovascular system is not well understood. Here, we describe MLF1 as a regulator of cardiomyocyte cell cycle progression. We first examined the expression pattern of MLF1 in neonatal mouse heart. MLF1 protein specifically expresses in the nucleus, and the expression level increases from P1 to P7 as revealed by quantitative PCR and Western Blot. To elucidate a potential role of Mlf1 in cardiomyocyte proliferation, we silenced Mlf1 in neonatal rat ventricle myocytes (NRVMs) which showed increased cardiomyocyte proliferation by staining with a mitosis marker phospho-Histone 3 (pH3). Then we generated an inducible cardiac-specific Mlf1 knockout mouse model and assessed for the prolongation of the proliferative window by echocardiography, cell size analysis, and immunostaining for cell cycle markers. Staining the Mlf1 KO hearts with proliferation markers, pH3 to determine mitosis and Aurora B kinase to determine cytokinesis, indicated that deletion of Mlf1 resulted in increased cardiomyocyte proliferation. It also showed improvement in left ventricular systolic function following myocardial infarction by echocardiography. In contrast, overexpression of MLF1 in heart showed decreased cardiac function as quantified by ejection fraction. These results identify MLF1 as a negative cell cycle regulator of cardiomyocyte proliferation.

F. Xiao: None. **J. Savla:** None. **A.C. Cardoso:** None. **A.M. Periera:** None. **S. Muralidhar:** None. **D. Canseco:** None. **H.A. Sadek:** None.

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Single Cell RNA-seq Analysis of Dynamic LIF Control of Adipose Derived Stem Cells Transition to Cardiomyocytes

Jiayi Yao, **Jiayi Yao**, UCLA, Los Angeles, CA

Heart failure continues to be a leading cause of human mortality worldwide and is the consequence of various forms of myocardial injuries that compromise the pumping ability of the heart. Cellular fate transition through manipulation of modifying factors holds great promise for large-scale production of cardiomyocytes required for therapy and for revealing principles of gene regulation in the process. In this study, we found leukemia inhibitory factor (LIF) acts directly on adipose derived cells to induce cardiomyogenesis by JAK/STAT3. LIF induces adipose-cardiac transdifferentiation in adipose derived stem cells, which exhibits morphology and function typical of beating cardiomyocytes. However, the cell population in adipose tissue that generates cardiomyocyte is not from adipocytes or stromal cells, as examined by Cre-Loxp system and FACs. Here, using single cell RNA sequencing, we identified a cell population in adipose tissue that undergoes a gradual

and simultaneous switch from relatively quiescent fate to proliferate cardiomyocyte like cells. By trajectory alignment, we compared the gene expression kinetics across fresh adipose cells and LIF induced cardiac transition process to identify crucial genes involved in the transition. Our findings may provide a better understanding of the mechanism underlying LIF-induced adipose-cardiac transdifferentiation, therefore reveal new therapeutic strategies for the treatment of myocardial injuries, and provide crucial information for regenerative medicine.

J. Yao: None. **J. Yao:** None.

Poster Session 2 and Reception

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Impaired Autophagic Off-rate Causes Cardiac Aging in Progeria Mouse Model

Yasuko K Bando, Takahiro Kamihara, Toyooki Murohara, Nagoya Univ, Nagoya Aichi, Japan

[Background/Introduction] Aging is known to be one of the primary causes of heart failure. Werner syndrome is one of the aging disorder due to dysfunction of DNA helicase-regulatory protein (WRN) and exhibiting progressive phenotype of natural aging. Ample evidences demonstrated progeria may promotes autophagic disorder. [PURPOSE] To elucidate whether autophagic disorder may be mechanistically responsible for cardiac aging.[Methods] We employed progeria mouse model harboring amino acid (AA) substitution of WRN at position 577 (WRN-K577M, 18 week-old male).

[Results] WRN-K577M exhibited diffuse left-ventricular (LV) hypertrophy, enhanced fibrosis, and diastolic LV dysfunction with preserved systolic ejection fraction. DNA microarray analysis of WRN-K577M heart revealed that 253 genes were upregulated compared to age- and gender-matched wild-type counterpart. Sixteen genes were increased > 4 fold higher than wild-type as follows: hypertrophy (Myh7, Kikb11), fibrosis (CTGF), inflammatory molecules (Ap1s3, Pla2g2e, Has1, MMP9), and oxidative stress (catalase). Cardiac aging markers (PARP-1, p53 and γH2AX) increased in heart of WRN-K577M with concomitant increase in oxidative stress (DHE staining) and apoptosis (TUNEL). Notably, autophagic turnover markers (p62 and LC3-II/I) were increased in myocardium of WRN-K577M, which was refractory to fasting-induced increase in on-rate of autophagy. Consistently, one of the key regulators of autophagy is the target of rapamycin, TOR kinase, which is the major inhibitory signal that shuts off autophagy with concomitant activation of Akt signaling. Collectively, these data indicates that the on-rate step of autophagy could be pathologically augmented with disruption of downstream process, i.e. impaired off-rate pathway that modulates turnover of autophagosome and lysosome under cardiac aging observed in WRN-K577M. Changes in autophagosomal and lysosomal accumulation were monitored by specific fluorescence indicators and chloroquine (50 microg/g body weight) augmented lysosomal accumulation with reduced LC3-II/I ratio.

[Conclusion(s)] In WRN-mutant progeria model, off-rate disorder of cardiac autophagy is, at least in part, the cause of cardiac aging.

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The Capillaries in Ischemic Border Zone: Origin And Role in Myocardial Infarction and Heart Failure

Jiqiu Chen, Lifan Liang, Roger J Hajjar, Mount Sinai Sch Med, New York, NY

It is a general believe that a blocked artery and its capillary are the main factor to determine myocardial infarction (MI) size and cardiac dysfunction. We have be plagued by the problem in repairing and regeneration of MI for many decades. How to improve the infarcted myocardium? By relieving the obstruction in coronary artery or promoting the angiogenesis of capillaries in the ischemic border zone? How to regrow the deteriorated capillary? From where? It is incredible to image the role of nearby non-ligated vessels in myocardial death and cardiac depression. In the present study, we show evidences that ischemic MI are not determined solely by the occluded artery, but also involved the nearby non-occluded artery, especially its capillaries that contributed nearly one-third effort to the MI. The mechanism is unclear yet, but this phenomenon is very interesting, offers a novel perspective to study heart failure. Arterial resistance to ischemia is a new object in heart failure research. It is unclear why some arterial vessel could survive in transmural MI, more interestingly it is unknown if capillaries could be regenerated from the residue arterial vessel in MI. The role of capillary both in ischemic artery and nearby non-ischemic branch in MI development is an amazing challenge. It is unknown why occlusion of left branch of LCA could

degenerate capillaries on right branch of LCA. Inflammation probably plays a role in this phenomenon, but the knowledge of its mechanism is totally lacking even it indicates a potential path to new treatment strategy of ischemic heart disease.

J. Chen: None. **L. Liang:** None. **R.J. Hajjar:** None.

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Rapamycin Can Ameliorate Antiretroviral Drug Mediated Cardiotoxicity

Spoorthi Alapati, **Manish K Gupta**, Ctr for Translational Med (CTM) in Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA

Introduction: In current therapy, due to application of combined antiretroviral drugs (cART), HIV related mortality significantly reduced, and AIDS became a chronic disease. Although, cART treatment increases life expectancy of AIDS patients, growing AIDS population suffer due to several organ diseases and heart failure is one of the leading causes of comorbidity and mortality.

Hypothesis: We tested the hypothesis that application of cART induces cardiotoxicity through inhibition of cellular protein quality control (PQC) and dysregulation of mitochondrial function.

Method and results: Cellular protein quality control was analyzed using cardiac tissue samples from HIV+ patients treated with cART and healthy donors. Western blot shows that there is upregulation of autophagy marker protein LC3 II in ART treated heart compared to healthy donor. Additionally, we found that there are abnormalities in the mitochondrial oxidative phosphorylation complexes (OXOPHOS) in the ART treated patient's heart, compared to healthy donor heart. Using neonatal rat ventricular cardiomyocytes, we tested the role of ART in cellular PQC and mitochondrial function. Our data shows that cardiomyocytes treated with the cART (Ritonavir+ Atazanavir+ abacavir+ Lamivudine) have increased expression of autophagy proteins LC3 II and P62 and higher level of pELF2 α . These suggest that, the cardiomyocytes treated with ART have dysregulation of autophagy and increased ER stress. Further, we found that cardiomyocytes treated with cART have high level of mitochondrial superoxide and decreased mitochondrial membrane potential. Mitochondrial function detected in whole cells shows that in ART treated cardiomyocytes there is significantly low OCR compared to control cells. Interestingly, application of pharmacological drug rapamycin can improve the cellular PQC and mitochondrial function in ART treated cells.

Conclusion: ART treatment induces cardiotoxicity through inhibition of cellular PQC and dysregulation of mitochondrial function and rapamycin might be important therapeutic drug to reduce the ART mediated cardiotoxicity.

S. Alapati: None. **M.K. Gupta:** None.

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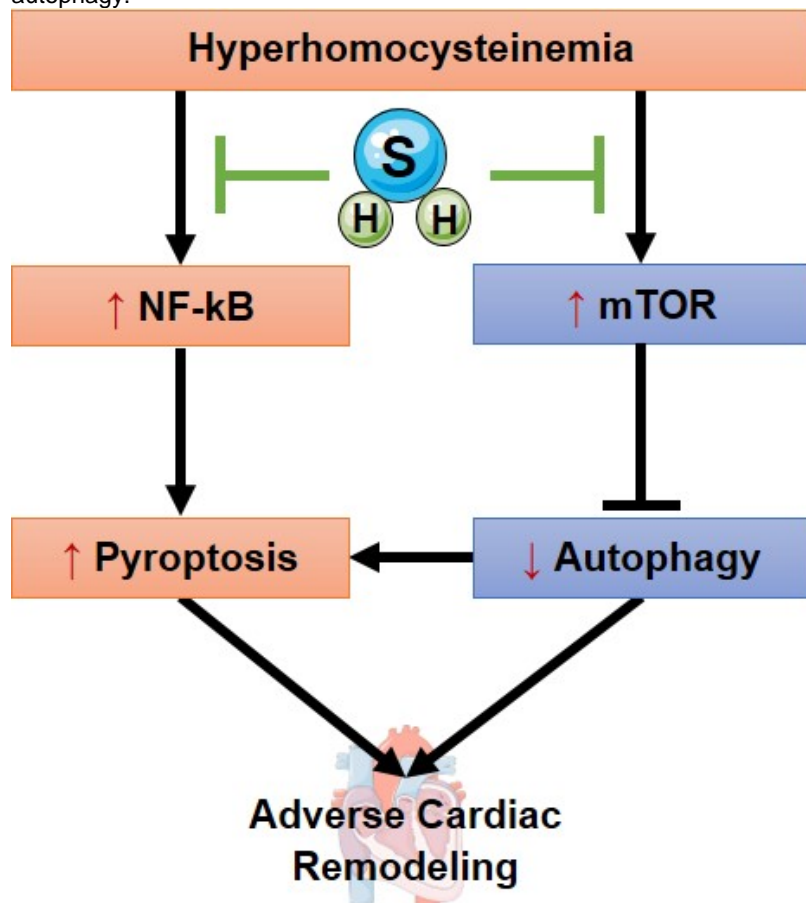
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Hydrogen Sulfide Protects the Heart Against Homocysteine-Induced Remodeling by Regulating Autophagy and Pyroptosis

Sumit Kar, Santosh K. Yadav, Univ of Nebraska Medical Ctr, Omaha, NE; Ravi Goyal, Univ of Arizona, Tuscon, AZ; David J. Lefer, Louisiana State Univ Health Sciences Ctr, New Orleans, LA; Paras K. Mishra, Univ of Nebraska Medical Ctr, Omaha, NE

Hydrogen sulfide (H₂S), a byproduct of homocysteine (Hcy) transsulfuration, protects against adverse cardiac remodeling in diabetic cardiomyopathy, hyperhomocysteinemia (HHcy), and other cardiomyopathies. However, the underlying molecular mechanisms are unclear. Pyroptosis is a caspase 1-mediated cell death mechanism that promotes remodeling. It is inhibited by autophagy, a lysosomal degradation process that maintains cellular homeostasis. We hypothesized that H₂S supplementation inhibits Hcy-induced pyroptosis by increasing autophagy in the heart. We treated CBS^{+/-} mice (a model of HHcy) and WT mice with SG1002 (slow-releasing H₂S donor) or control diet for 14 weeks and performed deep sequencing of their hearts (LV tissue). Ingenuity Pathway Analysis revealed SG1002 downregulated cardiac hypertrophy genes, plausibly by altering upstream regulators involved in pyroptosis and autophagy (IL-1 β and mTOR). Western blot results showed that SG1002 suppresses mTOR in CBS^{+/-} hearts (WT: 2.1 \pm 0.83, CBS: 10 \pm 2.4, CBS+SG: 3.8 \pm 2.3, WT+SG: 0.96 \pm 0.39) to induce autophagy. SG1002 decreases IL-1 β (WT: 0.61 \pm 0.08, CBS: 0.88 \pm 0.07, CBS+SG: 0.32 \pm 0.01, WT+SG: 0.38 \pm 0.03) and inhibits caspase-1 activity to suppress Hcy-induced pyroptosis. NF- κ B p65, which activates pyroptosis, is also downregulated by SG1002. We conclude that H₂S could alleviate cardiac remodeling by inducing autophagy and inhibiting NF- κ B-mediated pyroptosis. This is the first report that H₂S controls cross-talk of cardiac pyroptosis and

autophagy.



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Poster Session 2 and Reception

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Acidifying Nanoparticle Upregulates Autophagy and Enhances Cardiomyocyte Survival after Chemotherapy

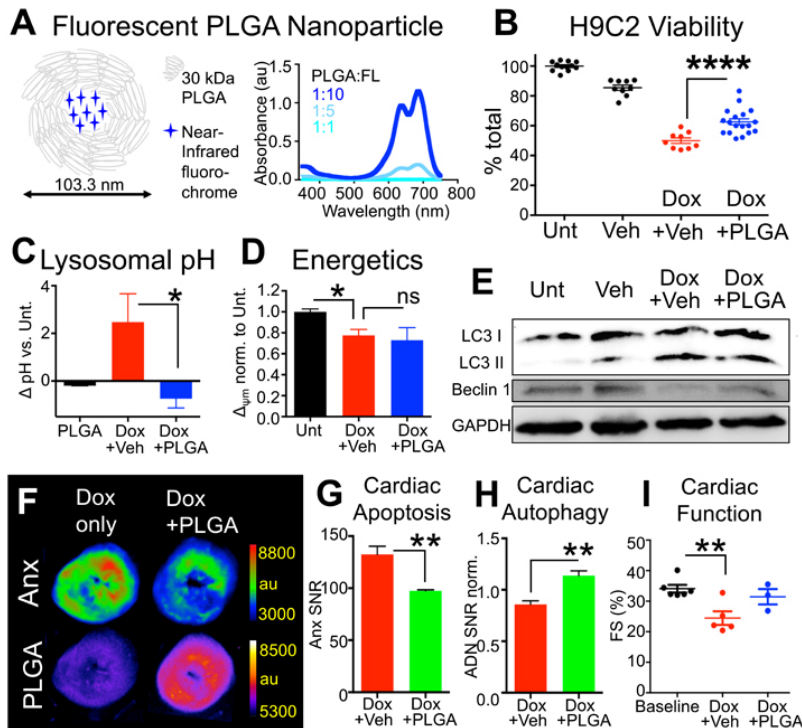
Zehedina Khatun, Lin Meng, Gregory Martin, Tanjheela Jahan, Lan Wei, Tufts Medical Ctr, Boston, MA; Hushan Yuan, Lee Josephson, Choukri Mekkaoui, David E. Sosnovik, Massachusetts General Hosp, Boston, MA; Robert M. Blanton, Howard H. Chen, Tufts Medical Ctr, Boston, MA

Introduction Chemotherapy-induced cardiotoxicity remains prevalent and deadly, with no effective therapy to slow the progression to irreversible heart failure, which in the case of doxorubicin (Dox) can reach 50% mortality. Dox impact on cardiomyocytes (CMs) is multifocal, including oxidative stress, lysosome alkalinization, and apoptosis. PLGAs, poly(DL-lactide-co-glycolide), are FDA-approved polymers, form defined nanoparticles (NPs). We hypothesize that PLGA NPs target the lysosomal compartments, restore lysosomal acidity after Dox, and confer cardioprotective effects.

Methods We synthesized fluorescent PLGA NPs by click chemistry. In H9C2 myocytes exposed to 1-5 μM of Dox +/- 1 mg/ml of PLGA, we measured cell viability (MTT), lysosomal pH (OG-514), mitochondrial membrane potential (JC-1), and autophagy proteins (western blot). We further co-injected C57Bl6 mice with PLGA (10 mg/kg, i.v.) and Dox (15 mg/kg, i.p.), to image apoptosis (Annexin) and autophagy (cathepsin-activatable autophagy probe, ADN, unpublished) after 24 hrs, or 4 mg/kg Dox weekly, 5x, i.p. and echo after 3-4 weeks.

Results PLGA NPs formulated are of controlled size and valency, emits near-infrared fluorescence (Fig. A). In H9C2s, PLGA significantly improved survival after Dox (Fig. B), acidified lysosomes (Fig. C), did not impact cell energetics (Fig. D), and further restored autophagic flux (Fig. E). Imaging in Dox mice revealed significant apoptosis reduction and autophagy activation (Fig. F-H), and cardiac function recovery (Fig. I).

Conclusions PLGA NPs may represent a novel class of cardioprotective therapeutics of early chemotherapy stress, and with translational potential.



A. PLGA NPs were functionalized with near-infrared fluorescence by covalent attachments, characterized by size and UV spectra, to achieve precise multivalency for robust readouts *in vivo*. **B.** In H9C2 cells challenged with Dox, PLGA significantly improved survival (triplicates, repeated 3x). **C.** PLGA is inert, with a dissociation constant of 3.8 close to normal lysosomal pH, and restored Dox-induced alkalinization (n=3). **D.** Mitochondrial membrane potential ($\Delta\psi_m$) was significantly reduced by Dox, but no change was seen with PLGA (n=3). **E.** Western blot showed Dox-induced autophagy impairment evident by elevated LC3 II and reduced Beclin-1. PLGA treatment reduced LC3 II accumulation and upregulated Beclin-1 levels (n=3). **F.** Representative mid-left ventricular view of Dox mouse hearts with robust Annexin V (Anx) uptake/apoptosis in the Dox only mice, but not in Dox mice treated with PLGA. PLGA fluorescence confirms uptake in the myocardium. **G-H.** Quantification of myocardial signal reveals a statistically significant 26.4% reduction in Annexin fluorescence/apoptosis, n=4 Dox only mice, n=5 Dox+PLGA mice, and a significant 32.7% increase in ADN signal/autophagy, n=7 Dox only mice, n=4 Dox+PLGA mice. **I.** Cardiac function in chronic Dox mice (n=5) 4 weeks after injection significantly reduced fractional shortening (FS), while no difference was seen with PLGA+Dox (n=3) compared to baseline (n=6). au=arbitrary unit. SNR=signal-to-noise ratio. Statistics: ANOVA (**B-D, I**) and t-test (**G-H**) were performed, *p<0.05, **p<0.01, ****p<0.0001, ns=not significant. PLGA=PLGA nanoparticle throughout the text.

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SUMOylation Site in MCL-1 Regulates its Anti-Apoptotic Activity

Leonardo J Leon 92111, Alexandra G Moyzis, Asa B Gustafsson, Univ of California San Diego, San Diego, CA

Myeloid Cell Leukemia-1 (MCL-1) is an anti-apoptotic BCL-2 protein upregulated in various types of cancer. It is also highly expressed in the myocardium. We previously found that MCL-1 plays a key role in maintaining mitochondrial homeostasis and myocyte survival. Cardiac specific deletion of MCL-1 in myocytes leads to mitochondrial dysfunction, loss of myocytes, and rapid development of heart failure. However, MCL-1's function in the heart is unclear and the exact mechanism by which

MCL-1 protects against apoptotic stimuli is not fully understood. Studies have reported that MCL-1 is subjected to phosphorylation and ubiquitination at several different residues which leads to changes in its stability. MCL-1 has a short half-life and proteasomal-mediated degradation of MCL-1 allows for apoptosis to proceed. Here, we screened the amino acid sequence of MCL-1 to identify novel sites of post-translational modifications involved in regulating MCL-1 function. We discovered the presence of a sumoylation site at lysine 219 that is conserved between species. To examine the function of the site, we generated a mutant of MCL-1 where the lysine residue was mutated to an arginine, MCL-1K219R. First, we investigated whether the sumoylation site was involved in regulating protein stability of MCL-1, but we found no differences in the degradation rates between MCL-1 and MCL-1K219R at baseline and after staurosporine treatment. Interestingly, MCL-1K219R failed to protect against staurosporine-induced apoptosis, while both MCL-1 and MCL-1K219R protected against doxorubicin-mediated cell death. This suggests that the mechanism of doxorubicin-mediated cell death is different from staurosporine. Moreover, MCL-1 has been reported to prevent activation of apoptosis via the mitochondrial pathway by sequestering pro-apoptotic BCL-2 proteins such as PUMA and NOXA. Hence, we examined whether MCL-1 sumoylation is involved in regulating its interaction with pro-apoptotic proteins. A better understanding of how MCL-1 regulates cell survival will not only aid the development of drugs that combat harmful cells, but will also help the production of drugs that effectively protect limited and valuable cells like myocytes.

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Role of Beclin1 in Regulating Parkin-mediated Mitophagy

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Autophagy is a cellular quality control mechanism involved in the selective elimination of damaged organelles and cytotoxic protein aggregates. Beclin1 is a component of the PI3 kinase complex that initiates autophagy in cells. Here, we have investigated the role of Beclin1 in autophagosome formation and Parkin-mediated mitophagy. Using Beclin1-deficient mouse embryonic fibroblasts (MEFs), we found that autophagosomes are still formed in the absence of Beclin1 in response to various treatments known to induce autophagy (FCCP, nutrient deprivation, rapamycin). Autophagic flux is also intact in the Beclin1^{-/-} MEFs, indicating an alternative mechanism of autophagy is induced. Next, we examined whether elimination of mitochondria via autophagy (mitophagy) was intact in Beclin1^{-/-} MEFs. Unexpectedly, we observed that Parkin-mediated mitophagy was significantly reduced in the absence of Beclin1. We confirmed that Parkin was recruited to depolarized mitochondria in both WT and Beclin1^{-/-} MEFs which correlated with increased ubiquitination of mitochondrial proteins. However, high resolution imaging revealed that autophagosomes failed to sequester mitochondria that had been labeled by Parkin. We observed that Parkin was degraded in Beclin1^{-/-} MEF's, but not WT, after mitochondrial stress. We also found that Beclin1 selectively localized as discrete puncta on Parkin-positive mitochondria suggesting a potential role for Beclin1 in linking mitochondria to autophagosomes. In contrast to our findings in MEFs, characterization of autophagy in mice with cardiac specific deletion of Beclin1 revealed that these hearts had a reduced number of autophagosomes which correlated with a reduction in autophagic flux as indicated by accumulation of cytosolic LC3I and p62. Beclin1-deficient hearts also had significantly reduced Parkin protein levels without changes in mRNA levels, suggesting an impairment in mitophagy. Overall, these findings suggest Beclin1 is required for the proper targeting of mitochondria into autophagosomes and that the adult heart is dependent on Beclin1 to induce formation of autophagosomes.

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PRMT5-induced Arginine Methylation Mediates Energy Stress-induced Autophagy in the Heart

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Autophagy is a self-degradative process that is important for protecting cells and organisms against stress and plays a protective role in the heart. However, the underlying mechanism by which autophagy protects the heart against stress is poorly understood. ATG proteins are required for autophagy and modulated by post-translational modifications (PTMs) such

as phosphorylation, ubiquitination, and acetylation. The level of arginine methylation, another form of PTM, is altered in the heart in response to stress. Our hypothesis is that arginine methylation plays an important role in the regulation of autophagy in the heart during stress. To this end, we examined the level of arginine methylation in response to energy stress *in vivo*. We observed a time-dependent increase in arginine methylation in the mouse heart under starvation conditions for 24 to 48 hours. Arginine methylation was increased (2.32-fold, $p < 0.01$) after 48 hours of fasting compared to in those without fasting. Expression of protein arginine methyltransferase 5 (PRMT5) was increased at both the mRNA and protein level (1.6- and 1.72-fold, $p < 0.05$) in the mouse heart after 48 hours of starvation. In order to investigate the role of endogenous PRMT5, we knocked down PRMT5 with adenovirus harboring sh-RNA. LC3-II, a marker of autophagy activity, was increased during glucose deprivation in sh-scramble-treated cardiomyocytes (CMs) (2.14-fold, $p < 0.01$), but not in sh-PRMT5-treated CMs. Arginine methylation was reduced in response to glucose deprivation in CMs when PRMT5 was downregulated. Mice were also injected intraperitoneally with a PRMT5-specific inhibitor (EPZ015666, 2 mg/kg) and hearts were harvested after 48 hours of starvation. Both arginine methylation levels and autophagy activity in the heart were decreased in the presence of EPZ015666 compared to in non-treated mice. Increased arginine methylation was also observed in the mouse heart in response to 1 hour of myocardial ischemia (1.87-fold, $p < 0.01$), which was accompanied by upregulation of PRMT5 (1.54-fold, $p < 0.05$). Taken together, these data suggest that arginine methylation mediated through PRMT5 may play a key role in mediating autophagy during energy stress conditions such as starvation and ischemia.

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GRAF1 is a Novel Mediator of Parkin-dependent Mitophagy

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Selective autophagic removal of damaged mitochondria by Parkin-mediated mitophagy is essential for metabolic homeostasis of long-lived cells and defects in this clearance pathway leads to the accumulation of toxic intermediates and accelerates the pathogenesis of numerous cardiometabolic diseases. This process is facilitated by the recruitment of the autophagosomal protein, LC3, by LC3-binding receptors that accumulate on damaged mitochondria. However, little is known about the precise LC3-receptors that mediate Parkin-dependent mitophagy in cardiomyocytes. Having previously identified a cardioprotective role for GRAF1, our current data indicate that GRAF1 plays critical role in promoting Parkin-dependent mitochondrial clearance. GRAF1 is expressed at high levels in the heart from E17 onwards and is poised to co-regulate actin- and lipid-dynamics by virtue of its multi-domain structure that includes a lipid binding/bending BAR domain, a phospholipid binding PH domain, a Rho-GAP domain, and a protein-interaction SH3 domain. Importantly, GRAF1 depletion in primary cardiomyocytes led to impaired mitochondrial OXPHOS-mediated ATP generation, mitochondrial membrane depolarization, increased mitochondrial-associated ROS, and increased ischemia/reperfusion-dependent myocyte death. These outcomes were accompanied by reduced LC3 mediated autophagic flux and accumulation of damaged mitochondria (and Parkin) in GRAF1 deficient cardiomyocytes and hearts. Mechanistically, we showed that GRAF1 facilitates Parkin-dependent mitophagy by serving as a novel LC3 receptor. GRAF1 is recruited to damaged mitochondria, is phosphorylated in a PINK1/Parkin-dependent fashion, and physically interacts with Parkin and LC3. While multiple LC3 receptors are recruited to depolarized mitochondria and act in a redundant function to promote mitochondrial engulfment, our studies reveal a unique and indispensable role for GRAF1 in this process. Our studies reveal that GRAF1 is distinctive because it facilitates mitochondrial clearance by further facilitating recruitment of Parkin and additional down-stream receptors to damaged mitochondria in a feed-forward fashion.

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The Role of Brown Adipose Tissue in Aging-Induced Cardiovascular Impairments

Eaman Abay, Megan M Mansour, Carmem Peres Valgas Da Silva, Lisa A Baer, Vikram Shettigar, Kristin I Stanford, Mark T Ziolo, Ohio State Univ, Columbus, OH

Adults age 65 and older are the fastest-growing demographic in the United States population and pose a significant healthcare challenge. Among several other physiological changes, aging is notably associated with an increased risk of developing cardiovascular disease. Aging leads to a decrease in brown adipose tissue (BAT), a thermogenic tissue rich in mitochondria that converts chemical energy into thermal energy. Importantly, BAT plays a role in combatting metabolic and cardiovascular impairments. Thus, while it is known that aging is associated with both impaired cardiovascular function as well as decreased BAT mass, the specific role of BAT in age-induced impairments in cardiac function remains unknown. For this study, we utilized an old C57BL/6 mouse population (18 months of age) that was divided to sham-operated (control), +BAT (mice surgically transplanted with BAT), and -BAT (or BATless; mice in which BAT was surgically removed) groups. Echocardiography was performed before (baseline) and 3 months after surgery (BAT transplantation or removal). Our data show that mice transplanted with BAT had enhanced cardiac function compared to control, suggesting that increasing BAT mass can reverse age-induced cardiac dysfunction. Moreover, removal of BAT not only resulted in a further deterioration of cardiac function but also adverse remodeling, suggesting that a decrease in BAT is detrimental to the heart. In conclusion, the decrease in BAT as we age plays a significant role in the cardiac dysfunction with senescence and that increasing BAT mass may be a novel therapeutic approach.

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Caregiver Burden, Stress, Depression, and Quality of Life in Family Caregivers of Patients with Heart Failure

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Background: Heart failure (HF) is a chronic disease, which is considered an important strategy to support HF patients for lifetime self-management and monitoring of worsening signs and symptoms of patients at home. However, the necessity of management throughout the whole life demands a great deal of challenge and coping of HF patients as well as their family caregivers. **Purpose:** We aimed to explore the levels of caregiver burden, stress, depression, and quality of life (QOL) in family caregivers of patients with HF, and to identify the relationships among the variables. **Methods:** A descriptive and exploratory study design was used. After approval of institutional review board, 64 family caregivers of patients with HF were recruited from a cardiovascular outpatient clinic at a university-affiliated hospital in Korea, between September 2018 and January 2019. We assessed the caregiver burden using Montgomery's scale, stress by heart rate variability measurement, depression using geriatric depression scale, and QOL using WHO-QOL scale. **Results:** Participants' mean age was 52.0 years, and the average length of care was 7.5 years. Most of them were the patient's wife or child (female), and half of them had their own health problems such as hypertension and back pain. The total mean score of caregiver burden, stress, depression, and QOL of family caregivers were 68.5 (out of 95), 39.8 (out of 100), 8.0 (out of 15), and 93.8 (out of 140), respectively. Their QOL showed negative correlations with caregiver burden and stress, and the levels of caregiver burden and stress increased with HF disease period. **Conclusion:** Healthcare providers should be aware of the importance of support for family caregivers of patients with HF. It is necessary to develop practical strategies to improve the QOL and to alleviate caregiver burden, stress and depression of family caregivers. And ultimately, it will enable them for positive participation in the comprehensive disease management with patients with HF.

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Long-Term Testosterone Deficiency Modifies Frailty and Cardiac Structure and Function in Aging Male Mice

Shubham Banga, Stefan Heinze-Milne, Susan E Howlett, Dalhousie Univ, Halifax, NS, Canada

Objective: Low circulating testosterone levels are correlated with heart diseases in older adults. The belief that low testosterone contributes to poor health and promotes frailty has dramatically increased testosterone prescriptions in recent years. Still links between low testosterone, frailty and the heart are unclear in part because preclinical studies typically use young, healthy animals. Here we investigated effects of chronic testosterone deficiency on frailty, body composition and cardiac structure/function in aging mice.

Methods: Male C57BL/6 mice (18-21 mos) underwent a gonadectomy (GDX) or sham surgery (4-wks of age) and then were aged naturally. Sham (n=10-13) and GDX mice (n=11) were tested for frailty (frailty index (FI) tool), echocardiography, electrocardiography (ECG), blood pressure, and serum testosterone (ELISA, facial vein). Body composition was measured with dual-energy X-ray absorptiometry. Mice were anaesthetized (isoflurane) during all procedures except FI scoring.

Results: Serum testosterone levels were lower in GDX mice than in sham controls (0.89 ± 0.13 vs. 0.29 ± 0.06 ng/mL; $p<0.05$). Systolic and diastolic blood pressures were unchanged between groups. Interestingly, GDX mice had lower lean mass than sham mice (23.5 ± 1.2 vs. 27.4 ± 0.7 g; $p<0.05$) but they were actually less frail (FI scores= 0.17 ± 0.05 vs. 0.23 ± 0.01 ; $p<0.05$). GDX also reduced heart mass (186 ± 11 vs. 148 ± 15 mg; $p<0.05$) and prolonged the QRS complex (8.5 ± 0.3 vs. 10.0 ± 0.4 ms; $p<0.05$) without altering heart rate. While end systolic volume was reduced by GDX ($p<0.05$), ejection fraction, fractional shortening, and cardiac output remained unchanged.

Conclusions: Chronic exposure to low circulating testosterone prolonged ventricular conduction time, reduced heart mass and lowered lean mass in aging male mice. This suggests that testosterone deficiency may promote electrical and contractile dysfunction in the setting of aging. However, despite adverse effects on the heart and lean muscle mass, frailty scores were actually lower in mice with chronic testosterone deficiency. This suggests that the impact of low testosterone on overall health is complex and raises questions about the benefits of testosterone supplementation in frail older adults.

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The Phase of Third Harmonic of Radial Pulse Predicts Cardiac Risk in Asymptomatic Patients With Type 2 Diabetes

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Patients with type 2 diabetes (T2DM) have a significantly increased risk of heart disease and adverse cardiac events (ACE). Many of asymptomatic patients with T2DM have myocardial perfusion defects or coronary artery disease and are not recognized by traditional risk factors. The third harmonic of radial pulse has been proved a risk factor for myocardial ischemia. We sought to further investigate if the third harmonic phase of the radial pulse (P3) could be an independent predictor of ACE and cardiac dysfunction in asymptomatic T2DM patients. The study consisted of 1968 asymptomatic T2DM patients who had no history of angina and cardiovascular disease. We conducted the pulse wave measurement at baseline and divided the enrolled patients into quartile groups based on P3 value (>3.2 , 3.0 to 3.2 , 2.7 to 3.0 , and <2.7). Participated patients received an average of 1.8 years of follow-up. To assess the risk of ACE, the primary outcomes were the composite of myocardial infarction, heart failure, and cardiovascular death. To measure the risk of cardiac dysfunction, the secondary outcomes were composed of newly discovered single or multi coronary stenosis, myocardial ischemia, and left ventricular dysfunction. Cox proportional hazards model were used. Reference to patients with $P3>3.2$, the hazard ratios (HR) of patients with $P3<2.7$ were as follows: (1) myocardial infarction (HR, 1.98; 95% CI, 1.32-2.98), heart failure (HR, 1.64; 95% CI, 0.88-3.06), and cardiovascular death (HR, 3.51; 95% CI, 0.73-16.9); (2) Single coronary stenosis (HR, 10.1; 95% CI, 1.29-78.7), multi coronary stenosis (HR, 9.06; 95% CI, 1.15-71.5), myocardial ischemia (HR, 3.05; 95% CI, 1.37-6.80), and low left ventricular ejection fraction (HR, 2.09; 95% CI, 1.13-38.7). The trend analysis further demonstrated that the reducing P3 leads to higher incidence of both primary and secondary composite outcomes ($P<0.001$). After adjusting for age, gender, smoking, systolic blood pressure, dyslipidemia, duration of diabetes, and Hba1c, P3 was still an independent predictor of ACE and cardiac dysfunction ($P_{trend}<0.005$). Conclusively, this report suggested that P3 provides independent predictive value for risk assessment of ACE and cardiac dysfunction in asymptomatic patients with T2DM.

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Monitoring the Hemodynamic Status During Three Trimesters of Pregnancy and Non-pregnancy Periods

Chih-Yu Chen, Renai Branch of Taipei City Hosp, Taipei, Taiwan; **Chi-Wei Chang**, Sheng-Hung Wang, Mii-Ann medical center, Taipei, Taiwan; Gin-Chung Wang, JinMu Health Technology, Taipei, Taiwan

Pregnancy is an important stage in women and is associated with delicate cardiovascular adaptation from non-pregnancy to pregnancy. Maternal hemodynamic changes are made to provide sufficient nutrients and oxygen to support the normal growth of the developing fetus. This study was designed to assess maternal hemodynamic status during three trimesters of pregnancy and non-pregnancy periods. Total 326 women (84 pregnant women and 242 age-matched non-pregnant women of childbearing age) between the ages of 20 and 44 participated in this non-invasive observational study. In this cross-sectional study, enrolled participants underwent blood pressure and radial pressure pulse measurements. In order to compare the hemodynamic status of different subjects, we recorded the systolic and diastolic blood pressure and calculated the first five harmonic amplitudes (C1~C5) and phases (P1~P5) of radial pulse wave using Fourier transform method. Compared with non-pregnant women, the maternal systolic and diastolic blood pressures were lower, and heart rate, C2, C4, and P1 were higher in pregnant women (Table 1). Those hemodynamic changes may be due to decreased arterial stiffness and peripheral arterial vasodilatation in pregnancy. In summary, a series of changes in maternal hemodynamic status can be observed during the trimesters of pregnancy, including lowering blood pressure and increasing harmonics of radial pulse. This study demonstrates the feasibility of non-invasive monitoring of the hemodynamic status of pregnant women, which could be used for early detection of pregnancy disorder.

Table 1 Clinical characteristics from pregnant and non-pregnant woman

Clinical characteristics	Non-Pregnancy	Pregnancy			
		All Pregnancy	First trimester (0 to 13 weeks)	Second trimester (14 to 26 weeks)	Third trimester (27 to 40 weeks)
N	242	84	16	34	34
Age (year)	35±7	34±5	35±4	34±4	34±5
BMI (kg/m ²)	22±4	22±4	21±4.2	22±3.9	23±2.8
Waist Circumference (cm)	74±11	81±11***	75±11	79±10*	87±10***
Heart rate (beats/second)	1.29±0.19	1.38±0.19***	1.30±0.19	1.39±0.19**	1.40±0.17***
SBP (mmHg)	114±16	110±13*	107±12*	113±17	109±9*
DBP (mmHg)	69±11	65±9***	65±9	66±11	63±7***
Gestation (weeks)		23±10	10±3	18±3	34±3
C1	0.855±0.082	0.852±0.095	0.861±0.126	0.861±.100	0.839±0.072
C2	0.463±0.086	0.526±0.115***	0.485±0.099	0.542±0.123***	0.528±0.111***
C3	0.276±0.075	0.287±0.095	0.283±0.085	0.293±0.108	0.284±0.087
C4	0.158±0.057	0.183±0.064***	0.183±0.046*	0.187±0.073*	0.179±0.063*
C5	0.148±0.054	0.152±0.066	0.174±0.062	0.149±0.076	0.144±0.057
P1	4.508±0.122	4.570±0.137***	4.582±0.110**	4.579±0.164*	4.554±0.121*
P2	3.960±0.238	4.027±0.282*	4.053±0.238	4.023±0.322	4.018±0.266
P3	3.239±0.377	3.250±0.387	3.416±0.345*	3.237±0.463	3.186±0.301
P4	3.082±0.396	3.077±0.445	3.260±0.282*	3.017±0.566	3.052±0.346
P5	2.636±0.552	2.527±0.561	2.795±0.407	2.470±0.674	2.457±0.029*

SBP= Systolic blood pressure, DBP= Diastolic blood pressure, BMI=Body mass index. The single, double, and triple asterisks indicate that the means of the variables in the pregnant group were significantly different from those in the non-pregnant group using the Student's t test (p < 0.05, P < 0.01 and p < 0.005, respectively).

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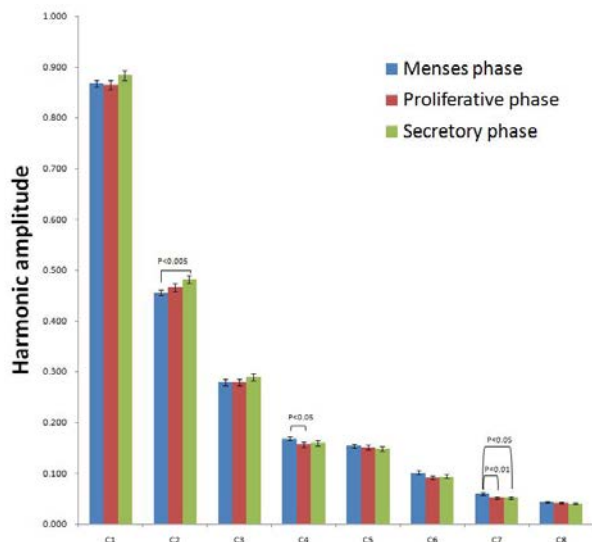
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The Impact of the Menstrual Cycle on the Cardiovascular System

Chih-Yu Chen, Renai Branch of Taipei City Hosp, Taipei, Taiwan; **Chi-Wei Chang**, Sheng-Hung Wang, Mii-Ann medical center, Taipei, Taiwan; Xiang-Yu Xie, Natl Taiwan Univ, Taipei, Taiwan; Gin-Chung Wang, JinMu Health Technology, Taipei, Taiwan

The stage of the menstrual cycle is an exquisite regulation of female hormone and cardiovascular adaptation to prepare for pregnancy. However, there are still many unclear areas in the study of the influence of the menstrual cycle on cardiovascular function. Since the arterial pulse wave provides insightful information about the cardiovascular system, this study aimed to study cardiovascular adaptation in three phases of the menstrual cycle through non-invasive blood pressure and radial pulse wave measurements. In this observational study, we recruited 113 healthy women with regular menstrual cycles (25±35 days). All participants underwent blood pressure and radial pulse measurements in three stages of the menstrual cycle, including menses period, proliferative phase, and secretory phase. The Fourier series method was used to calculate the first ten harmonic amplitudes of the radial pulse wave (C1~C10). The results showed no significant systolic and diastolic changes in the three phases of menstruation. However, compared to the other two phases, women in menses period had lower C2 and higher C4, C6 and C7. (Figure 1) C4 and C7 are two indices that are inversely related to arterial stiffness and peripheral arterial impedance. Thus, during the menstrual cycle, female hormones may be involved in the regulation of vascular elasticity and peripheral vasodilatation, which in turn changed the radial pulse spectrum. In summary, the menstrual cycle has an impact on the radial pulse spectrum and could alter arterial stiffness to accommodate the physiological needs of the pre-pregnancy period.



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Development of CD47-nanomedicine as Novel Treatment for Specific Mitigation of Thrombospondin 1-Induced Vascular Dysfunction

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The trans-membrane receptor CD47 is considered a central relay of thrombospondin 1 (TSP-1) mediated responses in human cells/tissues, and ubiquitously serves as a marker of self on hematopoietic cells.

Preliminary studies of TSP-1 binding with soluble human CD-47, serving as decoy recombinant protein (rhCD47p), demonstrated molar ratio ≥ 3 folds of rhCD47p abolished all TSP1-vascular CD47 receptor signaling. Accordingly, we attached multiple rh-CD47p ligands onto liposomes (NanoLip), as clinical prototype therapy. Novel rh-CD47p-NanoLip can avoid blood mononuclear cells, while binding many circulating TSP1 molecules, thus eliminating excessive plasma TSP-1, often implicated in pulmonary arterial hypertension (PAH) and ischemia-reperfusion injuries.

Direct coupling of rh-CD47p, onto NanoLip surface was achieved using a sulfo-NHS-reaction between amino groups on rh-CD47p and carbodiimide-activated NanoLip, followed by column purification and physico-chemical analysis.

Pharmaceutically, rh-CD47p-NanoLip formulation (av. size = 86 ± 6.0 nm) engaged $67 \pm 11 \mu\text{g/mL}$ of protein, while retaining 86% of native rh-CD47p activity. Western blots and TSP-1-ELISA revealed dosimetric binding of tirmeric human TSP-1: rh-CD47p-NanoLip. Activated macrophages showed substantial decrease in phagocytosis of labeled rh-CD47p-NanoLip, compared to plain- and IgG-NanoLip controls (5- and 4- folds, respectively), indicating self-marker function. Conversely, binding of exogenous TSP-1 caused significant upturn of internalized rh-CD47p-NanoLip ($p < 0.05$, $n=4$), due to TSP-1/nano-complex formation. Therapeutically, in isolated mouse thoracic aorta model, mimicking high plasma levels of TSP1 in PAH patients, titrated doses of CD47p-NanoLip neutralized TSP1-impaired vasodilation, back to the same level as controls ($p < 0.001$, vs. TSP-1, $n=4$).

This is the first report of its kind about CD47-based immunomodulatory nano-therapy specifically targeting TSP-1. The extracellular domains of native CD47 receptors of TSP1 were successfully "nano-modified" into a candidate systemic decoy pharmaceutical, rh-CD47p-NanoLip, to scavenge and eliminate pathologically elevated TSP-1 plasma levels, to abrogate TSP-1-associated vascular complications.

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Predicting Blood Pressure Response to Fluid Bolus Therapy Using Neural Networks with Clinical Interpretability

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Background: Fluid bolus therapy (FBT), the rapid infusion of fluid, has been recommended as the primary-line treatment for acute hypotensive episode (AHE) that occurs in about 41% of patients in ICU. However, previous studies have reported that approximately one-third of the acute hypotensive patients do not successfully respond to FBT treatment. Avoiding the administration of FBT that will not successfully resolve AHE might prevent an inappropriate increase of the total fluid volume administered to ICU patients, potentially reducing their risk for severe organ dysfunction and increased mortality.

Methods: Our study utilized regression models and attention-based recurrent neural network (RNN) algorithms and two large-scale information system databases, the multi-clinical MIMIC-ICU one and the multi-center Philips eICU CRD one, to predict the successful response to FBT among hypotensive patients in ICUs. We investigated both time-aggregated modeling and time-series modeling using RNN with the attention mechanism (AM) for clinical interpretability. The successful FBT is defined by intensive care experts as the presence of the maximum mean atrial pressure (MAP) $> 1.15 \times$ average (MAP) at least once, where maximum(MAP) is the maximal MAP from the FBT starting time to two hours after FBT, and average (MAP) is the average MAP from 30 minutes before FBT until 10 minutes after FBT.

Results: The stacked RNN with AM yielded the highest accuracy of 0.852 and area under the curve (AUC) value of 0.925 when trained and tested on the MIMIC-ICU dataset. The top features learned from regression include the patient's respiratory rate, diastolic pressure, temperature, and bicarbonate and base excess levels in blood. Preliminary results from training and testing the RNN on the Philips eICU-CRD database yielded an accuracy of 0.812 and AUC value of 0.769. We were also able to identify timesteps close to the time of FBT administration as clinically meaningful using the RNN models with AM.

Conclusion: This is the first study that utilizes machine learning for identifying hypotensive patients in ICUs who will have sufficient blood pressure recovery after FBT. Utilizing AM and identifying the top features learned also provided clinical interpretability to the models we used.

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Transplantation Alters Function and Clonality of Cytomegalovirus-Responsive T Cells

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Background: Cytomegalovirus (CMV) infection is a major complication of organ transplantation. CMV establishes lifelong latency in infected individuals. Healthy people are protected from CMV reactivation by immune memory, but immunosuppression limits protection in transplant recipients. Despite antiviral prophylaxis strategies, long-term survival is impaired. CMV-responsive memory CD8 T cells expand during the first year after transplantation in the absence of detectable viremia. The observed expansion correlates with protection against CMV. The objective of this study was to determine whether these cells are protective, utilizing innovative single cell sequencing analyses to probe their functional characteristics.

Methods: Mononuclear cells from peripheral blood of heart and kidney transplant recipients pre- and three months and one year post-transplant were stimulated with immunodominant CMV peptides. Single IFN gamma+ CMV-responsive T cells were index sorted into 96 well plates, and nested PCR, barcoding, and sequencing performed to analyze T cell receptor (TCR) and a panel of functional genes.

Results: Expanded CMV-responsive T cells tend to express a limited number of dominant CMV-responsive TCR clones. Clonally expanded CMV-responsive T cells express multiple antiviral proteins. The TCR repertoire may change from pre- to post-transplant. Once established at three months, clonal dominance is maintained until at least one year. The number of antiviral proteins expressed per clonally expanded T cell increases between these time points.

Conclusions: Protection from CMV post-transplant correlates with maintenance of TCR clonality and increased number of functions per cell. The stability of clonality post-transplant contrasts with the expansion of the total T cell population, and

suggests that clonal expansion in the absence of disease recapitulates the pre-existing repertoire. The increased number of functions suggests that the expanded population may become more protective. The potential impact on clinical practice would be to provide further information on the relationship between the T cell response to CMV and long-term outcomes after transplantation.

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Adipose Tissue Browning Induced by Natriuretic Peptide Exerts Thermogenic Actions and Improves Insulin Resistance in an in vivo Model of Diet-Induced Obese Mice

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Although natriuretic peptides (NPs) classically act on renal and cardiovascular systems, increasing evidence suggest that NPs also largely coordinate inter-organ metabolic cross-talk with adipose tissues. We recently reported that A-type NP (ANP) raises intracellular temperature in cultured adipocytes in a low-temperature sensitive manner. We herein investigated whether ANP exerts a significant impact on adipose tissues in vivo, using diet-induced obese mice. C57BL/6 mice were exposed to high-fat diet (HFD) or normal-fat diet (NFD) for 13 weeks, and treated with or without ANP infusion for 3 weeks (0.5 ug/kg/min via osmotic-pump). The ANP infusion significantly increased its serum concentration both in NFD and HFD group. Histological analyses revealed that HFD induced increased brown adipose tissue (BAT) cell size with accumulation of lipid droplets (whitening). ANP treatment significantly suppressed this whitening response (re-browning). Similarly, HFD induced enlarged inguinal white adipose tissue (WAT) lipid droplets, which was significantly reduced by ANP. This was associated with a substantial increase in uncoupling protein-1 (UCP1) expression in WAT confirmed by immunohistochemical analysis (browning, n=3 each) and UCP1 mRNA analysis (NFD+NP 2.7±0.4 fold vs NFD, P<0.01; HFD+NP 6.4±2.2 fold vs HFD, P<0.05, n=5 each). To determine the functional significance of browning effects of ANP, intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed and showed that ANP treatment substantially improved insulin resistance in HFD (p<0.05, n=9 each). Moreover, cold tolerance test (at 4°C for 4 hours) demonstrated that the ANP-treated mice were tolerant to cold exposure, which was more salient in HFD with ANP (Decrease in rectal temperature from baseline [°C]: NFD+ANP -2.4±0.6, HFD -2.5±0.4, HFD+ANP -2.1±0.4, versus NFD -9.2±2.2, P<0.01 each, n=9 each). In conclusion, ANP induces WAT browning and preserves BAT function in obese models, leading to in vivo thermogenesis as well as improvement in insulin resistance. These findings reveal the compensatory roles of natriuretic peptides against the core body temperature fall and insulin resistance in a state of heart failure.

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Pde1 Promotes Development of Pulmonary Arterial Hypertension and Could Be a Novel Treatment Target

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Backgrounds: Pulmonary arterial hypertension (PAH) is a rare but fatal disease, with an estimated mean survival period in untreated patients of approximately 3 years. Pulmonary vascular remodeling is a hallmark of PAH. Pulmonary vasculature shows increased proliferation, irrelevant migration and apoptosis resistance of vascular cells such as pulmonary arterial smooth muscle cells (PASMCs) and endothelial cells. However, the molecular mechanism in the pathogenesis of PAH remains unclear. We previously performed genome-wide association study (GWAS) in Japanese patients with idiopathic / heritable PAH and healthy controls and identified novel disease related SNPs in *PDE1A|DNAJC10* locus. PDE1A is ubiquitously expressed and would be associated with proliferation of smooth muscle cells. **Aim:** In this study, we aimed to clarify the relationship between PDE1 and PAH development. **Methods and Results:** We first examined the clinical significance of the SNPs identified in the GWAS. In a retrospective analysis of right heart catheterization, patients with the SNPs were more refractory to treatment with PDE5 inhibitor (PDE5i) compared with the patients without PDE5i, suggesting that treatment with PDE5i alone is inadequate possibly due to the increased PDE1A activity. In human PASMCs, inhibition of

PDE5 increased the expression of PDE1, whereas inhibition of PDE1 decreased the expression of PDE5, which suggests that PDE1 inhibitor (PDE1i) could decrease the expression of both PDE1 and PDE5, resulting in improvement of PAH. We then examined the influence of PDE1 inhibition on the cell proliferation and inflammation. PDE1i reduced cell number and proliferation of PASMCs in a dose-dependent manner. Also, elevation of inflammatory markers caused by TNF α was partially attenuated by PDE1 inhibition, suggesting that PDE1 is associated with exacerbation of PAH via PASMCs proliferation and inflammation. Finally, oral administration of PDE1i for PAH model mice improved pulmonary hypertension and right ventricular function. **Conclusion:** It was considered that PDE1 promotes development of PAH via PASMCs proliferation and inflammation. PDE1i in addition to conventional treatments could be a novel treatment for the patients with PAH showing resistant to PDE5i.

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A Unique Acylcarnitine Profile is Independently Associated with Diabetic Heart Failure and Associates with Adverse Clinical Outcomes: Insights from the FIGHT Trial

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Introduction: Heart failure with reduced ejection fraction (HFrEF) may be associated with unique metabolic derangements in patients with type 2 diabetes (T2DM). However, whether such derangements are associated with clinical outcomes remains unclear.

Purpose: 1) To determine metabolic pathways that are dysregulated in high-risk HFrEF with and without T2DM. 2) To identify metabolomic signatures which may be differentially associated with clinical outcomes in HFrEF patients with T2DM.

Methods: The FIGHT trial studied the effect of liraglutide on clinical stability in individuals with advanced HFrEF. Forty-five metabolites were quantified in 254 baseline plasma samples from the trial (T2DM: n=147, non-T2DM: n=107) using tandem flow injection mass spectrometry. Principal component analysis (PCA) was used to reduce the high number of correlated metabolites into uncorrelated factors. Linear regression was used to evaluate the association of T2DM with PCA-derived factor levels. Cox proportional hazards models, stratified by T2DM status, were used to test for time to mortality or HF hospitalization.

Results: Patients with T2DM were older, had higher BMI, and were more likely to have CAD, but had similar gender composition and severity of HF, as compared with non-T2DM patients. PCA identified 13 metabolite factors grouping in biologically consistent pathways. T2DM status was associated with two factors, one composed of the short chain dicarboxyl-acylcarnitine, C6-DC, and the long-chain acylcarnitine C22 ($\beta = -0.325$, $p = 0.016$), and one composed of aspartic acid/asparagine ($\beta = -0.373$, $p = 0.008$), in fully adjusted models. The same C6-DC, C22 factor was associated with time to mortality or HF hospitalization beyond adjustment for sex, race, liraglutide exposure, baseline age, systolic blood pressure, creatinine, BMI, NT-pro-BNP, and liver function (T2DM: HR 1.573, $p = 0.007$; Non-T2DM: HR 1.302, $p = 0.103$).

Conclusions: Amongst patients with high-risk HFrEF, those with T2DM had differential levels of a short chain dicarboxyl- and long-chain acylcarnitine, which were associated with adverse clinical outcomes. Such metabolites might serve as potential targets for diagnostic or therapeutic interventions in diabetic HFrEF.

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Phenotyping by ECG Dynamics During Sleep Predicts Cardiovascular Disease Risk Factors in a Multicenter Study of Asymptomatic Middle-aged Community Adults

Tracie K Lin, Johns Hopkins Univ, Baltimore, MD; **Daniel Wendelken**, Univ of Cincinnati, Cincinnati, MD; **Brian O'Rourke**, Steven R Jones, Naresh M Punjabi, Johns Hopkins Univ, Baltimore, MD; **Deeptankar DeMazumder**, Univ of Cincinnati, Cincinnati, OH

Introduction: ECG-based predictive analytics have typically focused on static assessment of beat-to-beat heart rate variability (HRV) during wakefulness. However, there is increasing evidence that quantification of time-related changes as well as non-beat-to-beat complexity indices, such as entropy, can provide important untapped insight. **Methods:** Analysis of HRV, QT variability (QTV), QT entropy (QTen), QT entropy rate (QTa) and RR entropy (RRen) in 5-min bins were performed on home polysomnographs of 6,300 asymptomatic community adults (Sleep Heart Health Study). Rate of change of ECG variables was quantified by linear regression in one- to four-hour epochs. Stepwise regression was used to identify age-, sex-, race-, comorbidity-, and sleep state-associated influences on ECG values. **Results:** Predictive multivariate models were generated for congestive heart failure, diabetes, obstructive sleep apnea, and history of myocardial infarction, each with sleep state-specific and ECG variable rate of change being of the highest predictive value in ECG-based models (Table 1). **Conclusion:** Although aggregate beat-to-beat HRV is associated with cardiovascular risk factors, unique time-varying and sleep stage-specific findings provide additional predictive power. By way of demonstrating that distinct classes of dynamic ECG features were predictive of each risk factor studied, our study suggests a potential role for patient-specific ECG phenotyping using a comprehensive analytical platform. Dynamic ECG assessment during sleep with attention to entropy-derived measures, in particular, may enhance precise risk stratification in otherwise low-risk individuals.

Table 1

Risk Factor	Strongest predictors	z-score	p-value
Diabetes	Mean RRen, arousal during first 2 hours after sleep onset	-3.77	<0.0001
	Minimum QTen	2.24	0.025
	QTa slope, sleep onset to 2 hours after sleep onset	2.42	0.016
History of myocardial infarction	Mean QT, sleep state transitions during first 2 hours after sleep onset	5.05	<0.0001
	Mean QTa, sleep state transitions during first 2 hours after sleep onset	-2.80	0.005
	Maximum RRen	-2.79	0.005
	Mean LFHF, during REM sleep in second 2 hours after sleep onset	-2.62	0.009
Congestive heart failure	Mean HR, sleep state transitions during second 2 hours after sleep onset	4.6	<0.0001
	Maximum QTen	3.04	0.002
	Minimum QTen	3.44	0.001
Obstructive sleep apnea	Range of QTa	3.11	0.002
	Mean SDNN, arousal during first 2 hours after sleep onset	-2.94	0.003

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Plasticizer Interaction with the Heart: Chemicals Used in Plastic Medical Devices Can Interfere With Cardiac Electrophysiology

Rafael Jaimes III, Damon McCullough, Bryan Siegel, Luther Swift, Daniel McInerney, James Hiebert, Children's Natl Medical Ctr, Washington, DC; Jiansong Sheng, CiPA Lab, Washington, DC; **Nikki G Posnack**, Children's Natl Medical Ctr, Washington, DC

Background: Phthalates are employed as plasticizers in the manufacturing of flexible, plastic medical products. Patients can be subjected to high phthalate exposure through contact with plastic medical devices. We aimed to investigate the cardiac safety and biocompatibility of mono-2-ethylhexyl phthalate (MEHP), a phthalate with documented exposure in intensive care patients.

Methods and Results: Optical mapping of transmembrane voltage and pacing studies were performed on isolated, Langendorff-perfused rat hearts to assess cardiac electrophysiology after MEHP exposure compared with controls. MEHP dose was chosen based on reported blood concentrations following an exchange transfusion procedure. 30-min exposure to MEHP increased the atrioventricular node (147 vs. 107msec) and ventricular (117 vs. 77.5msec) effective refractory periods, compared with controls. Optical mapping revealed prolonged action potential duration (APD) at slower pacing cycle lengths, akin to reverse use-dependence. The plateau phase of the APD restitution curve steepened and became monophasic in MEHP-exposed hearts (0.18 vs. 0.06 slope). APD lengthening occurred during late-phase repolarization resulting in triangulation (70.3 vs. 56.6 msec). MEHP-exposure also slowed epicardial conduction velocity (35 vs. 60 cm/sec), which may be partly explained by inhibition of $Na_v1.5$ (874 μ M and 231 μ M IC50, fast and late sodium current).

Conclusions: This study highlights the impact of acute MEHP exposure, using a clinically-relevant dose, on cardiac electrophysiology in the intact heart. Heightened clinical exposure to plasticized medical products may have cardiac safety implications – given that action potential triangulation and electrical restitution modifications are a risk factor for early after depolarizations and cardiac arrhythmias.

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A Novel XBP1s-FoxO1 Axis Regulates Lipid Metabolism and Cardiac Performance in Heart Failure With Preserved Ejection Fraction

Gabriele G Schiattarella, Francisco Altamirano, Dan Tong, Kristin French, Elisa Villalobos, Soo Young Kim, Xian Luo, Nan Jiang, Herman I May, Zhao V Wang, Theodore M Hill, UT Southwestern Medical Ctr, Dallas, TX; Dong Ik Lee, Virginia S. Hahn, Kavita Sharma, David A Kass, Johns Hopkins Sch of Med, Baltimore, MD; Sergio Lavandero, Univ of Chile, Santiago, Chile; Thomas G Gillette, Joseph A Hill, UT Southwestern Medical Ctr, Dallas, TX

Heart failure with preserved ejection fraction (HFpEF) is a common syndrome that is both morbid and mortal. Yet, no evidence-based therapies exist owing to lack of informative preclinical models. Existing evidence suggests that excessive body fat in HFpEF causes systemic inflammation triggering cardiomyocyte dysfunction. Lipid metabolism is regulated by several signaling molecules including the transcription factors Forkhead box protein O1 (FoxO1) and the spliced form of X-box binding protein 1 (Xbp1s). The role of the Xbp1s-FoxO1 axis in HFpEF is unknown. We have developed a novel preclinical model of HFpEF in which concomitant metabolic and hypertensive stress in mice elicited by a combination of high fat diet (HFD) and constitutive nitric oxide (NO) synthase inhibition by N^w-nitro-L-arginine methyl ester (L-NAME) recapitulates the numerous systemic and cardiovascular features of human HFpEF. Inflammation-dependent suppression of one of the unfolded protein response (UPR) effectors, Xbp1s, occurs in the myocardium of both experimental and human HFpEF coupled with increases in FoxO1 levels and lipid accumulation. Cardiomyocyte-specific over-expression of Xbp1s substantially ameliorated the HFpEF phenotype with reduction of cardiac lipid and FoxO1 levels. In isolated cardiomyocytes, over-expression of Xbp1s by adenovirus resulted in proteasomal degradation of FoxO1. FoxO1 is ubiquitinated upon Xbp1s over-expression and Xbp1s-induced proteasomal degradation of FoxO1 occurs through the activation of the E3 ubiquitin ligase STUB1. Cardiomyocyte-specific FoxO1 depletion limited lipid accumulation in HFpEF hearts and significantly ameliorated the cardiovascular phenotype. We have developed an informative preclinical model of HFpEF, unveiling a crucial role of an XBP1s-FoxO1 axis in lipid metabolism in the syndrome.

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Fundamental New Clinical Insight from Excitation-contraction Coupling Studies of the Cardiovascular System via Integrated ECG And Photoplethysmography (PPG) Analyses During Sleep

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INTRODUCTION: The surface ECG provides critical insights into cardiac excitation. The ECG waveform dynamics inform clinical decisions beyond heart rate and QT measures. Likewise, the PPG waveforms contain physiologically rich information about a person's health, but its clinical use is limited to peripheral oxygen saturation. Importantly, the complexity of ECG and PPG dynamics reflect multi-directional interconnected networks of cellular signaling pathways, tissues, organs and organ system interactions that regulate homeostatic function under changing conditions. While integrated dynamic analysis of ECG and PPG is rooted in fundamental principles of electromechanical coupling and systems biology, this has been unexplored for identifying subclinical illness.

HYPOTHESIS: Integrated dynamic analysis of ECG and PPG waveforms during sleep identify subclinical cardiovascular illness in a multicenter study of asymptomatic community subjects.

METHODS & RESULTS: We studied polysomnography recordings from 137 adults in a substudy of the multi-ethnic study of atherosclerosis (MESA-5). The cohort had mean age of 69±10 years, 43% women, 11% African Americans, 48% diabetes mellitus (DM) of which 33% received insulin therapy (DM-RxI), 21% prior myocardial infarction (MI), and 16% ostensibly healthy (i.e., unremarkable findings on history, physical exam, lab, etc). Dynamic analysis of PPG waveforms were performed for each sleep stage: (1) Awake before onset of sleep; (2) Non-REM sleep; (3) REM sleep; (4) Arousals during sleep; (5) Awake after completing sleep. In a multivariate model, the odds ratio (5th compared to 1st quintile) for MI for sleep stage 1 was 7.5 (2.1-27), for DM sleep stage 5 was 0.057 (0.0040-0.82), and for DM-RxI for sleep stage 5 was 0.23 (0.057-

0.91), with corresponding ROC curve areas ranging between 0.65-0.90 ($p < 0.05$).

CONCLUSIONS: Integrated dynamic analysis of ECG and PPG waveforms provide fundamental new insight for identifying an individual's health status, including MI, DM and treatment for DM. This novel strategy has potential for broad clinical application and warrants prospective validation.

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The Effect of Dietary Patterns on Cardiometabolic Risks in Healthy Asian Adults

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Rationale: Atherosclerosis often leads to acute myocardial infarction and stroke. Diet may be involved in innate immunity-mediated inflammation and the pathogenesis of atherosclerosis. **Objective:** To investigate the effects of various dietary patterns on metabolic profiles, inflammation and atherosclerosis. **Methods and Results:** We began by understanding the dietary pattern using questionnaire for 75 healthy adults who were enrolled in this study. To obtain the metabolic and inflammation profiles, we collected peripheral blood for analysis. The healthiness of vessels was evaluated by carotid intima-media thickness (CIMT). We found that participants who adhere to vegetarian diet more than ten years showed lower levels of serum total cholesterol compared to omnivores. However, we did not find any beneficial effect of vegetarian diet on other parameters as C-reactive protein, interleukins and CIMT were no difference between two groups. Among "junk foods", the amount of instant noodles and sugar drink consumption was positively associated with the levels of triglyceride and uric acid. Notably, the increment of sugar drink usage was correlated with higher expression of toll-like receptor (TLR) 2 and TLR4 on peripheral monocytes, but showed no impact on fasting serum glucose. As for the concern of "healthy foods", participants who took more fresh fruit had significantly better fasting glucose and higher high-density lipoprotein, as well as lower monocyte TLR2 and TLR4 expression. The amount of green vegetable consumption was inversely correlated with low-density lipoprotein and CIMT. In addition to food, water was also important. Participants who drank water more than 2000 ml per day had significant lower CIMT. On the other hand, we did not find any significant effects on coffee consumption. Moreover, for factors that may promote or prevent atherosclerosis, we found that trimethylamine N-oxide and S-adenosyl-L-homocysteine was positively correlated with CIMT whereas L-Lysine and L-Carnitine showed inverse association with CIMT. **Conclusions:** We discovered that consumption of healthy foods and water, but not vegetarian diets per se, is positively correlated with cardiovascular health from the perspective of metabolic panel and inflammation.

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Sex-Related Changes in Physiological Cardiac Hypertrophy in Beta Arrestin-2 KO mice

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β -arrestin-2 (ARRB2) has an integral role in G-protein-coupled receptor regulation and signaling. Our lab has reported that biased ligands acting at the AT1-R promote ARRB2 signaling that increases contractility and reduces maladaptations in dilated cardiomyopathy. Our hypothesis is that ARRB2 is necessary for the physiological eccentric cardiac hypertrophic response associated with voluntary exercise. **Methods and Results:** Our study compared ARRB2 knockout (KO) and age matched FVBN control mice (NTG). Twelve-week-old age mice were divided into 4 groups. For a 6-week period ARRB2-KO and NTG were either sedentary or subjected to voluntary running. Records from wheels were obtained continuously through Wi-Fi and the data were analyzed weekly. Before exercise, baseline echocardiographic analyses were performed showing no apparent differences in cardiac function among the groups. Initially no differences were found in running parameters between ARRB2-KO and controls. However, beginning in the third week through the end of the exercise duration we found a

decrease in the distance covered in ARRB2KO females compared with NTG females. After 6-weeks of exercise there was an increase in LA diameter, LV mass, LVIDd and HW/TL ratio only in NTG female compared with sedentary group suggesting ARRB2 sex-related differences in response to voluntary exercise. Although ARRB2 KO in C57/BL mice has been shown to alter myofilament Ca-sensitivity, we found no changes in myofilament Ca-sensitivity and post-translational modifications among all four groups. **Conclusion:** Our data suggest that ARRB2 is required for physiological hypertrophy caused by voluntary exercise only in female but not in male mice. Further studies are required to test whether ARRB2 is required for development of more stressful physiological hypertrophy during involuntary training regimens such as swimming or treadmill.

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Sodium Influx Modulates the Modality of Cardiac Relaxation

Daniel O Cervantes, Alejandro Andrade-Vicenty, Chaoyu Sun, Saketh Anand, Jillian Pope, Jessica R Dorilio, Dong Sun, Antonio Cannata, Eleonora Cianflone, Govindaiah Vinukonda, Thomas H Hintze, New York Medical Coll, Valhalla, NY; Heather O'Malley, Lori L Isom, Univ of Michigan, Ann Arbor, MI; Jason T Jacobson, Marcello Rota, New York Medical Coll, Valhalla, NY

Aging in experimental animals is coupled with protracted electrical recovery of the heart and increased late Na^+ current (I_{NaL}) in cardiomyocytes. These electrophysiological alterations are coupled with impaired cardiac relaxation, raising the possibility of a causative link between enhanced I_{NaL} and diastolic dysfunction. To test this hypothesis, genetic and pharmacological interventions were introduced to assess the consequences of enhanced Na^+ influx in myocytes on diastolic properties of the mouse heart, together with effects on mechanical properties of isolated cells. Using Langendorff preparations, acute enhancement of I_{NaL} with anemone toxin II increased diastolic and systolic pressure in the mouse heart. Importantly, a shift of the diastolic pressure-volume relationship toward higher pressure values was observed with activation of I_{NaL} . To test the in vivo effects of increased Na^+ influx, mice with inducible, cardiac restricted deletion of the beta1 subunit of the Na^+ channel (Scn1b-KO) were employed. Scn1b-KO male mice presented protracted electrical recovery with respect to control (Ctrl) animals, a condition that was reversed by administration of a specific I_{NaL} inhibitor (GS967, 0.5 mg/kg body weight). By invasive hemodynamics, left ventricular (LV) developed pressure was preserved in Scn1b-KO mice, but maximal velocities of pressure development and decay were attenuated by 16% and 25%, respectively. By echocardiography, LV end-diastolic volume and ejection fraction (EF) were preserved in Scn1b-KO. In contrast, using Doppler modality, LV filling pattern was altered and isovolumic relaxation time was prolonged by ~30%. I_{NaL} inhibition (GS967) in Scn1b-KO mice ameliorated LV filling and normalized isovolumic relaxation time, without effects on EF. Using isolated cardiomyocyte preparations, Scn1b deletion had no consequences on fractional cell shortening, but led to a ~5% prolongation of kinetics of contraction and relaxation. Inhibition of I_{NaL} (300 nM GS967) in Scn1b-KO myocytes accelerated contraction and relaxation kinetics and attenuated fractional shortening. In conclusion the late Na^+ current modulates the modality of myocyte contraction and relaxation with important effects on diastolic function of the heart.

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High Speed Imaging of Single Cardiomyocyte Action Potentials Using a Far-red Genetically Encoded Voltage Sensor

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The ability to image cardiac membrane potentials allows for the observation of cellular communication and electrical activity, both of which are important to maintain cardiac syncytium; these can be altered in diseases (e.g. Long QT Syndrome). Traditionally voltage dyes such as Di-8-ANEPPS have been used to optically measure action potentials (APs). However,

these dyes express transiently, have poor signal to noise ratios, and are toxic. More recently, genetically encoded voltage indicators (GEVIs) have been developed to replace state-of-the-art voltage dyes, but sensors currently used within the cardiac field exhibit poor kinetics and/or low signal to noise ratios (SNR). Recently, Archon1, a new genetically encoded voltage sensor, was developed in the neuroscience field; this sensor exhibits excellent membrane localization, temporal sensitivity, and SNR, enabling the optical detection of individual spikes in neurons. Here we use Archon1 for the first time in cardiac cells, to monitor single cell cardiac APs in 2D and 3D in vitro systems in response to different environmental stimuli. Human induced pluripotent stem cell-derived cardiomyocytes were infected with Archon1 and imaged using a one-photon fluorescence microscope equipped with a high speed sCMOS camera to demonstrate cardiac AP tracings. The kinetics and SNR of Archon1 are compared to traditional electrophysiology and Di-8-ANEPPS measurements. Additionally, E-4031 (K⁺ Channel Blocker) and Nifedipine (Ca²⁺ Channel Blocker) were used to demonstrate the sensitivity of this sensor in a drug dosage study. To study the APs of single cells within a 3D engineered microtissue, cardiomyocytes expressing Archon1 were seeded into a force transducing micro-pillar device and the APs for optically isolated cells were recorded. Demonstration of this new genetically encoded voltage sensor in cardiac cells enables the monitoring of single and multi-cell APs in 2D and 3D applications and can be extended to in vivo. This tool, newly applied to cardiac biology and tissue engineering will allow for better and more accurate observation of cardiac electrical activity to probe human cardiovascular disease.

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Poster Session 2 and Reception

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High Circulating Levels of 8-OHdG are an Independent Predictor of Subsequent MACE

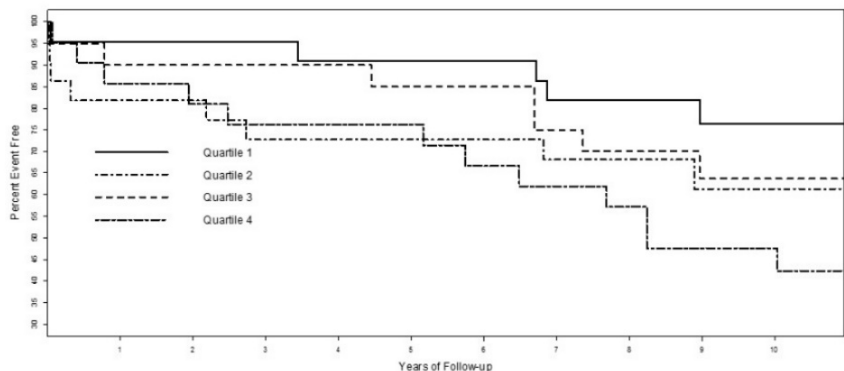
Oxana Galenko, Serene A Kergaye, Stacey Knight, Intermountain Heart Inst, Murray, UT; Joseph B Muhlestein, Intermountain Heart Inst, Univ of Utah, Salt Lake City, UT; Jeffrey L Anderson, Intermountain Heart Inst, Univ of Utah, Murray, UT; Kirk U Knowlton, John F Carlquist, Intermountain Heart Inst, Murray, UT

Objective: Oxidative stress is associated with several cardiovascular risk factors and cardiovascular disease. Damaged cells release cell-free DNA (cfDNA) into the circulation; oxidized cfDNA presence in the plasma indicates a pathological process in the context of oxidative stress. We hypothesized that biomarker of oxidative DNA damage 8-hydroxy- 2'deoxyguanosine (8-OHdG) may be associated with incident major adverse cardiovascular events (MACE).

Methods: Plasma samples from 84 consenting patients undergoing coronary angiography at Intermountain Healthcare were obtained at the time of the procedure and tested for 8-OHdG by ELISA. Study endpoints were subsequent myocardial infarction (MI), stroke and all-cause death (determined by electronic medical records and death certificates) within median follow-up of 10 years (IQR: 8.8, 10.6). Cox-proportional hazard analysis was used for examining MACE rate association by quartile of 8-OHdG.

Results: Mean 8-OHdG was significantly higher for individuals with a subsequent MACE vs those without (124.8 and 98.7 nM, respectively; $p < 0.006$, t-test). No other baseline demographic was significantly associated with 8-OHdG. MACE rates by 8-OHdG quartiles revealed a significant trend ($p = 0.0346$) (Figure). Hazard ratio increased by 1.653 for every 42 unit increase in 8-OHdG, $p = 0.006$.

Conclusion: High 8-OHdG plasma levels independently predict subsequent MACE. Whether 8-OHdG is contributes etiologically to event causation needs to be determined.



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Myopathy Causing Bag3^{P209L} Protein Leads to Restrictive Cardiomyopathy Caused by Aggregate Formation and Sarcomere Disruption in Cardiomyocytes

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The co-chaperone BAG3 (Bcl-2 associated athanogene 3) is strongly expressed in cross-striated muscles and plays a key role in the turnover of muscle-proteins as a member of the CASA (chaperone-assisted selected autophagy) complex. An amino acid exchange (P209L) in the human BAG3 gene, caused by a single base mutation, gives rise to a severe dominant childhood muscular dystrophy, restrictive cardiomyopathy, and respiratory insufficiency. To get deeper insights into the pathophysiological mechanisms of the disease, we generated a transgenic mouse model of the human mutation BAG3^{P209L}, in which a fusion protein consisting of the human BAG3^{P209L} and the green fluorescent protein eGFP can be conditionally overexpressed. Ubiquitous overexpression of BAG3^{P209L}-eGFP leads to a severe phenotype between the second and fourth week of life, including decreased body weight, skeletal muscle weakness, and heart failure. Echocardiography revealed that the BAG3^{P209L}-mice suffer from restrictive cardiomyopathy and Sirius-red-staining of heart tissue showed extensive fibrosis. In cardiomyocytes, isolated from hearts of transgenic mice overexpressing BAG3^{wt}-eGFP or BAG3^{P209L}-eGFP, BAG3^{wt}-eGFP stringently localizes to sarcomeres and intercalated discs, whereas cardiomyocytes from BAG3^{P209L}-eGFP mice displayed formation of BAG3 containing aggregates and disruption of sarcomeres *in vivo*. While BAG3^{P209L}-eGFP binding to α -Hsp70, Filamin C and α -HspB8 was unchanged it was less soluble than BAG3 and had a tendency to aggregate, thereby sequestering BAG3 and its clients. Depletion of the BAG3 pool leads to an impairment of CASA and accumulation of damaged proteins, causing sarcomere disintegration leading to restrictive cardiomyopathy.

K. Graf-Riesen: None. **K. Kimura:** None. **A. Unger:** None. **A. Lothar:** None. **L. Hein:** None. **J. Daerr:** None. **J. Braune:** None. **A. Ooms:** None. **G. Li:** None. **S. Wu:** None. **J. Höhfeld:** None. **W. Linke:** None. **D. Fürst:** None. **B. Fleischmann:** None. **M. Hesse:** None.

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Identification of Novel Pathogenic Mutations in Non-Canonical RNA Splice Sites in Congenital Heart Disease

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RATIONALE: Congenital heart disease (CHD) occurs in about 1 in 100 live births, yet known genetic causes explain less than 20% of CHD cases. While variants that cause frameshift, nonsense, start/stop site gain or loss, and canonical splice site alterations are readily categorized as being pathogenic or loss-of-function (LOF), interpreting the clinical significance of variants without obvious functional consequences remains challenging. Here, we aim to improve classification of variants of unknown significance (VUS) in non-canonical RNA splice sites that may be pathogenic for CHD.

METHODS: We tested candidate LOF *de novo* (DNV) and rare ($p < 2E-5$) inherited variants from whole exome sequencing of 4474 CHD probands and their parents in the NHLBI Pediatric Cardiac Genetics Consortium. Briefly, variants underwent computational selection to prioritize VUS in splice regions that are likely to alter splicing ("high-likelihood VUS"). These variants then underwent *in vitro* analysis including Minigene construction, transfection, RNA isolation, and sequencing to confirm splicing outcomes.

RESULTS: Preliminary results limited to DNV variants showed that 163 of 2678 (6.08%) were high-likelihood VUS. Subsequent analysis *in vitro* assay of high-likelihood VUS yielded 53 as splice-altering ($p < 0.05$) and thus LOF. Combined with previously identified 366 DNV LOF variants, the addition of these splice-altering variants represents a 15.3% increase in total LOF DNV variant calls. This includes new pathogenic mutations in known CHD genes such as *KMT2D*. In one case, a CHD proband with features of Kabuki Syndrome without a definitive diagnosis was found to have a splice-altering variant in *KMT2D*. We have extended this assay to 34518 rare, inherited variants in the same cohort, of which 868 (2.54%) are in genes previously associated with CHD.

CONCLUSION: Consideration of non-canonical RNA splice sites in this assay increased the yield of LOF mutations from traditional sequencing methods by 15.3% in the CHD cohort. Further analysis of splice-altering variants in both known and unknown pathogenic genes will improve diagnostic classification of VUS and gene-based diagnosis of CHD.

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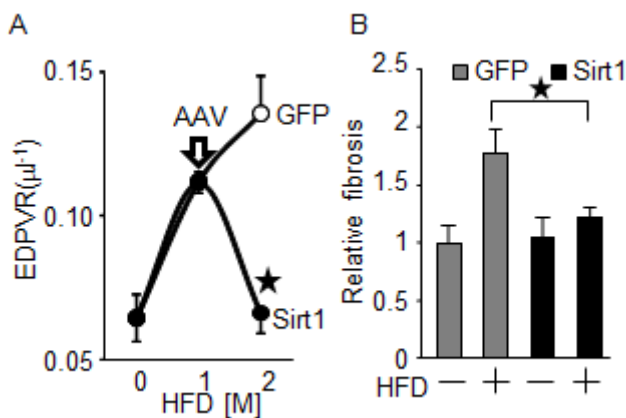
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Sirt1 Ameliorates High Fat Diet-induced Diastolic Heart Failure

Shinichi Oka, Peiyong Zhai, Junichi Sadoshima, Rutgers New Jersey Medical Sch, Newark, NJ

Approximately half of heart failure patients are diagnosed with diastolic heart failure, which is characterized by impaired relaxation. Cardiac fibrosis is observed in diastolic heart failure patients, which may promote diastolic dysfunction due to increased LV stiffness. Sirt1 is a protein deacetylase that regulates cardiac fibrosis. However, the role of Sirt1 in diastolic heart failure remains unknown. Since obesity is a significant risk factor for diastolic heart failure, we employed a high fat diet (HFD)-induced obese mouse model and cardiac-specific Sirt1 knockout (Sirt1cKO) and overexpression (Sirt1cTG) mice to define the role of Sirt1 in diastolic heart failure. Diastolic dysfunction induced by 1 and 3 months of HFD consumption was exacerbated in Sirt1cKO, evidenced by an increased end-diastolic pressure-volume relationship (EDPVR), an index of diastolic dysfunction obtained by pressure-volume loop analysis (EDPVR: wild-type mice (WT) Normal Diet (ND): 0.069; WT HFD (3 months): 0.11; Sirt1cKO ND: 0.063; Sirt1cKO HFD: 0.18*, $p < 0.05$ vs WT HFD). Consistently, HFD-induced diastolic dysfunction is ameliorated in Sirt1cTG mice (EDPVR: WT ND: 0.062; WT HFD: 0.11; Sirt1cTG ND: 0.061; Sirt1cTG HFD: 0.056*, $p < 0.05$ vs WT HFD). HFD-induced cardiac fibrosis was promoted in Sirt1cKO but ameliorated in Sirt1cTG mice. Moreover, exogenous expression of Sirt1 via adeno-associated virus vector ameliorated HFD-induced diastolic dysfunction and fibrosis (Figure). These results suggest that Sirt1 ameliorates diastolic heart failure, possibly through inhibition of cardiac fibrosis.



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Genomic Context Predicts Dilated but Not Hypertrophic Cardiomyopathy

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Rationale: Cardiomyopathies are a major cause of heart failure and have a strong heritable component.

Objective: Determine the role of both common and rare nonsynonymous genetic variation in hypertrophic (HCM) and dilated familial cardiomyopathy (DCM).

Methods and Results: Whole genome sequencing was used to determine common and rare nonsynonymous genetic variation in familial cases of HCM (n=56) or DCM (n=71). Variation was evaluated in 102 cardiomyopathy genes routinely assayed in clinical and commercial gene testing panels. We used cardiac gene expression data from GTEx (Genotype-Tissue Expression database) to define additional genes expressed in the heart. The number of nonsynonymous single nucleotide variants (nsSNVs), the majority of which were missense variants, was correlated with echocardiographic measurements. Principal component analysis (PCA) of left ventricular measures separated HCM and DCM. Regression of the first principal component using all nonsynonymous single nucleotide variants (nsSNVs) in cardiomyopathy genes showed the number of nsSNVs predicted DCM but not HCM. DCM probability in the cohort significantly increased as the number of cardiomyopathy gene nsSNVs increased ($p < 0.02$). The increase in nsSNVs in cardiomyopathy genes significantly associated with reduced left ventricular ejection fraction and increased left ventricular diameter in DCM. Resampling methods identified genes with deviant cumulative allele frequencies, identifying potential modifier genes for cardiomyopathy.

Conclusions: DCM subjects carry a greater burden of nsSNVs in cardiomyopathy genes. This genomic burden translates to impaired systolic cardiac function in DCM. In contrast, nsSNV burden in cardiomyopathy genes did not correlate with the probability or manifestation of left ventricular measures in HCM. These findings support a complex inheritance for DCM where increased variation in cardiomyopathy genes creates a genetic background that predisposes to DCM and increased disease severity. The distinct genetic landscapes of HCM and DCM suggest that greater genetic variation in cardiac genes provokes unfavorable ventricular remodeling with reduced systolic function.

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Reactivation of Fetal MicroRNAs Contribute to Dilated Cardiomyopathy

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Background

Dilated cardiomyopathy (DCM) is the most common cardiomyopathies that often leads to heart failure worldwide. MicroRNAs have emerged as an integral player in regulating diverse biological processes including those in the cardiovascular system. Previously, our lab has shown that the miR-424(322)/503 cluster is highly enriched during early cardiac fate determination and drives cardiomyocyte specification. However, the expression level of miR-424(322)/503 gradually decreases in the postnatal heart. Other studies have revealed the upregulation of miR-424(322)/503 in failing human hearts. Nevertheless, the association between miR-424(322)/503 and heart disease has not been studied yet.

Methods and Results

In this study, we created transgenic mice (TG) with tetracycline-controlled cardiomyocyte-specific miR-424(322)/503 expression. The TG mice and control mice (8 weeks) were fed with doxycycline-containing chow for up to a month. During this period, the weight of the mice and cardiac function were monitored. The weight of TG mice dropped significantly while controls grew normally. Echocardiography analysis showed continuous deterioration of cardiac function starting from 2 weeks. Ejection fraction and fractional shortening (n=8) decreased to $46 \pm 1.9\%$ and $22 \pm 1.09\%$ at 2 weeks and $15 \pm 2.7\%$ and $6 \pm 1.2\%$ at 4 weeks, respectively. In addition, left ventricular internal diameter (LVID,d and LVID,s) increased from 3.64 ± 0.098 and 2.5 ± 0.085 to 4.2 ± 0.2 and 3.9 ± 0.18 respectively. Morphologically, we found the hearts of TG mice to be larger than WT mice. H and E staining revealed dilated ventricular chambers and thinning of ventricular walls in TG. Likewise, fibrosis was observed in TG hearts through Masson trichrome stain. In addition, both atrial natriuretic peptides and brain natriuretic peptides increased by >8 folds in TG hearts (n=5). The level of β -MHC was higher while α -MHC was lower in TG hearts vs control. These indicate that TG phenotype is consistent with DCM.

Conclusion

Overall, this is the first study showing that upregulated miR-424(322)/503 in the heart is sufficient to lead to DCM. This study can potentially help establish miR-424(322)/503 as a novel therapeutic target to ameliorate heart failure progression.

S. Shrestha: None. **X. Shen:** None. **R. Liang:** None. **F. Wang:** None. **B.K. McConnell:** None. **Y. Liu:** None.

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Exploring Haploinsufficiency Drivers in a Set of cMyBP-C Missense Mutations Causing Hypertrophic Cardiomyopathy

Carmen Suay-corredera, Maria Rosaria Pricolo, Elías Herrero-Galán, Diana Velázquez-Carreras, David Sánchez-Ortiz, Ctr Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; Diego García-Giustiniani, Health in Code, A Coruña, Spain; Javier Delgado, EMBL/CRG Systems Biology Res Unit, Ctr for Genomic Regulation (CRG), The Barcelona Inst of Science and Technology, Barcelona, Spain; Silvia Vilches, Ctr de Investigación Biomédica en Red en Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; Fernando Domínguez, Ctr Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; Roberto Barriales-Villa, Unidad de Cardiopatías Familiares, Insto de Investigación Biomédica de A Coruña (INIBIC), Complejo Hospario Univrio de A Coruña, Servizo Galego de Saúde (SERGAS), Univ da Coruña, A Coruña, Spain; Giulia Frisso, Dept di Medicina Molecolare e Biotecnologie Mediche, Univ di Napoli Federico II, Napoli, Italy; Luis Serrano, EMBL/CRG Systems Biology Res Unit, Ctr for Genomic Regulation (CRG), The Barcelona Inst of Science and Technology, Barcelona, Spain; Pablo García-Pavia, Heart Failure and Inherited Cardiac Diseases Unit, Dept of Cardiology, Hosp Univrio Puerta de Hierro, Madrid, Spain; Lorenzo Monserrat, Health in Code, A Coruña, Spain; Jorge Alegre-Cebollada, Ctr Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease. Mutations in cardiac myosin-binding protein C (cMyBP-C) are a leading cause of HCM. However, as for many other genetic diseases, it remains challenging to define whether specific gene variants found in patients are pathogenic or not. Here, we have examined pathogenicity drivers in a group of clinically annotated exonic variants of MYBPC3, the gene encoding cMyBP-C. First, we did bioinformatics predictions of RNA splicing and of protein thermodynamic stability. To validate results, we studied RNA splicing of the MYBPC3 gene using peripheral blood from variant carriers, and circular dichroism measurements on purified recombinant proteins. Our results show that around half of the pathogenic exonic mutations alter RNA splicing or protein thermodynamic stability, both of which can lead to cMyBP-C haploinsufficiency. These molecular phenotypes are not found in control, non-pathogenic variants. Remarkably, the remaining pathogenic missense mutations appear to result in stable proteins, for which the cause of pathogenicity remains unknown. We propose that examination of protein haploinsufficiency drivers can define pathogenicity of genetic variants associated with HCM, decisive for the clinical management of patients and their families.

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Smyd1 Variants Regulate Distinct Areas of Chromatin in the Cardiomyocyte

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Heart failure is one of the most devastating conditions that contributes to 25% of all deaths in the USA every year. The development of heart failure, which is often preceded by cardiac hypertrophy resulting from an increased workload, is accompanied by global changes in gene expression that resemble a more fetal-like transcriptome. Although many epigenetic factors have been identified, which influence these underlying changes in transcription during disease, we still know very little about how this process is regulated on a molecular level. Smyd1 is a unique histone methyltransferase that modifies lysine residues to regulate gene expression in the cardiomyocyte. Originally, Smyd1 was identified as a necessary regulator of cardiac development, but recently we have shown that Smyd1 is differentially expressed during cardiac hypertrophy and failure and that loss of Smyd1 (in conditional, cardiomyocyte-specific knockout mice) leads to hypertrophic growth, metabolic dysfunction and ultimately heart failure. In mice, the *smyd1* gene produces two variants, Smyd1a and Smyd1b, which differ only by a 13 amino acid deletion. In addition, we have shown that overexpression of Smyd1a (but not Smyd1b) can prevent phenylephrine-induced hypertrophy, however, both variants are capable of suppressing ANF expression. Despite this interesting data, the specific genomic regions bound by these variants and the unique genes they regulate are unknown. Therefore, to identify the genes regulated by Smyd1 variants we performed ChIP-Seq in cardiomyocytes under normal and hypertrophic conditions. Interestingly, our analyses show that Smyd1a binds DNA more frequently than Smyd1b under basal

conditions (2,392 vs 1,004 binding events, respectively) but that Smyd1b exhibits greater binding in hypertrophic conditions (2,414 vs 5,045 binding events). Moreover, bioinformatics analysis of these sites confirmed both conserved and unique binding preferences and showed that while both variants are most abundant in distal intergenic regions (32-57%), Smyd1a is uniquely enriched (24%) at gene promoters (<1kb). These exciting results suggest novel and distinct regulatory functions of Smyd1 isoforms and begin to inform us how Smyd1 regulates cardiac morphology and physiology.

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Pharmacogenetics and Drug Discovery for Anthracycline-Induced Cardiotoxicity Enabled by Sinoatrial Node-like Cells Derived From Human Pluripotent Stem Cells

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The sinoatrial node (SAN) is the primary pacemaker of the heart. The human SAN is poorly understood due to limited primary tissue access and lack of robust *in vitro* derivation methods. We developed an efficient strategy, using a dual *SHOX2:GFP; MYH6:mCherry* knock-in reporter line, to generate and purify human pluripotent stem cell-derived SAN cells (hPSC-SAN), displaying molecular and electrophysiological characteristics of bona-fide nodal cells. We modeled cell type specific toxicity upon treatment with doxorubicin (DOXO) using hPSC-SAN generated from a library of induced pluripotent stem cells (iPSCs). We discovered 3 new genetic loci associated with increased sensitivity to DOXO-induced hPSC-SAN death. Genetic variants in these loci were associated with significantly higher early arrhythmia risk in patients receiving DOXO, confirmed by an unbiased PheWAS analysis. Finally, the *in vitro* DOXO assay enabled an unbiased drug screening platform and identification a candidate therapeutic that can partially block DOXO-mediated cardiac toxicity.

S. Chen: None.

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Role of Telomere Dysfunction in Duchenne Muscular Dystrophy Cardiomyopathy

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Duchenne muscular dystrophy (DMD) is a devastating X-linked genetic disorder that affects 1 in 3500 males. Characterized by progressive muscle degeneration that culminates in respiratory failure and dilated cardiomyopathy, DMD is caused by a lack of dystrophin, a protein that provides structural support between the sarcomeric cytoskeleton and the extracellular matrix. Loss of dystrophin leads to a leaky plasma membrane, contractile stress, and disruption of cellular homeostasis. However, the molecular mechanism that eventually leads to cell death remains to be explored. Recently, the Blau lab discovered a pathogenic link between DMD cardiomyopathy and telomere dysfunction. While mdx mice that lack functional dystrophin do not exhibit dilated cardiomyopathy as in human patients, when crossed with mTR mice that lack the RNA component of telomerase (TERC), mdx mice with "humanized" telomere lengths fully manifested the severe muscle wasting and cardiac failure seen in patients. Notably, the longer telomeres, characteristic of mice, appear to be cardioprotective. Importantly, we observed telomere shortening in cardiomyocytes, but not other cell types, of DMD patients compared to age-matched controls. Preliminary data suggests that contractile stress due to the lack of dystrophin leads to a pathogenic feed-forward loop which ultimately culminates in cardiomyocyte cell death. Using human iPS cells derived from DMD patients, we have modeled telomere shortening and aspects of cardiomyocyte dysfunction characteristic of DMD, including aberrant calcium transients, mechanical stress, and arrhythmia. By comparing cardiomyocytes derived from DMD iPS cells with those from CRISPR-corrected isogenic controls on patterned bioengineered hydrogel platforms of varying stiffness, we can study the role of fibrotic stiffening in the myocardium in the premature demise of DMD cardiomyocytes. Pinpointing the early molecular events that trigger the pathogenic feed-forward loop will provide strategies for intervention to ameliorate DMD cardiomyopathy.

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Poster Session 2 and Reception

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Identifying Signaling Pathways Regulated by Trophoblasts in Placental Vascular Development Using 3D Tissue Models

Olga Kashpur, Mary C Wallingford, Tufts Medical Ctr, Boston, MA

The placenta is an essential yet transient organ that supports healthy pregnancy and normal fetal development. Placental vascular dysfunction is associated with preeclampsia, HELLP (hemolysis, elevated liver enzymes, low platelets), fetal growth restriction, hemorrhaging, preterm birth, and loss of life. Placental vascular dysfunction can also negatively impact long-term maternal and offspring health, increasing risk for cardiovascular disease, coronary artery calcification, and stroke later in life. The vasculature of the maternal-fetal interface is uniquely characterized by the presence of trophoblast cells. Multiple trophoblast cell lineages serve essential functions in both the maternal vascular compartment and the fetoplacental angioarchitecture. Functional roles for FGF2, VEGF, and angiopoietin signaling pathways in endothelial vasculogenesis and angiogenesis are well supported by a large body of literature, but there is a gap in knowledge of placenta-specific molecular mechanisms that account for the unique interaction of trophoblasts and endothelial cells. I hypothesize that trophoblasts generate angiogenic signals that promote vascular development at the maternal-fetal interface in both an autocrine and paracrine manner.

Using 3D assays, I evaluated the role of endovascular cytotrophoblast and syncytiotrophoblast cell lines in establishment of a vascular compartment. Trophoblasts harbor intrinsic angiogenic competency evident by their ability to form angiogenic sprouts and generate 3D vascular networks under defined conditions. My current research implements 3D bioassays, bioengineered vessels, and chemical inhibition to further investigate paracrine angiogenic signaling between trophoblasts and endothelial cells.

Our work is the first to evaluate autocrine and paracrine angiogenic properties of trophoblasts under physiological 3D conditions that mimic development. When coupled with histological evaluation of human placenta, these systems will allow us to better evaluate and understand molecular mechanisms of placental vascular development and vascular dysfunction.

O. Kashpur: None. **M.C. Wallingford:** None.

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Cardiomyopathy Phenotypes Observed in Human Engineered Heart Tissue Depend on Functional Maturation

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Human iPSC-based models of cardiomyopathies have become increasingly prevalent. These models enable the study of genotype-phenotype relationships within a human context and have already led to new insights. A potential limitation of iPSC-derived cardiomyocytes (iPSC-CMs) is their varying degrees of functional maturation with respect to electrophysiology, calcium handling, and contractility. Variable levels of maturity may explain how different groups investigating hypertrophic cardiomyopathy (HCM) mutations in iPSC cardiomyocytes have arrived at conflicting results. Nevertheless, whether or not advanced functional maturation truly impacts disease phenotype in iPSC cardiomyocyte models remains an open question. Using a novel protocol that combines pacing and physiological media in a three-dimensional engineered heart tissue (EHT) derived from decellularized myocardium, we are able to mature human engineered heart tissues within three weeks to recapitulate key functional characteristics of adult heart tissue, such as a robustly positive force-frequency response, a strong post-rest potentiation, potent calcium handling and excitation-contraction coupling, and mature isometric twitches that closely match that of intact adult human ventricular myocardium. Using our platform, we investigated the effect of maturation on the functional consequence of a classic variant (E62Q) in the thin filament protein alpha-tropomyosin (TPM1). Initial characterization of TPM1 E62Q in EHTs show a hypercontractile phenotype compared to its isogenic wild-type line, with limited changes in calcium handling. Preliminary data on maturing TPM1 E62Q EHTs show that additional hallmarks of disease may develop with maturation such as arrhythmogenicity, hypertrophy, and fibrosis. In our work, we develop a method for advanced maturation of hiPSC-CMs in EHTs and show the dependence on maturation status of progressive hallmarks of HCM in human EHTs compared to EHTs grown under conditions standard for the field.

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A Novel Role for Telomerase in Calcific Aortic Valve Disease

Luis Hortells, Camille Boufford, Cailyn Regan, Claire Chu, William J Moorhead III, Genevieve Doyon, Dennis Bruemmer, **Cynthia St Hilaire**, Univ of Pittsburgh, Pittsburgh, PA

Intro: Telomerase (TERT) is an enzyme best known for its telomere-extending activities on the ends of chromosomes, however, less known are the non-canonical, transcriptional and epigenetic activities of TERT. We sought to assess if TERT, through non-canonical activities, contributes to the progression of calcific aortic valve disease (CAVD). It has been established that overexpression of TERT in mesenchymal stem cells primes these cells to differentiate into osteoblasts. Other studies have identified non-canonical roles for TERT in inducing the transcription of genes in inflammatory and cell differentiation pathways.

Methods: Using mesenchymal stem cells, primary valve interstitial cells (VICs) from control and CAVD patients, and smooth muscle cells (SMCs) from WT and TERT knockout mice, we performed in vitro biochemical assays to study the role of TERT in the calcification process.

Results: Our data shows that TERT is highly expressed in CAVD valves compared to Control valves. Under osteogenic differentiation conditions Control valve interstitial cells (VICs) upregulate TERT protein levels and calcify. Inflammatory signals induce TERT expression in VICs and exacerbates calcification. Similarly, WT vascular VSMCs readily calcify in vitro, but VSMCs from TERT knockout mice do not, and TERT deletion also reduced valve calcification in LDLR/TERT double knock mice. These studies provide evidence that TERT is necessary for the osteogenic switch of a healthy to a calcifying VIC. Knocking down TERT reduces expression of the osteogenic transcription factor RUNX2 and we further provided evidence that STAT5 may help to mediate TERT's effects on osteogenic gene transcription.

Conclusion: From these data we suggest that TERT is required for valve calcification by inducing the osteogenic transition of quiescent valve interstitial cells (VICs) into calcifying VICs, and that TERT and STAT5 co-regulate transcription of osteogenic genes. These results indicate that TERT is an active contributor to the calcification process of valve tissues and our future studies will delineate the mechanisms involved.

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Investigation into the Genetic Cause of Congenital Dilated Cardiomyopathy Using Human Induced Pluripotent Stem Cells

Kuo-chen Wang, Young Wook Chun, Charles C. Hong, Univ of Maryland, Baltimore, Baltimore, MD

Congenital dilated cardiomyopathy (cDCM) is a rare but typically fatal disease. In most cases, its etiology is unknown, but a genetic root cause is often suspected. The objective of current study is to determine whether a novel non-structural gene causes cDCM. We hypothesized that rare genetic mutations caused cDCM, which could be modeled using patient-derived induced pluripotent stem cells (iPSCs). We generated the cardiomyocytes from iPSCs of a cDCM proband and found significant impairment in contractility and mitochondrial function, compared to those of healthy controls. To identify the causal mutations, we performed a whole exome sequencing of the cDCM patient and his parents ("trio"). Based on the assumption of recessive mode of inheritance or a *de novo* mutation, 9 candidate causal genes were identified. On the ground of the expression profiles of trio and pathogenicity predictor algorithms, we identified an indel and a nonsynonymous point mutation in the rotatin (encoded by *RTTN*) gene as the putative genetic defect responsible for cDCM. CRISPR/Cas9-mediated knockout of *RTTN* in healthy control iPSCs recapitulated cardiomyocyte defects, and the correction of the missense mutation in the disease iPSCs restored cardiomyocyte structure and function, confirming causality. Thus, rotatin is a new causal molecule for cDCM and plays an important role in cardiac regulation.

K. Wang: None. **Y. Chun:** None. **C.C. Hong:** None.

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Modeling PKP2 Mutation Associated Arrhythmogenic Cardiomyopathy With CRISPR-edited iPSC-derived Cardiomyocytes in Engineered Cardiac Tissues

Kehan Zhang, Samuel Tomp, Jourdan K Ewoldt, Boston Univ, Boston, MA; Christopher N Toepfer, Harvard Medical Sch, Boston, MA; Anant Chopra, Boston Univ, Boston, MA; Christine E Seidman, Jonathan G Seidman, Harvard Medical Sch, Boston, MA; Christopher S Chen, Boston Univ, Boston, MA

Arrhythmogenic cardiomyopathy (ACM), also known as arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), is a leading cause of sudden death among young adults. Over half of ACM cases are associated with inherited desmosome gene mutations, most commonly in the gene PKP2 which encodes plakophilin-2. One of the obstacles to better understanding ACM pathogenesis is the lack of appropriate models which encompass the early stages of disease development; this imposes significant constraints on the advancement of clinical therapies. The recent advent of human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) has enabled the development of models for studying human cardiac cell biology and pathology. In this work, we combine hiPSC-CMs, CRISPR/Cas9 genome editing, and engineered cardiac tissue platforms to develop human *in vitro* systems for investigating the molecular mechanisms of ACM pathogenesis.

Isogenic control and mutant cell lines of hiPSC-CMs harboring ACM-associated PKP2 mutations were generated using CRISPR/Cas9 technology. Specifically, the PKP2tv cell line has an early truncation in plakophilin-2 that mimics PKP2 c.235C>T found in multiple family lineages. The effects of the PKP2tv mutation on cardiac tissue contractility were characterized using a 3D cardiac micro-tissue (CMT) platform. CMTs composed of PKP2tv cardiomyocytes were shown to have significantly decreased contractile forces compared to the control, which recapitulates the reduced ventricular systolic function seen in ACM patients. This result demonstrated the feasibility of using the hiPSC-derived tissue-engineering model to recapitulate ACM disease phenotype and allow for future investigation into the disease mechanisms.

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STING(ing) the Heart: How DNA Damage and Inflammation Orchestrate Cardiac Remodeling and Heart Failure

Mohammad H. Al-Khalaf, Gauri Akolkar, Peter P. Liu, uOttawa Heart Inst, Ottawa, ON, Canada

Background: We aimed to investigate the role of DNA breaks in inducing inflammation leading to adverse remodeling, premature senescence, and cardiac dysfunction leading to onset of heart failure. **Methods:** *in vivo*: Heart-focused IR (using XRad225Cx from PXi) on adult Sprague Dawley rats; 4-5 per group; dose: 0, 15, 30, 45 Gy (endpoint 12 weeks post-IR). Serial echocardiogram and blood collection, P/V at endpoint; whole heart harvest analysis. *in vitro*: Primary cardiomyocytes from adult rats; human derived cardiac AC16 cell line; UVB ionizing radiation; protein extraction, western probing; gene expression analysis; immunofluorescence staining and microscopy. **Results:** DNA damage response, as illustrated by XRCC1 protein expression, is upregulated in multiple heart injury/failure models (TAC, MI, aging) and differ depending on cell stage and/or age (*in vitro* proliferative vs differentiated AC16 cells). Using *in vitro* UVB radiation, exposure alters cardiomyocyte morphology, illustrated by α -actinin immunostaining, and activates innate inflammation and senescence, as illustrated by westerns and immunostaining of cGAS, STING, p16 and other relevant proteins. We demonstrate using heart-focused ionizing radiation a novel technique to induce aging related DNA damage and onset of diastolic heart dysfunction. Serial ECG show significant increase in E/A ratios at 8- and 12-week post irradiation of 30 and 45 Gy (E/A>2.0). We show P/V analysis supporting ECG data (~50% decrease in dPdt min, 2-fold increase in Tau, and significant [p<0.01] increase in End diastolic pressure, and no change in End systolic pressure). Using an inhibitor targeting cytosolic DNA sensor cGAS, we show attenuation of cardiac remodeling and expression of senescence. **Conclusions:** We elucidate the molecular pathway originating from the onset of DNA damage accumulation, leading to activation of specialized innate immunity proteins that ultimately upregulate inflammation and senescence. Here we uncover and connect key interactions between multiple pathways converging towards heart failure, and provide evidence for a novel therapeutic drug targeting cardiac aging.

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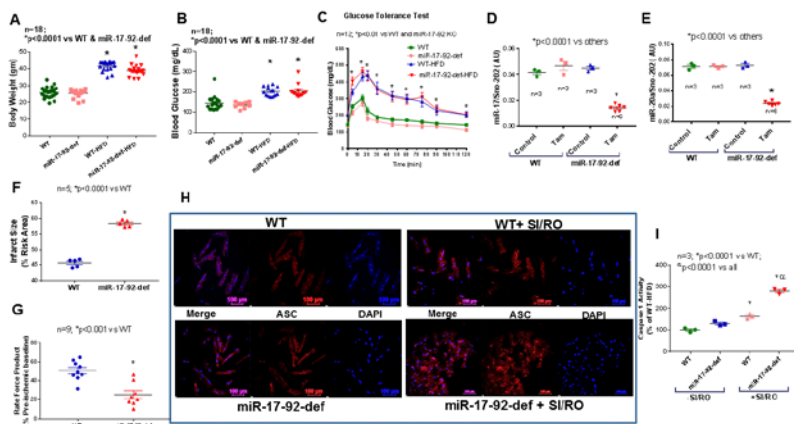
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Deficiency of Myocardial miR-17-92 Cluster Exacerbates Ischemic Injury in Diabetic Mice

Arun Samidurai, Sean K Roh, Rakesh C Kukreja, Fadi N Salloum, **Anindita Das**, Virginia Commonwealth Univ, Richmond, VA

Background: Dysregulation of miRNAs is associated with the pathogenesis of cardiovascular diseases. miR-17-92 cluster has been identified as a negative regulator of inflammation in rheumatoid arthritis. In diabetes, the persistent activation of NLRP3 inflammasome contributes to cardiac dysfunction and the severity of I/R. NLRP3 inflammasome consists of cryopyrin, apoptosis-associated speck-like protein (ASC), and procaspase-1 protein. The inflammasome triggers caspase-1 induced programmed cell death known as pyroptosis. Considering the pivotal role of NLRP3 in diabetes, we investigated the anti-inflammatory role of miR-17-92 cluster against I/R injury. **Methods and Results:** Adult male wild type and cardiac-specific miR-17-92-deficient mice were fed high-fat-diet (HFD) for 16 weeks before subjecting the heart to ex vivo I (30 min)/R (1 h) in Langendorff mode. HFD-fed mice exhibited impaired glucose tolerance with increased body weight and blood glucose levels (Figs. A, B, C). Tamoxifen-treatment (20 mg/kg, i.p. for 5 days) significantly reduced cardiac miR-17 and miR-20a in HFD-fed miR-17-92-deficient mice (Figs. D, E), which resulted in increased myocardial infarct size and cardiac dysfunction following myocardial I/R injury as compared to WT mice (Figs. F, G). ASC aggregation and caspase 1 activity following simulated ischemia/reoxygenation were increased in isolated adult cardiomyocytes from diabetic tamoxifen-inducible miR-17-92-deficient mice as compared to WT mice (Figs. H & I). **Conclusion:** miR-17-92 plays a critical role in attenuating exacerbated myocardial I/R injury in diabetic mice by regulating NLRP3 inflammasome.



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Immune Response Factors in Patients With Diabetes Mellitus and Acute Coronary Syndrome

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Purpose. To study cardiac remodeling in patients with ACS complicated with DM through analysis of laboratory parameters and echocardiogram. **Methods.** A total of 67 patients were prospectively enrolled (age range: from 36 to 79 years; average: 60,96 ± 9,22 years) with ACS who were admitted to Emergency Hospital. The patients were divided into 2 groups depending on the presence of DM: 23 patients with DM (age average: 65,09 ± 7,73 years) and 44 without DM (age average: 58,79 ± 9,19 years). Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), high sensitive C reactive protein (hs-CRP), antibodies to LPS General Enterobacteriaceae, antibodies to LPS Re-Glycolipid were tested for all patients. Echocardiograms were obtained on all the patients, interventricular septal thickness (IVSd) and others parameters were measured by taking the mean values of three continuous heartbeat cycles in the expiratory state. All indicators were calculated using the statistics program Statistica, a P-

value of $< 0,05$ was considered statistically significant. Results. There were no significant differences in the prevalence of smoking, family history of CVD, hypertension between groups. No differences were observed in the levels of serum hs-CRP, TC, HDL-C, LDL-C; but TG levels were significantly higher in patients with DM ($3,16 \pm 2,61$ mmol/l) than in controls ($1,75 \pm 1,33$ mmol/l, $p < 0,05$). Serum antibodies to LPS General Enterobacteriaceae levels ($441,52 \pm 303,70$ A.U.O.D) were also significantly higher in the DM group than in controls ($271,41 \pm 200,13$ A.U.O.D, $p < 0,05$). The levels of serum antibodies to LPS General Enterobacteriaceae were positively correlated with IVSd ($r = 0,765$, $p < 0,05$). Conclusion. The data demonstrated, that patients with DM have a greater immune response which correlates with indicators of left ventricular remodeling. The mechanism of the cardiac function impairment, however, has not been clearly explained yet. The role of the immune response in the development of cardiac muscle remodeling is not fully understood. It is currently considered that these changes may participate in the pathological process of left ventricular remodeling in patients with DM.

S. Glova: None. **S. Shlyk:** None.

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Overexpression of the Prostaglandin E2 EP3 Receptor Reduces Cardiac Function in an Angiotensin II Model of Hypertension

Timothy D Bryson, Jiang Xu, David Taube, Edward Peterson, **Pamela HARDING**, Henry Ford Hosp, Detroit, MI

Prostaglandin E2 (PGE2) EP receptor subtypes EP3 and EP4 are present in the heart and signal via decreased and increased cAMP production, respectively. Previously we reported that cardiomyocyte-specific EP4 KO mice develop a phenotype of dilated cardiomyopathy with reduced ejection fraction, that PGE2 decreases contractility via EP3 in vivo, in vitro and ex vivo; and that EP3 expression in the heart increases when mice are subjected to Angiotensin (Ang) II-hypertension (HTN). We therefore hypothesized that overexpression of EP3 in the heart would exacerbate Ang II-HTN. To test this hypothesis, we used 10-12 week old male EP3 transgenic (Tg) mice with EP3 overexpression in cardiomyocytes and their wild type (WT) controls infused with either vehicle or 1.4 mg/kg/d Ang II for 2 weeks. Echocardiography was performed on conscious mice at the end of the experiment and systolic blood pressure (SBP) was measured every 2 days by tail cuff. Results: Table 1. All data are reported as means \pm SEM. N= 3-6 mice per group. Statistical significance: * and *** $p < 0.05$ and $p < 0.005$ vs WT + vehicle, ++ $p < 0.01$ vs WT + Ang II, §§§ $p < 0.005$ vs Tg + vehicle.

As shown in the table, cardiac function was unaffected in WT mice after Ang II infusion. Baseline cardiac function was reduced in EP3 Tg mice receiving vehicle and in contrast to WT mice, was further diminished after Ang II infusion. These differences were not due to altered systolic blood pressure. In conclusion, EP3 Tg mice present with reduced cardiac function at baseline and in support of our hypothesis, exhibit an enhanced response to Ang II. Antagonism of the EP3 receptor may be a novel treatment for heart failure associated with hypertension.

	WT + Vehicle	WT + Ang II	Tg + Vehicle	Tg + Ang II
EF (%)	76.18 \pm 1.25	75.30 \pm 0.80	56.85 \pm 7.85	45.92 \pm 9.63**
SF (%)	61.69 \pm 2.94	63.21 \pm 1.37	42.92 \pm 7.86	34.77 \pm 7.33**
LVDs (mm)	1.03 \pm 0.06	0.88 \pm 0.04***	2.18 \pm 0.66***	2.40 \pm 0.54**
LVDd (mm)	2.62 \pm 0.01	2.42 \pm 0.05*	3.56 \pm 0.52*	3.50 \pm 0.42**
PWTd (mm)	1.05 \pm 0.04	1.11 \pm 0.03	0.94 \pm 0.01	1.00 \pm 0.05
Mass (mm ³ /10 g bw)	30.60 \pm 1.90	31.99 \pm 1.07	48.45 \pm 12.34	46.89 \pm 5.80
CO (ml/min/10 g bw)	13.84 \pm 1.04	15.97 \pm 1.47	12.91 \pm 0.88	10.86 \pm 1.11**
SBP (mm Hg)	107 \pm 6.4	169.4 \pm 7.9***	106 \pm 3.2	151.5 \pm 9.4§§§

T.D. Bryson: None. **J. Xu:** None. **D. Taube:** None. **E. Peterson:** None. **P. Harding:** None.

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CC Chemokine Receptor 5 Protects the Heart from Inflammation in Pressure Overload-induced Cardiac Dysfunction

Masato Ishizuka, Haruhiro Toko, Mutsuo Harada, Jiayi Guo, Satoshi Bujo, Haruka Yanagisawa-Murakami, Issei Komuro, The Univ of Tokyo, Tokyo, Japan

Objective: Sustained inflammation is a key pathological process in the development of heart failure and thus suppression of inflammation possibly leads to improvement of cardiac dysfunction. It has been suggested that CC chemokine receptor 5 (CCR5) scavenges pro-inflammatory mediators and resolves inflammation. The aim of this study is to uncover the role of

CCR5 in the pathogenesis of heart failure. *Methods:* Pressure overload heart failure was induced by transverse aortic constriction (TAC). Sham or TAC operation was performed in male 10-week-old CCR5 knockout (KO) mice and wild-type (WT) controls. Cardiac function and morphology were examined by echocardiography 1 week and 4 weeks after TAC surgery. After euthanization, the heart was extracted and embedded in paraffin for the measurements of cardiac fibrosis and cardiomyocyte hypertrophy with EVG stain and immunohistochemistry using WGA, respectively. The extent of inflammation was evaluated by the immunostaining against CD45 and F4/80 antigens, and by qRT-PCR for inflammatory cytokines and chemokines. *Results:* We found that heart weight was significantly larger and fractional shortening was significantly lower in CCR5 KO mice than those in WT 4 weeks after TAC, indicating CCR5-induced protective effects on heart failure development. Whilst there was no difference observed in the extent of fibrosis, the size of cardiomyocytes from CCR5 KO heart tended to be increased compared to WT. The number of CD45-positive inflammatory cells and F4/80-positive macrophage infiltration was increased 1 week after TAC in KO mice. The RNA expression levels of monocyte chemoattractant protein-1, transforming growth factor- β and tumor necrosis factor- α were significantly higher and NF κ B p65 was more phosphorylated in KO mice. *Conclusions:* We revealed that the inflammation in pressure overload-induced heart failure was exacerbated by the deletion of CCR5 and, therefore, CCR5 could contribute to resolution of inflammation and be a potential therapeutic target for the heart failure treatment.

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Sex-Specific Inflammatory Gene Expression in the Hypertrophied and Failing Human Heart

Georgios Kararigas, Daniel Lehmann, Charite Univ Hosp, Berlin, Germany; Christoph Knosalla, German Heart Inst, Berlin, Germany; Lea Gaignebet, Charite Univ Hosp, Berlin, Germany

Heart failure (HF) is a devastating syndrome with poor prognosis that is most often preceded by a left ventricular (LV) pressure overload (PO) disorder, such as aortic stenosis (AS). As pressure inside the LV increases, LV hypertrophy (LVH) develops. Persistent inflammation is known to be detrimental to the heart, which might promote HF. LVH manifests differently between men and women and there are pronounced sex differences in disease progression and outcome. However, the mechanisms that cause these differences are poorly understood. Hypothesizing significant sex differences, we aimed at studying the expression of inflammatory and remodeling genes in cardiomyocytes (CMs) isolated from the LV septum of AS patients ($n = 34$; 50% men; 67.5 ± 9.6 years old) undergoing aortic valve replacement. Echocardiography was performed 1 week before surgery and 20.06 ± 9.7 days after surgery. Ethics committee approval and patient consent were obtained. There was no significant difference between men and women in age, body mass index, systolic and diastolic blood pressure, co-morbidities and medication. Posterior wall thickness ($P = 0.02$), LV end diastolic diameter ($P = 0.007$) and mass index ($P = 0.03$) were higher in men than women. Preoperative ejection fraction (EF) was significantly lower in men than women ($P = 0.01$). Postoperative EF levels improved in men reaching those of women. Compared to female CMs, male CMs had significantly higher levels of *CTGF* ($P = 0.03$) and *NFKB1* ($P = 0.01$), as well as *ACTC1* ($P = 0.03$), *GATA4* ($P = 0.03$), *GJA1* ($P = 0.03$), *MYH6* ($P = 0.02$), *MYL4* ($P = 0.03$), *NPPA* ($P = 0.03$) and *NPPB* ($P = 0.05$). We found a negative association between gene expression and postoperative EF in men only for *GATA4* ($r = -0.77$, $P = 0.01$), *GJA1* ($r = -0.69$, $P = 0.03$), *MYH6* ($r = -0.69$, $P = 0.04$), *MYH7* ($r = -0.82$, $P = 0.008$), *MYL4* ($r = -0.71$, $P = 0.03$), *NPPA* ($r = -0.9$, $P = 0.001$) and *NPPB* ($r = -0.79$, $P = 0.01$). In end-stage HF, the levels of the chemokine receptor *CXCR4* were higher ($P = 0.04$) in LV samples of female ($n = 5$) vs. male ($n = 4$) patients. Collectively, in PO and HF, the expression of inflammatory and remodeling genes is regulated in a sex-specific manner, which may contribute to the mechanisms underlying sex differences in disease progression and outcome.

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Small Gtpase RhoE Regulates Inflammatory Response in Myocardial Infarction

Weijia Luo, Yuan Dai, TAMU, Houston, TX; Jiangping Song, Dept of Cardiac Surgery, State Key Lab of Cardiovascular Disease, Fuwai Hosp, Beijing, China; Jiang Chang, TAMU, Houston, TX

Background: Myocardial infarction (MI) is a severe clinical condition caused by coronary artery thrombotic occlusion. An acute inflammatory response to MI is essential for cardiac healing, while excessive and prolonged inflammation causes cytotoxic damages and provokes adverse cardiac remodeling. In the present study, we demonstrate a novel inflammatory mediator, RhoE, and its cardioprotective role in MI.

Methods: We use three genetic mouse lines in this study: global RhoE knockout, cardiac-specific RhoE haploinsufficient, and cardiac-specific RhoE overexpression mice. Cardiac function and inflammation in the first week post MI are assessed in mice receiving left anterior descending artery (LAD) ligation. Mechanistic study is conducted through a set of molecular signaling experiments including bimolecular fluorescence complementation (BiFC), immunoprecipitation, electrophoretic mobility shift assay and mRNA microarray analysis. Finally, we investigate the expression and clinical significance of RhoE in MI patients.

Results: RhoE is upregulated in mouse post MI. RhoE deletion leads to upregulation of pro-inflammatory genes in mouse embryo heart. RhoE haploinsufficiency causes excessive inflammatory response with deleterious cardiac function post MI, while RhoE overexpression restrains post-MI inflammation and preserves cardiac function and survival. Mechanistically, RhoE specifically inhibits NF- κ B activation in vivo and in vitro. RhoE binds to p65 and p50 individually in cytosol, blocking their dimerization and nuclear translocation. Consistent with findings in the mouse MI model, MI patients with higher RhoE expression show diminished cardiac inflammation and consequently a better prognosis.

Conclusions: This study identified RhoE as a fine-tuning factor modulating post-MI inflammation. RhoE expression level in heart positively correlates with the outcomes of MI patients.

Impact: The discovery of RhoE provides a potential therapeutic target for MI. RhoE abundance in the heart post-MI might be used for prognosis assessment.

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Adipolin/C1q/Tnf-related Protein 12 reduces Neointimal Formation and Atherosclerotic Lesion Development By Modulation Of Macrophage Inflammatory Response And Endothelial Cell Function

Koji Ohashi, Hayato Ogawa, Takashi Enomoto, Naoya Otaka, Toyooki Murohara, Noriyuki Ouchi, Nagoya Univ Graduate Sch of Med, Nagoya, Japan

Objectives: Obesity is causally linked to the progression of vascular disorders including atherosclerosis and post-angioplasty restenosis. Recently, we identified adipolin/C1q/Tnf-related protein 12 as an insulin sensitizing adipokine, which is downregulated in rodent models of obesity. Here, we investigated whether adipolin modulates the development of vascular diseases using loss-of-function genetic manipulations. **Methods and Results:** Under basal conditions, no significant differences were observed in body weight, organ weights, blood pressure and fasting plasma glucose levels between wild-type (WT) and adipolin-knockout (APL-KO) mice. APL-KO mice exhibited enhanced neointimal thickening after femoral artery injury compared with WT mice, which was accompanied by increased expression levels of pro-inflammatory mediators including tumor necrosis factor (TNF)- α and interleukin (IL)6 in injured vessels. Conversely, systemic administration of adipolin to WT mice using adenoviral vector expression systems ameliorated injury-induced neointimal thickening and inflammatory response. APL-KO mice also showed impaired re-endothelialization after vascular injury compared with WT mice. Furthermore, adipolin deficiency increased atherosclerotic lesion area in an apolipoprotein E-knockout mouse model, which was accompanied by enhanced inflammatory response in the arterial vessel walls. Treatment of cultured macrophages with recombinant adipolin protein attenuated lipopolysaccharide-stimulated expression of inflammatory mediators, such as TNF- α and IL6. The anti-inflammatory actions of adipolin in macrophages were reversed by inhibition of transforming growth factor- β receptor II (TGF- β RII)/Smad2 signaling. In addition, treatment of human umbilical vein endothelial cells with adipolin protein promoted migratory activity and reduced serum starvation-induced apoptosis. **Conclusion:** These data suggest that adipolin suppresses injury-induced neointimal formation and atherosclerosis development through its abilities to modulate macrophage inflammatory responses and endothelial cell functions in vascular walls.

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Multidirectional Analysis of Anti-atherosclerotic Effect Caused by Korean Radish in Apo E $^{-/-}$ Mice Model

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Atherosclerosis, a leading cause of worldwide mortality and morbidity, is mainly controlled with statins, however, the statins have severe side effects like rhabdomyolysis. In search of an alternative for statin, we investigated anti-atherosclerotic and anti-hyperlipidemic effects of two different species of *Raphanus sativus* Linn (also known as radish), Jeju (white radish) and Ganghwa (purple radish) cultivated in South Korea. An atherosclerosis model in *ApoE* knockout mice was firstly established with high fat diet, followed by feeding the mice with extracts of each radish. Concentration of plasma lipids and histological changes were evaluated. Metabolomics analysis of urine was determined to see the effect of radish. Ganghwa-radish treated mice were observed with significantly decreased levels of total cholesterol, low-density lipoprotein, triglyceride, atherogenic index and cardiac risk factor while high-density lipoprotein was significantly increased. Steroid biosynthesis and steroid hormone biosynthesis were although significantly altered metabolic pathways among both radish treated groups, however, significant changes in these pathways were mainly observed with the treatment of Ganghwa-radish. Crucially, estrogens, estrone and estradiol, were significantly decreased in Ganghwa-radish treated group compared to control. In conclusion, we confirmed the prominent antilipidemic effect of Ganghwa-radish compared with Jeju-radish and such results might be due to the inhibition of estrogens by Ganghwa-radish treatment.

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Role of Slug / PIP Axis in Pulmonary Hypertension Secondary to Pulmonary Fibrosis

Gregoire Ruffenach, Mylene Vaillancourt, Jason Hong, Nancy Cao, Christine Cunningham, Rajan Sagggar, Srinivasa Reddy, Soban Umar, Gregory Fishbein, Mansoureh Eghbali, Univ of California, Los Angeles, CA

Pulmonary hypertension (PH) secondary to pulmonary fibrosis (PF-PH) is the second most common cause of PH. While never studied in detail, pathology reports suggest that vascular remodeling (VR) differs between PF and PF-PH. In PF, VR is mainly limited to fibrotic areas, whereas in PF-PH, it also exist in non-fibrotic areas. These histological differences suggest potential molecular differences. To better understand this mechanism, we investigated the expression of the transcription factor Slug which is known to play a role in PH and PF.

Using explanted rat (n=7/group) and human (n=7-14/group) lungs, we compared lung fibrosis, VR and Slug expression between non-fibrotic and fibrotic areas in PF and PF-PH. Online microarray data (GSE24988) were used to find the targets of Slug that are implicated in VR. A new animal model recapitulating our findings in patients was used to test the therapeutic potential of Slug inhibition (n=10/group). This model is based on intra-tracheal instillation of bleomycin (2.5mg/Kg) at day 0 and 2weeks later, an injection of monocrotaline (60mg/kg). PH was assessed by right ventricular systolic pressure (RVSP). P<0.05 are considered significant.

In both PF and PF-PH patients, fibrotic areas (PF29±4; PF-PH37±3) exhibit significantly increased VR when compared to non-fibrotic areas of the lung (PF 22±1; PF-PH 31±6). PF-PH patients have increased pulmonary vascular thickening in both areas vs PF patients. This is concomitant with an increased number of Ki67+ vascular cells in PF-PH (12±2%) vs PF (8±1%) as well as an upregulation of Slug in PF-PH patients (2.3±0.5) vs PF (1±0.1). Co-immunolabeling with CD68 demonstrate that macrophages are the main cell type responsible for Slug up-regulation in PF-PH. Human microarray data reveal an up-regulation of the Prolactin-induced protein (PIP) in PF-PH vs PF (9±3 vs 1±0.4). PIP is an extracellular transcriptional target of Slug, known to promote cell proliferation. In-vitro, PIP significantly increases pulmonary arterial smooth muscle cell proliferation in a dose dependent manner. Finally, Slug inhibition decreases RVSP (47±3 vs 62±3mmHg) in an animal model of PF-PH.

There are histological differences between PF-PH and PF lungs that are at least in part mediated by a Slug/PIP axis leading to VR.

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Determinants of Atrial Fibrillation Mechanisms Using Metabolomic Profiling

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Introduction: Atrial fibrillation (AF) represents one of the most common arrhythmias seen clinically, yet, current treatment paradigms have proven largely inadequate. One of the main contributors to the pathophysiology of AF is inflammation. Hence, reduction of inflammation associated atrial remodeling represents a novel therapeutic strategy for the treatment of AF. Oxylipins are derived from arachidonic acid, which is metabolized through three pathways including the cytochrome P450 (CYP450) pathway. The CYP450 products, epoxyeicosatrienoic acids (EETs), are major anti-inflammatory metabolites with several cardioprotective effects. The central hypothesis to be tested is that patients with AF will demonstrate an increase in oxylipin profiles towards inflammatory features compared to patients with normal sinus rhythm (NSR). We further hypothesize that lipid mediators may represent a new therapeutic target for AF. **Methods:** In the cross sectional study, human atrial appendage specimens and blood from informed and consented patients undergoing coronary artery bypass graft surgery were obtained in accordance with the approved UC Davis IRB protocol. Metabolomic profiling and inflammatory cytokines were assessed from the plasma of patients using LC-MS/MS. The underlying mechanisms of AF were determined using an integrated approach with molecular biology, flow cytometry, and electrophysiology. **Results:** Metabolomic profiling shows a significant decrease in the EETs/DHETs ratios (Fig 1A) and EpOMEs/DiHOMEs ratios (Fig 1B) in the cohort with AF compared to patients in NSR. There was a significant increase in inflammatory cytokine, chemokine levels and oxidative stress in patients with AF compared to patients in NSR. There was a significant increase in nuclear translocation of NF- κ B (nNF- κ B) from patients with AF compared to NSR using western blot analysis. Assessment of total I κ B and phosphorylated-I κ B (pI κ B) levels showed a decrease in I κ B level and an increase in the pI κ B levels associated with the activation of NF- κ B in AF compared to the NSR cohort. **Conclusions:** The study provides important insights into metabolomic profiles in patients with AF and reveals the prospect of EETs/DHETs being a novel therapeutic target in the treatment of AF.

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Poster Session 2 and Reception

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Inhibition of the Rna Binding Protein Hur Protects Against Cardiac Ischemia/reperfusion Injury by Reducing Inflammatory Gene Expression

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Despite medical advances, cardiac ischemia/reperfusion (I/R) injury remains a leading cause of morbidity and a huge economic burden in the United States. RNA binding proteins are becoming recognized as potential mediators of cardiac physiology and pathology, but the role of HuR, an RNA binding protein highly expressed in myocytes, in acute cardiac I/R injury is unknown. HuR has been shown in other tissues to be a critical post-transcriptional mediator of pro-inflammatory chemokine and cytokine gene expression, and we have shown HuR to be activated (nuclear-to-cytoplasmic translocation) in cardiomyocytes 2 hours post-ischemia/reperfusion injury. To address the functional role of HuR in I/R, cardiomyocyte-specific HuR deletion mice (iCM-HuR^{-/-}) were subjected to 30 minutes of LAD (left anterior descending) coronary artery ligation followed by 24 hours of reperfusion. In parallel, a separate group of wild-type mice were subjected to 30 minutes ischemia with a pharmacological inhibitor of HuR given just prior to reperfusion. Analysis of infarct size showed a smaller infarct with both HuR genetic deletion (~10% decrease in infarct size compared to control, N=3, P<0.05) and pharmacological inhibition (~11% decrease in infarct size compared to vehicle, N=4, P=0.069). Similarly, HuR inhibition significantly reduced cell death, caspase-3 activity, and inflammatory gene expression in an *in vitro* model of simulated I/R using neonatal rat ventricular myocytes. HuR inhibition results in a significant blunting of IL-6 and TNF-alpha gene expression two hours post-reperfusion *in vivo*, suggesting that HuR activity is necessary for the early induction of inflammatory gene expression networks. In addition, it appears that HuR inhibition attenuates macrophage infiltration following ischemia/reperfusion injury. In conclusion, our results suggest that inhibition of HuR is protective against cardiac I/R injury through a reduction in inflammatory gene expression.

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Poster Session 2 and Reception

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Studying Cardiovascular Effects of Marijuana on Healthy Individuals Using Human Derived Induced Pluripotent Stem Cells

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Marijuana is the most widely used illicit drug worldwide. Epidemiological studies indicate that marijuana increases the risk of coronary artery disease (CAD). Adverse cerebrovascular and peripheral vascular effects are also associated with marijuana use. In addition, three synthetic cannabis drugs have been approved by the FDA for treating chemotherapy-induced nausea and vomiting, which also show cardiovascular side effects. Thus, both medical and recreational marijuana have adverse cardiovascular side effects. Cannabinoid CB1 receptor signaling is involved in a variety of pathophysiological processes and selective CB1 antagonists show therapeutic potential. However, the current repertoire of CB1 antagonists has psychiatric side effects and limited application. Therefore, developing new CB1 antagonists are an unmet and growing clinical need with marijuana use on the rise. Here we found compound JW-1, an isoflavone abundantly presenting in soybeans, partially docked into the CB1 receptor and inhibited CB1 activity, suggesting that compound JW-1 was a novel CB1 antagonist. Human endothelial cells were more sensitive to Δ^9 -tetrahydrocannabinol (Δ^9 -THC) than cardiomyocytes and cardiac fibroblasts. To determine the mechanism of Δ^9 -THC pathological effects on the vasculature, we generated human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) from 5 healthy individuals. CB1 receptor was expressed in all hiPSC-ECs, whilst CB2 expression was low. Δ^9 -THC induced inflammation and oxidative stress via NF- κ B signaling activated in hiPSC-ECs. Knockdown of CB1 receptor with siRNA, abrogation of receptor expression with CRISPRi and compound JW-1 treatment could rescue the effect of Δ^9 -THC. Furthermore, compound JW-1 blocked Δ^9 -THC-induced endothelial dysfunction in mice models. Our investigations reveal that Δ^9 -THC causes endothelial dysfunction via the CB1 receptor. Compound JW-1 is a novel CB1 antagonist that can be used for preventing Δ^9 -THC-induced side effects.

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The Effects of Low-carbohydrate High-fat Diet on Vascular Remodeling

Haruka Yanagisawa, Haruhiro Toko, Mutsuo Harada, Jiaxi Guo, Satoshi Bujo, Masato Ishizuka, Issei Komuro, The Univ of Tokyo, Tokyo, Japan

Background: Low carbohydrate diet (LCD) has been reported to reduce body weight and cardiovascular risk factors, including diabetic mellitus and dyslipidemia. Therefore, LCD was once expected to prevent cardiovascular events. However, recent meta-analysis reported that LCD was related to an increase in cardiovascular events. Moreover, other cohort studies reported that higher all-cause and cardiovascular mortality were observed only in LCD with animal derived-fat (LCD-A), not in LCD with plant derived-fat (LCD-P). Precise mechanisms of these effects have not been clarified, so we explored the potential roles of LCD-A and LCD-P in blood vessel remodeling by using two types of vascular disease model mice, atherosclerotic model and vascular injury model. **Method:** We generated two types of LCDs, beef tallow-based LCD (LCD-A) and cacao butter-based LCD (LCD-P). Six-week-old male ApoE Knockout (ApoE KO) mice (atherosclerosis model mice) and wild-type mice were subjected to LCD-A, LCD-P or normal chow for 14 weeks, and %plaque area of aortic root and serum lipid profile were measured. For vascular injury model, mice were inserted with a wire in a femoral artery of male 10-week-old wild-type mice and subjected to the three-types chow. After 4 weeks, femoral arteries were excised and embedded in paraffin and assessed with the extent of vascular injury severity. The femoral artery without injury on the other side was served as control. **Results:** In ApoE KO mice, serum levels of total cholesterol (TC) and triglyceride (TG) were decreased in LCD group (TC: $p < 0.01$, TG: $p < 0.05$ vs normal chow), but there was no significant difference between LCD-A to LCD-P. In wild-type mice, serum TC level was increased in LCD-A group ($p < 0.0001$ vs normal chow). LCD had no effect on %plaque area of aortic root in ApoE KO mice. In vascular injury model mice, there was no significant difference in the extent of neointimal hyperplasia induced by wire-injury among groups. **Conclusion:** Our preliminary data showed that LCD had no effect on vascular remodeling despite the presence of significant alteration in lipid profiles. Additional experiments are needed to confirm the results and clarify the precise mechanisms.

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Poster Session 2 and Reception

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Molecular, Cellular and Systemic Mechanism of Nonlinear Dynamic Patterns of Ventricular Repolarization and Spontaneous Arrhythmic Sudden Death in Non-ischemic Heart Failure

Daiana C. O. Vieira, Jeffrey S Crocker, Krina Desai, Neha Reddy Sanagala, Daniel R Wendelken, Kenneth G Parks, Div of Cardiology, Univ of Cincinnati Coll of Med, Cincinnati, OH; Ting Liu, Brian O'Rourke, Swati Dey, Div of Cardiology, Johns Hopkins Univ Sch of Med, Baltimore, MD; Deeptankar DeMazumder, Div of Cardiology, Univ of Cincinnati Coll of Med, Cincinnati, OH

INTRODUCTION: Sudden cardiac death (SCD) is the leading cause of death in USA. Heart failure (HF) confers high SCD risk, but most SCD victims do not have HF or well-defined genetic abnormalities. The underlying mechanisms are poorly understood, precluding the design of more effective strategies for risk stratification and therapy. We previously showed that distinct non-linear patterns of cardiac repolarization strongly and independently predict SCD in HF patients [PMID: 27044982]. We also showed in a unique guinea pig HF model that increased levels of mitochondrial reactive oxygen species (mROS) drive spontaneous SCD, even before HF onset [PMID: 29898892]. Herein, we further dissect the underlying mechanisms. **HYPOTHESIS:** Increased mROS levels in pressure overloaded hearts reduce K⁺ currents, destabilize the resting membrane potential, increase repolarization lability and cause SCD. **METHODS:** Guinea pigs were randomized to Sham, aortic banding (AC), or AC with daily brief low-dose β -adrenergic stress (ACi) with and without *in vivo* mROS scavenger MitoTEMPO (ACi+MT). We performed time series analyses on recordings of: (1) 24-hour ECG QT interval in freely ambulating animals before sacrifice at 4 weeks; (2) pressure-volume and electrophysiology (EP) in excised perfused hearts; and (3) duration of action potential (APd), calcium transient (CaTd) and sarcomere shortening (SSd) in isolated LV myocytes. **RESULTS:** About 50% of ACi animals had SCD by 4 weeks. Increased QT entropy in ACi (at 1 week) predicted SCD over followup, similar to HF patients. Compared to Sham and ACi+MT, LV myocytes isolated from AC and ACi had reduced inward (IK1) and delayed slow (IKs) and rapid (IKr) rectifier currents; and increased beat-to-beat lability in APd, CaTd and SSd. The late Na and L-type Ca currents were similar between models. Acute exposure to isoproterenol (Iso) and carbachol (CCh) increased lability in LV myocytes compared to Iso or CCh alone. **CONCLUSIONS:** High mROS levels reduce repolarization reserve in LV myocytes and contribute, at least in part, to the non-linear dynamics of ventricular repolarization (reflected by high QT entropy), leading to spontaneous arrhythmic SCD. These findings provide important new mechanistic insight with direct clinical implications.

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Progression of Chronic Ischemia-Mediated Arrhythmogenesis in a Rat Model of Heart Failure

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Background: Worldwide, the prevalence of heart failure (HF) is increasing and HF patients are living longer. Chronic ischemic HF presents with increased vulnerability to ventricular tachycardia and fibrillation. However, the mechanism of these arrhythmias differ between the acute infarction phase and the chronic, namely via myocyte membrane potential instability in the acute phase versus substrate-mediated reentry in the chronic phase. We propose our chronic heart failure (CHF) model to recapitulate the potentially progressive nature of arrhythmogenesis in the chronic phase.

Methods: Adult male Sprague Dawley rats underwent permanent left coronary artery ligation and were maintained for six or ten weeks; SHAM operations were also performed. At each respective endpoint, SHAM and CHF rats underwent transthoracic echocardiography and invasive hemodynamic evaluation. Finally, a median sternotomy was performed for epicardial electrophysiology (EP) assessment. Programmed electrical stimulation was utilized to assess susceptibility to induced ventricular tachycardia (VT) and also to determine effective refractory period (ERP).

Results: Six-week CHF (n=50) induction proved successful as evidenced by the decrease in left ventricular ejection fraction (LVEF) ($76\pm 3\%$ to $35\pm 2\%$, $p<0.0001$) and the increase in left ventricular end-diastolic pressure (LVEDP) (6 ± 1 mmHg to 20 ± 2 mmHg, $p=0.0008$) compared to SHAM (n=15). Progression to ten-week CHF (n=18) also proved successful via LVEF and LVEDP maintenance ($35\pm 2\%$ to $38\pm 3\%$, $p=0.3909$) (20 ± 2 mmHg to 15 ± 3 mmHg, $p=0.1578$). EP studies revealed an increase in the incidence of induced VT between SHAM and six-week CHF (0% to 60%, $p<0.0001$) and a prolongation of the ERP (54 ± 4 milliseconds to 69 ± 3 ms, $p=0.0228$). The incidence of induced VT and ERP did not continue to increase between six- and ten-week CHF (60% to 50%, $p=0.5804$), (69 ± 3 ms to 68 ± 3 ms, $p=0.8221$).

Conclusion: Myocardial infarction-mediated arrhythmogenesis is known to exist in an acute and a chronic phase. Here, we examine the chronic phase of ischemic HF and show evidence to support that once the adverse remodeling-mediated substrate stabilizes, proarrhythmic deterioration ceases.

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Selective *in vivo* Modulation of Vagal-Muscarinic Signaling Prevents Sudden Cardiac Death in a Pressure Overload Model of Heart Failure

Jeffrey S Crocker, Daiana C. O. Vieira, Kenneth G. Parks, Div of Cardiology and of Pharmacology and Systems Physiology, Univ of Cincinnati Coll of Med, Cincinnati, OH; Brian O'Rourke, Swati Dey, Div of Cardiology, Johns Hopkins Univ Sch of Med, Baltimore, MD; Deeptankar DeMazumder, Div of Cardiology and of Pharmacology and Systems Physiology and of The Artificial Intelligence Ctr of Excellence, Univ of Cincinnati Coll of Med, Cincinnati, OH

INTRODUCTION: Sudden cardiac death (SCD) from ventricular tachyarrhythmias (VT/VF) claims a quarter millions lives/year in USA. Patients with heart failure (HF) have high SCD risk. We previously demonstrated that differential remodeling of cardiac muscarinic receptors plays a critical role in pathogenesis and for effective therapy of HF and SCD risk [PMID: 25733594]. Ongoing clinical trials of chronic vagus nerve stimulation (cVNS) aimed at improving left ventricular (LV) function in HF patients have had mixed preliminary results. However, the effect of cVNS on SCD risk is unexplored. While *acute* VNS terminates VT/VF, cVNS likely alters the arrhythmogenic substrate. A better understanding of the underlying mechanisms is necessary for the design of new, more effective therapies. **HYPOTHESIS:** cVNS prevents SCD by reducing calcium, autonomic and redox derangements in LV myocytes and improving repolarization reserve in pressure overloaded hearts. **METHODS:** Guinea pigs are randomized to Sham or aortic constriction (AC) with daily brief (30-min) low-dose β -adrenergic stress (Shami; ACi), with and without *in vivo* cVNS therapy via an implanted device, with and without *in vivo* end-organ muscarinic receptor blockade (MRB) using glycopyrrolate. Echocardiography and continuous 24-hour ECG analyses are performed. Animals are sacrificed at 4 weeks for biochemical, excised perfused heart (pressure-volume, electrophysiology, optical mapping) and isolated LV myocyte studies at 37°C. **PRELIMINARY RESULTS:** Compared to Sham, the M2 subtype of muscarinic receptors are upregulated in AC and ACi, along with reduced calcium cycling, sarcomere shortening and β -adrenergic responsiveness. Whereas ACi+MRB markedly increased SCD incidence, ACi+cVNS prevented SCD. Interestingly, cVNS prolonged but also stabilized the ECG QT interval by reducing dispersion of repolarization in ACi model. Echocardiographic fractional shortening was also higher in ACi+cVNS ($52\pm 1\%$; 4 weeks) compared to ACi ($27\pm 2\%$; 4 weeks) and ACi+MRB ($23\pm 5\%$; 2 weeks). **CONCLUSIONS:** Chronic *in vivo* perturbation of cholinergic signaling may be an effective strategy for SCD therapy. Ongoing experiments are aimed at dissecting underlying mechanisms and identifying potential targets for SCD therapy.

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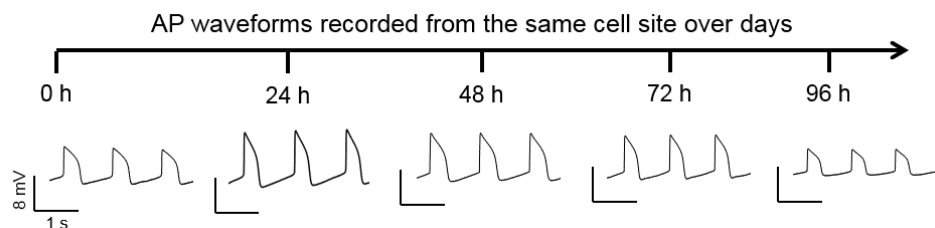
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Reversible Cardiac Action Potential Recordings to Track Cellular Electrophysiology Over Days

Viviana Zlochiver, Stacie Edwards, Jonathan A. Cook, Rosy Joshi-Mukherjee, Aurora Res Inst, Milwaukee, WI

Long-term action potential (AP) studies from the same cell site over days in multicellular preparations are currently lacking, thus limiting our understanding of cardiac physiology in development and disease. We recently reported a novel high-throughput platform for label-free AP measurements using multielectrode array (MEA) technology to gain intracellular access by way of electroporation. We hypothesized that multiple reversible electroporations of the same cell site will allow high-resolution recordings of AP waveforms without detrimental effects to the cell membrane. A total of 19 hiPSC-CM constructs on multiwell MEAs were simultaneously electroporated by employing a train of low voltage pulses (1 V, 1 ms, 1 Hz) for 30 s to elicit a transient transformation of field potentials (FPs) to APs. Waveforms were recorded from 12 electrodes per construct for electrophysiological characterization of the network. Recurrent electroporations of the same cell site at 0, 24, 48, 72 and 96 h enabled tracking of the electrophysiological evolution of the AP waveforms (see figure). We analyzed millivolt AP signals 10 s following the end of electroporation before APs reverted to FP signals strongly suggesting cell membrane recovery and network integrity for subsequent recordings. This methodology allows for screening transmembrane APs from cardiomyocyte networks over days. Overall, we present a spatio-temporal model capable of tracking FPs and APs in a non-invasive approach for cardiomyocyte functional maturation, developmental and pharmacological studies.



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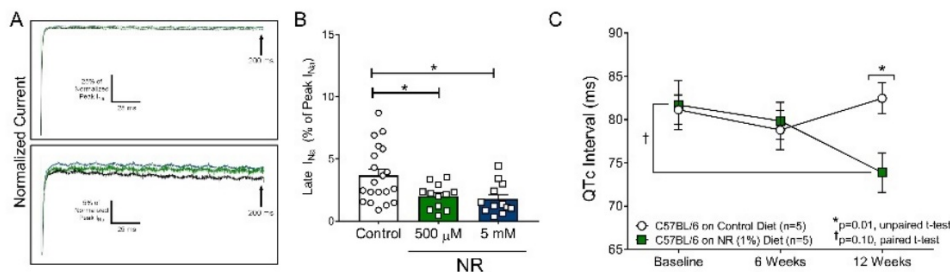
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Nicotinamide Riboside Modulates Late Sodium Current and Cardiac Repolarization

Daniel S Matasic, Pravda Quinones, Charles Brenner, Barry London, The Univ of Iowa, Iowa City, IA

Background: Boosting NAD⁺ content within the heart has emerged as a cardioprotective strategy against heart failure-related remodeling, ischemic injury, and arrhythmias. Our group recently demonstrated that the bioavailable NAD⁺ precursor Nicotinamide Riboside (NR) drives Sirtuin 1-mediated deacetylation of the main cardiac sodium channel Na_v1.5 to increase peak sodium current (I_{Na}), a major determinant of cardiac excitability and action potential conduction. However, the effects of NAD⁺ precursors and NR on cardiac repolarization are relatively unexplored. **Objective:** To evaluate the effects of NR on late Na⁺ current and cardiac repolarization. **Methods:** To study the *in vitro* effects of NR, whole-cell patch clamp was utilized to measure I_{Na} and late sodium current (I_{NaL}) in myocytes isolated from 1-3 day old neonatal rats (NRCMs). To study the *in vivo* effects of NR, 4-5 month-old C57BL/6 mice were placed on either a control diet or a diet supplemented with NR (1%). EKGs were performed at baseline, 6 weeks, and 12 weeks post-diet. **Results:** NR increased peak I_{Na} (500 μM: +60.1 ± 26%, 5 mM: +74.7 ± 39%) with a non-significant change in absolute I_{NaL} (500 μM: -12.8 ± 9%, 5 mM: -32.4 ± 20%), leading to decreased I_{NaL} when normalized to peak current (500 μM: -45.2 ± 7.8%, 5 mM: -51.6 ± 9.8%, Figure A,B). Additionally, 12-week dietary NR supplementation shortened QT_c in NR-treated mice (-9.1 ± 4.6%) but not in control mice (+1.7 ± 1.3%, Figure C). **Conclusion:** NAD⁺ supplementation with NR is potentially anti-arrhythmic by decreasing I_{NaL} and shortening QT_c. Potential mechanisms include mitigating the effects of oxidative stress and/or modulation of PKA and PKC phosphorylation of Na_v1.5.



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AAV9-Mediated Overexpression of TRPM4 Increases the Incidence of Ventricular Arrhythmias in Living Mice

Andy Pironet, Frone Vandewiele, Ninda Syam, Greetje Vande Velde, Rik Gijsbers, Rudi Vennekens, Catholic Univ of Leuven, Leuven, Belgium

Cardiac conduction disorders are a common cause of lethal cardiac arrhythmias and can be caused by mutations in ion channels. Several studies suggest that mutations in the Transient Receptor Potential subfamily M member 4 (TRPM4) are responsible for hereditary forms of cardiac conduction disorders. The majority of mutations that are described in TRPM4 lead to gain-of-function of channel activity. Analysis of the first identified gain-of-function mutation, p.E7K in a cellular overexpression system suggests that attenuated deSUMOylation of the protein, leads to impaired endocytosis and consequently an increase in the number of functional channels in the plasma membrane. To test the idea that an increased number of TRPM4 channels leads to cardiac arrhythmias, we created a functional overexpression model of the TRPM4 channel in living mice. To this end, we overexpressed TRPM4 in mice using adeno-associated virus serotype 9 (AAV9) particles, which has been described as the most cardiotropic of AAV serotypes. Overexpression of TRPM4 was achieved via tail vein injection of AAV9 particles. Subsequently, we performed telemetric ECG-measurements in freely-moving mice to determine their *in vivo* cardiac phenotype. In baseline conditions, the heart rate and ECG-parameters were similar between TRPM4-overexpressing and WT mice. Additionally, the number of arrhythmic incidents in resting mice was not different between TRPM4-overexpressing mice and WT mice. Instead, WT mice overexpressing TRPM4 exhibited more premature ventricular ectopic beats during exercise-induced β -adrenergic stress. Conduction abnormalities were rare and was not increased in mice overexpressing TRPM4. Taken together, mice overexpressing TRPM4 were more prone to develop premature ventricular ectopic beats during exercise-induced β -adrenergic stress.

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Poster Session 2 and Reception

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Adenovirus Targets Connexin43 Gap Junction Expression and Function During Infection

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INTRODUCTION: Myocarditis leads to pathologic remodeling of the myocardium and arrhythmia. A leading etiological agent of viral myocarditis is human Adenovirus type 5 (Ad5). Individuals affected are at a higher risk of arrhythmogenesis which can lead to sudden cardiac death. Gap junctions, comprised of connexin proteins, provide direct intercellular communication between cardiomyocytes to facilitate electrical coupling. Connexin43 (Cx43) is the predominant connexin expressed in the working myocardium and is tightly regulated by post-translational modifications. Gap junctions are also known to propagate the innate antiviral immune response but the impact of Ad5 infection on Cx43 expression and function is essentially unexplored.

HYPOTHESIS: During Ad5 infection Cx43 gap junctions are directly targeted to limit intercellular communication thus precipitating an arrhythmogenic substrate.

METHODS: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), keratinocytes (HaCaT), and lentivirus-transduced HaCaTs stably overexpressing Cx43 were infected with Ad5. Biochemical fractionation and pulse chase assays were implemented to determine gap junction and Cx43 stability. Cx43 protein levels and phosphorylation status were determined by western blotting and immunoprecipitation experiments. Confocal immunofluorescence microscopy was employed to determine localization and functional of Cx43 during Ad5 infection.

RESULTS: Cx43 expression is targeted early in infection with remaining protein localized at the cell-cell boarder during infection. Phosphorylation of Cx43-Ser368 was detected during early Ad5 infection implicating reduced gap junction conductance. In contrast phosphorylation of Cx43-Ser262 is reduced during Ad5 infection with a concomitant increase in protein half-life despite losses in functional gap junction intercellular communication.

CONCLUSION: Cx43 gap junction structures are stabilized during Ad5 infection but directly targeted by specific phosphorylation events to effect channel closure. Insight provided into mechanisms by which Ad5 manipulates Cx43 regulation will provide novel therapeutic avenues to target gap junction function in a broad spectrum of heart disease states.

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Trpm4 Contributes to Ca²⁺-dependent Triggered Arrhythmias in Pathological Conditions

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Rationale: TRPM4 is a Ca²⁺-activated non-selective cation channel that is abundantly expressed in the heart. Mutations in the *Trpm4* gene are associated with human cardiac conduction disorders, including Progressive Familial Heart Block type I (PFHBI) and Brugada Syndrome. However, the mechanistic role and *in vivo* significance of TRPM4 in the triggering of cardiac arrhythmias is still completely unclear. **Objective:** To investigate the role of TRPM4 during pathological Ca²⁺ handling in the heart. **Methods and results:** Using three *in vivo* pro-arrhythmic assays, we found that *Trpm4*^{-/-} mice show a reduced arrhythmic burden compared to control mice. First, aconitine intoxication resulted in severe cardiac arrhythmias, both in WT and *Trpm4*^{-/-} animals, but *Trpm4*^{-/-} mice developed significantly less ventricular ectopic beats (VEBs) and showed a lower arrhythmic score. Second, during ischemia-reperfusion, induced by 30 min of LAD ligation, significantly more WT animals developed arrhythmias compared to *Trpm4*^{-/-} mice. Third, catecholaminergic polymorphic ventricular tachycardia (CPVT) mice, carrying mutations in RyR2, were subjected to a stress test. Significantly more *RyR^{+/R2474S}-Trpm4^{+/+}* animals developed arrhythmias compared to *RyR^{+/R2474S}-Trpm4^{-/-}* mice. **Conclusion:** Our data establish that TRPM4 represents a novel target in the prevention and treatment of cardiac arrhythmias.

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Activation of Transient Receptor Potential Canonical Channel Currents in Iron-Overloaded Cardiac Myocytes

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Background: Iron (Fe) overload cardiomyopathy is the leading cause of death in hemochromatotic patients, yet the mechanistic insight is still incomplete and controversial. We investigated alterations of action potentials (APs), ionic currents, and intracellular Ca²⁺ (Ca²⁺_i) in Fe-loaded mouse cardiomyocytes, as well as functional impacts of Fe overload on single-cell contraction and whole-heart arrhythmias.

Methods: Cardiomyocytes were isolated from left ventricles of mouse hearts and were superfused with Fe³⁺/8-hydroxyquinoline complex (5-100 μM). APs, L-type Ca²⁺ currents (I_{Ca,L}), total outward K⁺ currents (I_K), and transient receptor potential canonical (TRPC) channel currents were recorded by the patch-clamp technique. Ca²⁺_i was evaluated by using Fluo-4. Cell contraction was measured by a video-based edge detection system. Arrhythmias were evaluated in Langendorff-perfused hearts under S₁-S₂ stimulation protocol.

Results: Persistent Fe (15 μM) treatment prolonged AP duration at 90% repolarization (APD₉₀: 46.8 ± 2.8 vs. 203.6 ± 63.4 ms, p<0.05), induced early and delayed afterdepolarizations (EADs: 0 % vs. 45.0 ± 15.0 %; DADs: 4.3 ± 1.4 vs. 27.0 ± 7.0 %, p<0.05, respectively) in mouse cardiomyocytes. Consistently, arrhythmia incidence was increased in Fe³⁺/8-HQ-perfused hearts. Fe treatment decreased peak I_{Ca,L} (16.5 ± 1.7 vs. 11.4 ± 1.3 pA/pF, p<0.01) and I_K (59.2 ± 3.3 vs. 50.4 ± 3.0 pA/pF, p<0.01), altered Ca²⁺_i transient patterns and decreased contractility (4.8 ± 0.5 vs. 3.5 ± 0.4%, p<0.01). During the late phase of Fe treatment, fast Ca²⁺ waves and sustained depolarization were induced to generate a secondary (shallow) resting membrane potential (RMP: from -68.8 ± 0.6 to -25.0 ± 3.7 mV) where the myocytes became unexcitable. Gadolinium, a TRPC channel blocker, abolished fast Ca²⁺ waves and reversed RMP to the deep level (-62.9 ± 3.5 mV). The involvement of TRPC activation was determined for the first time by recording TRPC current and assessing the effect of functional TRPC

channel antibodies.

Conclusions: In mouse cardiomyocytes, Fe overload induced arrhythmogenic APD prolongation and EADs/DADs, aberrant Ca^{2+} dynamics, and impaired contractility. The activation of TRPC channels accounts for an important underlying mechanism.

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Spatial Transcriptomics Unveil ZBTB11 as a Regulator of Cardiomyocyte Degeneration in Arrhythmogenic Cardiomyopathy

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Introduction: Arrhythmogenic cardiomyopathy (ACM) is an inherited cardiac disorder characterized by progressive loss of contractile myocardium which is replaced by fibrous and adipose tissue, ventricular arrhythmias, and sudden cardiac death. Fibrofatty replacement extends transmurally with an epi-to-endocardial gradient, yet molecular differences between the transition regions are poorly characterized. **Methods and Results:** To explore molecular mechanisms underlying ACM we obtained an explanted heart of a 35-year-old male with a known pathogenic *PKP2* mutation, c.2544G>A. We utilized Tomo-Seq to acquire a genome-wide transcriptional profile with 100 μ m-spatial resolution, generating a transmural expression atlas of the ACM heart. Tracing transcriptional differences across the ventricular wall enabled us to identify clusters of genes specific to the myocardium and fibrofatty region. In addition, we detected restricted sites of active remodelling within the myocardium with a clearly distinct gene program. Amongst genes specifically enriched in this composite region was the Zinc Finger and BTB Domain-Containing Protein 11 (ZBTB11). Overexpression of ZBTB11 in human cardiomyocytes induced a differential gene expression profile with striking similarities to the composite region from the Tomo-Seq analysis. Most notably, ZBTB11 induced a TP53 mediated stress response including activation of apoptosis and autophagy. The presence of ZBTB11-positive cardiomyocytes flanking fibrofatty islands was confirmed in ACM patients harbouring other *PKP2* mutations, as well as ACM and DCM hearts with mutations in non-desmosomal genes. We are currently exploring the ZBTB11 transcriptional network, and its role in disease progression in human cardiomyopathies. **Conclusions:** Using a spatial transcriptomics approach on a human ACM heart we have identified ZBTB11 as a novel marker of cardiomyocyte degeneration in cardiomyopathies. ZBTB11 induces autophagy and apoptosis and therefore may be relevant for the development of biomarker assays and in identifying novel targets for therapies against cardiomyopathies.

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Regulation of p53 Protein Levels Drives Activation of Cardiac Fibroblasts in Response to Pressure Overload

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Heart disease is accompanied by the accumulation of resident cardiac fibroblasts (CF) that subsequently become myofibroblasts and secrete copious amounts of extracellular matrix (ECM), impeding cardiac function and driving the progression of heart failure. Understanding the mechanisms coordinating CF accumulation and myofibroblast activation may reveal novel therapeutic strategies to block pathologic fibrosis. We recently found that small proline rich protein 2b (SPRR2B) drives CF proliferation in response to pathological cues by facilitating MDM2-dependent proteasomal degradation of p53. Surprisingly, although *Spr2b* gene deletion or targeted mutation of the USP7/MDM2 interacting domain in mice (*Spr2b-KO* and *Spr2b-USP^m*, respectively) stimulated the expression of p53-dependent cell cycle arrest genes, mutant animals also developed excessive fibrosis in LV pressure overload. The development of fibrosis in *Spr2b* mutant mice was

traced primarily to a more robust myofibroblast activation response, providing evidence that CF accumulation and myofibroblast activation may be mutually antagonistic. To investigate the contribution p53 to CF accumulation and fibrosis in mouse heart disease models, we deleted floxed p53 alleles specifically in adult CF using the tamoxifen-inducible *Tcf21^{MerCreMer}* mouse line (called p53-CKO). Surprisingly, while p53-CKO animals display exaggerated accumulation of Tcf21⁺/PDGFR α ⁺ CF in response to left ventricle pressure overload, we observed a biphasic physiological response; initially, p53-CKO animals are resistant to systolic functional decline, only developing more severe fibrosis and functional decline than littermate controls at later time points. Time course studies using primary adult mouse CF revealed that p53 positively correlates with myofibroblast activation, while reduction in p53 levels correlates with accelerated cell cycle and the suppression of myofibroblast activation until the subsequent induction of p16/19 - Rb-mediated cell cycle arrest. Taken together, this study offers detailed insight into the transition of CF from a proliferative to an activated state that may accelerate the development of anti-fibrotic strategies.

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Extracellular Matrix Components Isolated From Diabetic Mice Alters Cardiac Fibroblast Function Through the AGE/RAGE Signaling Cascade

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Roughly 30 million Americans suffer from diabetes and these individuals are at an increased risk of developing cardiovascular complications. A common complication is heart failure which occurs due to the stiffening of the left ventricle brought on by cardiac fibroblast "activation" that results in the remodeling the extracellular matrix (ECM). Fibroblast "activation" can be triggered by the AGE/RAGE signaling cascade. Advanced Glycated End products (AGEs) are produced and accumulate in the ECM overtime, but under hyperglycemic conditions this process is accelerated. We aim to investigate how the presence of AGEs in the either diabetic or non-diabetic ECM can affect fibroblast ECM remodeling as well as determine the role of AGE/RAGE signaling during this process. In order to assess this question diabetic and non-diabetic fibroblasts were embedded in 3D matrices composed of collagen isolated from either diabetic or non-diabetic mice. Non-diabetic fibroblasts displayed similar matrix contraction and α -SMA expression to diabetic fibroblasts when embedded in diabetic collagen. In addition, increasing the AGE/RAGE cascade leads to increase gel contraction indicating increase in fibroblast "activation". These results indicate 1) the ECM from diabetic and non-diabetic mice differ from one another, 2) diabetic ECM can impact fibroblast function and shift them towards an "active" state, and 3) that fibroblasts can modify the ECM through activation of the AGE/RAGE signaling cascade. These results suggest the importance of understanding the impact diabetes has on the ECM and fibroblast function.

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Exosomes Derived From Podoplanin Positive Cells Induce Fibrosis and Inflammation in Healthy Mouse Heart

Maria Cimini, Venkata Naga Srikanth Garikipati, Chunlin Wang, May Truongcao, Grace Huang, Vandana Mallareddy, Cindy Benedict, Raj Kishore, Temple Univ, Lewis Katz Sch, Philadelphia, PA

Superseding fibrosis is the leading cause of the adverse remodeling after myocardial infarction (MI); inflammation and paracrine signals enhance the fibrosis and ventricular dysfunction and inhibit the favorable repair. It has been reported that cells expressing Podoplanin (PDPN), a platelet aggregation-inducing type I transmembrane glycoprotein, appear around 2 days after MI as a signal of activation. We hypothesized that exosomes derived from these cells may actively affect the biology of fibrosis and inflammation. PDPN⁺ cells were isolated from hearts of mice 2 days after MI, expanded in a selective media and treated with TNF α , Angiotensin II or the combination of both. Exosomes derived from activated PDPN⁺ cells were isolated from the conditioned media and used in vitro for the treatment of mouse cardiac endothelial cells (mCECs), mouse embryonal fibroblast (MEF) and monocytes and in vivo for the treatment of healthy mouse hearts. Data from q-PCR showed that stimulated PDPN⁺ cells derived exosomes reprogrammed mCECs to the endothelial lymphatic phenotype enhancing the

expression of the major lymphatic lineage markers and upregulated the expression of fibrotic markers suggesting an endothelial-mesenchymal transition. Furthermore, stimulated PDPN+ cells derived exosomes drove the fibroblast to myo-fibroblast phenotype and activated monocytes toward pro-inflammatory lineage with an increased expression of TNF α and IL-1 β . In vivo, stimulated PDPN+ cells derived exosomes were initially injected in to the left ventricle of healthy mouse hearts followed with additional boosters delivered by retro-orbital vein injection. Treated mice developed an extended epicardial fibrosis with a subsequent impairment in the contractility and increase in the end diastolic and systolic volumes. In conclusion stimulated PDPN+ cells derived exosomes may impair the biology of mCECs, fibroblast and monocytes leading to adverse remodeling after MI.

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Proteomic Characterization of Extracellular Matrix Regulation in Human Aortic Valve Development and Disease

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We present the first report of the collagen protein interactome in human aortic valve (AV) development and disease. AV disease affects up to 13% of the world population. As no therapies are available, patients must “watch and wait” until surgical valve replacement is necessary to prevent heart failure. Extracellular matrix (ECM) forms the basis for correct valve function but alterations of the ECM scaffold during development and disease are not well defined. Here, we report ECM protein regulation from a cohort of 26 valvular tissues in four pathological categories: normal functioning AVs, pediatric end-stage congenital aortic valve stenosis (CAVS), adult end-stage fibrocalcific aortic valve stenosis (FAVS), and a pulmonic Ross operation valve. A novel proteomic method was used to target ECM proteins, primarily collagens, for sequence analysis from FFPE 5- μ m thin tissue sections. Collagens represented 19/49 proteins identified (39%); A total of 46/49 ECM proteins (94%) were implicated in direct interactions in collagen synthesis, regulation or modification. Data suggested dynamic regulation of collagen types during age and disease, e.g., Col15a1 was found only in normal AV, whereas Col4a1 was reported only in patients younger than 5 years. Upstream regulators of the collagen interactome included COLQ1, IGFBP2, and SPARC. Detailed peptide sequence analysis reported 70 specific collagen peptide sequences containing up to multiple hydroxylated prolines (HYP), a post-translational modification critical to stabilizing the collagen triple helix. Quantitative data analysis on HYP peptides reported differential regulation across patient categories – i.e., Col3a1 peptides show 52% increased HYP in CAVS compared to age matched normal valve. Interestingly, the pulmonic Ross valve represented unique collagen HYP signatures distinct from all other aortic valve tissues. Tissue from pediatric end-stage CAVS patients showed a higher percentage of hydroxylated peptides compared to non-hydroxylated peptides (33%) as compared to normal (25%) or adult end-stage FAVS (16%). We anticipate that the completed study will be useful to targeted therapies aiming to inhibit fibrosis and ECM remodeling, and may better inform engineered options for valve replacement.

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WNT the Right Ventricle's Had Enough

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Background: A greater understanding of the gene programs that drive adaptive and pathologic right ventricle (RV) remodeling are needed given the poor response of the RV to standard reverse remodeling agents and the absence of RV-specific therapies. Left ventricular failure (LVF) provides an ideal model to study human RV remodeling given the spectrum of secondary RV involvement. Methods: Dilated (DCM) and ischemic cardiomyopathy (ICM) hearts in the Penn Human Heart Tissue Bank were identified as having preserved RV function (pRV) or RVF using preexplant right atrial to wedge pressure

ratio. Using RT-PCR and a candidate gene approach, we assessed RV differential expression of 28 *WNT*-related genes and NPPA/NPPB as positive controls between nonfailing (NF, n = 29), DCM-pRV (n = 47), DCM-RVF (n = 26), ICM-pRV (n = 31), and ICM-RVF (n = 10). Genes which met significance for differential expression between NF, pRV, and RVF for both DCM and ICM using Kruskal-Wallis were further assessed by Mann Whitney U comparing pRV to RVF after combining ICM and DCM groups (Benjamini-Hochberg $p < 0.05$ for significance). Results: Most (20/28) *WNT*-related genes were differentially expressed in at least one cardiomyopathy group, but only 13, as well as NPPA, were significant for both DCM and ICM. Only, CREBBP, NFATC2, and ROR2 were differentially expressed between pRV and RVF (Table). Discussion: *WNT*-related genes show significant DGE in RV remodeling. But, of those tested, only CREBBP, NFATC2, and ROR2 appear to be implicated in the progression from adaptive to pathologic RV remodeling. Additional studies are underway to confirm differential protein expression.

Differential Expression between RVF and pRV in DCM and ICM		
Gene	RVF/pRV expression	<i>p</i>
AXIN2	1.27	0.42
CREBBP	1.55	0.0042
DAAM2	1.16	0.78
FZD1	1.00	0.83
FZD7	1.11	0.43
JNK1	1.02	0.83
NFATC2	1.48	0.040
NPPA	2.37	0.08
PDK1	1.19	0.63
ROR2	1.26	0.040
SFRP1	0.78	0.26
SFRP3	1.16	0.42
WISP2	1.50	0.23
WNT5A	1.13	0.23

Significantly differentially expressed genes noted in bold using Benjamini-Hochberg corrected p value < 0.05

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GRK5 Regulates Cardiac Fibroblast Differentiation and Fibrosis

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Pathological remodeling of the heart is a hallmark of heart failure (HF) and these structural changes further perpetuate the disease. Cardiac fibroblasts are the critical cell type that is responsible for maintaining the structural integrity of the heart. Stress conditions, such as a myocardial infarction (MI), can activate and induce the transdifferentiation of quiescent fibroblasts into synthetic and contractile myofibroblasts. G protein-coupled receptor (GPCR) kinases (GRKs) are important mediators of cardiovascular homeostasis through dampening of GPCR signaling. GRK5 is one isoform of these kinases that is expressed in the heart and has been shown to be up-regulated in human HF. Of note, GRK5 has been demonstrated to translocate to the nucleus in cardiomyocytes in a calcium-calmodulin (Ca^{2+} -CAM)-dependent manner, promoting hypertrophic gene transcription through activation of NFAT and NF κ B. Interestingly, these transcription factors are also involved in fibroblast activation. GRK5 is highly expressed and active in cardiac fibroblasts (CFs), however its pathophysiological role in these crucial cardiac cells is unknown. Utilizing adult mouse CFs *in vitro*, our preliminary data suggests a relevant role for GRK5 in the activation of fibroblasts. We observed that genetic deletion of GRK5 (GRK5KO) in CFs attenuated α -smooth muscle actin (α -SMA) expression, a myofibroblast marker, after stimulation with Angiotensin II (AngII). In addition, deletion of GRK5 diminished the expression of myofibroblast genes and proteins such as Collagen I and III after AngII stimulation. GRK5KO CF's were also refractory to AngII mediated collagen gel contraction and protein translation. *In vivo*, mice with fibroblast-specific deletion of GRK5 were protected against cardiac fibrosis and hypertrophy after AngII infusion and post-MI compared to wild type mice. Mechanistically, GRK5 translocates to the nucleus after AngII stimulation and pharmacological inhibition of this event (via inhibition of Ca^{2+} -CAM) prevented AngII mediated fibroblast

transdifferentiation. These data support the hypothesis that GRK5 is an essential regulator of fibroblast activation and could be a novel therapeutic target to decrease adverse myocardial remodeling after injury.

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Glutaminolysis is Required to Initiate Myofibroblast Differentiation and Persistence During Stress

Andrew A Gibb, Alyssa A Lombardi, Emma K Murray, Temple Univ, Philadelphia, PA; Yinfei Tan, Fox Chase Cancer Ctr, Philadelphia, PA; Pawel K Lorkiewicz, Univ of Louisville, Louisville, KY; Anh T Huynh, Devin W Kolmetzky, Temple Univ, Philadelphia, PA; Zolt Arany, Daniel P Kelly, Kenneth B Margulies, Univ of Pennsylvania, Philadelphia, PA; Bradford G Hill, Univ of Louisville, Louisville, KY; John W Elrod, Temple Univ, Philadelphia, PA

Cardiac fibrosis occurs in ischemic heart failure, genetic cardiomyopathies, diabetes, and aging. While initially considered reparative, resident cardiac fibroblasts (CFs) activation/differentiation to myofibroblasts provide contractile and integral support; however, persistent activation leads to progressive cardiac dysfunction and maladaptive remodeling. Recent reports implicate acute and/or chronic changes in metabolism as a central driver for many cellular differentiation programs. Indeed, metabolite bioavailability is directly linked to the activity of epigenetic-modifying enzymes implicated in lineage commitment and differentiation. We recently identified α KG-dependent lysine demethylases as key contributors to myofibroblast formation. Here, TGF β stimulated fibroblasts isolated from adult mouse hearts were subjected to next-gen sequencing methodologies (RNA-seq, ATAC-seq, and RRBS-seq) to identify dynamic modifications in chromatin architecture and DNA accessibility at gene loci critical to the myofibroblast gene program. Utilizing unbiased and stable-isotope metabolomics, we correlated chromatin remodeling with a significant decrease in abundance/utilization of s-adenosylmethionine, the methyl donor for cytosine and histone methylation. In addition, a significant increase in α KG abundance driven by enhanced glutaminolysis was observed. To investigate the significance of glutaminolysis and enhanced α KG biosynthesis, we treated CFs with a glutaminase inhibitor (CB-839) and evaluated differentiation. Treatment with CB-839 in the presence of TGF β prevented activation of the fibrotic gene program (RT-qPCR) and myofibroblast formation. Furthermore, following TGF β -induced differentiation, inhibition of glutaminolysis was sufficient to revert activated myofibroblasts to a quiescent non-fibrotic phenotype, even during sustained stress. Importantly, this phenomenon is reproducible in CFs derived from human HF patients. Collectively, these results suggest a primary role for metabolism in not only initiating differentiation, but also the persistence of myofibroblasts, potentially through epigenetic-dependent gene transcription, providing new therapeutic targets to treat fibrosis.

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Pharmacological Inhibition of Human Antigen R (HuR) Blunts fibroblast Activation and Cardiac Fibrosis

Lisa C Green, Sarah R Anthony, Samuel Slone, Perwez Alam, Univ of Cincinnati, Cincinnati, OH; Liang Xu, Univ of Kansas, Lawrence, KS; Michael Tranter, Univ of Cincinnati, Cincinnati, OH

Myocardial fibrosis is associated with many cardiac diseases and often leads to a worsened prognosis. In a healthy heart, cardiac fibroblasts are responsible for maintaining the extracellular matrix (ECM). In response to stress or injury, however, the fibroblasts activate to myofibroblasts and begin proliferating, migrating, and depositing excess ECM. This leads to decreased compliance and contractility of the myocardium, furthering the progression to heart failure. Dampening the chronic activation of fibroblasts, therefore, is an attractive target for future cardiac therapeutics. Human Antigen R (HuR) is an RNA binding protein known to stabilize mRNA through binding to AU rich regions in the 3'UTR. Our lab has previously utilized a myocyte-specific knockout of HuR to show that HuR plays an important role in cardiac fibrosis through mediating TGF β gene expression in the myocytes. Additionally, pharmacological inhibition of HuR markedly reduced interstitial cardiac fibrosis in an 11-week transverse aortic constriction (TAC) mouse model of heart failure, quantifying the fibrosis with Masson's Trichrome Stains, western blots, and qPCR of profibrotic markers.

The goal of this work is to identify the functional role of HuR in mediating pro-fibrotic gene expression and ECM remodeling activity in cardiac fibroblasts. Our results demonstrate that HuR inhibition blunts the activation and wound healing activity of primary adult cardiac fibroblasts. Specifically, utilizing an *in vitro* scratch/wound healing assay we show that HuR inhibition significantly prolongs wound closure time and blunts the expression of profibrotic genes (e.g. collagen1a1, periostin, TGF β , fibronectin). Ongoing studies will determine the effect of HuR inhibition in a two-week isoproterenol pump (30mg/kg/day) model. Quantification of fibrosis and upstream signaling of fibroblast activation, such as TGF β -dependent phosphorylation of SMAD2/3, will be assessed to determine the role of HuR in the initiation of fibrotic signaling.

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Desmin Protects Cardiomyocyte Nuclei from Microtubule-dependent Collapse

Julie Heffler, Parisha P Shah, Patrick Robison, Sai Phyo, Kimberly Veliz, Alexey Bogush, Joshua Rhoades, Rajan Jain, Benjamin L Prosser, Univ of Pennsylvania, Philadelphia, PA

Alterations to mechanical forces have been long-appreciated to be sufficient to drive cardiac pathophysiological remodeling; however, the mechanism by which cardiomyocytes sense and transduce mechanical stressors remains poorly understood. In part, mechanical forces can be transduced to transcriptional responses by direct strain transmission to the nucleus via the Linkers of the Nucleo- and Cytoskeleton (LINC) complex, which couples the cytoskeleton to the nuclear lamina and DNA. While LINC complex mutations are known to cause cardiomyopathy, cytoskeletal-LINC interactions are understudied in the cardiomyocyte. To probe these interactions, we acutely disrupted the LINC complex as well as microtubules, actin, and intermediate filaments and assessed the consequences on baseline nuclear homeostasis in the cardiomyocyte. Our result show that a balance of microtubules and desmin intermediate filaments is required to maintain nuclear shape and the fidelity of the nuclear envelope and lamina. Upon acute depletion of desmin (or Nesprin-3, its binding partner in the LINC complex), microtubules drive infolding of the nuclear membrane. This results in DNA damage, a loss of genome organization, and broad transcriptional changes. Desmin knockdown also causes compromised excitation-contraction coupling and contractile function. Together, our data suggest that a balance of forces imposed by intermediate filaments and microtubules is required to maintain nuclear structure and genome organization in the cardiomyocyte, and that this process is important for maintaining proper myocyte health and function.

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Cavin-2/SDPR in Cardiac Fibroblasts Modulates TGF- β /Smad Signaling and Promotes Pressure Overload-induced Fibrosis

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Introduction: Heart failure (HF) is a debilitating disease associated with high morbidity and mortality. The high mortality rate reflects the inadequacy of modern therapy and calls for new treatments. A major cause of HF is the adverse tissue remodeling with fibrosis. Excessive extracellular matrix (ECM) turnover is involved in the poor outcome. Resident fibroblasts are responsible for cardiac fibrosis on pressure-overload heart failure. Caveolins and Cavins are known as caveolar-related proteins. Caveolin-1 inhibits TGF- β 1-induced fibrosis in the previous reports. Although Cavin-2/Serum deprivation response protein (SDPR), which is one of the caveolar-related proteins, is also abundant in fibroblasts, the role of Cavin-2 in cardiac fibrosis and function remains unknown. **Methods and Results:** To clarify the role of Cavin-2 in the cardiac fibroblasts in the pressure-overloaded heart, we performed transverse aortic constriction (TAC) operation on SDPR^{flox/flox} mice and fibroblast-

specific Cavin-2 cKO mice (Postn^{Cre}; SDPR^{flox/flox}). Four weeks after TAC, left ventricular fractional shortening (LVFS) was preserved with a significant reduction of cardiac fibrosis in Postn^{Cre}; SDPR^{flox/flox} mice. Fibrosis-associated mRNA expressions (*Col1a1*, *Ctgf*, *Col3*), $\alpha 1$ type I collagen deposition, and α SMA-positive cells were also reduced in the hearts of Cavin-2 cKO mice after TAC. Trans-differentiation of fibroblasts into activated myofibroblasts is a defining feature of fibrosis. Myofibroblasts express α -smooth muscle actin (α SMA) and secrete ECM proteins via Smad signaling. In mouse embryonic fibroblasts (MEFs), Cavin-2 deficiency reduced the levels of α SMA protein and fibrosis-associated mRNA expressions by TGF- β 1 stimulation. Furthermore, TGF- β 1-induced Smad2 phosphorylation was attenuated in Cavin-2 KO MEFs compared to WT MEFs. On the other hand, adenovirus-mediated Cavin-2 overexpression significantly increased α SMA and the fibrosis-associated mRNA expressions. **Conclusions:** Our observations suggest that Cavin-2 contributes to the development of cardiac fibrosis through the differentiation from fibroblasts into myofibroblasts via TGF- β /Smad signaling. Cavin-2 may be a novel therapeutic target for cardiac fibrosis.

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Poster Session 2 and Reception

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Cardiac Fibroblasts are Activated During Postnatal Extracellular Matrix Remodeling

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Objectives: During the postnatal period, P0 to P30 in the mouse, the myocardial extracellular matrix (ECM) transitions to a mature profile necessary for increased cardiac output. We hypothesize that, during the postnatal period, cardiac fibroblasts (CF) become activated, as indicated by Periostin (Postn) expression, and remodel the ECM, followed by quiescence in the mature heart. Our goal is to study CF activation and its role in postnatal heart development.

Methods: CF activation was monitored by Postn^{MerCreMer(MCM)};Rosa^{GFP} lineage analysis and the resident CF were studied using a TCF21^{MCM};R26^{GFP} model. Tamoxifen induction of Cre^{MCM} was initiated in the postnatal period and lineage-specific CF were quantified. Cardiac ECM maturation and CF proliferation, together with bone marrow-derived and endothelial cells, were assessed by Immunofluorescence (IF). *TCF21*, *Postn*, *Col1a1*, *Tnni* and *CD31* transcripts were examined by RNAscope, and Fibronectin (FN) compartmentalization, an indicator of ECM maturation, also was examined.

Results: FN, Postn, and Postn^{MCM}R26^{GFP} lineage cells were more prominent at P0-7 than at P30. *Postn*-expressing cells also co-express *Tcf21* and *Col1a1*, but not *Tnni* (myocytes) or *PECAM* (endothelial cells), as determined by RNAscope. In agreement, Postn^{MCM}R26^{GFP} CF do not express CD31, CD45, or the myofibroblast marker α SMA at P7. At P30, Postn, FN, and *Col1a1* expression is reduced in CF, but TCF21^{MCM};R26^{GFP} is maintained, suggesting a transient period of CF activation followed by quiescence. Similarly, proliferation rates of the Postn^{MCM}R26^{GFP} cells are higher than TCF21^{MCM};R26^{GFP} CF at P7, followed by decreased proliferation at P30.

Conclusions: Activation and proliferation of CFs and ECM gene expression is increased in the week after birth relative to quiescent CFs at P30. Postn+ cells also peak at P7 but are not detected in appreciable numbers at P30, suggesting a critical role in ECM remodeling and myocardial maturation in the postnatal period. Studies are ongoing to determine the codependence of postnatal activated CF, ECM maturation and myocardial regeneration.

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IL-10 Knockout Bone Marrow Fibroblast Progenitor Cells-derived Exosomes Activate Cardiac Fibroblast and Exaggerate Pressure Overload-induced Fibrosis in Mice Heart

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Background: Inflammatory mediators play important role in cardiac remodeling. In heart, fibrosis is mainly mediated by activated fibroblasts (myofibroblasts), however, origin of myofibroblasts in diseased heart remains unclear. Recently, we

have shown that bone marrow fibroblast progenitor cells (FPCs) significantly contribute in cardiac fibrosis in IL10 KO (KO) mice, but its molecular mechanism is not known. Here, we hypothesize that BM-FPCs activate resident cardiac fibroblast via their paracrine mediators (packaged in exosomes) to exaggerate the cardiac fibrosis in KO mice post- transverse aortic constriction (TAC). **Method and Results:** Cardiac hypertrophy and fibrosis was induced in Wild-type (WT) and IL10-knockout (IL10KO) mice by TAC surgery. TAC-induced left ventricular (LV) dysfunction and fibrosis were further exaggerated in KO mice. Recombinant IL10 administration markedly improved LV function and inhibited PO-induced cardiac fibrosis. PO enhanced FPC mobilization and homing in KO mice compared to WT mice. Furthermore, to identify the paracrine signaling, exosomes were isolated from WT and KO-FPCs culture media and characterized for fibrotic miRNA and proteins. Our data suggests that KO-FPC-exosomes are enriched with profibrotic miRs (miR21 and miR27) and proteins (RhoA). To explore whether KO-FPC-exosomes modulates resident fibroblasts biology and activation, adult cardiac fibroblasts (WT) were isolated and treated with WT and IL10KO-BMFPC-exosomes for 48 hrs. myoFB-associated gene (Collagen1 α , Collagen3 α and α - SMA) expression was notably increased in KO-FPC-exosome treated cells. Finally, immunostaining data further corroborated with our gene expression data and showed significantly enhanced α -SMA expression after KO-FPCs exosome treatments in cFBs. **Conclusion:** In conclusion, our data suggest that fibrotic remodeling factors (miRs and/or proteins) packaged in KO-FPC exosomes are sufficient to enhance the resident cardiac fibroblast activation and mediate cardiac fibrotic remodeling.

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Myocardial Hypertrophy and Fibrosis Regulated by Nogo-ROCK-norepinephrine Transporter Signaling Pathway in Spontaneously Hypertensive Rats

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Objective To investigate the regulation of Nogo/ROCK- norepinephrine transporter (NET) signaling pathway on myocardial hypertrophy and fibrosis in spontaneously hypertensive rats (SHR). **Methods** Twenty male SHR rats aged 7-week-old were randomly divided into four groups: control group (n=5), NEP1-40 group (n=5), fasudil group (n=5) and fluoxetine group (n=5), and fed for 7 weeks. Left ventricular mass (LVW) was separated and weighed. Left ventricular mass index (LVMI) was calculated. Real-time quantitative PCR was used to detect the gene expression of myocardial hypertrophy and fibrosis and NET. **Results** Compared with the control group, the left ventricular mass index (LVMI), the gene expression of ANP, BNP and β -MHC were significantly increased in NEP1-40 group, and were significantly decreased in Fasudil group. Compared with the control group, the gene expression of CTGF, Collagen I and Collagen III increased significantly in NEP1-40 group, while decreased significantly in Fasudil group. Compared with the control group, the gene expression of norepinephrine transporter (NET) significantly increased in fasudil group. Compared with the control group, the gene expression of ANP, BNP, β -MHC, CTGF and Collagen I were significantly increased in fluoxetine group. **Conclusion** Nogo/ROCK-NET signaling pathway is involved in the regulation of myocardial hypertrophy and fibrosis in spontaneously hypertensive rats.

S. Li: None.

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Activated Fms-like Tyrosine Kinase 3 Receptor Prevents Ventricular Remodeling Induced by Ang II Through the Attenuation of Autophagy

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Aim: FMS-like tyrosine kinase 3 (Flt3) is a receptor tyrosine kinase. This study was to determine whether activation Flt3 could regulate autophagy ameliorate AngII-induced heart cardiac remodeling and to elucidate the mechanisms of action. **Methods:** In vivo cardiac remodeling was induced in male C57BL/6 mice by implanting subcutaneously osmotic minipumps releasing Angiotensin II (Ang II) for 28 days (1100ng/kg/min). Flt3 ligand (FL) was administered intraperitoneally

(5µg/mouse/2days). Heart hypertrophy, fibrosis and function were evaluated based on echocardiography, histological and biochemical measurements. *In vitro*, hypertrophy was induced by Ang II on adenovirus vector-mediated overexpression Flt3 receptor in H9c2 cells, and NRCMs. Crystal violet staining, flow cytometry and TUNEL were used for *in vitro* experiments. The levels of signaling proteins were measured using western blotting, while the expression of the relevant genes was analyzed using real-time qRT-PCR.

Results: Echocardiography demonstrated that Ang II induced marked damaged heart function, while cardiac Flt3 receptor activation significantly decrease left ventricular internal dimension at end-diastole (LVIDd), left ventricular posterior wall thickness at end-diastole (LVPWd), and increase ejection fraction (EF%) and fractional shortening (FS%). FL-treated mice showed a significant attenuation of cardiac hypertrophy, ratios of heart weight/body weight (HW/BW) and heart weight/tibia length (HW/TL), as well as cardiac fibrosis and apoptosis. Additionally, the autophagic levels in cardiomyocytes of FL-treated mice were distinctly decreased as evidenced by expression of LC3 in immunohistochemical staining and western blotting. In H9c2 cells with adenovirus

vector-mediated overexpression of Flt3 and neonatal rat cardiomyocytes, FL treatment significantly decrease the cell size, apoptosis and autophagy induced by Ang II. Additionally, using rapamycin or metformin, respectively canceled FL protective effects.

Conclusion: Flt3 activation suppresses Ang II-induced cardiac hypertrophy and apoptosis via AMPK /mTOR/ FOXO3a autophagy signaling pathway. These results provide evidence supporting Flt3 as a novel therapeutic target in cardiac remodeling.

W. Ma: None.

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In vitro Reverse Remodeling After Mechanical Unloading With Engineered Heart Tissue

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LVADs serve as a bridge to transplantation for many dilated cardiomyopathy (DCM) patients with advanced heart failure. In rare cases, mechanical unloading can lead to reverse myocardial remodeling sufficient for the removal of LVAD. We recreated the first reverse remodeling process *in vitro* in order to examine the role of active actin-myosin contraction and fibroblasts in contributing to this recovery, and to test the efficacy of drugs commonly prescribed for concurrent use with LVAD implantation. Engineered heart tissue (EHT) were prepared from decellularized porcine ventricular scaffolds in a proof-of-concept animal model with neonatal rat cardiomyocytes and subsequently with human induced pluripotent stem cell-derived cardiomyocytes and adult human cardiac fibroblasts. Mechanical stretch and myosin inhibition were used to simulate the pathological remodeling of DCM. Preliminary animal data showed that our model was able to capture the key features of DCM and reverse remodeling process including improved slack length, tissue stiffness, and active kinetics following mechanical unloading. β -adrenergic receptor response, protein assay, immunohistochemistry, and genetic analysis are being carried out to comprehensively investigate reverse remodeling in this artificial human system. Additional drug studies aim to investigate how reverse remodeling is affected by concurrent administration of β_1 antagonists and β_2 agonists (typical drug regimen for heart failure) and may inform new potential treatment during mechanical unloading to promote beneficial reverse remodeling for patients.

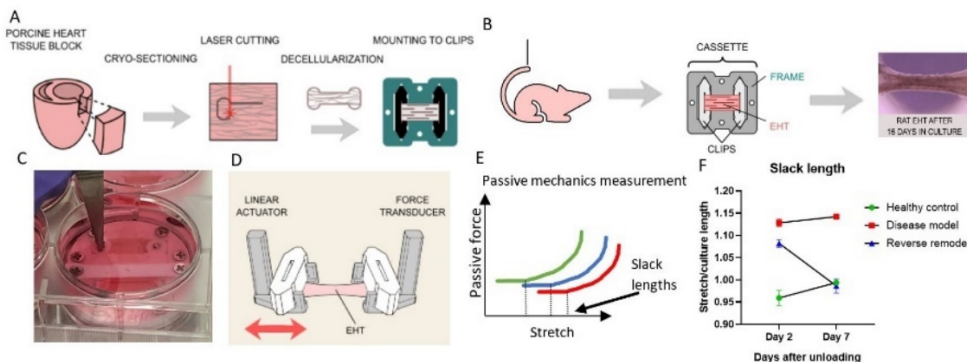


Figure 1A. Process of make decellularized porcine ventricular scaffold and mount onto laser-cut Teflon frame. 1B. Workflow of isolating neonatal rat ventricular cardiomyocytes and making rat cardiac tissue. 1C. Stretch of a single tissue in a laser-cut CAM device. 1D. Schematic of tissue mechanics measurement. 1E. Schematic of passive tissue measurement and the determination of tissue slack length (the point where passive force starts to develop). 1F. Preliminary animal data showing reverse remodeling following mechanical unloading.

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A Transcriptomic Analysis of Cardiac Myofibroblasts Reveals Novel Anti-fibrotic and Immunomodulatory Genes Modulated by CDC-Derived Exosomes

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Background: Cardiac fibroblasts are the most prevalent cell in the heart and play an important role in both the healthy and diseased myocardium. Following myocardial infarction, TGF- β secretion promotes a phenotype transition of fibroblasts to activated myofibroblasts. We have previously shown that CDC-derived exosomes promote cardiomyocyte proliferation and survival, stimulate angiogenesis, and polarize cardiac macrophages to a unique Arg1-high phenotype. We hypothesized that CDC-derived exosomes modulate the gene expression of myofibroblasts to attenuate fibrosis in the remodeling heart.

Methods: We took a genomics-based approach to investigate CDC-exosome mediated differential gene expression and regulation of operant signaling pathways in TGF- β induced ventricular myofibroblasts. Cardiac fibroblasts were isolated from C57BL/6 mice (n=3) using a Langendorff-free method of *in situ* enzymatic dissociation. Cells were plated at 1×10^5 cells/mL, serum depleted to induce cell cycle arrest and exposed to 4 different conditions: media alone, TGF- β (4 ng/ml) "myofibroblasts", CDC-exosomes (20 μ g/ml), or TGF- β and CDC-exosomes. After 24 hours, RNA was extracted and analyzed by RNA-seq.

Results/Conclusions: Gene ontology analysis showed attenuation of a pro-fibrotic gene expression profile in the TGF- β + CDC-exo group relative to TGF- β treatment alone. Genes involved in blunting of the pro-fibrotic response (*Mmp14* and *Qsox1*), as well those implicated in attenuating the transition of fibroblasts to myofibroblasts (*Gsto1*) were also upregulated in CDC-exosome treated myofibroblasts (edgeR; FDR-corrected $p < 0.05$). Interesting, we found a highly significant enrichment of genes involved in macrophage migration (*Kars*, *Mmp14*, and *Tnfsf18*) in the CDC-exosome treated myofibroblasts relative to the TGF- β group (GO:1905523, $p = 8.42 \times 10^{-6}$). These data suggest active cross talk between myofibroblasts and macrophages and highlight a novel immunomodulatory role of CDC-derived exosomes on myofibroblasts post MI.

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Cardiac Fibroblast Ablation in Chronic Fibrosis

April Stempien-Otero, Deri Helterline, Steve Farris, Univ Washington Sch Med, Seattle, WA

Cardiac fibrosis is a common feature of heart failure independent of etiology. Although much is known about initiation of fibrosis in acute injury, factors leading to the persistence of fibrosis are poorly understood. We sought to determine if cardiac fibroblasts are necessary for the persistence of fibrosis by ablating resident cardiac fibroblasts in a mouse model of cardiac fibrosis. **Methods:** We generated mice with inducible ablation of cardiac fibroblasts (fb) by crossing the *tcf21-cre* gene into a *MerCreMer-DTA* mouse. These mice were then crossed into *SR-uPA* transgene which promotes diffuse interstitial fibrosis by 12 weeks of age. Control *tcf21-DTA* and *tcf21-DTA-SRuPA* mice were treated with tamoxifen for 1-4 weeks to activate DTA and ablate cardiac fibroblasts. Echocardiography, histology and qPCR of isolated fb and macrophages (mac) was performed. **Results:** Treatment of wildtype *tcf21-DTA* mice with tamoxifen for 1-4 weeks resulted in a decrease in alpha-SMA positive cells in the heart but no change in left ventricular size or function. There was no excess mortality. Hearts showed no evidence of increased inflammation or fibrosis. *Tcf21-DTA-SRuPA* mice were viable but exhibited increased mortality starting at 10 days following initiation of tamoxifen. There were no changes in echocardiographic parameters, histologic inflammation or fibrosis. Fb had no change in expression of *Col1a1* normalized to GAPDH. However, mac from *Tcf21-DTA-SRuPA* mice had a significant decrease in expression of M2 genes *Arg1* and *YM1*. **Conclusion:** Ablation of fibroblasts was toxic to mice with established fibrosis, without changes in cardiac structure or function. Mac phenotype shifted from pro-fibrotic/M2 to pro-inflammatory/M1 with fibroblast ablation. These data point to important compensatory mechanisms that may maintain fibrosis in the heart.

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Restricted Proteasome Heterogeneity Promotes Premature Heart Failure and Deterioration of Associated Protein Levels Upon Continuous β -Adrenergic Stimulation

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Introduction. The ubiquitin proteasome-system is a main contributor to cellular proteostasis. The dynamic regulation of proteasome composition and function during cardiac remodeling has been of high interest. To what extent discrete proteasome subunits in turn modify cardiac remodeling, is poorly understood.

Objective. Aim of the study was to investigate the contribution of the non-essential proteasome subunit low molecular mass peptide 2 (Lmp2) on cardiac function, tissue and proteome upon induced hypertrophic remodeling.

Methods & Results. We found induction of cardiac Lmp2 during isoproterenol (Iso)- and TAC-induced remodeling and hypertrophic cardiomyopathy. Unchallenged adult mice congenitally lacking Lmp2 (KO) were indistinguishable from their WT littermates. After 7 days of continuous Iso administration (30 mg/kg/d) though, cardiac function was significantly reduced in Lmp2 KO mice as compared to WT (fractional shortening: -32% vs. WT, $p < 0.01$, $n \geq 9$). Interestingly, reduced cardiac function was accompanied by augmented cardiac remodeling as shown by heart weight to body weight ratio, wall thickness, cardiomyocyte cross-sectional area and interstitial collagen content. Cardiac-restricted re-expression of Lmp2 utilizing AAV9-mediated gene transfer rescued cardiac function and restricted remodeling to WT levels, attributing those findings to cardiac Lmp2. Hence, we analyzed the cardiac proteome utilizing 2-D DIGE. Out of approximately 1500 detected protein spots, 114 were showing significant differences upon cardiac remodeling depending on absolute Lmp2 protein expression ($p \leq 0.05$). All spots were manually inspected for correct matching across 10 gels. Subsequently, spots were picked with priority on those with the most prominent changes in abundance and identified by mass spectrometry. For example, we found significantly less carbonic anhydrase 2 and manganese superoxide dismutase in the remodeled myocardium of WT compared to Lmp2 KO mice.

Conclusions. Cardiac lack of Lmp2 exacerbates cardiac remodeling, which is reflected in the cardiac proteome by deteriorated levels of proteins with a known pivotal role in human cardiac disease.

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Circulating Pro Fibrotic Protein Promotes Fibrosis in Liver and Heart

Yuki Tsukano, Ippei Shimizu, Yohko Yoshida, Ryutaro Ikegami, Yuka Hayashi, Masayoshi Suda, Goro Katsuomi, Takayuki Wakasugi, Masaaki Nakao, Tohru Minamino, Niigata Univ Graduate Sch of Medical and Dental Sciences, Niigata, Japan

Analyzing two sets of DNA micro array data with bioinformatics, we identified a secreted form pro-fibrotic protein (sPFP) expressed in dysfunctional brown adipose tissue (BAT) in mice. Testing our biobank samples, we found this protein increased in plasma of non-alcoholic steatohepatitis (NASH) patients or aged individuals. We generated a murine obese NASH model by imposing a high fat diet in C57BL/6Ncr mice for 9-10 months since 4 weeks of age, and found that sPFP is produced predominantly by BAT. In this model, we also found that sPFP increased in plasma. We generated a murine systemic sPFP knockout (KO) model and found that liver fibrosis ameliorated in sPFP-KO model. We also suppressed circulating sPFP with a peptide vaccine targeting this molecule, and found that sPFP vaccination therapy inhibited liver fibrosis. Next, we generated sPFP gain of function (GOF) model by the administration of plasmid encoding sPFP into skeletal muscle. Liver fibrosis augmented in sPFP-GOF model, and these results suggested that sPFP has causal role for the progression of fibrotic response in liver. In the obese NASH model, we found that cardiac fibrosis also developed and it ameliorated in sPFP-KO model, indicating that sPFP may have pathological roles for heart failure with preserved ejection fraction (HFpEF) related with age-related disorders. In addition to an increase in circulating sPFP in aged individuals, we found that sPFP increased in BAT of chronological aged mice model. In vitro studies with differentiated brown adipocytes showed that c-Fos upregulated sPFP in transcript level. Our results suggest that sPFP contributes for the progression of fibrotic responses in obese or aged models. Inhibition of sPFP may become a therapy for NASH or HFpEF.

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Cardiac Fibroblast GSK-3 α Contributes to Ventricular Remodeling and Dysfunction of the Failing Heart

Prachi UMBARKAR, Anand Prakash Singh, Qinkun Zhang, Hind Lal, Vanderbilt Univ Medical Ctr, Nashville, TN

Background: Heart failure is the leading cause of mortality, morbidity, and healthcare expenditures worldwide. Numerous studies from our lab and others have implicated Glycogen Synthase Kinase-3 (GSK-3) as a promising therapeutic target for cardiovascular diseases. GSK-3 isoforms appear to play overlapping, unique and even opposing functions in the heart. Recently our group has identified cardiac fibroblast (CF) GSK-3 β as a negative regulator of fibrotic remodeling in the ischemic heart. However, the role of CF-GSK-3 α in cardiac pathophysiology is poorly understood. **Methods and Results:** To determine the role of CF-GSK-3 α in the pathogenesis of heart failure, GSK-3 α was deleted specifically from mouse resident cardiac fibroblast or myofibroblast with tamoxifen-inducible TCF21- or periostin (Postn)- promoter-driven Cre recombinase. At 2 months of age, WT and KO mice were subjected to cardiac injury and heart functions were monitored by serial echocardiography. Histological analysis and morphometric studies were performed at 8 weeks post-injury. In TCF21-KO mice, deletion of GSK-3 α from resident cardiac fibroblasts significantly restricted pressure overload-induced adverse cardiac remodeling and improved cardiac function. Consistently, in Postn-KO mice, deletion of GSK-3 α from myofibroblasts remarkably reduced LV scar circumference and prevented cardiac dysfunction post-MI. To gain the mechanistic insights of observed GSK-3 α mediated fibrotic remodeling, we examined the effect of GSK-3 α deletion on myofibroblast transformation and profibrotic TGF- β 1-SMAD3 signaling *in vitro*. WT and GSK-3 α KO mouse embryonic fibroblasts (MEFs) were treated with TGF- β 1 (10 ng/mL). Indeed, a significant reduction in cell migration, collagen gel contraction, and α -SMA expression in TGF- β 1 treated GSK-3 α KO MEF confirmed that GSK-3 α is required for myofibroblast transformation. Surprisingly, GSK-3 α deletion had no effect on TGF- β 1 induced SMAD3 activation indicating the potential involvement of GSK-3 α in eliciting SMAD3 independent profibrotic response. **Conclusion:** These findings suggest the causal role of CF-GSK3 α in the cardiac remodeling of the injured heart that could be a therapeutically targeted for the future clinical applications.

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Urolithin A Suppress Cardiac Fibrosis via Autophagy Pathway in the Diabetic Cardiomyopathy

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Objective: The cardiovascular protective effect of Urolithins remains poorly understood, despite wide-spread human exposure via the dietary consumption of their metabolic precursors, the ellagitannins, which is found in the pomegranate fruit. We identified Urolithin A (UA) as a natural compound that induces mitophagy and protects against diabetic cardiomyopathy both *in vivo* and *in vitro*. Healthy male C57BL/6 mice were fed with a High-fat diet for 6 months combined with a small dose of STZ injection to establish the T2DM model. Compared with the control, the diabetic mice suffered from cardiac dysfunction characterized by decrease EF, FS and E/A ratio, which was reversed with UA treatment. Masson staining and collagen marker Immunoblots showed that cardiac fibrosis was also decreased with UA treatment in the diabetic mice both *in vivo* and *in vitro*. Interestingly, we found UA significantly activated mitophagy and increased the co-localization of Myto-tracker and Lysotracker. However, we found UA increased Drp1 expression but not PINK1 or Parkin phosphorylation indicating a non-canonical mitophagy pathway. We treated the cardiac fibroblast with Drp1 siRNA and found the protective effect of UA against cardiac fibrosis was abolished. Conclusion: UA protected against diabetic cardiomyopathy by activating mitophagy via a non-canonical pathway. Keywords: Diabetic cardiomyopathy; Cardiac fibrosis; Urolithin A; Autophagy; Mitophagy

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Methamphetamine-induced Cardiomyopathy Associated With Mitochondrial Dysfunction, Cardiac Fibrosis and Hypertrophy

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Introduction: Methamphetamine (METH) is one of the most commonly abused illicit drugs in the United States, exerting a range of adverse effects upon multiple organ systems. Cardiovascular complications are among the major causes of death in METH users. METH-induced cardiomyopathy is a poorly characterized disease entity as METH-induced molecular perturbations, and histopathological changes in the heart remain under-explored. **Objectives:** We studied histopathology in the hearts of human METH users. We also observed the histological alteration and changes in mitochondrial function in mice that received 'binge' administration of METH. **Methods and Results:** We obtained 32 autopsy heart samples from humans with positive toxicology for chronic METH use and performed Sirius Red and Masson's Trichrome (MT) staining on left ventricular (LV) sections. Notably, chronic METH user hearts showed intense perivascular and interstitial fibrosis in LVs. 'Binge' METH administration in mice for 4 weeks showed an increase in heart weight-to-tibia length and increase in myocyte cross-sectional area in WGA stained LVs compared to saline-treated mice. Sirius red and MT staining also showed an increase in perivascular and interstitial fibrosis in METH mice heart. Isolated mitochondria from METH-treated mice heart showed suppressed mitochondrial bioenergetics measured by Seahorse Analyzer. Immunoblotting in heart lysates and mitochondrial fractions showed altered mitochondrial dynamics regulatory proteins expression in METH mice compared to control saline group. METH-treated cultured neonatal rat ventricular cardiomyocytes also showed suppression of mitochondrial respiration and mitochondrial network disorganization indicating a direct effect of METH on cardiomyocytes. **Conclusions:** We report that maladaptive cardiac fibrotic remodeling is typical in a human and pre-clinical mouse model of METH abuse. 'Binge' METH exposure in mice induces cardiac hypertrophy, cardiac fibrosis, and suppression of mitochondrial respiration. Thus, chronic METH use induces maladaptive cardiac remodeling associated with mitochondrial dysfunction.

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Sigma 1 Receptor-dependent Regulation of Mitochondrial Respiration and Function in Cardiomyocytes

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Background: Sigma 1 receptor (Sigmar1) is an abundantly expressed molecular chaperone protein in cardiomyocytes. Sigmar1 ligands have been shown to have protective function in animal models of cardiac hypertrophy, heart failure, and many other cardiovascular disorders. Despite extensive studies, the physiological and molecular functions of Sigmar1 in cardiomyocytes remains elusive. **Objective:** We investigated the molecular function of Sigmar1 in regulating mitochondrial respiration and function in cardiomyocytes. **Methods and Results:** To define the molecular function of Sigmar1 in regulating mitochondrial respiration and function in cardiomyocytes, we used small interfering RNA-mediated Sigmar1 knockdown and adenovirus-mediated overexpression in cultured neonatal rat ventricular cardiomyocytes. Sigmar1 knockdown in neonatal cardiomyocytes showed significantly decreased mitochondrial respiration measured by Seahorse Analyzer. Mitochondria

stained with mitoTracker green showed a significantly increased number of hyperfused mitochondria in Sigmar1 knockdown cardiomyocytes. Similarly, mitochondria isolated from Sigmar1 global knockout mouse hearts also showed decreased mitochondrial respiration and the accumulation of larger size mitochondria measured by flowcytometry. Sigmar1 overexpression in cardiomyocytes significantly increased mitochondrial respiration and also showed increased mitochondrial fragmentation. **Conclusions:** Our findings suggested that Sigmar1 plays an essential role in maintaining normal mitochondrial morphology, respiration, and function in cardiomyocytes.

R. Aishwarya: None. **M. Bhuiyan:** None.

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Uncovering the Physiological Role of Mitochondrial Respiratory Supercomplexes in the Heart

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The assembly of mitochondrial electron transport chain (ETC) supercomplexes (SCs), particularly the respirasome containing complexes I, III, and IV have been shown to participate in facilitating electron transport, reducing ROS production and maintaining the structural integrity of individual ETC complexes. However, the physiological role of SCs in high energy demanding tissues such as the heart remains unknown. Here, we elucidated whether disassembly of SCs affects the cardiac function. Hearts isolated from adult male Sprague Dawley rats were perfused using a Langendorff-mode perfusion with Krebs-Henseleit solution (KHS) for the first 20 min (equilibration period) followed by perfusion in the following groups: (i) 40-min perfusion with KHS (n=4), (ii, iii) 20-min perfusion with rotenone (an inducer of SC dissociation) or vehicle (ethanol, n=8 for both), and (iv, v) 20-min perfusion with rotenone or vehicle followed by a 20-min perfusion with KHS without rotenone or vehicle (n=6 for both). Cardiac function was monitored throughout the entire perfusion period. At the end of each protocol, the mitochondria were isolated for analysis of SCs, permeability transition, respiration rates, and ROS production. We found that cardiac function between rats perfused with KHS (i) and ethanol (i, iii) had no significant difference, though rotenone perfused rats (ii and iv) had a significant reduction (<40%) in cardiac function associated with reduced oxygen consumption rates in these groups. Furthermore, a significant increase in ROS production (ii) and permeability transition pore opening (iv) was observed when the hearts were perfused with rotenone with and without subsequent washout, respectively. Analysis of SCs by blue native PAGE displayed a significant reduction in SC levels in both ethanol (iii and v) and rotenone (ii and iv) perfused rats. Surprisingly, there were no differences in SC levels between the ethanol (iii and v) and rotenone (ii and iv) perfused groups. In addition, incubation of isolated intact mitochondria with ethanol and acetaldehyde did not demonstrate any direct effects of these compounds on ETC SC assembly. In conclusion, our data demonstrate a lack of an associative link between mitochondrial ETC SCs and cardiac function in rats.

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The Metabolites of Human Cardiac Precursors Enhanced Cardiomyogenic Differentiation of hiPSC

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BackgroundWnt signaling pathways are involved in cardiomyogenic differentiation. Besides that, nutrients and metabolism may also influence cell differentiation. However, it is still unclear whether the cell lineage commitment could be controlled by the metabolites of its precursor cells. The aim of this study is to examine whether the metabolites of cardiac precursors could determine cardiomyogenic differentiation fate of hiPSC. **Method**hiPSC was maintained in serum-free and feeder-free monolayer condition. After CHIR99021-induced mesendodermal differentiation for two days, IWP4 was added to inhibit Wnt signaling and to induce myogenic differentiation for another two days. After wash, the culture medium was collected during cell differentiation period of day 5-10 (d5-10-diff-medium). ExoQuick-TC kit was used to extract the exosomes in the medium. Cardiomyocytes were identified by immunocytochemistry staining with mouse monoclonal antibody against cardiac sarcomeric actinin alpha, and were counted the percentage by flow cytometry. **Results**The exosome-free d5-10-diff-medium was found to be able to dramatically induce cardiomyogenic differentiation only in CHIR99021-induced mesendodermal

cells, rather than directly in hiPSC, in the absence of Wnt inhibitor. The metabolic profiling of the exosome-free d5-10-diff-medium was further characterized by LC-MS. After functional validation, the effective metabolites for the induction of cardiomyogenic differentiation were identified and the possible signaling pathways were analyzed. Conclusion This study demonstrated that the metabolites of cardiac precursors could control cardiac cell commitment by driving the development of cardiac metabolic system.

W. Chen: None. Y. Li: None.

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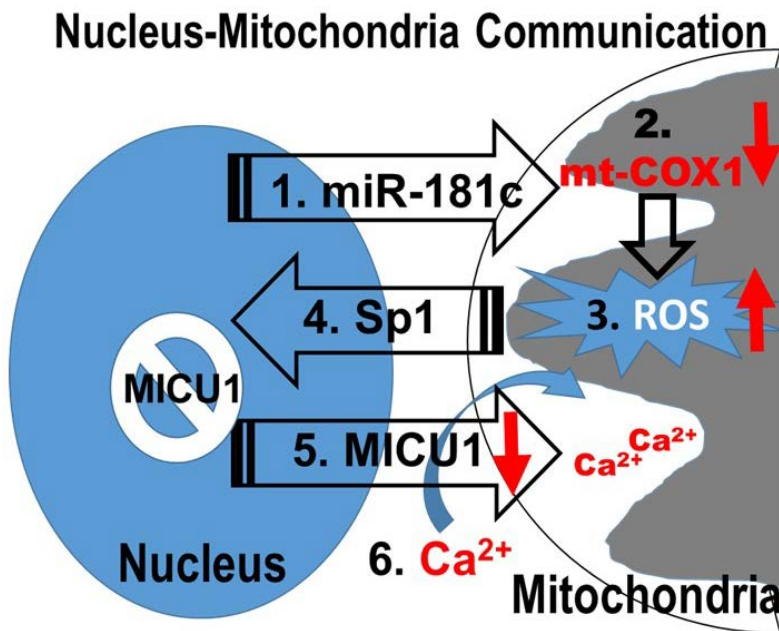
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microRNA181c Activates Mitochondrial Calcium Uptake by Regulating Micu1 in the Heart

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Translocation of miR-181c into heart-mitochondria downregulates the mitochondrial gene, mt-COX1. miR-181c/d^{-/-} hearts experience less oxidative stress during I/R and are protected against I/R injury. Additionally, miR-181c overexpression can increase mitochondrial matrix Ca²⁺ ([Ca²⁺]_m); the mechanism is unknown. By RNA-Seq, here we show that hearts from miR-181c/d^{-/-} mice overexpress nuclear-encoded Ca²⁺ regulatory genes, suggesting that alterations in miR-181c and mt-COX1 perturb mitochondria-to-nucleus retrograde signaling and [Ca²⁺]_m regulation. qPCR validation of transcription factors that are known to initiate retrograde signaling revealed significantly higher Sp1 expression in the miR-181c/d^{-/-} hearts. Furthermore, an association of Sp1 with the promoter region of MICU1 was confirmed by ChIP-qPCR and higher expression of MICU1 was found in the miR-181c/d^{-/-} hearts. Conversely, downregulation of Sp1 by siRNA decreased MICU1 expression in NMVM. Changes in PDH activity provided evidence for a change in [Ca²⁺]_m via the miR-181c/MICU1 axis. Moreover, this mechanism was implicated in the pathology of I/R injury. When MICU1 was knocked down in the miR-181c/d^{-/-} heart by lentiviral expression of an shRNA against MICU1, cardioprotective effects against I/R injury were abrogated. Furthermore, using an *in vitro* I/R model in miR-181c/d^{-/-} NMVM, we confirmed the contribution of Sp1-MICU1 in ischemic injury. In summary, miR-181c regulates mt-COX1, which in turn regulates MICU1 through the Sp1-mediated mitochondria-to-nucleus retrograde pathway. Loss of miR-181c can protect the heart from I/R injury by modulating [Ca²⁺]_m through the upregulation of MICU1.



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A Multifunctional Genetic Probe for Investigating Mitochondrial Calcium Dynamics in Cardiac Cells

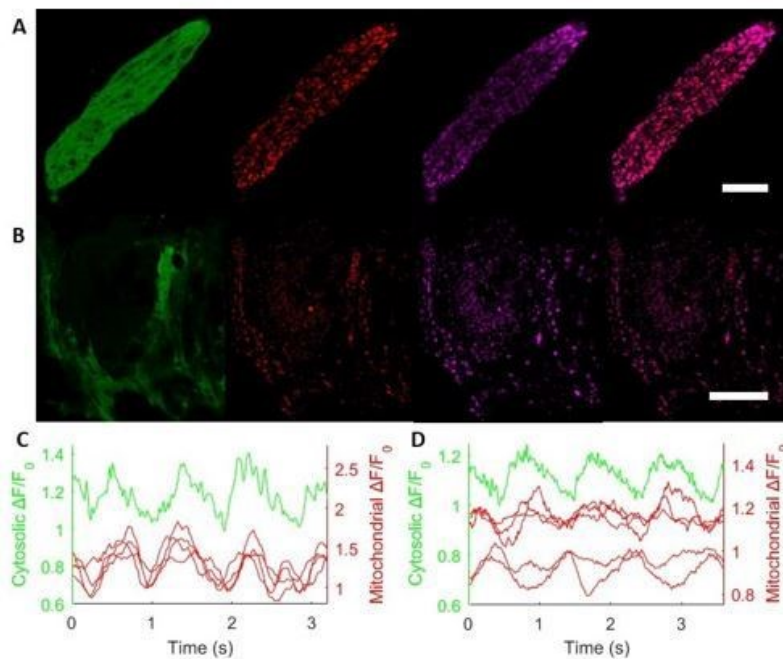
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Introduction: Calcium cycling plays a critical role in regulating function of cardiomyocytes (CMs) under both physiological and pathological conditions. Among their many important functions, mitochondria are known to involve in calcium handling. However, little is known about the calcium dynamics of human stem cell-derived cardiomyocytes (hiPSC-CM) and what little is known shows much greater variability than what is seen in adult cardiomyocytes.

Methods: We produced adenovirus encoding the red Ca^{2+} indicator RCaMP prepended with a mitochondrial targeting sequence fused to the green Ca^{2+} indicator GCaMP with a cleavable linker. We then used this virus to induce expression of this calcium probe in CMs & recorded calcium activity with a confocal microscope.

Results & Discussion: Mitochondrial targeting was confirmed by confocal imaging showing GCaMP (green), mRCaMP (red), and costaining with Mitotracker Deep Red (Magenta) (Figure 1A, 1B). Probe functionality was validated by observing beat-by-beat calcium dynamics in paced neonatal rat CMs with and without blocking mitochondrial calcium uptake with the mitochondrial calcium uniport inhibitor Ru360. Recording GCaMP and mRCaMP dynamics in adult CMs (Figure 1C) showed synchronous beat-by-beat calcium activity in the cytosol and mitochondria. However, iPSC-CMs (Figure 1D) showed variable mitochondrial activity, with some synchronous mitochondria and some showing inverse activity.

Conclusions: hiPSC-CMs and adult CMs show differences in beat-by-beat mitochondrial calcium dynamics, offering a potential investigatory target for improving the therapeutic effect of stem cell-derived heart therapies.



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Exploring MCUb Function in the Heart and Its Role in Ischemia Reperfusion Injury

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Mitochondrial calcium alterations can promote oxidative metabolism to match increasing functional demands during stress stimulation. However, mitochondrial calcium overload-induced cell death contributes to the pathogenesis of several cardiac disorders including ischemia reperfusion injury. The mitochondrial calcium uniporter (MCU) complex is the only identified transporter that permits rapid calcium uptake into mitochondria. While the biological function of the MCU pore-forming subunit has been annotated, much less is known about the MCUb protein, a high similarity paralog that is part of the greater MCU complex. The goal of our study was to investigate the biological function of MCUb, its role in mitochondrial calcium uptake, and its contribution to the pathogenesis of ischemia reperfusion injury in the heart. To address these questions, we generated both MCUb overexpressing mice as well as MCUb null mice.

We observed that the cardiac-specific overexpression of MCUb inhibited mitochondrial calcium uptake, although basal mitochondrial calcium levels remained unchanged. Genetic ablation of MCUb, conversely, did not influence mitochondrial calcium uptake in the heart. MCUb null mice did not have differences in cardiac function by echocardiography, nor were tissue histological changes observed. This lack of an overt phenotype in MCUb null mice may be attributable to very low or even absent expression in the heart at baseline.

However, induction of MCUb protein was observed in the hearts of mice subjected to ischemia reperfusion injury. Thus, mice were challenged with one-hour ischemia followed by 24-hour reperfusion and the ischemic area/area at risk was analyzed. Deletion of MCUb did not change the initial infarct size of the heart. However, MCUb null mice showed decreased fractional shortening 4-weeks after the ischemic injury. Gravimetric analysis as well as histological examination further supported the conclusion that MCUb deletion exacerbated damage in the heart after ischemia reperfusion injury. We are currently investigating the underlying mechanisms of this greater functional deficit.

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Metabolomic and Transcriptomic Profiling of Rat Offspring Exposed to Gestational Diabetes Reveals Altered Cardiac Gene Expression and Metabolism

Stephanie Kereliuk, Prasoon Agarwal, Laura Cole, Kyle Cheung, Bo Xiang, Mario Fonseca, Grant Hatch, Jonathan McGavock, Vernon Dolinsky, Univ of Manitoba, Winnipeg, MB, Canada

Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Children exposed to GDM are at an increased risk of developing cardiometabolic diseases later in life, though the mechanisms responsible are unknown. We hypothesize that fetal exposure to GDM induces alterations in cardiomyocyte metabolism and concomitant left ventricular (LV) dysfunction with age. GDM was induced in female rats with a high fat (45% kcal) and sucrose diet prior to mating, throughout pregnancy and lactation. Lean control females received a low fat (10% kcal) diet. Fetal rat ventricular cardiomyocytes (FRVC) were isolated from e20.5 offspring for U-13C glucose metabolic flux analysis and mitochondrial respiration. Serum metabolites and cardiac transcriptome profiles were measured in 3-month old offspring. LV morphology and function was assessed through the life course of the offspring (e18 to 12-months of age) by transthoracic ultrasound. Offspring exposed to GDM exhibited increased LV posterior wall thickness across their life course (fetal to 12-months of age; $p < 0.05$) and impaired LV filling beginning at 6-months of age ($p < 0.05$). U-13C glucose metabolic flux through glycolysis and the citric acid cycle was reduced in FRVC from GDM offspring when treated with isoproterenol, and compared to Lean FRVC. Basal and maximal mitochondrial oxygen consumption was reduced for glucose (35% and 68%) and fatty acid (49% and 52%) substrates in FRVC isolated from GDM offspring ($p < 0.05$). In 3-month old GDM offspring, serum metabolomics revealed elevated levels of beta-hydroxybutyrate (2.4-fold, $p < 0.05$) and reduced levels of several citric acid cycle intermediates, which also corresponded to alterations in gene expression patterns identified by RNASeq transcript analysis. Large-scale profiling revealed GDM induced alterations in the cardiac gene expression profile leading to modified serum metabolite levels in the offspring. These alterations corresponded with mitochondrial dysfunction, impaired cardiomyocyte metabolic flux and contractility, in concert with LV hypertrophy and diastolic dysfunction in the rat offspring. Our findings identify several mechanisms that link early-life GDM exposure to the development of cardiovascular disease later in life.

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Cardiomyocyte-KLF5 Expression is Increased by FOXO1 and Accounts for Cardiomyopathy in Type-1 Diabetes

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Cardiomyopathy in type 1 diabetes (T1D) is accompanied by altered cardiac energetics, impaired mitochondrial function and oxidative stress. We showed previously increased cardiac expression of Krüppel-like factor 5 (KLF5) and Peroxisome Proliferator Activated Receptor (PPAR) α at late T1D stage in mice. We confirmed that KLF5 expression is higher in cardiomyocytes of diabetic patients than in non-diabetic individuals. Mechanistic analyses in human cardiomyocyte cells (AC16) and in mice with cardiomyocyte-specific FOXO1 deletion (α MHC-*Foxo1*^{-/-}) revealed that FOXO1 activation accounts for the increased KLF5 expression via direct binding on *Klf5* promoter. Both pharmacologic and cardiomyocyte-specific inhibition of KLF5 alleviated diabetic-related cardiac dysfunction. Accordingly, mice with doxycycline-mediated cardiomyocyte-specific KLF5 constitutive expression (α MHC-rtTA-*Klf5*) recapitulated cardiomyopathy even in the absence of T1D. We also showed that diabetic PPAR α ^{-/-} mice had elevated cardiac KLF5 expression and cardiac dysfunction, suggesting that KLF5-driven diabetic cardiomyopathy is PPAR α -independent. Furthermore, cardiomyocyte KLF5 upregulation was associated with oxidative stress, increased NADPH oxidase (NOX)4 expression, lower NADPH levels and impaired cellular Ca²⁺ handling. Conversely, KLF5 inhibition prevented NOX4 upregulation and alleviated cardiac superoxide formation. Lipidomic analysis followed by Euclidean clustering showed strong correlation of lipidome profiles between α MHC-rtTA-*Klf5* mice and diabetic C57BL/6 mice, while diabetic α MHC-*Klf5*^{-/-} mice and diabetic C57BL/6 mice that were treated with pharmacologic KLF5 inhibitor grouped with the non-diabetic C57BL/6 mice. Further analysis of individual lipid species showed increased ceramide accumulation in diabetic C57BL/6 and α MHC-rtTA-*Klf5* mice that was reversed upon KLF5 inhibition. Treatment of α MHC-rtTA-*Klf5* mice and diabetic C57BL/6 mice with the antioxidant LGM2605 improved partially cardiac dysfunction. In conclusion, cardiomyocyte KLF5 expression is activated by FOXO1 and drives diabetic cardiomyopathy in a non-PPAR α -dependent manner, suggesting KLF5 inhibition as a therapeutic intervention in T1D cardiomyopathy.

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Cardiac Aging is Associated With Impaired Mitophagy and Formation of Megamitochondria

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Aging is associated with increased risk of developing cardiovascular disease. Currently, the molecular mechanisms contributing to the cardiac aging process and the resulting pathogenesis are unclear. Here, we have characterized changes in mitochondria and mitochondrial autophagy (mitophagy) in aging mouse hearts. To evaluate structural and functional changes that occur with cardiac aging, we compared young (4 months) and old (24 month) mice. We found that aging led to cardiac hypertrophy as characterized by increased left ventricular (LV) mass index and heart weight/body weight ratio as well as elevated myosin heavy chain 7 mRNA levels. Although echocardiography analysis showed no detectable changes in systolic function, the aged mice showed evidence of diastolic dysfunction as there was a significant decrease in the E/A ratio. We also found increased levels of fibrosis and inflammation in the aged hearts. Evaluation of autophagy in aged hearts revealed impaired autophagic flux, as indicated by accumulation of the autophagy substrate p62. Treatment of mice with the mTOR inhibitor Rapamycin, led to the expected increase in LC3II-positive autophagosomes in young hearts but not in the aged hearts. Parkin is an E3 ubiquitin ligase involved in labeling mitochondria for mitophagy by ubiquitinating proteins on the outer mitochondrial membrane. We found a significant increase in Parkin at the mitochondria in aged hearts which was accompanied with a significant increase in mitochondrial protein ubiquitination and p62 levels. However, LC3II protein levels in the mitochondrial fraction did not increase. This suggests that although mitochondria have been labeled for mitophagy,

they are not eliminated due to decreased formation of autophagosomes. Finally, ultrastructural analysis of hearts showed the presence of enlarged mitochondria in aged hearts. Although we observed no changes in mitochondrial fusion proteins Mfn1/2, we found decreased activation of the mitochondrial fission protein Drp1. Overall, these data suggest that which might contribute to the development of age-related cardiac pathologies.

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Differences in GCN5L1 Expression Between Male and Female Hearts Are Associated With Increased Mitochondrial Protein Acetylation

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Introduction: Historically, most preclinical heart research has relied predominantly on male subjects, despite the identification of significant differences in signaling, metabolism, and pathology between males and females. Delineating the mechanisms behind these sex differences is critical to the effective application of novel findings to the general population as a whole. GCN5L1 is a mitochondrial-targeted protein that drives the acetylation and activation/deactivation of several metabolic proteins, and which has recently been reported to regulate cardiac metabolism and recovery from ischemia-reperfusion. It is unknown whether GCN5L1 plays a role in sex-specific differences in the myocardium. **Hypothesis:** Differential regulation of myocardial GCN5L1 expression between male and female animals results in differences in the acetylation and activity of proteins that regulate glucose and fatty acid oxidation. **Methods:** Heart lysates from male and female mice were probed for the expression of GCN5L1, and the acetylation status of whole cell and mitochondrial protein fractions was analyzed. Proliferating cardiac cells were treated with 17 β -estradiol, and the expression of GCN5L1 was evaluated by immunoblotting. The acetylation status of mitochondrial proteins was measured using immunoblotting and immunoprecipitation. **Results and Conclusions:** Significant differences in cardiac GCN5L1 protein expression were found between male and female mice. In contrast, there was no difference in the protein expression of SIRT3, the mitochondrial deacetylase enzyme previously shown to counter GCN5L1 activity. Mitochondrial acetylation levels were found to be different between male and female mice. Treatment of cardiac cells with estradiol resulted in increased GCN5L1 expression, with a concurrent increase in the acetylation status of GCN5L1 targets localized to mitochondria. We conclude that sex-based differences in mitochondrial protein acetylation may be mediated by hormone-induced changes in GCN5L1 expression.

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Bone Morphogenetic Protein-3b Deficiency Induces Metabolic Syndrome and Modulates Adipogenesis

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INTRODUCTION: Bone morphogenetic proteins (BMPs) are mediators in a wide range of cellular functions, including the regulation of cell metabolism. The objective of this study was to investigate the effects of BMP-3b, a glycoprotein synthesized and secreted by adipose tissue, on cardiovascular risk factors. **METHODS:** BMP-3b knock-out (KO) and age-matched male and female wild-type (WT) mice were weighed weekly until 6 months of age. At 6 months, fasting levels of total cholesterol, HDL-C and triglycerides were measured and glucose and insulin tolerance tests were performed. Telemetry was used to determine blood pressure and heart rate in anesthetized mice. Echocardiograms were performed under light sedation (isoflurane 1%). Mice were sacrificed and tissues were resected for gene expression analysis by qPCR. Transient transfections were performed in 3T3-L1 cells to study the interactions of BMP-3b and the *C/ebpa* promoter. **RESULTS:** BMP3b KO mice gained more weight than WT mice. The plasma levels of total cholesterol, HDL and triglycerides were higher in BMP-3b KO mice than in WT mice. Insulin and glucose tolerant tests reveal insulin resistance and glucose intolerance in BMP-3b KO mice compared to WT mice. The basal heart rate was higher in BMP-3b KO mice than in WT mice, but no differences were observed in blood pressure. Echocardiography revealed a decrease in relative wall thickness

(RWT) and left ventricular ejection fraction (LVEF), and an increase in the left ventricle end diastolic volume (LVEDV) in BMP-3b KO mice, demonstrating eccentric remodeling. Expression of genes (*Ppar γ* and *C/ebp α*) involved in adipogenesis in white adipose tissue was higher in BMP-3b KO mice than in WT controls. Finally, transient transfection assays with 3T3-L1 cells showed that BMP-3b modulates *C/ebp α* promoter activity. **CONCLUSIONS:** The results of this study suggest that BMP-3b has an important role in the regulation of age-related weight gain, glucose metabolism and LV remodeling. Interventions that increase the level or function of BMP-3b may protect the heart from the adverse effects of cardiovascular risk factors and pathological cardiac remodeling.

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Comprehensive Arteriovenous Metabolomics in the Human Heart

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Introduction: Despite broad interest in the role of metabolism in the pathogenesis of heart failure, a definitive account of fuel use by the beating human heart *in situ* is lacking. We therefore determined the uptake and release of over 270 metabolites by simultaneous sampling of arterial, coronary sinus, and femoral venous blood in 100 patients. **Methods:** We enrolled 100 patients (60% male, 12% diabetic, age = 63.6 +/- 13.5 years, BMI = 29.75 +/- 6.1, EF = 57.14 +/- 6.48) undergoing ablation of atrial fibrillation. We used liquid chromatography-mass spectrometry to quantify over 270 metabolites in plasma from coronary sinus, femoral vein, and arterial blood. We also measured O₂ and CO₂ concentration, as well as atrial pressure. **Results:** We detected uptake or release of 77 metabolites by the heart and 136 metabolites across the leg (p < 0.05 after Benjamini-Hochberg correction). We determined the cardiac extraction of all major fuel sources, including 29 fatty acids, 11 amino acids, lactate, acetoacetate, and 3-hydroxybutyrate. Several patterns emerge: first, while the leg extracts glucose (FV/A = 0.95, q = 4.7x10⁻¹⁴) and releases lactate (FV/A = 1.11, q = 2.87x10⁻¹⁴), the heart extracts lactate (CS/A = 0.95, q = 3.77x10⁻⁹) and neither extracts nor secretes glucose. Second, while the leg releases nearly all fatty acid and amino acid species, the heart consumes most fatty acids and certain non-essential amino acids. Third, extraction of unsaturated acylcarnitines correlates with left atrial pressure (p < 0.01). Finally, we find a significant positive correlation between myocardial O₂ consumption and extraction of ketones (p < 0.01), but not other major cardiac fuels. **Conclusion:** We present the most comprehensive study to date of metabolite use and secretion by the human heart. We find the heart to be an avid consumer of fatty acids and ketone bodies, but less so of glucose and essential amino acids. Consumption of ketones increases with O₂ consumption, suggesting that ketones may be a preferential fuel source under high work loads. Extraction of certain acylcarnitines increases with left atrial pressure, indicating metabolic reprogramming under high filling pressures. In summary, we provide the first comprehensive model of fuel use by the beating human heart *in situ*.

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Cardiac TIGAR Reduces Myocardial Energetics and Cardiac Function in the Pressure Overload Heart Failure Model

Yoshifumi Okawa, Atsushi Hoshino, Tomoya Kitani, Ryoetsu Yamanaka, Daichi Hato, Sakiko Honda, Yohei Fushimura, Ryota Urata, Yoshito Minami, Shyo Hashimoto, Ayumi Matsuki, Shunta Taminishi, Toshiyuki Nishiji, Tomohiro Hino, Nobuichiro Yagi, Eri Iwai-Kanai, Satoaki Matoba, Kyoto Prefectural Univ of Med, Kyoto, Japan

Back ground: Although metabolic alterations were observed in heart failure (HF), only recently have the mechanisms underlying these changes been identified. Tumor suppressor p53 responds to metabolic changes through several mechanisms. One of the p53 targets, TIGAR (TP53-induced glycolysis and apoptosis regulator) reduces glycolysis and suppresses autophagy, which augments ischemic damage, however its role on HF is unclear. **Method and Results:** In order

to investigate TIGAR's function in HF, we compared myocardial metabolic and functional outcomes between TIGAR deficient (TIGAR^{-/-}) mice and wild-type (TIGAR^{+/+}) mice subjected to chronic thoracic transverse aortic constriction (TAC), a pressure-overload HF model. In wild-type mice hearts, p53 and TIGAR increased markedly during HF development. Eight weeks after TAC surgery, the left ventricular (LV) dysfunction, fibrosis, oxidative damage, and myocyte apoptosis were significantly advanced in wild-type than in TIGAR^{-/-} mouse hearts. Further, myocardial high-energy phosphates in wild-type hearts were significantly decreased compared to those of TIGAR^{-/-} mouse heart. Glucose oxidation and glycolysis rates were also reduced in isolated perfused wild-type hearts following TAC than those in TIGAR^{-/-} hearts, which suggest that the upregulation of TIGAR in HF causes impaired myocardial energetics and function. The effects of TIGAR knockout on LV function were also replicated in tamoxifen (TAM)-inducible cardiac-specific TIGAR knockout mice (TIGAR^{flox/flox}/Tg(Myh6-cre/Esr1) mice). **Conclusion:** The ablation of TIGAR during pressure-overload HF preserves myocardial function and energetics. Thus, cardiac TIGAR targeted therapy to increase glucose metabolism will be a novel strategy for HF.

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Response of the Ischemic Myocardium to Adrenergic Stimulation as Detected by Hyperpolarized [1-¹³C]pyruvate

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Metabolic imaging by nuclear methods is widely used to access ischemic myocardium but provide limited information about individual enzyme-catalyzed reactions. ¹³C hyperpolarization methods are sensitive to flux in specific key enzymes such as lactate dehydrogenase and pyruvate dehydrogenase. We tested the hypothesis that LDH flux is increased while PDH flux is abolished in low-flow ischemia. Metabolism of HP [1-¹³C]pyruvate was studied in four groups: (1) normal perfusion pressure (NPP), 100 cm H₂O; (2) NPP with epinephrine; (3) low-perfusion pressure (LPP), 25 cm H₂O; (4) LPP with epinephrine. Hearts excised from male Sprague-Dawley rats were perfused (37°C) at an initial pressure of 100 cm H₂O using standard Langendorff method with Krebs-Henseleit buffer containing 0.75% bovine serum albumin, 0.4 mM non-labeled free fatty acid, 5.5 mM [U-¹³C]glucose, 1 mM [3-¹³C]pyruvate, 0.1 mM [3-¹³C]lactate. Perfusion pressure was reduced to 25 cm H₂O after 30 min of perfusion for ischemia group and epinephrine added at 50th min of perfusion. HP [1-¹³C]pyruvate was injected to heart and subsequently, ¹³C NMR spectra were acquired. The freeze clamped heart tissues were extracted with perchloric acid and analyzed by high-resolution ¹H and ¹³C NMR (14.1 T). The ³¹P NMR spectroscopy confirmed significant myocardial ischemia under LPP conditions, demonstrated by a marked increase in the [Pi]/[ATP] ratio. Surprisingly, increased LDH flux was observed in the ischemic hearts (+18.4%) while the metabolism of HP [1-¹³C]pyruvate by PDH remained unchanged as indicated by bicarbonate production. To investigate the oxidative capacity of mitochondria, epinephrine was used to stimulate hearts. As anticipated, epinephrine increased heart rate (+23%) and coronary flow (+11%) under NPP. Epinephrine increased heart rate (-60%) but did not alter coronary flow under LPP. We found that epinephrine had no effect on the rate of bicarbonate production under LPP. The study demonstrated that the conversion of pyruvate to lactate increased in ischemic hearts but the degree of pyruvate oxidation was not affected by the low-flow ischemia. Adrenergic stimulation did not increase PDH flux in these ischemic hearts. Translation to human is likely to provide new data from low-flow myocardium.

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Branched Chain Ketoacid Dehydrogenase Kinase Inhibition Alters Substrate Utilization and Gene Expression in Myocytes

Parker Siddall, Eliza Bollinger, Matthew Peloquin, Bina Albuquerque, Zhongyuan Sun, Gang Xing, Michelle Clasquin, Darren Dumlao, Mary Piotrowski, Jake Delmore, Evanthia Pashos, Angela Hadjipanayis, Teresa Cunio, Chang Zou, Bei B. Zhang, Russell A. Miller, Rachel J. Roth Flach, Pfizer, Cambridge, MA

Circulating increases in branched chain amino acid (BCAA) levels have long been associated with type II diabetes and metabolic syndrome. Emerging data also suggest that impaired BCAA catabolism may play a role in heart failure progression. BCAA are catabolized via the branched chain ketoacid (BCKA) dehydrogenase enzyme complex (BCKDH). BCKD kinase (BCKDK) is a negative regulator of BCAA catabolism through its inhibitory phosphorylation of the BCKDHE1a subunit, and the phosphatase PPM1k dephosphorylates this same site to activate BCAA catabolism. Using an inhibitor of BCKDK (BT2), BCAA catabolism is increased in vivo. Here, we utilized metabolomics to evaluate the contribution of BCAA catabolism to substrate preference in heart and skeletal muscle. Surprisingly, BCKDK inhibition with BT2 had no effect on incorporation of glucose into TCA cycle intermediates in heart or skeletal muscle. Because others have recently shown that the primary site of BCAA catabolism is skeletal muscle, we knocked down BCKDK and PPM1k in human skeletal myocytes to further investigate how BCKDK loss or inhibition affects substrate utilization. Similar to our in vivo observations, knockdown of BCKDK and PPM1k had no effect on glucose and pyruvate utilization in a mitochondrial function assay. However, an increase in maximal respiration was observed after BCKDK knockdown when fatty acids were used. To evaluate the mechanisms underlying this increase we then performed RNAseq in these cells after BCKDK and PPM1K knockdown and observed changes in a number of genes that may explain these alterations in substrate utilization. Finally, we performed C13 BCAA metabolomics in human skeletal myocytes after BT2 treatment or knockdown of BCKDK and PPM1k. Using BT2, we observed a dose-responsive reduction in BCKA production from C13 BCAA by the muscle cells as expected; however, though BCKA production was increased after PPM1k was knocked down, we surprisingly did not observe a decrease in BCKA production after BCKDK knockdown. Collectively these data suggest that BCKDK inhibition may improve metabolism and cardiac function by altering substrate preference in skeletal myocytes.

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Fgf21 as a Biomarker for Metabolic Stress in Heart Failure

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FGF21, an important metabolic regulator, has recently been suggested as a biomarker for heart failure (HF). FGF21 is involved in the integrated mitochondrial stress response, and has been shown to be upregulated with mitochondrial DNA damage, which occurs more frequently in dilated cardiomyopathy. In this study, we investigated whether FGF21 can be used as a biomarker for metabolic stress in HF. We collected blood and cardiac tissue samples from ischemic and non-ischemic HF patients who have undergone VAD transplantation. We also collected blood and tissue from mice with HF due to 1) combination of transverse aortic constriction and coronary artery ligation (TAC+Lig) or 2) cardiac-specific knockout of the mitochondrial transcription factor A (Tfam). Serum FGF21 levels were measured using Enzyme-linked immunosorbent assay (ELISA). Messenger RNA was extracted from the tissue and FGF21 gene expression was measured using real-time quantitative PCR (qPCR). Immunohistochemical staining was performed on tissue sections (either paraffin embedded or frozen) to observe FGF21 levels. Serum FGF21 was elevated in human HF patients compared to healthy controls, as well as in both mouse models of HF. In human patients, cardiac FGF21 gene expression was upregulated 2.2-fold compared to donors. In the TAC+Lig mouse model we observed a 3.37-fold increase, while the Tfam knockout model which has severe mitochondrial damage exhibited a 218-fold increase in cardiac FGF21 gene expression. Further qPCR assays revealed changes in FGF21 gene expression in the liver and white fat of TFAM-KO, indicating metabolic stress on other organs resulting from HF. In conclusion, serum FGF21 is elevated in multiple models of HF, and appears to have both cardiac and extra cardiac sources. Future work will investigate 1) whether there is a correlation between FGF21 levels and mitochondrial damage, and 2) the signaling pathway resulting in metabolic stress to other organs in HF.

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Effects of Burn-induced Cardiac Released Factors on Cardiomyocyte and Profiles of Differentially Expressed Cardiac Mitochondrial Proteins

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Introduction: Previous studies have indicated that cardiac dysfunction worsens in the first 24 hours post burn (pb) and improved over the next 48 hpb. We have found that cardiac mitochondria were damaged at 24 hpb and 24 hpb serum-treatment caused cardiomyocyte mitochondrial dysfunction.

However, the differentially expression of mitochondrial proteins in the early post burn period have still not been explored.

Aims: To test if 3 hpb serum-treatment will interfere cardiomyocyte growth/proliferation similar to 24 hpb serum-treatment *in vitro*; To identify the cardiac mitochondrial protein expression profile, relating to burn-induced cardiac mit dysfunction.

Methods: Male Sprague Dawley rats were randomized into sham control, 3 hpb and 24 hpb groups, with six rats in each group. 60% TBSA burn was induced by immersing in boiling water. In this study, heart function cardiac mit function, and cardiac mitochondrial protein profiles were measured. Burned rat serum were used to test our hypothesis *in vitro*.

Results: Cardiac functions was significantly worse in 3 hpb (Fig 1). Cardiac mitochondrial dysfunction was observed via mit OXPHOS or mit complexes activities after burn. However, no change of CII-driving OXPHOS in 24 hpb and no change of CIII activity in 3 hpb were seen, suggesting that different

mechanisms may be involved (Fig.2). Burn-serum treatment increased oxidative stress, apoptosis, and nitrite/nitrate, while decreasing antioxidants and cell proliferation in cardiomyocytes (Fig. 3). Nano LC MS/MS identified 13569 peptides and 1275 proteins from burn-induced heart lysates (Fig.4.A), which were associated with cardiomyopathy (Fig.4.B). Further analysis showed that burn injury induced the alterations of 25 up regulated signaling pathway and 7 down regulated signaling pathway (Fig.5.A). 147 mitochondrial proteins were identified to be differentially expressed after burn. Among the 147 proteins, 32 proteins were continually down-regulated, 37 continually up-regulated. In addition, 16 were first up regulated then down-regulated while 38 had the opposite expression perform (Fig.5.B).

Conclusions: Burn-induced heart dysfunction is associated with cardiac mitochondrial damage by altering mitochondrial protein expression.

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Functional Benefits of Muscle PGC-1alpha in Aged Animals^{1,2,3}

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Metabolic homeostasis requires a complex network of transcriptional programs. PGC-1 (peroxisome-proliferator-activated receptor-γ coactivator-1) alpha is a potent transcriptional coactivator that coordinates the activation of a large number of nuclear-encoded genes, which, in turn, regulate numerous metabolic processes. Exercise strongly induces muscle PGC-1alpha in both humans and rodents. Over-expression of PGC-1alpha in skeletal muscle activates mitochondrial oxidative metabolism, fast-to-slow fiber-type switching, and neovascularization, leading to markedly increased endurance. In light of these findings, PGC-1alpha has been proposed to protect from age-associated sarcopenia, bone loss, and whole-body metabolic dysfunction, although these findings have been controversial. We therefore comprehensively evaluated muscle and whole-body function and metabolism in 24 month-old transgenic mice that over-express PGC-1alpha in skeletal muscle. We find that the powerful effects of PGC-1alpha on promoting muscle oxidative capacity and protection from muscle fatigability persist in aged animals, although at the expense of muscle strength. However, skeletal muscle PGC-1alpha does not prevent bone loss and in fact accentuates it, nor does it have long-term benefit on whole-body metabolic composition or insulin sensitivity. Interestingly, we see protection from sarcopenia in male animals with over-expression of PGC-1alpha in skeletal muscle but not in female animals. In summary, muscle-specific expression of PGC-1alpha into old age has

beneficial effects on muscle fatigability and may protect from sarcopenia in males, but does not improve whole-body metabolism and appears to worsen age-related trabecular bone loss.

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Mechanism of Mitochondrial Calcium Uniporter Regulation

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Calcium uptake by mitochondria plays an important role in sequestering cytosolic calcium, regulating ATP production and maintaining the redox homeostasis. Mitochondrial calcium uniporter (MCU), a macromolecular channel complex that resides in the inner mitochondrial membrane, is the primary electrogenic pathway for calcium influx into the matrix. Because calcium uptake by mitochondria is dictated by the kinetic equilibrium between the influx and efflux machinery, the patch clamp is the best method to measure electrogenic MCU activity in isolation from the efflux mechanisms. Here we report the development of a system for heterologous expression of MCU and its associated subunits, essential MCU regulator (EMRE), MCUB and MICU1–3. Using this system, we investigated the biophysical properties of the MCU pore and its regulation. The system is based on a recombinant cell line (Drp1^{-/-} mouse embryonic fibroblasts) in which knockouts for all subunits of the MCU complex have been generated. This system allowed both optical and direct electrophysiological studies of the structure-function relationships in the MCU complex with a relative ease in comparison to other cell lines. We confirmed the essential role of pore-forming MCU and EMRE subunits in mediating the MCU current. Importantly, fast-step solution exchange and single-channel recordings allowed us to gain novel insights into the biophysical properties of MCU e.g. inward rectification, kinetics of activation and selectivity to monovalent vs divalent ions.

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Loss of Function Variant in CYB5R3 Associates with Exacerbated Cardiac Hypertrophy in Mice

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African Americans (AA) are 20 times more likely to be diagnosed with heart failure (HF) before the age of 50 and 2 times more likely to die from heart failure. Previous reports have shown AA with HF have diminished nitric oxide (NO) signaling, a pathway critical for cardiac contractility. NO signals, in part, via binding reduced heme iron (Fe²⁺) in soluble guanylyl cyclase (sGC) leading to cyclic guanosine monophosphate (cGMP) generation. Recently, our lab reported that cytochrome b5 reductase 3 (Cyb5R3) reduces oxidized sGC heme-iron from the oxidized (Fe³⁺) to the reduced (Fe²⁺) state, thereby sensitizing sGC to NO. However, the role of Cyb5R3 in the setting of HF remains elusive. It is known that a high frequency Cyb5R3 T117S single nucleotide polymorphism (23% minor allele frequency) exists and is enriched in individuals with African ancestry. To determine the impact of T117S in HF outcomes, we completed a retrospective study from AHEFT and GRACE trials. Our data show that Cyb5R3 T117S carriers have significantly accelerated time to death/transplant. Additionally, biobank HF samples from AA samples show an enrichment of Cyb5R3 T117S carriers from 0.23 to 0.4. To assess the impact of Cyb5R3 T117S on sGC/cGMP signaling in the heart, ventricular cGMP levels in AA with HF were examined. Pooled Cyb5R3 T117S carriers have significantly decreased cGMP relative to non-carriers. Next, we determined if this variant impacts sGC heme redox state. Using purified protein activity assays, we found that Cyb5R3 T117S results in a 60% loss-of-function and an inability to reduce oxidized sGC. Lastly, to test the *in vivo* impact of the Cyb5R3 T117S variant in heart failure, we generated a novel Cyb5R3 T117S mouse. Transverse aortic constriction (TAC) studies in Cyb5R3 T117S mice show significantly accelerates cardiac hypertrophy relative to wild-type TAC controls. Taken together, these data suggest Cyb5R3 T117S may be a disease modifying variant that augments hypertrophic signaling through an sGC-dependent mechanism.

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Phosphodiesterase 2 in Cardiac Arrhythmias and Heart Failure

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Heart failure (HF) patients often suffer from lethal ventricular arrhythmias leading to sudden cardiac arrests. Phosphodiesterase 2 (PDE2), a cGMP activated enzyme hydrolyzing cAMP, is upregulated in HF. Cardiac-specific PDE2 overexpression revealed strong anti-arrhythmic and heart rate lowering effects of PDE2. Here, we validated the cardioprotective role of PDE2 upon modulating cardiac PDE2 activity, first, via assessing cardiac function of cardiac-specific PDE2-KO mice and second, via potentiating PDE2 mediated cGMP/cAMP crosstalk using natriuretic peptide type C (CNP), which are upstream of the cGMP/PDE2 axis. Cardiac function and arrhythmia susceptibility of PDE2-KO were assessed via echocardiography and ECG telemetry under basal conditions and after myocardial infarction (MI). Patch clamp technique was used to investigate CNP effects on action potentials and ion currents in WT isolated cardiomyocytes and in-vivo via ECG-telemetry. Already under basal conditions, PDE2-KO displayed severe irregularities in RR-intervals. 14 days post MI, PDE2-KO showed lower cardiac function and delayed increase in heart rate after arrhythmia provocation (double isoprenaline (ISO) injection (2 mg/kg i.p.) compared to WT controls, indicating diminished cardiac responsiveness. On the other hand, CNP treatment in WT displayed a PDE2-dependent antiarrhythmogenic effect. CNP (1 μ M) significantly reduced the total number of delayed afterdepolarizations and spontaneous action potentials upon acute ISO (10 nM) stimulation. Furthermore, CNP significantly reduced the ISO-mediated increase of the L-type Ca^{2+} and late Na^{+} currents as well as SR Ca^{2+} waves. These effects were reversed upon specific PDE2 inhibition with BAY 60-7550. Finally, in vivo, CNP (33 μ g/g i.p.) significantly reduced the number of ventricular extrasystoles after arrhythmia provocation. This effect was significantly attenuated after PDE2 inhibition with BAY 60-7550. Here we provide evidence for acute antiarrhythmic effects of CNP upon downstream stimulation of cGMP-stimulated PDE2 and a critical role of PDE2 on heart rate regulation. Therefore, pharmacologically enhancing myocardial PDE2 activity may represent a novel cardioprotective strategy in cardiac arrhythmia and HF.

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Matricryptin p1158/59 Improves Cardiac Function Post-myocardial Infarction by Reducing Adverse Remodeling

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Matricryptins are biologically active peptides, generated from extracellular matrix proteolysis, able to regulate cell function and survival. We recently identified a collagen-derived matricryptin (p1158/59) that gradually forms post-myocardial infarction (MI). In humans, p1158/59 plasma levels negatively correlate with left ventricle (LV) filling pressure, indicating that therapeutic elevation of this matricryptin could benefit functional remodeling. Previously, we showed p1158/59 binds to cardiac fibroblasts in vitro to stimulate migration and mice treated with p1158/59 for 7 days post-MI display reduced fibrosis, suggestive of reduced LV remodeling. Our goals were to establish the therapeutic effects of p1158/59 in long term cardiac remodeling and function using a rodent MI model. We used a murine permanent occlusion MI model (male and female C57Bl/6 mice, 4-6 months old, n=6/group) to assess the effects of p1158/59 treatment. Three hours after MI, mice received matricryptin (950 μ g/day) or saline solution via an osmotic mini-pump for 28 days (D28). Parameters of cardiac remodeling and function were analyzed by serial echocardiography (D0, D14, D28) and LV remodeling gene arrays. As measured by ejection fraction (EF), matricryptin p1158/59 significantly blunted the reduction in cardiac function observed post-MI (saline 12% EF, p1158/59 31% EF, p=0.004). Additionally, p1158/59 reduced LV dilation displaying decreased end-systolic and

end-diastolic volumes compared to controls (ESV $p=0.014$, EDV $p=0.037$). Similarly, internal diameter (ID) in diastole and systole were also reduced versus saline (IDd $p=0.010$, IDs $p=0.014$). Finally, we observed less thinning of the posterior wall (PW) in p1158/59-treated mice compared to vehicle controls (LVPWd $p=0.018$, LVPWs $p=0.047$). These results indicate that p1158/59 attenuated cardiac dysfunction and remodeling post-MI by preserving cardiac compliance and structure. Our data suggest that matricryptin p11/58/59 treatment reduces adverse remodeling post-MI resulting in improved cardiac function.

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Brg1 Protects Cardiomyocytes Against Oxidative Damage Through Activation of the Nrf2 Signaling Pathway in Acute Myocardial Infarction

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Background: Brahma-related gene 1 (Brg1), the core ATPase subunit of a large chromatin remodeling complex, plays critical role in the regulation of gene expression during cardiac growth, differentiation. In adults, Brg1 is turned off in cardiomyocytes and reactivated by cardiac stress. How Brg1 in myocardial infarction (MI) is poorly understood. The Keap1-Nrf2-ARE pathway plays an important role in the development of MI. However, by which Nrf2 activation is mediated in MI still remains to be determined.

Methods and results: In vivo, adult male C57BL/6 mice were subjected to ligation of the left anterior descending coronary artery for MI model. Our data demonstrated that in peri-infarct zone, the protein of Brg1 was significantly increased 7 days after MI compared with the sham group, accompanied by Nrf2 nuclear translocation, and upregulation of the expressions of NQO1, GSTP1, HO1. We further revealed that with adenoviral intramyocardial injection, the Brg1 overexpression reduced the percentage myocardial infarct, improved cardiac dysfunction, decreased the relative fluorescence intensity of ROS with fluorescent probe DHE in MI mice. Conversely, shRNA-mediated knockdown of Brg1 enlarged the percentage myocardial infarct, exacerbated cardiac dysfunction, increased the relative fluorescence intensity of ROS. More importantly, the Brg1 overexpression significantly induced Nrf2 translocation from cytoplasm to nuclear and upregulated NQO1, GSTP1 and HO1 expressions. Whereas Brg1 knockdown decreased the level of Nrf2 protein in the nucleus, suppressed NQO1, GSTP1 and HO1 expressions. In vitro, Oxygen-Glucose deprivation (OGD) on neonatal cardiomyocytes was established. The effects of Brg1 on OGD and Nrf2 activation were observed with gain- and loss-of-function approaches to regulate Brg1 expression. The results were consistent with *in vivo* study. Moreover, the down-regulation of Nrf2 by brusatol inhibited the increase of these antioxidative genes induced by Brg1 overexpression.

Conclusions: This study indicated that Brg1-induced cardiac protection was partially mediated through transcription activator Nrf2. The Brg1-Nrf2-ARE pathway may represent a novel therapeutic target for preventing cardiac dysfunction in patients with MI.

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Involvement of the Alpha 1A-Adrenergic Receptor in the Cardiac Adaptation to Physiological Stress

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The 'fight or flight' response, involving sympathetic nervous system activation and catecholamine release, is central to survival. Resulting activation of adrenergic receptors (ARs) drives physiological responses, such as positive inotropy, which to date has been attributed solely to β_1 -AR/Gs/adenylyl cyclase/protein kinase A pathway-activation. We previously demonstrated that overexpression of the α_{1A} -AR is cardioprotective in rodents in the setting of pathological cardiac stress; a finding in agreement with studies of α_{1A} -AR inactivation. However, it remains unknown if α_{1A} -ARs also protect the heart from physiological stress. Using a tamoxifen-inducible, cardiomyocyte-specific α_{1A} -AR knockdown mouse model, we evaluated cardiac responses to swim exercise. Treatment of adult (8-12 week) mice with 4-OH tamoxifen (Tam) resulted in a 7.4 fold decrease ($p<0.001$) in cardiac α_{1A} -AR mRNA levels, with no compensatory changes in expression of β_1 -, β_2 - or α_2 -AR

subtypes. A 90 minute, twice daily, four-week swim challenge induced eccentric cardiac hypertrophy, with Tam- and vehicle-treated mice displaying similar increases in heart weight/tibia length (mean increase: 1.4 and 1.6mg/mm, respectively; $p < 0.001$ vs sedentary mice) and left ventricular chamber radius (mean increase: 0.16 and 0.17mm, respectively; $p < 0.001$ vs sedentary mice) with no change in wall thickness. Consistent with physiological hypertrophy, *Igf-1* mRNA increased 1.7 and 0.7 fold ($p = 0.004$ vs sedentary mice), respectively, with no increase in *Nppb*, *Myh7* or *Acta1* mRNA. Cardiac function, assessed by micromanometry and echocardiography, showed increases in dP/dt min/EDV (mean: -117 vs -150 mmHg/s/ μ L; $p = 0.011$), end-diastolic volume (mean: 76.9 vs 68.0 μ L; $p = 0.038$) and end-systolic volume (mean: 29.4 vs 21.9 μ L; $p = 0.018$), as well as a decrease in ejection fraction (mean: 63 vs 68%; $p = 0.046$) in Tam- compared with vehicle-treated mice, respectively. Thus, despite a similar hypertrophic response, mice with cardiomyocyte-specific α_{1A} -AR inactivation showed an impaired contractile response to a sustained swim challenge. These findings are consistent with cardiomyocyte α_{1A} -ARs playing an important role in maintaining cardiac contractility in response to physiological stress.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Cardiovascular Risks of Oxidative DNA Damage

Kaitlin Lowran, Ann Fuelle, Phillip Popp, Colin G Wu, Oakland Univ, Rochester, MI

G-quadruplexes (or G4s) are nucleic-acid structures formed by guanine-rich sequences. G4s must be timely unfolded in cells; otherwise, they can interfere with DNA replication, RNA transcription, and other essential processes. Guanine bases are susceptible to forming 8-oxoguanine (8oxoG) as a result of oxidative damage. Although 8oxoG-modified DNA sequences can still fold into stable G4s, it is not known how 8oxoG4s are removed in human cells. We have shown previously that the FANCI DNA helicase targets G4s using an AKKQ amino acid motif and unfolds them with its motor activity. Here, we have examined the interactions of FANCI with various 8oxoG4s using biolayer interferometry and fluorescence spectroscopy. We show that a FANCI AKKQ peptide alone can recognize G4s independently. Moreover, this motif binds to 8oxoG4s with greater affinities. A detailed description of the mechanisms by which 8oxoG4s are repaired is essential for understanding how human hearts respond to oxidative stress. To test the importance of FANCI AKKQ-G4 interactions in cells, we measured the total extent of oxidative DNA damage in human cardiomyocytes by single-cell electrophoresis. Cells that overexpress FANCI can readily overcome the chemical stress induced by hydrogen peroxide treatment and the G4-stabilizing compound telomestatin. On the contrary, cells that produce a FANCI ADDQ mutant, which cannot interact with G4s, resulted in an accumulation of 8oxoG4s. Based on this evidence, FANCI plays an important role to alleviate the damage caused by oxidative stress. In future experiments, we plan to further examine the cardiovascular risks of DNA damage caused by FANCI malfunctions.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Hyperglycemia Acutely Increases Cytosolic Reactive Oxygen Species (ROS) via O-linked GlcNAcylation Activation of CaMKII in Mouse Ventricular Myocytes

Shan Lu, Zhandi Liao, Julie Bossuyt, Donald Bers, Univ of California, Davis, Davis, CA

Diabetes mellitus (DM) is a complex, multisystem disease, affecting large populations worldwide. Chronic CaMKII activation in autonomous states can occur in DM and contribute to arrhythmogenesis. Diabetic hyperglycemia has been shown to activate CaMKII by (1) O-linked attachment of N-acetylglucosamine (O-GlcNAc) at S280 leading to arrhythmia and (2) a ROS-CaMKII pathway, and these can increase post-infarction mortality. However, the mechanism by which high extracellular [glucose] (Hi-Glu) promotes ROS generation that may synergize with CaMKII activation remains unknown. Here we tested the mechanism by which extracellular Hi-Glu influences ROS production in adult ventricular myocytes, using H₂DCFDA to measure ROS. Hi-Glu (30 mM) significantly increased the rate of ROS generation, which was prevented in myocytes pretreated with CaMKII inhibitor KN-93 or from both global CaMKII δ -KO and cardiac specific CaMKII δ -KO. CaMKII-KO or inhibition prevented Hi-Glu induced sarcoplasmic reticulum (SR) Ca²⁺ release events (Ca²⁺ sparks and waves). Thus,

CaMKII activation is required for Hi-Glu induced ROS generation in cardiomyocytes.

Next, we tested the involvement of O-GlcNAc-CaMKII pathway. Inhibition of de-GlcNAcylation by 100nM Thiamet G (Thm-G) mimicked the effect of Hi-Glu, enhancing the rate of ROS. Also, inhibition of GlcNAcylation (OSMI-1) blocked the ROS induction triggered by either Hi-Glu or Thm-G. Moreover, in new CRSPR-based knock-in mouse in which the functional GlcNAc site on CaMKII δ was ablated (S280A) neither Hi-Glu nor Thm-G induced myocyte ROS generation. So CaMKII δ -S280 is required for the Hi-Glu-induced (and GlcNAc-dependent) ROS production. To identify the ROS source(s), we used different inhibitors: NOX2 inhibitor (Gp91-ds-tat peptide), NOX4 inhibitors (GKT137831), Mito Tempo and NOS pathway inhibitors (L-NAME, L-NIO and L-NPA). Only NOX2 inhibition prevented Hi-Glu/Thm-G induced ROS generation. In summary, our data suggests that diabetic hyperglycemia acutely induces ROS production via an O-GlcNAc-CaMKII dependent pathway and the ROS induced by hyperglycemia is mainly from cytosolic NOX2 complex.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Obg-like AtPase 1 (OLA1) Regulates Cardiomyocyte Hypertrophic Response via Gsk3 β Signaling

Hien T Luong, Gayathri Narasimhan, John Henderson, Phuong Quach, Divya Annamalai, Univ of Alabama at Birmingham, BIRMINGHAM, AL; Prasanna Krishnamurthy, Univ of Alabama at Birmingham, Birmingham, AL

Background: OLA1 that possesses both GTP and ATP hydrolyzing activities has been reported as a translational regulator of cell growth and survival. However, its role in cardiac hypertrophy, a process involving enhanced protein synthesis and increased cell size has not been studied. Hence, we hypothesized that OLA1 expression is regulated by oxidative stress and inflammatory responses in angiotensin-II induced cardiac hypertrophy and its aberrant expression mediates altered GSK3 β - β -Catenin signaling.

Methods: Protein expression of OLA1, GSK3 β /P-GSK3 β , β -Catenin/P- β -Catenin in total protein extracts of human cardiomyocytes with and without angiotensin II (500nM) treatment were studied. Knockdown of OLA1 expression in cardiomyocytes was achieved by siRNA transfection and total protein extracts were isolated. In addition, phosphokinase array in OLA1 knockdown cells with and without angiotensin II was performed. H₂O₂ and LPS treatment in cardiomyocytes for 24h was performed in culture and their total protein extracts were isolated to study the expression of OLA1, GSK3 β /P-GSK3 β and β -Catenin/P- β -Catenin.

Results: A significant increase in OLA1 expression from 1 to 3.86 \pm 0.72 in angiotensin II treated cells was observed. GSK3 β phosphorylation was also increased to 1.27 \pm .10 in angiotensin II treated cells. While, β -Catenin phosphorylation was reduced, total β -Catenin level was significantly increased to 2.26 \pm .34 in angiotensin II treated cells. OLA1 knockdown cells showed a significant reduction (0.12 \pm 0.0006) in GSK3 β phosphorylation both with and without angiotensin II treatment. Also, phosphorylation of β -Catenin (1.28 \pm .16) was induced in OLA1 knockdown cells.

OLA1 expression was up-regulated in both H₂O₂ and LPS treated cardiomyocytes after 24h. GSK3 β phosphorylation and total β -Catenin levels were increased in these cells similar to angiotensin II treated cells. We also found cell proliferation and survival-related phosphoproteins were up-regulated in OLA1 knockdown cells with and without angiotensin II treatment.

Conclusion: OLA1 was up-regulated during inflammation and reactive oxygen species production which then lead to GSK3 β phosphorylation and β -Catenin accumulation in angiotensin II treated cardiomyocytes.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Single Cell Analysis of Monocytes and Macrophages in the Infarcted Heart

Richard P Ng, Dave Calcagno, Avinash Toomu, Zhenxing Fu, Univ of California, San Diego, San Diego, CA; Aaron Aguirre, Ralph Weissleder, Massachusetts General Hosp, Boston, MA; Kevin King, Univ of California, San Diego, San Diego, CA

Introduction: Ischemic Heart Disease (IHD), the leading cause of death in the world, is often initiated by myocardial infarction (MI). In mice, death typically occurs on day 4 after acute MI is induced by coronary artery ligation. At this time the interferon regulatory factor 3 (IRF3)/type I interferon (IFN) pathway is activated almost exclusively in mononuclear cells in the

infarct. We have shown that the genetic or pharmacologic disruption of critical components of this innate immune pathway reduces inflammation, limits ventricular dilation and contractile dysfunction, and improves survival.

Hypothesis: Treatment with the IFNAR neutralizing antibody will bias the differentiation of cardiac macrophages and in doing so establish a cardioprotective environment in the infarct.

Methods: We conducted single cell RNA-sequencing analysis of mononuclear cells isolated from infarct tissue collected on day 4 after MI from mice that had been treated with an IFNAR neutralizing antibody (Ab) as well as untreated mice.

Results: Analyses demonstrate the presence of 3 core cell populations in the untreated sample: monocytes expressing *Plac8* and *Ms4a4c*, phagocytic macrophages expressing *Mrc1* and *Ccl8*, and reparative macrophages expressing *Arg1* and *Fn1*. In addition, the dramatic co-expression of related genes in response to environmental stimuli, such as secreted type I IFNs or reactive oxygen species (ROS), resulted in the formation of two more clusters of cells. One cluster was marked by the expression of interferon stimulated genes. The other was marked by the expression of an established anti-oxidant transcriptional program. Anti-interferon therapy with IFNAR Ab inhibited both the interferon stimulated genes and the anti-oxidative transcriptional program.

Conclusion: We have defined the differentiation patterns of monocytes and macrophages within the infarcted heart on day 4 after MI. Treatment with the IFNAR Ab after MI alters the composition of macrophage subsets.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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DJ-1 Confers Protection Against Ischemic Injury by Preserving the Activity of Thioredoxin

Yvanna Pantner, Emory Univ, Atlanta, GA; Yuuki Shimizu, Nagoya Univ Graduate Sch of Med, Nagoya, Japan; Rohini Polavarapu, Lian Li, Lih-Shen Chin, John Calvert, Emory Univ, Atlanta, GA

Background: DJ-1 is a cytoprotective protein expressed in the heart. It can act as an antioxidant in oxidative stress conditions where it is cleaved and converted into an active protease. DJ-1 and Thioredoxin (Trx1), another antioxidant, are known to act on related targets, both playing a role in opposing the apoptosis signaling kinase 1 (ASK1) signaling pathway. We recently found that the cleaved form of DJ-1 attenuates ischemia-reperfusion (I/R)-induced heart failure by reducing glycation stress. Thus, the purpose of this study was to examine the role of DJ-1's antiglycation activity on Trx1 and how this effects the ASK1 signaling pathway after I/R injury. **Methods and Results:** Initial studies found that DJ-1 deficient mice (DJ-1 KO) showed decreased levels of Trx1 activity after I/R injury compared to wild-type control mice. To explore a possible explanation for this decrease, we assessed the glycation of Trx1. Analysis revealed higher levels of carboxymethyl-lysine (CML) glycation of Trx1 in hearts of DJ-1 KO mice after I/R injury. This was accompanied by an increase in ASK1 phosphorylation and activity, as well as an increase in the phosphorylation of JNK, a downstream target of ASK1. To further determine if DJ-1 directly influences this signaling cascade, we employed an adenoviral approach (AAV9-CMV-DJΔc) to over-express the cleaved form of DJ-1. Compared to mice treated with a control virus, the overexpression of cleaved DJ-1 decreased the glycation of Trx1. Further, Trx1 activity was increased and ASK1 signaling was attenuated. **Conclusion:** These data suggest that the antiglycation activity of DJ-1 plays a role in preserving Trx1 activity after I/R injury. By expanding our perspective of how DJ-1 interacts with Trx1, these data bring us closer to uncovering a therapy that targets the ASK1 signaling pathway in the heart.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Role of Splice Variant Clic5B in Cardiac Mitochondrial Localization and Function

Devasena Ponnalagu, The Ohio State Univ, Columbus, OH; Ahmed Tafsirul Hussain, Drexel Univ Coll of Med, Philadelphia, PA; Erhe Gao, Lewis Katz Sch of Med, Philadelphia, PA; Harpreet Singh, The Ohio State Univ, Columbus, OH

Blocking chloride channels (Cl⁻) with indanyloxyacetic acid (IAA-94) abrogated the cardio-protective effects of ischemic preconditioning (IPC) and increased myocardial infarction suggesting the possible contribution of IAA-94 sensitive Cl⁻ channels in IPC mediated cardio-protection. Unlike cation channels, insights into the role of Cl⁻ channels in

cardioprotection is limited due to lack of information on their molecular identity. Chloride intracellular channel (CLIC) proteins are one of the known targets of IAA-94. They exist in both soluble and integral membrane form. Amongst the six known mammalian paralogs, CLIC1, CLIC4 and CLIC5 are most abundant in heart. Previously, we showed differential localization of CLIC4 and CLIC5 to outer and inner mitochondrial membrane respectively where as CLIC1 is present in the endoplasmic reticulum. Both CLIC4 and CLIC5 are important for mitochondrial functional integrity as well as cardioprotection from ischemia-reperfusion injury. Interestingly, none of the CLICs possesses conventional mitochondrial targeting sequence, which intrigued us to understand the mechanism of their localization to mitochondria. In this study we demonstrate that the mitochondrial localization of CLIC5 is related with the presence of splice variant CLIC5B (~50 kDa) in heart but not in lung lysates. Two different bands of CLIC5 at ~30 kDa (CLIC5A) and ~50 kDa, were observed in the cardiac mitochondrial lysates. Lung lysates showed the presence of only CLIC5A. In addition, mass spectrometric analysis revealed a peptide coverage of ~30% corresponding to CLIC5B at 50 kDa in heart but not in lung lysates further confirming its presence in heart. Moreover CLIC5 localized to mitochondria in p3 neonatal cardiomyocytes isolated from mouse but not in mouse lung epithelial (11 ± 2.1 %) cells which lacked CLIC5B indicating that the splice variant CLIC5B has a role in IMM localization. Interestingly, cardiac mitochondria from *clic5*^{-/-} mice exhibited increased ROS (p< 0.05, n=3) production whereas the *clic5*^{+/-} lung mitochondria did not show any change, suggesting the role of CLIC5B in regulating the mitochondrial function as well. Our study establishes splice variation as a mechanism for targeting ion channels to mitochondria.

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Poster Session 2 and Reception

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VA-ECMO Increases Urinary Levels of the Biomarker Kidney Injury Marker-1 (KIM-1) in a Preclinical Model of Acute Myocardial Infarction

Xiaoying Qiao, Lija Swain, Lara Reyelt, Cody Machen, Andrew Jarrah, Aditya Chennjorwala, Paige Crowley, Shiva Annamalai, Sina Foroutanjazi, Allen Razavi, Navin Kapur, Tufts Medical Ctr, Boston, MA

Background: Acute kidney injury (AKI) is associated with increased morbidity and mortality in patients with acute myocardial infarction (AMI). Use of trans-valvular pumps and veno-arterial extracorporeal membrane oxygenation (VA-ECMO) for patients with AMI is growing. Trans-valvular pumps transfer rotational kinetic energy to blood and generate flow from the left ventricle into the ascending aorta. VA-ECMO drains blood from the venous system and returns oxygenated blood into the descending aorta, thereby increasing systemic perfusion. The impact of these support strategies on renal blood flow and function remains poorly understood. We hypothesized that compared to a trans-valvular pump, VA-ECMO is associated with increased renal injury in AMI. **Methods and Results:** Adult male swine were subjected to left anterior descending artery occlusion for 90 minutes followed by either immediate reperfusion (IRI), trans-valvular pumping (Impella CP) or VA-ECMO starting 30 minutes before reperfusion, or sham-operated controls (n=4/group). Compared to IRI, urinary levels of the biomarker kidney injury molecular 1 (KIM-1) were increased by VA-ECMO, not Impella. Inflammatory factors IL6 and IL1beta were increased by VA-ECMO, not Impella in both plasma and cortex tissue by ELISA analysis. KIM-1 protein expression for precursor KIM-1 and the extracellular domain (soluble) of KIM-1, as well as STAT3, HIF1alpha were analyzed by Western blot. Compared to sham, IRI and VA-ECMO reduced levels of soluble KIM-1 and increased levels of the KIM-1 protein precursor, pSTAT3 and HIF1a in the renal cortex. Impella had no impact on these protein levels. **Conclusion:** This is first study to identify that VA-ECMO, not Impella, increases urinary levels of KIM-1, a highly sensitive biomarker of acute kidney injury. The shedding of KIM-1 extracellular domain from the renal cortex is associated with systemic inflammatory response. These findings may identify novel approaches to limit renal injury in AMI patients requiring mechanical circulatory support.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Beta-blockers Reverse Beta Adrenergic Receptors Desensitization Under Hypoxia

Yu Sun, Manveen Gupta, Kate Stenson, Sathyamangla Prasad, Cleveland clinic foundation, Cleveland, OH

Hypoxia to heart or brain is a primary cause of heart failure or stroke. Studies have shown hypoxia increases the beta-adrenergic receptors (β ARs) phosphorylation and dysfunction (Cheong et. al., 2016). These observations provide evidence that β ARs can directly be regulated by hypoxia but less is known about the underlying mechanisms. We postulated that hypoxia shifts the homeostasis between kinase and phosphatase driven mechanisms may underlie β AR dysfunction. β 2AR HEK 293 cells were exposed to hypoxia (2% O₂) and assessed the mechanisms underlying desensitization (G-protein coupled receptor kinases, GRKs) and resensitization (Protein phosphatase 2A, PP2A). Six hours of hypoxia treatment resulted in increase of β AR phosphorylation and GRK2 expression. Assessment of β AR phosphorylation in the plasma membrane and endosomal fractions surprisingly, showed marked increase in β 2AR phosphorylation in the endosomal fraction. Furthermore, we also observed that receptor associated PP2A activity was inhibited in the endosomes following hypoxia with minimal changes of activity at the plasma membranes. Similarly subjecting normal mice to 20 hours of hypoxia resulted in significant cardiac dysfunction (% FS: Normoxia 38.83% vs. Hypoxia 32.38%, P=0.0055; % EF: Normoxia 69.71% vs. Hypoxia 60.76%, P=0.0105) and was associated with significant increase in β 2AR phosphorylation associated with significant loss in β AR function as measured by G-protein coupling adenylyl cyclase activity. Given that β -blockers confer beneficial effects, we tested whether β -blocker (propranolol) would prevent β ARs phosphorylation under hypoxia or normoxia. Consistently, β -blocker treatment in normoxia results in increased β 2AR phosphorylation however, remarkably β -blocker treatment in hypoxia results in loss of β 2AR phosphorylation, reduction in GRK2 expression and increase in β AR-associated PP2A. These studies show that agonist-independent hypoxia-driven β 2AR dysfunction can be ameliorated by β -blockers and the underlying mechanisms for this expected findings will be discussed in the presentation. These findings have significant clinical implications as understanding these mechanisms could provide novel insights into the benefits provided by β -blockers.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Left Ventricular Unloading and Delayed Coronary Reperfusion Protects the Structural and Functional Integrity of Mitochondrial Complex 1 in a Preclinical Model of Acute Myocardial Infarction

Lija Swain, Xiaoying Qiao, Lara Reyelt, Shiva Annamalai, Paige Crowley, Courtney Boggins, Navin K Kapur, Tufts Medical Ctr, Boston, MA

Background: Mitochondrial Complex 1 (MC1) plays a key role in energy metabolism during myocardial ischemia-reperfusion injury (M-IRI). Disruption of MC1 promotes reactive oxygen species (ROS) generation and myocardial damage. We recently reported that first reducing left ventricular (LV) workload using a trans-valvular pump and delaying coronary reperfusion, known as Primary Unloading (PU), reduces infarct size in preclinical models of M-IRI. The Door to Unload Pilot Trial recently demonstrated the safety and feasibility of PU in patients with ST-segment elevation myocardial infarction (STEMI), however the cardioprotective mechanisms of PU remain poorly understood. We hypothesized that compared to immediate reperfusion (IR); PU preserves mitochondrial function in M-IRI. **Results:** M-IRI was induced by balloon occlusion of the left anterior descending artery for 120 minutes in adult swine followed by reperfusion for 180 minutes. After 90 minutes of occlusion, animals were assigned to 30 minutes of continued occlusion (IR, n=5) or 30 minutes of support with an Impella CP pump (PU, n=5) with persistent occlusion before reperfusion. Compared to IR, PU reduced LV stroke work by 38.4% (p=0.047) and infarct size by 41.3±5.6% (p=0.0052). Compared to IR, PU preserved gene and protein expression of specific MC1 subunits (NDUFA8 and NDUFS3). Using the Agilent Seahorse Platform, we identified that compared to IR, PU preserved function of MC1 and ATP production in mitochondria isolated from the infarct zone by 82.45±12.8% (p=0.0040, IR vs PU). Compared to IR, PU reduced levels of ROS and preserved mitochondrial membrane potential. Next, we studied the activation state of MC1 and observed that compared to IR alone, PU increased the ratio of activated A form versus de-activated D form from 2.79 ± 0.29 to 7.58 ± 0.80 (p=0.005) levels of MC1 within the infarct zone. **Conclusion:** We report for the first time that compared to IR, mechanically unloading the LV and delaying reperfusion protects the structural and functional integrity of mitochondrial complex 1. These findings provide new insight into the cardioprotective mechanisms of Primary Unloading and support the development of novel therapeutic approaches to improve clinical outcome for patients with STEMI.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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EphrinA1-Fc Attenuates Dysfunction and Fibrosis in Nonreperfused Myocardium at 4 weeks Post-MI in WT B6 but Not EphA2-R Mutant Mice

K'Shylah S Whitehurst, Heather K Estes, Robert C Chase, Uma M Sharma, **Jitka A Virag**, ECU, Greenville, NC

Each year, an estimated 805,000 US citizens have a new or recurrent myocardial infarction (MI), causing over 360,000 deaths. We have previously reported that a single intramyocardial administration of recombinant ephrinA1-Fc, a member of the ephrinA/EphA family of membrane-anchored receptor tyrosine kinases, significantly reduced ischemic injury and remodeling 4 days in nonreperfused WT B6 mice via reduced apoptosis and inflammation and increased autophagic flux. After acute ischemia/reperfusion (30 min I/24hr or 4 days R) in WT B6 mice, ephrinA1-Fc demonstrated anti-inflammatory effects coupled with complete functional preservation due to modulation of cardiomyocyte ultrastructure and metabolism. The goal of this study is to assess the capacity of a single intramyocardial administration of ephrinA1-Fc at the time of permanent coronary artery ligation to maintain the structural and functional salvage effects through the wound healing process to stable scar formation in both B6 and EphA2-R-M (EphA2 receptor null mutant) mice. At 4 weeks post-MI in WT B6 mice, ephrinA1-Fc improved ejection fraction by 11%, attenuated cardiomyocyte hypertrophy by 38%, and decreased interstitial fibrosis by 35%. In contrast, these parameters were not different or worsened in EphA2-R-M mice treated with ephrinA1-Fc, suggesting that this receptor plays an important role in ephrinA1-Fc-mediated mitigation of the deleterious ventricular remodeling caused by permanent coronary artery occlusion. To further investigate the effects of ephrinA1-Fc directly on fibroblast function, isolated primary mouse cardiac fibroblasts were cultured with ephrinA1-Fc both with and without pro-fibrotic TGF- β stimulation to determine the direct effects of ephrinA1-Fc on col I and III production, remodeling mediators MMP-2, MMP-9, and TIMP-1, as well as signaling molecules DDR2 and SMADs2/3 as potential mechanistic pathways involved. The results of these assays are forthcoming. This study demonstrates the potential of a single administration of ephrinA1-Fc to not only attenuate acute ischemic injury but also mitigate pathological accumulation of interstitial fibrosis and remodeling, thereby reducing ventricular stiffening and the resultant progression to heart failure.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Improving Cardiovascular Health Through DNA Repair

Colin G. Wu, Oakland Univ, Rochester, MI

Accumulated DNA damage and oxidative stress play a critical role in the etiology of cardiovascular diseases. Malfunctions of human DNA repair proteins can lead to increased myocardial infarctions, ischemic heart disease, and congestive heart failure. The FANCD1 helicase and the BRCA1 tumor suppressor are essential proteins that coordinate the repair of interstrand DNA crosslinks and double-stranded breaks in human cells. Their activities are especially needed in heart muscle, which undergoes tremendous physiological and chemical stress. Our limited understanding of how FANCD1 and BRCA1 target damaged DNA structures remains a major barrier to research progress. As an independent investigator, my primary focus is to assess the impact of oxidative DNA damage on cardiovascular health through systematic dissection of the DNA repair pathways maintained by FANCD1 and BRCA1. A detailed understanding of these molecular mechanisms would provide new diagnostic and therapeutic strategies to detect and to treat ischemic heart disease and congestive heart failure. Here, we have examined the DNA binding properties of FANCD1 and BRCA1 using fluorescence spectroscopy and biolayer interferometry. We have also measured the viabilities of human cardiomyocytes and probed their sensitivity to reactive oxygen species by automated cell counting and single-cell electrophoresis. The DNA binding results, which describe precisely how FANCD1 and BRCA1 organize onto damaged DNA sites, are compared with the cell-based analysis to correlate their DNA interaction patterns with cardioprotection. Our future work will utilize this platform to predict the risks of FANCD1 and BRCA1 single-nucleotide polymorphisms that still have unknown cardiovascular effects.

C.G. Wu: None.

Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Cardioprotection During Ischemia by Induced Coronary Collateral Growth

Anurag Jamaiyar, Cody Juguilon, Devan Cumpston, Weiguo Wan, Tao Wang, Zhiyuan Wang, James Gadd, Molly Enrick, Sofia Chinchilla, Caige McCabe, Autumn Y Pu, William Chilian, **Liya Yin**, Northeast Ohio Medical Univ, Rootstown, OH

Patients with poorly developed coronary collateral networks have a poorer prognosis after a cardiovascular event than those with well-developed collaterals. The outcome of patients with metabolic syndrome (MetS) was worse than the patient without MetS. Interestingly, coronary collateral growth is impaired in MetS. We hypothesize that coronary collaterals are critical for the cardiac protection in ischemic heart diseases (IHD) and induction of coronary collateral growth (CCG) might ameliorate the outcome of patients with MetS. We study the cardiac protection by coronary collaterals in IHD and the underlying mechanism of impaired CCG in the MetSin a mouse model of CCG. A pneumatic snare was implanted and situated around the LAD. After the mice recovery from the surgery, periodic inflation of the snare occludes the LAD; thus, producing repetitive ischemia (RI). Cardiac function was measure with echocardiography. CCG was measured by myocardial blood flow with contrast echocardiography and by collateral numbers with micro-CT or by vascular density with fluorescence scope. Our preliminary results showed that with occlusion of the LAD, there was no blood flow in the ischemic area. However, after stimulation of CCG by RI, the blood flow the ischemic area was compensated by the grown coronary collaterals and cardiac function was reserved during ischemia. Diabetic mice failed to grow coronary collaterals after RI stimulation and there was no cardiac protection from coronary collaterals during ischemia. Moreover, we studied the roles of growth differentiation factor 11 (GDF11) and miR21 in the regulation of CCG and cardiac protection in IHD. GDF11 was downregulated and miR21 was upregulated in the hearts of Zucker Obese Fatty (ZOF) rats during CCG. We used the Gdf 11 knockout mice and miR 21 knockout mice to study the underlying mechanism. GDF11 is highly expressed in myocardial blood vessels, which suggested the role of GDF11 in vascular growth. Impaired CCG of mice on high fat and high sugar was restored by miR-21 knockout. These data suggest that miR-21 is involved in the impairment of CCG in MetS.

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Poster Session 2 and Reception

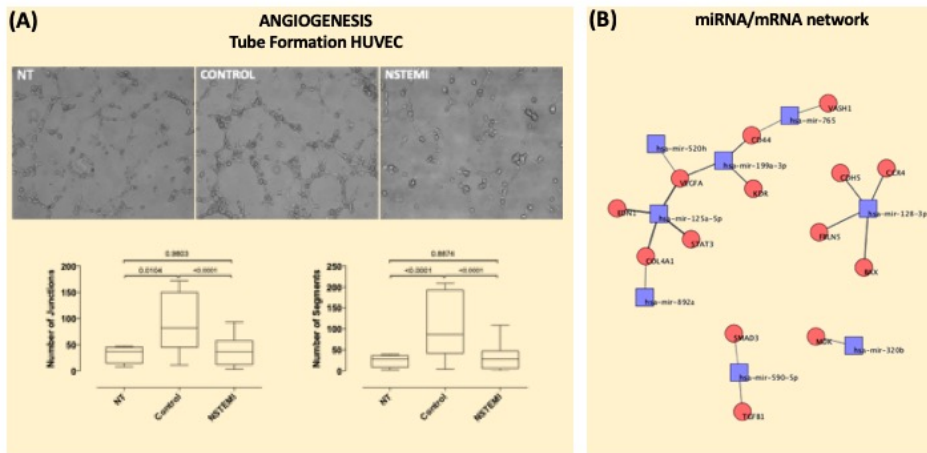
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600

Anti-Angiogenic Effects of Circulating Exosomes from Patients With Acute Coronary Syndrome: Potential Role of miR-199a and miR-125a

Ana Paula V Dantas, Joaquim Bobi, Luis Ortega-Paz, Ahmed Amin, Margarida Pujol-Lopez, Iolanda Lazaro, Manel Sabate, Salvatore Brugaletta, Inst of Biomedical Res August Pi Sunyer (IDIBAPS), Dept of Cardiology, Hosp Clinic, Barcelona, Spain

Circulating exosomes have a great impact in human health as a biomarker or as messengers in intercellular signaling. In this study we aimed to determine how miRNA profile in circulating exosomes interfere with angiogenic phenotype of endothelial cell (EC) during acute event of coronary syndrome. Exosomes were purified from serum of patients with non-ST segment elevation myocardial infarction in the acute phase (NSTEMI, n=34) during percutaneous coronary intervention. Healthy donors (n=23) were included as control. Purified exosomes were quantified and characterized by nanoparticle tracking analysis (Nanosight). Healthy EC (HUVEC) were treated for 48h with labeled exosomes (5×10^5 particles/ 1×10^6 cells) and tested for their angiogenic potential (migration and tube formation) and mRNA expression. Exosome miRNA profile was determined by miRNA array. Exosomes levels were markedly increased in NSTEMI (1.3×10^9 particle/ml) vs control (7.5×10^8 particle/ml; $p=0.02$). HUVEC treatment with healthy exosomes improve migration and tube formation compared to untreated HUVEC. Nevertheless, exosomes from NSTEMI patients, in the acute phase lack their pro-angiogenic potential (Fig A). Network analysis of exosome miRNA and HUVEC mRNA expression revealed a correlation of increased miR-199a and miR-125a expression with decrease of components involved in EC sprout and stabilization (Fig B). Circulating exosomes have an important role in the control of angiogenesis. However, in the acute phase of NSTEMI intercellular communication via exosome is modified and exert an inhibitory effect on angiogenesis. These results could contribute to the progression and outcomes of the disease.



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Radiation Therapy Induces Changes in the Endothelial Methylome Affecting Pro-angiogenic Lncrna Expression

Cristina Espinosa-Diez, RaeAnna Wilson, Rishima Mukherjee, Marlee Feltham, Clayton Hudson, Rebecca Ruhl, Sudarshan Anand, Oregon Health & Science Univ, Portland, OR

Radiation therapy is a big part of standard of care in oncology. However, in the long-term patients treated with radiation are at higher risk of suffering cardiovascular events.

It is well described that the genotoxic stress induced by ionizing radiation in normal cardiovascular tissue triggers genetic and epigenetic changes. In addition to coding mRNAs, non-coding RNAs are also highly regulated by changes in methylation and transcription, leading to a tight regulatory response to damage. Genotoxic stress induces global hypomethylation due to decreased expression of DNA methyltransferases (DNMT). We observed that DNMT1, DNMT3A, and DNMT3b are downregulated in response to radiation treatment, in a dose-response manner in human endothelial cells. We have also observed that methylation changes produced by radiation affect a specific ncRNA cluster, DLK1-DIO3. Previous results from our lab indicate that ncRNAs from this cluster are highly responsive to different genotoxic agents, including radiation. Given the essential role of microRNAs and Long non-coding RNAs (lncRNAs) from the DLK1-DIO3 cluster in cardiovascular development and aging, we are interested in understanding how the epigenetic changes induced by radiotherapy affect to lncRNAs expression, and how they influence cardiovascular health in the long term after treatment. We have identified that a specific lncRNA from the DLK1-DIO3 cluster, MEG9, increases in ECs after exposure to ionizing radiation. Interestingly, knockdown of the individual DNMTs enzymes indicates a significant upregulation of MEG9 when DNMT3b is inhibited, more so after radiation treatment. To explore the role of this lncRNA, we performed loss-of-function studies. MEG9 inhibition not only diminished proliferation but also increased apoptosis through caspase 3/7 activation. Consistent with this phenotype, knockdown of MEG9 decreases growth factor-dependent angiogenesis in a 3D fibrin gel angiogenesis assay. Taken together, our findings illustrate how DNA methylation at particular lncRNA loci can regulate their expression and drive endothelial cell fate decisions. Our work illustrates how epigenetic changes may affect the long-term cardiovascular function of cancer patients submitted to radiation therapy.

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Mir-182-5p is a Conserved Downstream Effector of Tbx5 Involved in Heart Development and Arrhythmia in Zebrafish

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Background: TBX5 mutations cause Holt-Oram syndrome (HOS) characterized by upper limb and cardiac malformations, but can also contribute to early-onset of atrial fibrillation. Focusing on miRNAs involved in TBX5 regulatory circuits with a cardiac relevant role, we identified miR-182-5p, belonging to miR-183 cluster, found upregulated in Tbx5-depleted hearts of mouse and zebrafish embryos.

Methods: To functionally analyse the miR-182-5p role in developing heart, miR-182-5p was dysregulated in zebrafish zygotes of Tg(Myl7:EGFP) and Tg(myl7:gCaMP) transgenic lines. To stably deregulate miR-182-5p in zebrafish heart we exploited the Gal4/UAS system to restrict the miR-182 expression into cardiac context. For physiological analyses we performed the mechanogram of cardiac contraction and electrocardiogram recording. To understand miR-182-5p downstream regulation, in silico analyses, followed by ddPCR/real-time quantifications on dissected zebrafish hearts and rescue experiments both in transient and stable miR-182-5p overexpressing zebrafish embryos were performed.

Results: Depletion of Tbx5 from cardiomyocytes increased the expression of miR-182 cluster family that is controlled by Kruppel-like factor 4 (KLF4), a transcription factor repressed by Tbx5. Both transient and stable upregulation of miR-182 in zebrafish affect heart morphology, calcium handling and the onset of arrhythmia while its cardiac-specific downregulation decreases cardiac defects in zebrafish HOS hearts. Expression analyses on selected miR-182-5p putative targets revealed that several calcium channel proteins resulted downregulated in miR-182-5p overexpressing hearts. Transgenic zebrafish line stably overexpressing miR-182-5p in the heart manifested arrhythmia overtime with or without cardiac structural defects. **Conclusion:** We identified miR-182-5p as a potential suitable target to interfere in the circuit between upstream genetic abnormalities and downstream effectors leading to arrhythmia occurrence.

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Differential Proteoform Regulation in Cardiac Aging

Erin Yu Han, Rushita Bagchi, Div of Cardiology, Sch of Med, Univ of Colorado Denver, Aurora, CO; **Edward Lau**, Stanford Cardiovascular Inst, Dept of Med, Stanford Univ, Palo Alto, CA; **Maggie PY Lam**, Div of Cardiology, Sch of Med, Univ of Colorado Denver, Aurora, CO

Background. The aging heart is prone to diseases and injuries, and is associated with changes in gene expression and alternative splicing. The goal of our study is to identify changes in protein isoforms in cardiac aging and explore their roles in age-related processes.

Methods. Left ventricles (LV) from young (12 weeks) and old (78 weeks) C57BL/6J mice (male and female) were collected and stored at -80 °C until use. LV proteins were extracted, reduced, and alkylated. Proteins were digested with trypsin or chymotrypsin and the tryptic or chymotryptic peptides were desalted using C18 spin columns followed by injection into an Easy-nLC 1200 liquid chromatography system coupled to a high-resolution Q-Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer for LC-MS/MS analysis. To quantify isoform expression, MS spectra were searched against a custom peptide isoform database generated from a publicly available ENCODE RNAseq dataset (accession ENCSR000BYQ) on normal C57BL/6J heart (8 weeks) through an in-house computational workflow. Protein isoform expression was compared between the young and old heart samples.

Results. Our experiments identified over 1,200 proteins (FDR < 0.05) in young and aged mouse LV tissue, including 65 isoform-specific tryptic and chymotryptic peptide sequences originating from 45 alternatively spliced genes that are not currently documented in the commonly utilized protein sequence database SwissProt. The detected protein isoforms include TMED2, TOM1 and TACC2. Our preliminary analysis suggests widespread proteome changes between young and aged hearts, which may have implication on energetics and fibrotic changes in cardiac aging.

Conclusion. We report the first large-scale survey on the expression of canonical and alternative protein isoforms in the aging LV proteome. The identification of age-associated alternative isoforms suggests a potential mechanism for protein function regulation in the aging heart and may have implications for age-related disease processes.

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604

Doxorubicin-induced microRNA-377 Alters Cardiomyocyte and Endothelial Cell Function

John Henderson, Hien T Luong, Suresh K Verma, Prasanna Krishnamurthy, Univ of Alabama at Birmingham, Birmingham, AL

Background: microRNAs (miRNA/miR) dysregulation has been implicated in cardiac remodeling after injury or stress. We have previously shown that miR-377 negatively affects bone marrow progenitor cells function in response to inflammation/myocardial ischemia. However, its role in doxorubicin (DOX)-induced cardiotoxicity, cell death and endothelial dysfunction is not known.

Objective: The purpose of this study is to evaluate the role of miRNA-377 in DOX-induced cardiomyocytes (CMs) stress and endothelial dysfunction.

Methods: miR-377 expression was assessed in myocardial tissue from human patients with heart failure (HF) and mouse model of ischemia-reperfusion injury. *In vitro*, we determined the effect of pre-miRNA (pre-miR-377, stimulates miRNA) on Dox-induced cardiomyocyte cell death, endothelial cell migration (using modified Boyden chamber), and vascular tube formation (using matrigel assay).

Results: Human cardiac biopsies from HF patients show significant increase in miR-377 expression in comparison to non-failing control hearts. Cardiac ischemia-reperfusion (I/R) injury in mice increases myocardial miR-377 expression (vs. sham-operated mice). Interestingly, preparation of single cell suspension of mouse ischemic heart and qPCR analysis show a robust increase in miR-377 expression specifically in the cardiomyocyte as compared to other cardiac cell types. Following DOX-induced cellular stress, miR-377 expression in CMs was elevated, and inhibition of miR-377 attenuates DOX-induced cell death in CMs *in vitro*. Furthermore, transfection of miR-377 mimics in HUVECs significantly inhibits VEGF induced migration and vascular tube formation. Intriguingly, proteome profile of human CD34⁺ cells transfected with miR-377 mimics show significant decrease in numerous proangiogenic proteins as compared to nonspecific control transfected cells.

Conclusions: These findings suggest that doxorubicin-induced cardiotoxicity is associated with an increase in miR-377 expression in the myocardium and that miR-377 overexpression is detrimental to cardiomyocyte viability and endothelial cell function. Therefore, we anticipate that anti-miR-377 treatment might have a beneficial effect against DOX-induced cardiotoxicity.

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Identification of Differential Roles of MicroRNA-33a and -33b During Atherosclerosis Progression with Genetically Modified Mice

Takahiro Horie, Satoshi Koyama, Yui Miyasaka, Takeshi Kimura, Koh Ono, Kyoto Univ Hosp, Kyoto, Japan

Backgrounds: MicroRNA (miR)-33a targets cholesterol transporter ATP-binding cassette protein A1 or other anti-atherogenic targets and contributes to atherogenesis. Its inhibition or deletion is known to result in the amelioration of atherosclerosis. However, mice lack the other member of miR-33 family, miR-33b, which exists in humans. Thus, precise evaluation and comparison of the responsibilities of these two miRs during the progression of atherosclerosis has not been

investigated. **Methods and results:** The difference between miR-33a and miR-33b were analyzed from multiple directions using genetically modified mice. We generated four strains with or without miR-33a and miR-33b. Comparison of these strains showed distinct distribution and regulation of miR-33 family. In particular, comparison between mice with only miR-33a (wild-type mice) and mice with only miR-33b (miR-33a^{-/-}/miR-33b^{+/+}) revealed the prevalence of miR-33b in the liver. Such differential expression resulted in a worsened serum cholesterol profile in mice with only miR-33b. On the contrary, in macrophages the expression level of miR-33 family genes was similar and their effects on cholesterol efflux capacity were almost comparable. To evaluate the whole body atherogenic potency, we developed ApoE^{-/-}/miR-33a^{+/+}/miR-33b^{-/-} mice and ApoE^{-/-}/miR-33a^{-/-}/miR-33b^{+/+} mice. ApoE^{-/-}/miR-33a^{-/-}/miR-33b^{+/+} mice developed increased atherosclerotic plaque compared with ApoE^{-/-}/miR-33a^{+/+}/miR-33b^{-/-} mice, in line with the predominant expression of miR-33b in the liver and worsened serum cholesterol profile. On the contrary, a bone marrow transplantation study showed no significant difference, and this was consistent with the relevant expression levels of miR-33a and miR-33b in bone marrow cells. **Conclusions:** miR-33 family exhibited differences in distribution and regulation, and particularly in the progression of atherosclerosis, miR-33b would be more potent than miR-33a.

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Stimulation of Cardiomyocyte α - and β -Adrenoceptors Uniquely Regulates Exosome Generation and miRNA Content

Jin-Sook Kwon, Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA; Walter Junior Koch, a Ctr for Translational Med, Dept of Pharmacology, Philadelphia, PA

Background and Purpose. Influence of adrenoceptor (AR) signaling for the regulation of exosomes secreted from cardiomyocytes is unknown and since catecholamines are increased in heart failure (HF), there is interest in uncovering whether α - or β -ARs can induce specific changes in circulating blood exosomes in HF. In this study, we have evaluated whether α - or β -AR stimulation of neonatal rat ventricle myocytes (NRVMs) can alter the number, size or microRNA (miR) content of secreted exosomes.

Methods and Results. The number and size distribution of exosomes from NRVMs were evaluated by Nanosight NS300. Phenylephrine (PE, 10 μ M for 3 days), an α -AR agonist, increased exosome number significantly of NRVM compared than vehicle (PBS) treatment group (Veh: $7.82 \times 10^8 \pm 5.06 \times 10^7$ and PE: $10.09 \times 10^8 \pm 15.0 \times 10^7 / 1 \times 10^8$ NRVMs, n=3) but Isoproterenol (ISO, 1 μ M), a β -AR agonist, did not. Both agonists had no effect on exosome size distribution. After purifying total RNA from secreted exosomes, we evaluated the expression level of 37 candidate miR's, which were selected from previous microarray data. ISO treatment decreased 14 miR's (miR-222, -106a, -292a, -181b, -210, -489, -214, -1947, -195, -17, -7b, -93, -532 and -19a) and PE increased 5 miR's (miR-20a, -210, -17-1, -Let7a, and 146b). Importantly, PE or ISO regulated any identical miR's. As a control for treatment effects, we found that PE and ISO elevated myocyte mRNA levels of ANP and BNP to similar degrees.

Conclusions. Both α - and β -AR agonists are can stimulate cardiomyocyte hypertrophy to similar degrees, however they have distinct regulatory patterns for secreted exosomes. This includes the number produced and more importantly, miR content. These miR's and exosomes found in circulating blood may be able to distinguish and be biomarkers for different modes of maladaptive cardiac hypertrophy and HF.

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Effects of Blocked MicroRNA-221 and 222 in Differentiation of Human Pluripotent Stem Cells Derived Hematopoiesis and Hemogenic Endothelium via c-KIT Upregulation

Ji Yoon Lee, 1Dept of Biomedical Science, CHA Stem Cell Inst, CHA Univ, Gyeonggi-do, Korea, Republic of; Seok-Ho Hong, 2Dept of Internal Med, Kangwon Natl Univ, Chuncheon, Korea, Chuncheon, Korea, Republic of

This author has requested that the abstract content not be published

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Long Non Coding RNA Miat Contributes to Cardiac Hypertrophy and Regulates Ribosomal Genes

He Wang, UCLA, Los Angeles, CA; **Zhihua Wang**, Wuhan Renmin Hosp, Wuhan, China; **Yifan Wang**, Christopher Rau, Shuxun Ren, Yibin Wang, UCLA, Los Angeles, CA

Cardiac hypertrophy is characterized by elevated protein synthesis and pathological cardiomyocyte enlargement. The mechanisms underlying the stress response in ribosomal remodeling at organelle level during cardiac hypertrophy is not fully established. We have identified a long non-coding RNA, Myocardial Infarction Associated Transcript (MIAT), that is significantly associated with pathological cardiac hypertrophy through a systems genetic analysis. MIAT expression is induced by hypertrophic signal, and silencing of the MIAT gene significantly blocks myocyte hypertrophy in response to hypertrophic stimuli associated with diminished global protein synthesis activities. Furthermore, MIAT inactivation significantly reduces ribosomal genes at mRNA and protein levels, and reduces protein synthesis activity at basal and post-hypertrophic stimulation *in vitro* and *in vivo*. Using a MIAT knockout mouse model, we investigated the *in vivo* pathophysiological impact of MIAT in pressure-overload induced cardiac hypertrophy and heart failure. Remarkably, we find that the MIAT knockout mice show significantly blunted cardiac hypertrophy and preserved cardiac function following pressure-overload. This study highlights a novel mechanism of translational regulation by a lncRNA MIAT. The outcome will expand functional role of lncRNAs in heart diseases and advance our current understanding of the complexity of regulatory circuits in cardiac hypertrophy and heart failure. It will provide novel insights to protein synthesis and ribosomal regulation and reveal potential therapeutic targets for pathological hypertrophy.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Automated Analysis of Displacement from Intravital Multiphoton Microscopy in Mouse Ventricle

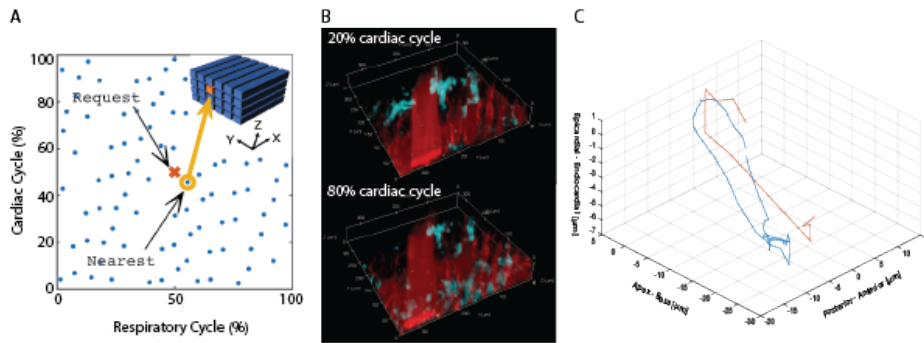
Nathaniel H Allan-Rahill, David M Small, Michael RE Lamont, Salomon Djakpa, Marvarakumari G Jhala, Yvette Zhu, Nozomi Nishimura, Cornell Univ, Ithaca, NY

Background: Multiphoton microscopy (MPM) has enabled *in vivo* time-lapse imaging of the heart that shows motion of cells within the tissue with micrometer resolution. We developed automated analysis techniques to quantify cellular motion from *in vivo* cardiac MPM images throughout the cardiac cycle.

Methods: Intravital cardiac MPM of the beating mouse heart was performed on 26 week-old, C57Bl6 mice (n=6). Image volumes (100 μ m deep) were acquired at 30 frames per second while recording the electrocardiogram and respiratory pressure. An image volume was reconstructed by assembling lines acquired nearest to a specified point in the cardio-respiratory phase space (Fig. a). Motion was calculated as the three-dimensional transformation required to register the reconstructed images to the image at the most stable cardiac phase.

Results: Volumes were reconstructed in 50 intervals across the cardiac phase that show vasculature (intravenous Texas-red dextran, red) and cardiomyocytes (rhodamine 6G, cyan) moving across the field of view (Fig. b). Automated analysis indicated a maximum displacement occurring at 16 % (anterior-posterior), 36 % (base-apex) and 38 % (epi-endocardial) of the cardiac cycle defined by R-wave. Comparison by manual tracking of features across the cardiac cycle at a subset of phases (10 cardiac phases at peak exhalation) validated the automated measurement (Fig. c). Automated motion tracking shows superior performance in the spatial resolution and speed of analysis.

Conclusions: We have shown a novel, fast, and accurate technique for characterizing cardiac motion from *in vivo* cardiac MPM to study the performance of the contractile cells in health and disease.



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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

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Isolating the Pathological Contribution of Detyrosinated Microtubules in Human Myocardial Mechanics

Matthew A Caporizzo, Christina Y Chen, Kenneth Bedi, Kenneth B Margulies, Benjamin L Prosser, Univ of Pennsylvania, Philadelphia, PA

Detyrosinated microtubules (dTyr MTs) provide a viscoelastic resistance to myocyte motion and are proliferated in end-stage heart failure. In isolated myocytes, proliferation of the MT network leads to reduced shortening and relaxation, consistent with an underlying viscoelastic resistance, yet a clear contribution of MTs to the passive mechanical properties of myocytes or myocardium in heart failure remains to be determined. To this end, intact human myocytes were isolated from failing and non-failing hearts and their viscoelasticity was measured using a physiological stretch assay where strain-rates were consistent with diastolic filling. In myocytes from heart failure patients, we find an increase in myocyte viscoelasticity and slowing of passive relaxation during cell stretch compared to myocytes from non-failing hearts. Depolymerizing MTs with colchicine, or suppressing dTyr MTs either pharmacologically with parthenolide or genetically by overexpression of tubulin tyrosine ligase reduces myocyte passive viscoelasticity, accelerates passive relaxation time and leads to consistent improvements in contractile and relaxation dynamics in failing cardiomyocytes. Manipulation of dTyr MT levels does not significantly alter the calcium transient during unloaded contraction, suggesting that the observed gain in function is mechanical in nature. We additionally have begun to assay the MT contribution to mechanics at the tissue level. In intact trabeculae from failing hearts, colchicine treatment to depolymerize MTs reduces passive myocardial viscoelasticity upon diastolic stretch. The results indicate that microtubules contribute to myocardial mechanical properties in the context of end-stage heart failure.

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Using Intact Trabeculae to Determine the Effect of Myosin-Modifying Drugs on Work, Power, and Mechanical Control of Relaxation

Brianna M Schick, Melissa J Bukowski, **Charles S Chung**, Wayne State Univ, Detroit, MI

Background: Heart failure and especially heart failure with preserved ejection fraction are significant burdens on health. Intact cardiac trabeculae may reveal functional changes that cannot be seen in other ex vivo (skinned, single molecule) preparations. For example, using intact trabeculae, we have shown that relaxation is mechanically controlled by the lengthening strain rate at end systole, not afterload. **Objective:** We sought to evaluate the effect of myosin activator Omecamtiv Mercarbil (OM) and inhibitor Mavacamten (Mav) on physiologic function in intact trabeculae. **Methods/Results:** Afterload-clamp protocols were applied to intact cardiac trabeculae from Sprague Dawley rats

to simulate physiologic work-loops and evaluate mechanical control of relaxation. Both OM and Mav reduced stroke work (force x length) by >50% and power (force x velocity) by ~50% at doses reducing developed force by 50%. These were mediated by dose-dependent reductions in both force and shortening length. We have recently reported preliminary results that OM improves contraction-relaxation coupling and makes the relaxation rate more sensitive to strain rate in a dose-dependent manner. Mav does not lead to significant changes in contraction-relaxation coupling at any dose; Mav alters the sensitivity of relaxation rate to strain rate only at doses that reduce developed force by 50%. **Summary/Perspective:** Intact rat cardiac trabeculae reveal function mimicking physiology. OM and Mav show remarkable similarities in force, work, and power, which may be due to the high expression of alpha-myosin heavy chain. OM treatment not only modified contractility but increased sensitivity of the relaxation rate to strain rate in a dose dependent manner, which may explain why diastolic dysfunction is not more prevalent in clinical studies. Mav appears to only modify the attachment of crossbridges as expected.

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The Cardiac Myosin Inhibitor, CK-3773274, Reduces Contractility in the R403q Mouse Model of Hypertrophic Cardiomyopathy

Darren T Hwee, Yangsong Wu, Peadar Cremin, Bradley P Morgan, Fady I Malik, Eva R Chin, Cytokinetics, Inc., South San Francisco, CA

Cardiac sarcomere hypercontractility appears to underlie pathological hypertrophy and fibrosis in select genetic hypertrophic cardiomyopathies. The small molecule, CK-3773274, is a novel cardiac myosin inhibitor that decreases contractility *in vitro* and in healthy animals *in vivo*. The objective of this study was to evaluate the effect of CK-3773274 in the genetic R403Q mouse model of hypertrophic cardiomyopathy. At approximately 40 weeks of age, left ventricular wall dimensions were determined by echocardiography in male wild type (WT) and heterozygous R403Q mice. As an indicator of cardiac hypertrophy, R403Q mice had significantly greater septal and posterior wall thickness than WT mice (septal wall WT: 0.93 ± 0.03 mm vs. R403Q: 1.22 ± 0.08 mm; posterior wall WT: 0.84 ± 0.04 mm vs. R403Q: 1.09 ± 0.04 mm; mean \pm SEM, $p < 0.05$). R403Q mice were treated with single oral doses of CK-3773274 ranging from 0.25 to 1.5 mg/kg and fractional shortening (FS) and heart rate were assessed at select time points over a 24-hour period. One hour after dose administration, CK-3773274 significantly reduced FS in a dose-related fashion relative to pre-dose baseline values (FS % R403Q baseline: $55.5 \pm 2\%$; 0.25 mg/kg: $43.9 \pm 2\%$; 1 mg/kg: $27.3 \pm 2\%$; 1.5 mg/kg: $13.7 \pm 1\%$; mean \pm SEM, $p < 0.05$ vs. baseline at all doses) without any changes to heart rate. At all dose levels, fractional shortening returned to baseline values by 24 hours. The plasma concentration at 10% and 50% reduction of FS relative to baseline (IC_{10} and IC_{50}) was 0.11 and 0.78 μ M, respectively. In summary, single oral dose administration of CK-3773274 reduced fractional shortening in a dose and concentration-dependent manner in the genetic R403Q mouse model of hypertrophic cardiomyopathy. Cardiac myosin inhibition may be a viable approach to reduce underlying hypercontractility of the cardiac sarcomere in hypertrophic cardiomyopathies.

D.T. Hwee: 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **Y. Wu:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **P. Cremin:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **B.P. Morgan:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **F.I. Malik:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position. **E.R. Chin:** 1. Employment; Significant; \$10,000 or more during last 12 months. 7. Ownership Interest; Modest; Equity position.

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Glycogen Synthase Kinase 3b Localizes to and Modulates Sarcomere Function in Health and Disease

Jonathan A Kirk, Loyola Univ Chicago, Maywood, IL

Ischemic damage to a region of myocardium renders it weaker than the surrounding tissue, leading to contractile heterogeneity. This heterogeneity causes abnormal stress and strain patterns, further reducing systolic function. We

hypothesized glycogen synthase kinase 3 β (GSK-3 β) may be a therapeutic target for improving function in patients with ischemic damage, as we previously discovered its activity is reduced by abnormal mechanical stress and it can phosphorylate myofilament proteins. We measured GSK-3 β levels in LV whole tissue lysate from patients with ischemic cardiomyopathy (ICM) and non-failing rejected donor hearts (NF), but detected no differences. However, we found GSK-3 β present at the myofilament, and ICM patients had a ~70% reduction in this localized pool compared to NF controls. Furthermore, GSK-3 β phosphorylated at Y216 (pY216), an understudied site, was highly enriched in the myofilament. We used immunofluorescence, co-immunoprecipitation, and adenoviral constructs of Y216 phospho-mimetic or -blocked, in human, mouse, or neonatal rat ventricular myocytes to confirm Y216 phosphorylation results in GSK-3 β binding to the myofilament. pY residues can dock to SH2 domains. The only SH2-containing protein that associates with the myofilament is tensin-1 (discovered by us in the myofilament by mass spectrometry). Here we further found tensin-1 localizes to the cardiac z-disk. GSK-3 β and tensin-1 co-IP together in myocytes and in HEK cells (in the absence of the myofilament), but this interaction is reduced if Y216 phosphorylation is blocked. To identify the functional consequences of this interaction, we utilized an inducible myocyte specific GSK-3 β knock-out mouse. Skinned myocytes from GSK-3 β KO mice had significantly depressed calcium sensitivity compared to WT mice. Furthermore, treating skinned myocytes isolated from ICM patients with GSK-3 β significantly increased calcium sensitivity, but had no effect in NF cells. These findings identify a novel functional role for GSK-3 β in the ischemic human heart. GSK-3 β is a promiscuous kinase, but we have discovered a specific regulatory mechanism (Y216 phosphorylation) that could allow precise therapeutic intervention to improve contractile function in ICM patients.

J.A. Kirk: None.

Poster Session 2 and Reception

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In vivo Titin Oxidation as a Modulator of Sarcomeric Contractibility

Ines Martinez-martin, Elías Herrero-Galán, Natalia Vicente, Cristina Sánchez-González, Diana Velázquez-Carreras, Molecular Mechanics of the Cardiovascular System, Ctr Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; Elena Bonzón-Kulichenko, Cardiovascular Proteomics, Ctr Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; Enrique Calvo, Proteomics Technical Unit, Ctr Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; Jesús Vázquez, Cardiovascular Proteomics, Ctr Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; Jorge Alegre-Cebollada, Molecular Mechanics of the Cardiovascular System, Ctr Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

Titin is the sarcomeric protein responsible for the passive elasticity of muscle. Its function is based on the unfolding and refolding of the immunoglobulin-like (Ig) domains located in the I-band, thus described as the mechanically active region of the protein. Previous *in vitro* studies have shown that oxidation of cysteines located in these domains modulates the mechanical properties of the protein. However, the extent of these redox modifications and the identity of the modified residues *in vivo* remains unexplored. Here we show for the first time that titin is oxidized *in vivo* and that oxidative modifications target conserved cysteines of the mechanically active Ig domains. We have set up a biochemical method for in-gel determination of oxidized thiols by which we found that titin is remarkably oxidized when compared to myosin, which is not a target of cysteine redox modifications. By mass spectrometry, we also detected that the conserved cysteines previously described as mechanically relevant are in fact oxidized. We propose that characterizing the oxidation of titin Ig domains will lead to a better understanding of the regulation of muscle elasticity, and could explain the pathological effects of an imbalanced redox status, such as during myocardial infarction or ischemia.

I. Martinez-martin: None. **E. Herrero-Galán:** None. **N. Vicente:** None. **C. Sánchez-González:** None. **D. Velázquez-Carreras:** None. **E. Bonzón-Kulichenko:** None. **E. Calvo:** None. **J. Vázquez:** None. **J. Alegre-Cebollada:** None.

Poster Session 2 and Reception

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Overexpression of Rrm2B Elevates dATP and Cardiac Function

Jason Murray, Sabrina Do, Tim McMillen, Michael Regnier, **Farid Moussavi-harami**, Univ of Washington, Seattle, WA

Cardiac myosin can use 2-deoxy-ATP (dATP) instead of ATP as energy substrate, significantly enhancing the rate and magnitude of force development and relaxation. Our group has shown repeatedly that overexpression of the enzyme ribonucleotide reductase (RNR; the rate-limiting step in *de novo* dNTP synthesis) in cardiomyocytes, by either transgenic or viral vector approach, enhances cardiac function by significantly elevating cytosolic concentrations of dATP. We are exploring different promoter, gene constructs and engineered mutations of RNR to be able to vary the amount of dATP maintained in cardiomyocytes.

RNR activity is partially governed by levels of the small subunit, Rrm2, whose concentration oscillates with the cell cycle via degradation by the ubiquitin-proteasome system. The p53-inducible small subunit Rrm2B is naturally present in quiescent cells and does not contain the amino acid sequence that is targeted by the ubiquitin-conjugating enzyme. We hypothesized that Rrm2B overexpression may be more stable than Rrm2 through its resistance to degradation by this pathway. Transgenic mice that overexpress Rrm1 and Rrm2B have a ~60 fold increase in myocardial [dATP] as measured by liquid chromatography-mass spectrometry compared to wild-type littermates. This translates to significantly elevated left-ventricular developed pressure as well as higher rates of contraction and relaxation in perfused isolated hearts, as well as enhanced shortening and relaxation in isolated cardiomyocytes.

Isolated rat cells also have an increase in [dATP] and corresponding greater fractional shortening and rates of contraction and relaxation when transduced *in vitro* with adenovirus encoding Rrm1 and Rrm2B when compared to cells transduced with a control vector. We are currently in the process of comparing the effectiveness of adeno-associated viruses encoding Rrm2 and Rrm2B *in vivo*. Our ultimate goal is to develop an improved vector to enable stable and consistent overexpression of RNR and elevation of dATP in cardiomyocytes transduced *in vivo*, thereby improving both systolic and diastolic function for treatment of cardiomyopathies.

J. Murray: None. **S. Do:** None. **T. McMillen:** None. **M. Regnier:** None. **F. Moussavi-harami:** None.

Poster Session 2 and Reception

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Cardiac Function in Electronic Cigarette-Exposed Adolescent Mice

Evan W Neczypor, Vineeta Tanwar, Jeremy M Adelstein, Michael D Muffler, Chadi Pellegrini Anaruma, Loren E Wold, Dorothy M. Davis Heart and Lung Res Inst, The Ohio State Univ Coll of Med, Columbus, OH

Objective: Electronic cigarettes (e-cigs) are the most commonly used tobacco product by teens and young adults. The aim of the present study was to determine the potential effects of e-cig vapor exposure on cardiac function in adolescent mice. **Materials and Methods:** Adult FVB/NJ male and female mice were bred in our housing facility. Following birth, male offspring were raised in room air until weaning. At 3 weeks of age, mice were separated into two exposure groups: 1) E-cigarette exposure with nicotine (EC(+), N=5) (10.1 mg nicotine/1mL 50:50 propylene glycol:vegetable glycerin), or 2) E-cigarette exposure without nicotine (EC(-), N=4) and exposed for two months. A puff profile consisting of one 70-mL vapor puff per minute of exposure was administered for 4 h/day, 5 days/week over the course of two months. Cotinine concentration in the urine of mice from either group was determined using a cotinine ELISA kit. Echocardiography was used to examine global cardiac function after 1 and 2 months of exposure. **Results:** The average cotinine concentration in the urine of the EC(+) group after two months of exposure was 113.33 ng/mL, while there was not a detectable concentration of cotinine in the urine of EC(-) mice. Echocardiography showed no significant alterations in ejection fraction (%EF), fractional shortening (%FS), left ventricular end systolic diameter (LVES_d), or posterior wall thickness (PWT) between the EC(+) and EC(-) groups, nor any significant alterations between the first month and the second month timepoints for either group. **Future Directions:** Exposures will continue for longer durations until a cardiac phenotype becomes apparent. Once *in vivo* cardiac dysfunction becomes evident, we will investigate *in vitro* cardiomyocyte function.

E.W. Neczypor: None. **V. Tanwar:** None. **J.M. Adelstein:** None. **M.D. Muffler:** None. **C. Pellegrini Anaruma:** None. **L.E. Wold:** None.

Poster Session 2 and Reception

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Molecular Mechanisms and Therapeutic Approaches to Myofilament Glycation as a Result of Diabetes

Maria Papadaki, June Theerachat, Jonathan Alder Kirk, Loyola Univ Chicago, Maywood, IL

Methylglyoxal (MG), a glycolysis byproduct, is significantly elevated in the blood of patients with diabetes and modifies proteins by reacting with arginine and lysine residues to form irreversible adducts. We previously found that patients with diabetes and heart failure (dbHF) exhibited an increase in MG-modifications on cardiac myofilament proteins compared to non-failing hearts. We discovered these modifications reduced myofilament calcium sensitivity and maximal calcium-activated force, and despite being primarily on actin and myosin, did not affect their direct binding. The aim of this study was to elucidate MG's molecular effects on muscle mechanics as well as to identify effective therapeutic options for dbHF patients. We first utilized a custom loaded fiber system to simultaneously measure isometric force and ATPase activity. When mouse fibers were treated with 100 μ M Mg for 20 mins, similar to previous studies, there was a decrease in force production, but there was a concomitant decrease in ATPase activity as well. Thus, the tension cost was not altered by MG, supporting our hypothesis that MG modifications on myosin do not alter myosin function, instead altering its interactions with thin filament proteins. MG modifications are irreversible so therapeutic approaches should compensate for the functional defects induced. We pre-treated mouse skinned myocytes with MG, and then measured force-calcium relationships before and after treatment with different drugs. We first used 1 mM aminoguanidine (AG), an MG scavenger that has been efficacious in basic science studies but failed in clinical trials. Agreeing with the clinical trial, we show AG cannot reverse MG's effects. We then treated the myocytes with omecamtiv mecarbil (OM), a myosin activator currently in clinical trials. In myocytes not pretreated with MG, OM increased calcium sensitivity and decreased maximum force, as reported previously. Interestingly, OM had no effect of calcium sensitivity but still decreased Fmax, suggesting OM may have less effect in patients with diabetes. Overall, in this study we further elucidate the mechanism of MG effect's on the myofilament, and also show these modifications may reduce the efficacy of OM, a small molecule currently in clinical trials.

M. Papadaki: None. **J. Theerachat:** None. **J.A. Kirk:** None.

Poster Session 2 and Reception

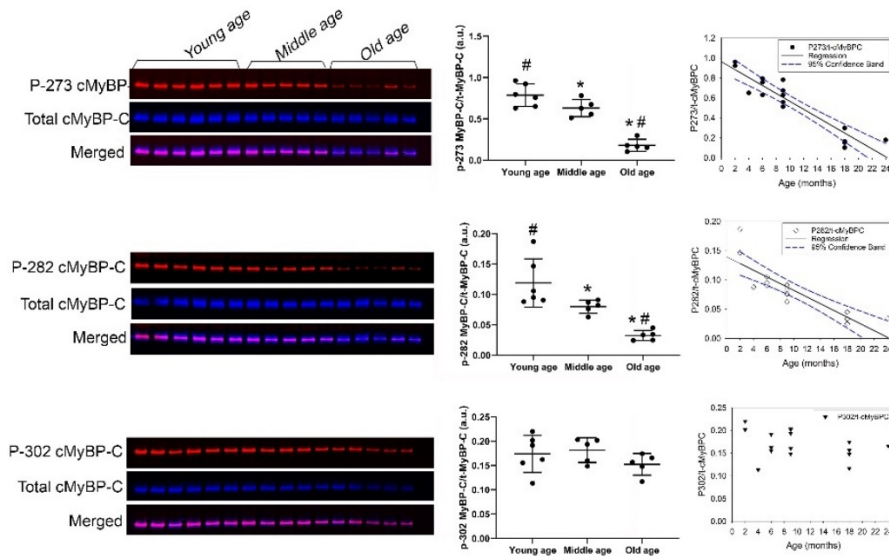
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Cardiac Myosin Binding Protein-C Phosphorylation in Ser-273 and Ser-282 is Critical to Maintain Cardiac Function During Aging

Paola C Rosas, Chad M Warren, Ashley Batra, UIC, Chicago, IL; Heidi Creed, Texas A&M, College Station, TX; R John Solaro, UIC, Chicago, IL; Carl W Tong, Texas A&M, College Station, TX

Background/hypothesis: Heart failure (HF) afflicts 6.2M Americans as of 2019 and its prevalence is expected to increase due to a growing aging population. HF progression involves depression in contractility and relaxation, which are strongly controlled by phosphorylation of cardiac myosin binding protein-C (cMyBP-C). We hypothesize that phosphorylated cMyBP-C supports normal heart function during aging. **Methods:** We aged mouse models of cMyBP-C de-phosphorylation mimetic (t3SA: S273A/S282A/S302A mutations), phosphorylation mimetic (t3SD: S273D/S282D/S302D mutations) and WT equivalent expressed onto cMyBP-C(-/-) background (tWT). **Results:** Western blotting (N=16, 2-24 months) showed that cMyBP-C phosphorylation decreases with aging at S273 and S282, showing strong Pearson correlations with age (see figure; S273P $r = -0.931$, $p < 0.0001$; S282P $r = -0.868$, $p < 0.0001$). Simultaneous force and $[Ca^{2+}]_i$ measurements on intact papillary muscles showed that phosphorylation-mimetic t3SD has greatest $dFR = (-dF/dt)_{min}/(+dF/dt)_{max}$, signifying better lusitropy with similar calcium decay rate constant (k_{Ca}) as others. Echocardiography at 3, 12, 15, 18 months showed that t3SD has preserved ejection fraction, faster peak myocardial relaxation velocity e' and preserved posterior ventricular wall thickness with aging. Moreover, t3SD demonstrated superior 600-days survival than others (t3SD 87.5% $n=96$; tWT 69.3% $n=122$; t3SA 53.2% $n=97$; overall LogRank $p=0.0002244$). **Conclusions:** De-phosphorylation of cMyBP-C at S273 and S282 contributes to worsening of cardiac function with aging. Conversely, phosphorylated cMyBP-C mitigates aging-related cardiac dysfunction.



*p < 0.05 vs. Young age, #p < 0.05 vs. Middle age. Young age: 2-6 months (n=6), Middle age: 9 months (n=5), Old age: 18-24 months (n=5).

P.C. Rosas: None. C.M. Warren: None. A. Batra: None. H. Creed: None. R.J. Solaro: None. C.W. Tong: None.

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Load-Independent Systolic Dysfunction and Altered Z-Disc Protein Phosphorylation in Swine with Stretch-Induced Myocardial Stunning

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Objective: We recently demonstrated that a transient rise in left ventricular (LV) preload leads to a reversible reduction in LV ejection fraction in the absence of ischemia ("stretch-induced stunning"). The present study was designed to determine whether this phenomenon is characterized by impaired load independent LV systolic function and alterations in sarcomeric protein phosphorylation.

Methods: Swine (n=9) received a 1 hour infusion of phenylephrine (PE; 18 mg/hour iv) to transiently elevate LV end-diastolic pressure (EDP). Load independent systolic function was derived from LV pressure volume loops collected during inferior vena cava occlusion at baseline and 30 min after PE (Transonic SciSense). In a subset of animals (n=5), LV tissue was collected 1 hour after PE for TMT-labeled, LC-MS-based quantitative phosphoproteomics using Orbitrap.

Results: PE elicited a rise in LV EDP (9 ± 2 to 29 ± 3 mmHg, $p < 0.01$), followed by a reduction in LV ejection fraction and LV dP/dt_{max} 30 min later. At this time, load independent indices of LV systolic function tended to exhibit a reduced slope with a significant rightward shift of the volume-axis intercept (V_0), indicative of decreased contractility (Table). This was accompanied by altered phosphorylation of 16 sarcomeric Z disc proteins at 27 phosphosites, including increased phosphorylation of α actinin, α crystallin B chain, desmin, and myotilin.

Load-Dependent Systolic Function		
	Baseline (n=9)	Stretch-Induced Stunning (n=9)
LV Ejection Fraction (%)	57 ± 3*	36 ± 5*
LV dP/dt_{max} (mmHg/sec)	2008 ± 70	1448 ± 87*
Load-Independent Systolic Function		
	Baseline (n=9)	Stretch-Induced Stunning (n=9)
ESPVR Slope (mmHg/mL)	4.8 ± 0.9	3.4 ± 0.6 [#]
ESPVR V₀ (mL)	20.4 ± 7.0	42.3 ± 10.1*
PRSW Slope (mmHg)	53.4 ± 6.5	47.7 ± 4.2
PRSW V₀ (mL)	22.5 ± 20.8	65.5 ± 15.5*
Preload-Adjusted dP/dt_{max} Slope (mmHg*sec⁻¹/mL)	20.3 ± 6.7	15.5 ± 3.9
Preload-Adjusted dP/dt_{max} V₀ (mL)	-24.1 ± 17.7	28.4 ± 12.8*

Data are mean ± SEM. *p<0.05 vs. Baseline; [#]p=0.08 vs. Baseline. LV indicates left ventricular; ESPVR, end-systolic pressure volume relationship; PRSW, preload-recruitable stroke work; V₀, volume-axis intercept.

Conclusions: Stretch-induced stunning following transient preload elevation is characterized by load independent systolic dysfunction. Phosphoproteomics implicate alterations in Z disc protein phosphorylation as a mechanism underlying contractile dysfunction in this condition.

B.R. Weil: None. **S. Rasam:** None. **F. Konecny:** 1. Employment; Significant; Transonic Scisense, Inc.. **C. Smith:** None. **J. Qu:** None. **J.M. Canty:** 8. Consultant/Advisory Board; Modest; Lantheus Medical Imaging.

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Mixed Lineage Kinase 3 Regulates Blood Pressure Through Kinase Independent Effects in the Vasculature

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Background: Mixed lineage kinase 3 (MLK3) opposes pathologic cardiac remodeling, but its role in blood pressure (BP) has not been studied. MLK3 activates JNK signaling through kinase-dependent effects, and opposes RhoA activation through kinase-independent mechanisms, but the relevance of these mechanisms to BP is unknown. We investigated the effect of genetic deletion of MLK3 on BP. **Methods and Results:** Using ambulatory telemetric monitoring in 3 month old male mice, MLK3 ^{-/-} mice had significant hypertension compared to wild type (WT) littermates (WT systolic BP 121 ± 2 mmHg, MLK3 ^{-/-} 162 ± 5 mmHg; n=3; p<0.05). The MLK3 kinase inhibitor URM-099 (10 mg/kg IP) did not affect BP in WT mice. By contrast, inhibition of downstream RhoA dependent Kinase (ROCK) with Y-27632 (15 mg/kg) fully normalized BP in MLK3 ^{-/-} mice (SBP WT baseline 126 ± 2 mmHg; WT ROCK inhibitor 94 ± 2 mmHg; MLK ^{-/-} baseline 163 ± 6 mmHg; MLK3 ^{-/-} ROCK inhibitor 94 ± 12 mmHg; n=5 WT, 9 MLK3 ^{-/-}). Aortic pulse wave velocity was elevated in MLK ^{-/-} mice (2.7 ± 0.1 mm/ms WT vs 3.6 ± 0.2 mm/ms MLK3 ^{-/-}; p<0.05) indicating increased aortic stiffness. Pressure myography in mesenteric resistance arterioles of MLK3 ^{-/-} mice revealed reduced distensibility compared to WT. Both pressure myography and direct histological measurement in resistance arterioles demonstrated reduced passive luminal diameter but preserved wall cross sectional area in MLK3 ^{-/-} arterioles, indicating eutrophic remodeling. Compared with dispersed aortic smooth muscle cells from WT littermates, MLK3 ^{-/-} cells had increased actin stress fiber accumulation and cell area. **Summary and Conclusions:** These data demonstrate that MLK3 deletion leads to hypertension with increased arterial stiffness, reduced distensibility and eutrophic remodeling of resistance vessels, but retained BP responsiveness to downstream ROCK inhibition. Together with previous work, these findings support that MLK3 modulates cardiac remodeling through a kinase dependent mechanism while modulating blood pressure through kinase-independent effects. Because hypotension limits many heart failure

therapies, delineating vascular versus cardiac mechanisms of MLK3 signaling has the potential to suggest novel approaches to heart failure treatment.

T.D. Calamaras: None. **R.A. Baumgartner:** None. **M. Aronovitz:** None. **J. McCarthy:** None. **K. Tam:** None. **S. Kim:** None. **G. Martin:** None. **D.A. Richards:** None. **P. Baca:** None. **I.Z. Jaffe:** None. **R.M. Blanton:** None.

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Pro-Inflammatory Signaling by Cardiomyocytes Involves Activation of a RhoA Dependent Gene Expression Pathway

Cameron S Brand, Hoyoung Moon, Joan Heller Brown, Univ of California San Diego, La Jolla, CA

Cardiac inflammation, particularly the recruitment of inflammatory cells following injury, precedes cardiac fibrosis. A chronic inflammatory state in the heart increases risk of progression to heart failure. Activation of the small G-protein RhoA occurs in response to cardiac injury, and chronic activation of RhoA has been shown to facilitate maladaptive cardiac fibrosis. We tested the hypothesis that RhoA activation induces early pro-inflammatory signals in the cardiomyocyte which can lead to progression of inflammatory responses and adverse remodeling. Mice were infused with angiotensin-II, an agonist that can activate RhoA signaling and which rapidly induces cardiac inflammation and fibrosis. After one day of infusion, hearts were assessed for RNA levels of various inflammatory genes in wildtype versus cardiomyocyte-specific RhoA knockout mice. A set of genes for pro-inflammatory secreted cytokines associated with macrophage infiltration, including CCL2, IL-6, and IL-1 β were upregulated in hearts from control mice infused with angiotensin-II, but these responses were abolished in hearts from RhoA knockouts. These findings indicate that RhoA activation in the cardiomyocyte contributes to the upregulation of these inflammatory signals. Our lab and others have demonstrated the importance of transcriptional responses to RhoA activation, and have shown concomitant downstream activation of the transcriptional co-activators Myocardin-related Transcription Factor A (MRTF-A) and Yes-activated Protein (YAP). With intravenous injection of cardiomyocyte-specific adeno-associated virus serotype 9 (AAV9) expressing Cre recombinase in mice with floxed YAP, we thus tested involvement of YAP in inflammatory gene expression regulated by angiotensin-II activation of RhoA. In summary, we propose that activation of RhoA in the cardiomyocyte facilitates a gene program involved in cardiac inflammatory response, and are examining the role of signaling from cardiomyocytes through MRTF-A and YAP regulated genes for inflammation and immune cell infiltration.

C.S. Brand: None. **H. Moon:** None. **J. Brown:** None.

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Primary Cilia of the Cardiac Neural Crest Orchestrate Critical Aspects of Ventricular Maturation and Postnatal Cardiac Function: A Role for Hedgehog Signaling

Lindsey A Fitzsimons, Graduate Sch of Biomedical Science & Engineering, Univ of Maine, Orono, ME; Adrian M Moran, Dept of Pediatric Cardiology, Maine Medical Ctr, Portland, ME; Kerry L Tucker, Coll of Osteopathic Med, Univ of New England, Biddeford, ME

Primary cilia are tiny, plasma-membrane bound organelles that function to modulate intra- and extracellular signaling, in particular Hedgehog signaling, implicated in both normal development as well as in various disease pathologies, including congenital heart disease (CHD). Cardiac neural crest cells (CNCC) display primary cilia and function as a major progenitor cell population contributing to the developing heart. We hypothesized that damage to or loss of primary cilia in CNCC would impair normal heart development, leading to CHD. We present 1) a precise description of CHD's resulting from loss of primary cilia of CNCC *in vivo*; 2) characterization of the functional, electrophysiological aspects of the perinatal phenotype; and 3) a preliminary investigation of the molecular mechanisms leading to this phenotype. Using a Wnt1:Cre-2, Ift88-targeted conditional elimination of primary cilia, and a Td-tomato reporter to track CNCC, we observed loss of cilia in CNCC at embryonic day E9.5, with notable CNCC contribution to the ventricular myocardium and pronounced disorganization of the endocardium by E10.5. The phenotype resulting from cilia loss in CNCC was characterized by a variety predicted CHDs in addition to disorganization of the ventricular endocardium, pronounced noncompaction of the ventricular myocardium, and perinatal lethality. To investigate potential changes in Hedgehog signaling resulting from cilia loss, we implemented an *in vivo* conditional knockout in which Wnt1:Cre-2 drives expression of a mutant, constitutively-active Hedgehog receptor. Noncompaction observed in all Wnt1:Cre-2/Ift88 mutants became exacerbated in a primary-cilia dependent fashion with the

introduction of forced Hedgehog signaling in CNCC. These results support a critical role for both primary cilia and Hedgehog signaling in the development of the ventricular myocardium. Further electrophysiological assessments of Wnt1:Cre-2/Ift88 newborn mutant mice revealed bradycardia, conduction defects, and ECG tracings consistent with ventricular hypertrophy. These data collectively support a role for Hedgehog signaling via primary cilia of CNCC in the pathogenesis of outflow tract defects, VSD, and most notably, ventricular maturation CHDs.

L.A. Fitzsimons: None. **A.M. Moran:** None. **K.L. Tucker:** None.

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Diabetes-Mediated Vascular Calcification is RAGE-Dependent

Amber M. Kay, James A. Stewart Jr., Univ of Mississippi, University, MS

Type II diabetes mellitus (DM) is characterized by chronic hyperglycemia, and medial vascular calcification is a common cardiovascular complication of DM. This leads to aortic stiffening, which can leave patients at an increased risk for heart attack or stroke. Advanced Glycation End-Products (AGEs)/Receptor for AGEs (RAGE) signaling cascade has been implicated as a potentiator of diabetes-mediated vascular calcification, but it is not well understood. AGE/RAGE signaling influences both cellular and systemic responses to increase bone matrix proteins in hyperglycemic and calcification conditions and has also been shown to increase oxidative stress by promoting diabetes-mediated vascular calcification. This causes a phenotypic switch of vascular smooth muscle cells (VSMCs) to osteoblast-like cells and the hypothesized activation of adventitial fibroblasts (AFBs) to a myofibroblast phenotype. The purpose of this research is to understand AGE/RAGE mediated vascular calcification as a complication of diabetes. Calcification was induced for 7 days in primary mouse VSMCs and AFBs of non-diabetic, diabetic, non-diabetic RAGE knockout (RKO), and diabetic RKO, and then treated with AGEs to activate RAGE. Alizarin Red S staining was utilized to visualize calcification. Intracellular calcium levels were quantified and normalized to cell number (DAPI). Pronounced calcification was observed in non-diabetic VSMCs and the loss of RAGE resulted in decreased calcification in the non-diabetic RKO VSMCs. AFBs were exposed to the same experimental conditions as the VSMCs and calcification was increased in the diabetic AFBs while calcification was significantly decreased in the diabetic RKO AFBs. These data demonstrated that diabetes-mediated vascular calcification was RAGE-dependent in both cell types. Literature has cited the VSMC as the primary mediator for vascular calcification, but we have shown that the AFBs in the outer layer of the aorta have the ability to calcify and this is mediated by RAGE signaling, which elucidates a role for RAGE in diabetes-mediated vascular calcification. Understanding the role of AGE/RAGE signaling in diabetes-mediated vascular calcification will allow for possible targets for pharmacological intervention.

A.M. Kay: None. **J.A. Stewart:** None.

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Txlnb-Deficient Mice Reveal That Ubiquitin-Proteasome-System Dysfunction is Not Sufficient to Impair Cardiac Performance

Jared M McLendon, Xiaoming Zhang, Colleen S Stein, Nathan H Witmer, Gabrielle M Abouassaly, Ryan L Boudreau, Univ of Iowa, Iowa City, IA

Prior reports show that impaired cardiac function is associated with, if not caused by, Ubiquitin-Proteasome-System (UPS) dysfunction. Rodent loss-of-function studies for proteins involved in protein folding/ER stress responses, ubiquitin ligation, and proteasomal degradation are frequently associated with accumulation of misfolded/ubiquitinated proteins, decreased proteasomal activities, worse outcomes to cardiac stress, and premature death. By contrast, gain of function models that improve protein folding/trafficking or activate the proteasome prevent these molecular phenotypes and improve cardiac function. We have recently found an exception to this association while characterizing beta-taxilin (TXLNB), an understudied muscle- and heart-enriched protein with unknown function. In cardiomyocytes, TXLNB overexpression decreased ubiquitinated proteins and increased proteasome activity, whereas knockdown promotes proteasomal insufficiency. Similarly, hearts from TXLNB knockout (TXLNB-KO) mice show increased ubiquitinated proteins (>2-fold, n=8, p=.007) and decreased 26SB5 proteasome activity (~40%, n=8, p=.025), starting by 12 weeks of age and persisting through life. Despite robust cardiac proteasomal insufficiency, TXLNB-KO mice surprisingly exhibit normal cardiac function [echo/ekg measures done in

several independent cohorts (n>7 per sex/genotype) up to ~24 months of age]. In addition, these mice do not show worse responses to mild pressure-overload induced by thoracic aortic constriction (n>13 per sex/genotype). While their cardiac function is normal, TXLNB-KO males do display slight reductions in heart growth with age (10 months, p=.023, n=7; 18 months, p=.07, n=11-17; 24 months, p=.026, n=3-4), suggesting that TXLNB function may interface with hypertrophic signaling. Together, our findings indicate that TXLNB serves to bolster cardiac UPS, and more importantly, that *UPS dysfunction and proteasomal insufficiency is not sufficient to cause cardiac dysfunction in mice*. Future studies will need to examine if the incongruity with prior reports relates to distinct proteosomal pools (e.g. sarcomeric vs. nuclear vs. ER-associated) and/or the specific identities of the accumulated ubiquitinated proteins.

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Complementary Roles for 3-mercaptopyruvate Sulfurtransferase and Cystathionine γ -lyase in Angiogenesis

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Introduction. Hydrogen sulfide (H₂S) is a signaling molecule with important actions in the cardiovascular system. The major endogenous sources for H₂S in the endothelium are cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulfotransferase (3MST). We have previously shown the CSE-derived H₂S enhances angiogenesis, however, the role of 3MST in new blood vessel formation remains largely unexplored. **Aim.** To evaluate the impact of 3MST on angiogenesis and to study the potential mechanisms involved. **Methods & Results.** 3MST deficient EC exhibited attenuated cell growth, reduced mobility in the scratch wound assay and less sprouting in the fibrin gel bead assay. All of the above-mentioned deficits were less prominent than in cells lacking CSE. In an ex vivo sprouting assay, fewer microvessel-like structures were generated by aortic rings treated with a siRNA directed against 3MST compared to a control oligonucleotide. Similarly, sprouting from 3MST KO aortic rings was less than from wild-type mice. In line with the in vitro responses, angiogenesis in vivo in the Matrigel plug assay and in the retina was reduced in 3MST KO mice, but the decrease was of smaller magnitude compared to that seen with CSE KO mice. The angiogenic switch of EC relies mainly on glycolysis, therefore glycolytic intermediates and TCA metabolites were assessed. Glucose-6P, pyruvate, lactate, α -ketoglutarate and citrate were reduced in CSE KO cells, but not in 3MST KO cells, while fumarate and malate were reduced in 3MST KO, but not CSE KO cells. Moreover, extracellular acidification was reduced in EC lacking CSE, but was only minimally affected in 3MST KO EC. In contrast, oxygen consumption rate was more affected in 3MST KO compared to CSE KO cells. In vivo, 3MST expression was mainly observed in the stalk cells, while CSE was highly expressed in the tip cells. Interestingly, only 3MST ablation resulted in reduced pericyte coverage of retinal vessels. **Conclusion.** We conclude that 3MST and CSE are both required for angiogenesis. 3MST preserves the quiescence of stalk cells by targeting oxidative phosphorylation, while CSE supports tip cell behavior by enhancing their glycolytic capacity.

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MTOR-Proteasome Imbalance Upon Deletion of Pras40 Inhibits Cardiac Growth but Results in Cardiac Failure

Eva RIECHERT, Moritz H. Kern, Agnieszka A. Gorska, Christoph Hofmann, Lonny Jürgensen, Kira Gür, Thanh C. Ho, Univ Hosp Heidelberg, Heidelberg, Germany; Joanna Kirkpatrick, Norman Rahnis, Leibniz Inst on Aging- Fritz Lipmann Inst, Jena, Germany; Hugo A. Katus, Mirko Völkens, Univ Hosp Heidelberg, Heidelberg, Germany

Background The mammalian target of Rapamycin complex 1 (mTORC1) increases cell size by initiating translation as well as by inhibiting catabolic functions such as proteolysis or autophagy. We have previously proposed Proline-rich Akt substrate 1 (Pras40) as a cardioprotective endogenous inhibitor of mTOR-dependent protein synthesis during pathologic growth.

Pras40 is released from mTORC1 during growth, but other interactions are largely unknown. In a proteomic screen we have now found a novel interaction of Pras40 with the 26S proteasome during hypertrophy. We hypothesize that Pras40 directly links the mTOR-dependent protein synthesis network and the proteasomal degradation machinery to balance both processes.

Methods and Results To determine the growth-dependent Pras40-interactome, we identified binding partners by proximity-dependent biotin labeling using APEX2 and subsequent mass-spec. Interestingly, aside from interactions with the mTOR-dependent translation machinery, we find Pras40 binding to subunits of the 26S proteasome only during growth. To test consequences of Pras40 deletion on cardiac function *in vivo*, we generated cardiomyocyte-specific knock-out mice, that we subjected to pathologic hypertrophy (TAC). Conversely to Pras40 overexpression, we find growth significantly blunted in KO animals, function reduced and fibrosis elevated. mTORC1 signaling as well as autophagy and proteasomal function are severely disturbed in KO animals. Mechanistically, chymotrypsin-like 26S proteasome activity is blunted in KO hearts as well as isolated cardiomyocytes from KO animals, whereas overexpression shows reciprocal effects in myocytes. Disturbed proteasomal function in KO mice leads to severe alterations in metabolic functions highlighting the importance of both intact mTORC1 signaling and proper proteasomal maintenance during cardiac stress.

Conclusion In this study we find a novel mechanism how mTOR and proteasomal function are linked in the diseased heart. We provide evidence that Pras40 links anabolic protein synthesis and catabolic proteolysis in the heart: At rest, Pras40 binds and inhibits mTOR, but when released during pathologic growth, Pras40 directly interacts with the 26S proteasome and modulates its activity.

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Semaphorin3e-Plexind1 Signaling is Required for Cardiac Ventricular Compaction

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Left ventricular noncompaction (LVNC) is one of the most common forms of genetic cardiomyopathy characterized by excessive trabeculation and impaired myocardial compaction during fetal development. LVNC patients are at higher risk to develop left or right ventricular failure, or both. While key regulators for cardiac chamber development are well studied, the role of Semaphorin-Plexin signaling in this process remains poorly understood. Here, we demonstrate that genetic deletion of Plxnd1, a class 3 semaphorin receptor in endothelial cells, leads to severe cardiac chamber defects, as characterized by excessive trabeculation and non-compaction similar to LVNC patients. Loss of Plxnd1 results in decreased expression of extracellular matrix (ECM) proteolytic genes leading to excessive deposition of cardiac jelly. We demonstrate that Plxnd1 deficiency is associated with an increase in the expression of Notch and its downstream target genes. In addition, inhibition of Notch signaling pathway can partially rescue the excessive trabeculation and non-compaction phenotype present in Plxnd1 mutants. Furthermore, we demonstrate that Semaphorin 3e (Sema3e), one of Plxnd1's ligands is expressed in the developing heart and is required for myocardial compaction. Collectively, our results demonstrate that the Sema3e-Plxnd1 pathway is essential for myocardial trabeculation and compaction.

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Role of Microglial Calcium Signaling in Ischemic Stroke

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Objectives: During ischemic stroke, the initial injury leads to inflammation and activation of the immune system. Many processes in immune cells are regulated by intracellular calcium signaling. We have asked if microglia, the principal immune

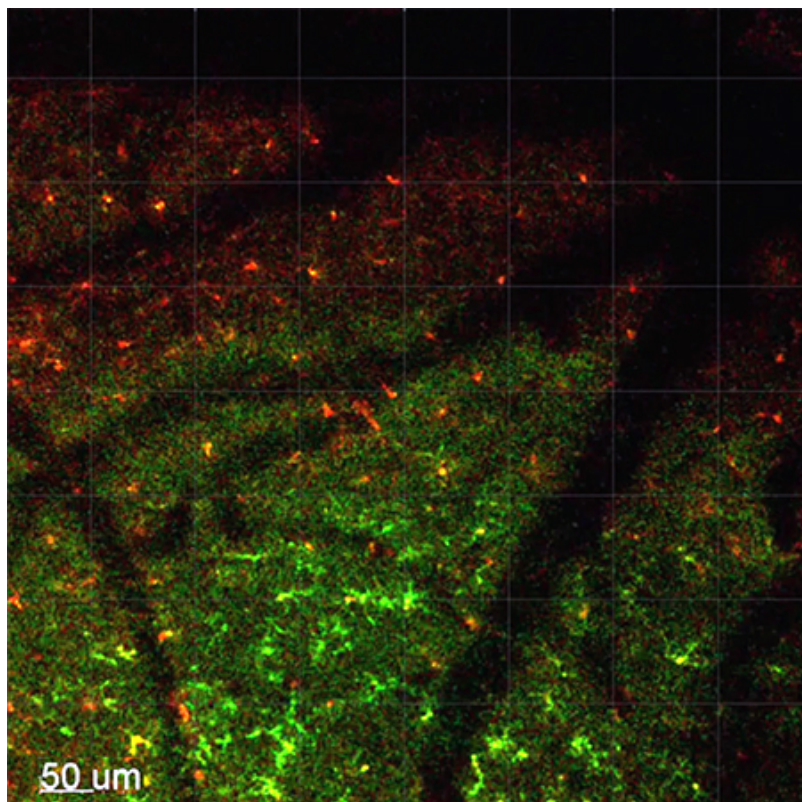
cells of the brain, undergo changes in intracellular calcium signaling during the acute phase of ischemic stroke.

Methods: To address this question, we have developed a mouse model expressing genetically encoded indicators of calcium in microglia. In this system, we induce ischemic stroke by intraluminal occlusion of the middle cerebral artery and investigate microglial activity through sealed craniotomy with fast two-photon laser scanning microscopy.

Findings: We have discovered periodical waves of calcium activity in microglia in the acute phase of stroke. These waves occur frequently in the ischemic penumbra of the cerebral cortex and appear to correlate with cortical spreading depolarizations. We show that amplitudes of these calcium transients increase during stroke progression. We also demonstrate that these transients can be blocked with novel inhibitors of calcium influx developed by CalciMedica, Inc.

Conclusions: We report a novel mechanism of microglial activation during stroke. Our experimental system enables testing of the hypothesis that the inhibition of calcium waves will reduce inflammatory responses, improve neuronal survival and reduce infarct size.

Figure Legend: A two-photon image of a spreading calcium wave in cortical microglia during ischemic stroke. High calcium levels are indicated with green color of the calcium reporter (GCaMP), pre-wave microglia are red (tdTomato) and blood vessels are dark.



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β_2 -adrenergic Stimulation Compartmentalizes β_1 Signaling into Nanoscale Local Domains by Targeting the C-terminus of β_1 -adrenoceptors

Lipeng Wang, Huaqian Yang, Yunyun Gong, Xuexin Fan, Siyu Zhu, Xiaoting Wang, Yupu Wang, Linlin Li, Xin Xing, Xiaoxiao Liu, Guangshen Ji, Tingting Hou, Yan Zhang, Ruiping Xiao, Shiqiang Wang, Peking Univ, Beijing, China

β -adrenergic receptors (β ARs) are prototypical G-protein coupled receptors that play a pivotal role in sympathetic regulation. In heart cells, β_1 R signaling mediates a global cAMP response, regulating both L-type Ca^{2+} channels in the sarcolemma/T-tubules (SL/TTs) and ryanodine receptors in the sarcoplasmic reticulum (SR). In contrast, β_2 AR mediates local cAMP signaling with little effect on the function of SR proteins. Accumulating evidence in pathological and transgenic models has suggested a functional complexity of β_2 AR beyond its regulation of L-type Ca^{2+} influx. So we investigated the signaling relationship between β_1 ARs and β_2 ARs. Using whole-cell patch clamp combined with confocal calcium imaging, we simultaneously measured L-type Ca^{2+} currents and Ca^{2+} transients in adult cardiomyocytes. β_1 AR stimulation with

norepinephrine or isoproterenol increased both Ca²⁺ currents and Ca²⁺ transients. β_2 AR stimulation with Zinterol or Salbutamol only increased Ca²⁺ currents (onside compartmentalization) and surprisingly inhibited the ability of β_1 AR in upregulating Ca²⁺ transients (offside compartmentalization). Further evidence showed that β_2 AR activation recruited G-protein coupled receptor kinase 2 (GRK2) to cell membrane, which phosphorylated the 'SSES' serine cluster on β_1 AR carboxyl terminal. Phosphorylated β_1 AR coupled to β -arrestin1 and further recruited phosphodiesterase-4, leading to localized cAMP degradation. Immunocytochemical and biochemical analysis showed the β_2 AR-mediated offside compartmentalization was confined to the nanoscale domain. A knock-in rat model harboring mutations of the three serine residues in 'SSES' cluster of the β_1 AR C-terminus, a component of the putative β -arrestin1 binding site and GRK2 phosphorylation site, eliminated the offside compartmentalization conferred by β_2 AR activation. This finding reveals a fundamental "negative feed-forward" mechanism that serves to avoid the cytotoxicity of circulating catecholamine and to sharpen the transient β_1 AR response of sympathetic excitation, providing many potential novel targets for drug discovery against cardiovascular diseases.

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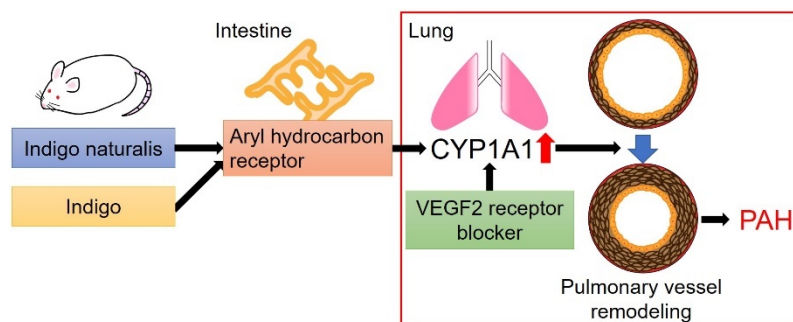
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Indigo Naturalis, a Promising Herbal Medicine for Ulcerative Colitis, Can Induce Experimental Pulmonary Arterial Hypertension via Aryl Hydrocarbon Pathway

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Background: Indigo naturalis is a Chinese herbal medicine, whose dramatical effects for treatment of ulcerative colitis have been reported. However, some patients who took indigo naturalis developed pulmonary arterial hypertension (PAH), which is a poor prognostic disease with right ventricular (RV) failure and progressive remodeling of pulmonary vessels. **Aims:** We examined the reproducibility in experimental animals and elucidate the mechanisms for indigo naturalis to induce PAH. **Methods and Results:** Rats with 12-week intake of low-dose indigo naturalis (60 mg/kg body weight; corresponding to therapeutic dose for patients) did not induce PAH. However, RV and pulmonary vessel remodeling were identified in rats fed with high-dose indigo naturalis (600 mg/kg body weight) for 12 weeks. Significantly elevated RV systolic pressure was identified in rats administered with vascular endothelial growth factor-2 receptor blocker (Sugen 5416) and fed with indigo naturalis for 8 weeks compared to that of rats fed with normal diet (38.6 [38.2, 40.0] mmHg) vs. 31.9 [31.4, 34.6] mmHg, $p < 0.001$). Indigo, a major compound of indigo naturalis, also induced mild PAH. Indigo works as a ligand of aryl hydrocarbon receptor (AhR), and the mRNA levels of CYP1A1, downstream of AhR signal, were elevated in lungs. Rats with indigo naturalis and AhR antagonist gavage developed less pulmonary vessel remodeling than rats without AhR antagonist. **Conclusions:** The combination of Sugem 5416 and indigo naturalis can be a novel unique approach to generate rat PAH model without a hypoxic chamber. The effects of indigo naturalis to PAH might be mediated at least in part through activation of AhR-CYP1A1 pathway.



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Drosophila Adipogenesis Regulates Cardiac Function

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High-calorie diets are associated with obesity, type 2 diabetes, and cardiovascular disease. Recently, several investigators have proposed a model of "maximum adipose expandability," where the limited ability of animals to store fat as triglycerides results in cellular toxicity via fatty acid overflow into alternative fates, or lipotoxicity. Tissue-specific loss-of-function studies targeting metabolic pathways in the *Drosophila* fat storage organ, the fat body, were used to determine the relationship between fat body lipogenesis and cardiac physiology. Our data support a model where when caloric excess overwhelms fat body lipid storage capacity, cardiac lipotoxicity ensues. Metabolomics studies in hearts and blood from these animals may help us to identify specific lipotoxins that are "conserved" between flies and humans.

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Single-Cell RNA-seq Unveils Unique Transcriptomic Signatures of Organ-Specific Endothelial Cells

David T Paik, Lei Tian, Ian M Williams, Chun Liu, Hao Zhang, Damon Williams, Ridhima Mishra, Sean M Wu, Joseph C Wu, Stanford Univ, Palo Alto, CA

Endothelial cells (ECs) display considerable functional heterogeneity depending on the vessel and tissue in which they are located. While these functional differences are presumably imprinted in the transcriptome, the pathways and networks which sustain EC heterogeneity have not been fully delineated. To investigate the transcriptional control of EC specification, we analyzed single-cell RNA-sequencing (scRNA-Seq) data from tissue-specific mouse ECs generated by the *Tabula Muris* consortium. We found a strong correlation between tissue-specific EC transcriptomic measurements generated by either scRNA-Seq or bulk RNA-Seq, thus validating the approach. Using a graph-based clustering algorithm, we found that certain tissue-specific ECs cluster strongly by tissue (e.g. liver, brain) whereas others (i.e. adipose, heart) have considerable transcriptional overlap with ECs from other tissue. Using gene set enrichment analysis, we identified novel markers of tissue-specific ECs and signaling pathways that may be involved in maintaining their identity. By performing pseudotime trajectory analysis, we found that ECs from endoderm-derived tissues appear to be more developmentally immature when compared with the highly specialized ECs of ectoderm-derived tissues such as brain. In addition, we compared these data from mouse with human fetal heart scRNA-seq data for interspecies correlation in organ-specific EC gene expression. Finally, we identified potential angiocrine interactions between tissue-specific ECs and other cell types by analyzing ligand and receptor expression patterns. In summary, we have utilized scRNA-Seq to uncover transcriptional networks which maintain EC identity and identify novel developmental and angiocrine relationships between tissue-specific ECs.

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Integrated Systems Genetics Analysis of Tmem43 Mouse Model and Murine Genetic Reference Population of Bxd Strains Defines Novel Genetic Modifiers and Pathogenic Mechanisms in Arrhythmogenic Cardiomyopathy

Undral Munkhsaikhan, Qingqing Gu, Ramona Sabau, Univ of Tennessee Health Scie, Memphis, TN; Joseph F. Pierre, UNIVERSITY OF TENNESSEE HEALTH SCIE, Memphis, TN; Joseph F. Pierre, Univ of Tennessee Health Scie, Memphis,

Background: Arrhythmogenic cardiomyopathies (ACMs) are heritable diseases characterized by arrhythmias, related sudden death, fibro-fatty infiltration and progressive heart failure. Although the pathogenic variant p.S358L in TMEM43/LUMA causes fully penetrant ACM (ARVC5) in patients, little *in vivo* evidence has been reported and the roles of Tmem43 in normal cardiac function and disease remain obscure and controversial. This study explored Tmem43-associated genetic modifiers using forward, reverse, and systems genetics analyses *in vivo*. **Methods:** Microarray of cardiac and jejunal tissues were performed in a newly created Tmem43^{S358L} knock-in mouse and in forty BXD strains, used as a murine genetic reference population (GRP). Levels of plasma and fecal lipids and Ppar γ activities were defined in heart and jejunum of Tmem43 animals. Immunohistochemistry was performed in the myocardium of patients with non-TMEM43 origin ACM. **Results:** BXD strain cardiac tissues demonstrated high levels of *Tmem43* expression that was significantly negatively correlated with heart mass and heart rate, while positively correlated with plasma HDL and LDL levels. Expression of *Tmem43* was also significantly ($r>0.5$, $p<0.00075$) correlated with *Ppargc1a* (*Pcg1a*) in the heart and intestine of BXDs and Tmem43 mutants. In the Tmem43^{S358L} mouse heart, *Tmem43* and *Ppargc1a* were downregulated resulting in reduced Ppar γ activities. Conversely, *Tmem43* and Ppar α -regulated genes, including *Mogat2*, responsible for cholesterol, bile acid, and lipid absorption and re-esterification, were upregulated in the jejunum, resulting in elevated lipid absorption in the gut lumen and hyperlipidemia in Tmem43^{S358L} mutants. Expression of TMEM43 was disrupted in cardiomyocyte intercalated disks in ACM patients' myocardium in contrast to normal controls. **Conclusions:** *Tmem43* is an essential gene for cardiac and small intestine signaling and function. The S358L-Tmem43 pathogenic variant is significantly associated with downregulation of *Tmem43*, *Ppargc1a* and *Ppar γ* in the myocardium and upregulation of Ppar γ and Ppara signaling in small intestine *in vivo*. Testing plasma lipid levels and TMEM43 expression in ACM patients may provide critical information for personalized predictive care.

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Enhancing Lymphangiogenesis Ameliorates Chronic Heart Failure

Loujin Song, Terri Swanson, Xian Chen, Teresa Cunio, Brianna LaViolette, Shoh Asano, Katherine Hales, Jincheng Pang, Andrew Robertson, Casey Ritenour, Magalie Boucher, Rachel J. Roth Flach, Pfizer Inc, Cambridge, MA

Previous studies have indicated that cardiac lymphatics are involved in the pathogenesis of acute cardiac injuries. However, the role of cardiac lymphatics in the pathogenesis of chronic heart failure remains unclear. The goal of this study is to investigate the role of cardiac lymphatics in the development of chronic heart failure, and to test the hypothesis that improving lymphatic vascular function will have therapeutic benefits in chronic heart failure. First, we characterized lymphatic endothelial marker expression in human hearts from normal or heart failure patients, and a trend towards reduced lymphatic marker expression was observed. These data suggested that lymphatic vessels may be impaired in chronic heart failure. To test this hypothesis further, angiotensin II infusion was used to induce cardiac dysfunction and hypertrophy in mice. Using lymphangiography, we discovered impaired lymphatic transport in angiotensin II-infused mouse hearts compared with control mouse hearts, suggesting impairments in cardiac lymphatic function. Interestingly, when the angiotensin II infused mice were co-administered VEGFCc156s protein to enhance lymphangiogenesis and improve lymphatic vascular function, the VEGFCc156s-treated mice demonstrated improved cardiac function and reduced cardiac hypertrophy compared with angiotensin II infused mice, indicating a potential beneficial effect of VEGFCc156s treatment on angiotensin II-induced cardiac dysfunction and hypertrophy. Overall, these results demonstrate that cardiac lymphatic vessels were impaired in chronic heart failure, and improving lymphatic vascular function may have therapeutic effects on chronic heart failure. The outcome of the study may provide new insights into developing new therapeutics for chronic heart failure.

L. Song: 1. Employment; Significant; Pfizer. **T. Swanson:** 1. Employment; Significant; Pfizer. **X. Chen:** 1. Employment; Significant; Pfizer. **T. Cunio:** 1. Employment; Significant; Pfizer. **B. LaViolette:** 1. Employment; Significant; Pfizer. **S. Asano:** 1. Employment; Significant; Pfizer. **K. Hales:** 1. Employment; Significant; Pfizer. **J. Pang:** 1. Employment; Significant; Pfizer. **A. Robertson:** 1. Employment; Significant; Pfizer. **C. Ritenour:** 1. Employment; Significant; Pfizer. **M. Boucher:** 1. Employment; Significant; Pfizer. **R.J. Roth Flach:** 1. Employment; Significant; Pfizer.

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CUBIC as an Alternative “Clearing” Method for Creating Hydrogel-based Structure in Organs

Meghana Tallam, Qize Yuan, Meg Greer, Matthew W Kay, The George Washington Univ, Washington, DC

Cardiac nerves have a major influence on cardiac health, heart failure, and sudden cardiac death. Understanding the impact of the cardiac nervous system requires in depth cell-specific analysis of the heart, which can be done using tissue clearing followed by 3D microscopic imaging. “Clearing” is the creation of hydrogel-based structures of organs where the lipids are removed without damaging the proteins of the organ. We are interested in using clearing to analyze cardiac neural anatomic pathways with high accuracy. Recently the CUBIC clearing method has gained momentum since it can clear tissue quicker than other methods. While previous research has shown qualitative images and descriptions to describe CUBIC efficacy, quantification of the clearing performance is lacking. The purpose of this study was to compare sequential images of cardiac tissue immersed in CUBIC solution alongside tissue absorbance spectra to measure the efficiency of the CUBIC method. To visualize cardiac neurons, we selectively expressed a fluorescent probe: an enhanced yellow fluorescent protein (EYFP) conjugated with the light-activated channel (channelrhodopsin (ChR2)). Mice were crossbred to express EYFP+ChR2 in parasympathetic cardiac neurons which was driven by a choline acetyltransferase (ChAT) promoter using the Cre-Lox system. Hearts from these mice were excised and perfused with PFA as a fixative and perfused with the CUBIC solution. The tissue then was immersed in CUBIC solution and kept in an insulated shaker at 37° C. Each day, the tissue holder that held the cardiac tissue was taken out of the CUBIC solution and a white light from an LED was directed through the heart and the light intensity was recorded by a spectrometer. Beer-Lambert’s Law related light intensity and transmittance to absorbance to measure daily changes in the absorbance spectrum. Finally, we imaged the right atria of a cleared heart to capture the parasympathetic neural anatomic pathways using 2-photon confocal microscopy. We found that, that over the course of 14 days, the absorbance spectrum of the heart sample decreased over time, with the greatest reduction occurring between days 1 and 4. Future work would include decreasing environmental factors when measuring light intensity.

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Myocardial Ischemia/Reperfusion Impairs Adipocyte Endocrine Function via Exosome-Mediated Endoplasmic Reticulum Dysfunction

Lu Gan, Dina Xie, Wayne Lau, Theodore Christopher, Bernard Lopez, Ling Zhang, Jing Liu, Thomas Jefferson Univ, Philadelphia, PA; Erhe Gao, Walter Koch, Temple Univ, Philadelphia, PA; **Yajing Wang 19107-6731**, Xinliang Ma, Thomas Jefferson Univ, Philadelphia, PA

By incompletely understood mechanisms, MI patients sustain systemic metabolic disorder. Adipocytes are an important cellular type regulating energy homeostasis. The impact of MI upon adipocyte function remains unknown. Exosomes (Exo) are critical vehicles mediating organ-organ communication. However, whether and how Exo may mediate post-MI cardiomyocyte/adipocyte communication have not been previously investigated. Adult male mice were subjected to MI/R. Serum Exo were isolated 3 hours after R and incubated with 3T3L cells for 24 hours. Compared to control, MI/R Exo significantly altered the expression of 17 genes known to be important in adipocyte function. GO analysis revealed that genes associated with endoplasmic reticulum (ER) function and adipocyte endocrine function are the primary two pathways altered by MI/R Exo. Venn analysis identified 11 mi-RNAs as cardiac-enriched, adipocyte-poor, and ER function-related miRNAs. RT-qPCR confirmed the miR-23a/27a/24-2 family members are the most markedly increased mi-RNAs in MI/R Exo. Incubation of 3T3L cells with mi-R27a mimic significantly downregulated EDEM3, DsBA-L, and PPAR γ , and upregulated PERK and CHOP. Conversely, mi-R27a inhibitor significantly decreased the impact of MI/R Exo upon ER function genes. Additional studies demonstrated EDEM3 and PPAR γ (two critical molecules maintaining ER function and adipocyte endocrine function) to be direct targets of mi-R27a. One of the most significant endocrine molecules of adipocyte origin, adiponectin is regulated by PPAR γ at the transcriptional level and by DsBA-L at the post-translational level. We next determined whether MI/R Exo may affect adiponectin expression/assembly. Incubation of 3T3L cells with MI/R Exo significantly inhibited total and high molecular weight adiponectin expression, an effect blocked by miR27a mimic. Finally, in vivo administration of GW4869 (Exo biogenesis inhibitor) or miR27a inhibitor attenuated adipocyte ER dysfunction and restored plasma adiponectin level in MI/R animals. We demonstrate for the first time that MI/R causes significant adipocyte ER and endocrine dysfunction by Exo mediated cardiomyocyte/adipocyte communication via miR-23a/27a/24-2.

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Identification and Characterization of a *Titin* Enhancer using CRISPR/Cas9 Genome Editing and hiPSC-Derived Cardiomyocytes

Meraj Neyazi, Manuel Schmid, Arun Sharma, Christopher N. Toepfer, Yuri Kim, Lauren K. Wasson, Seong Won Kim, Daniel M. DeLaughter, Jon A. L. Willcox, Radhika Agarwal, Angela Tai, Joshua M. Gorham, Steven DePalma, Jonathan G. Seidman, Christine E. Seidman, Harvard Medical Sch, Boston, MA

Dilated cardiomyopathy (DCM) is a leading cause for heart failure and is associated with a rate of mortality of 20% within 5 years of diagnosis. The most common genetic causes for DCM are mutations of the sarcomere protein titin (encoded by *TTN*), which occurs in 10-20% of DCM cases. Dominant DCM mutations truncate titin (TTNtv) and result in haploinsufficiency. Thus, strategies to increase the expression of the wild type *TTN* allele could attenuate damaging effects of TTNtv. Utilizing bioinformatic tools, we identified a putative enhancer for *TTN* in its intron 1. We deleted a 658 bp region from intron 1 which encompasses the region of interest in human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas9 genome editing to validate its function. Utilizing RNA sequencing and qPCR of RNA harvested from hiPSC-derived cardiomyocytes (hiPSC-CMs), we demonstrated that a homozygous deletion in this region leads to a drop in *TTN* expression compared to the wild type (WT) control (0.344-fold change, $p < 0.001$). To further characterize this region, we subdivided it into three parts which we called E1 (296 bp), E2 (206 bp), and E3 (139 bp). E1 includes a highly conserved region and a region of open chromatin as identified by the Assay for Transposase-Accessible Chromatin Sequencing (ATAC-Seq) performed on hiPSC-CMs. A homozygous E1 deletion resulted in a decreased *TTN* expression of 0.63-fold compared to the WT control ($p < 0.001$) when performing RNA sequencing on hiPSC-CMs. Both homozygous E2 and E3 deletions resulted in an increased *TTN* expression (1.56-fold change, $p < 0.001$; 1.19 fold change, $p < 0.001$). Utilizing a published sarcomere tracking platform, SarcTrack, to investigate hiPSC-CM physiology, we saw a decreased contractility of 6.6% in hiPSC-CMs carrying a homozygous E1 deletion compared to 10.1% in the WT control ($p < 0.001$). Cells carrying homozygous E2 or E3 deletions were hypercontractile (13.8%, $p < 0.001$; 13.7%, $p < 0.001$). Given our results, we hypothesize that *TTN* expression depends on the E1 region. If confirmed, we expect that increasing the activity of this enhancer using small molecules may provide a novel therapeutic target for DCM caused by TTNtv.

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Dose-response Effect of Hyperglycemia in Maternal Diabetes Mediated Congenital Heart Defects

Corrin Mansfield, Sathiyarayanan Manivannan, Emily Morris Cameron, Vidu Garg, **Madhumita Basu**, Nationwide Children's Hosp, Columbus, OH

Congenital heart defects (CHDs) are the leading cause of infant morbidity and mortality. The etiology is multifactorial and maternal pre-existing diabetes is a non-genetic risk factor, which perturbs embryonic development and increases the risk of CHDs by 3- to 5-fold. We have previously demonstrated that maternal hyperglycemia (matHG) leads to an increase in reactive oxygen species and reduced nitric oxide bioavailability and found an epigenetic mechanism for altered expression of *Notch1*, a gene critical for endocardial cushion formation, cardiomyocyte (CM) differentiation, outflow tract development, and ventricular trabeculation. We also noted spectrum of cardiac defects in developing embryos when exposed to increased severity of matHG, the underlying molecular basis of which remains unclear. To this end, we hypothesized that matHG alters gene-expression of cardiac progenitor cells in a spatiotemporal manner to predispose embryos to diabetes-induced CHDs. To test cell-specific responses to HG, murine embryonic cell-lines [AVM, atrioventricular cushion mesenchymal and ECC1,

endothelial] were cultured in normal (NG, 5.5mM) and different doses of HG (10, 25 and 40mM). RNA-seq was performed after 48hrs of treatment and gene-set enrichment analysis of AVM NG and HG (25mM) identified genes associated with metabolic processes, response to oxidative-stress (OS), chromatin regulation and altered Wnt/Notch signaling pathways with HG. Similarly, RNA-seq analysis of protein-coding genes in ECC1-HG revealed increasing numbers (8, 311, 1072) of differentially expressed genes compared to NG, and associated with OS, ATP-synthesis, cardiac development, suggesting a dose-response relationship. Additionally, a streptozotocin-induced murine model of diabetes was used to characterize the influence of matHG dose on CHD susceptibility. Examination of E9.5, E11.5, E13.5 embryos displayed defects in CM proliferation, ventricular trabeculation, thin ventricular myocardium and septal defects with matHG. Together, our in vitro transcriptomic analysis and in vivo histologic characterization support a variable molecular and phenotypic response to hyperglycemic stress during cardiac development that contribute to matHG associated CHDs.

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Single-nucleus Transcriptome Analysis of Patients' Heart Tissue Reveals Disease-specific Transcriptional Signatures in Heart Failure

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Single-cell transcriptome analysis is a powerful strategy uncovering heterogeneities of cells and their responses and molecular mechanism in developmental, physiological, and pathological processes. We have applied this technology to cardiac tissue, single-cardiomyocyte RNA-seq analysis of heart failure model mice and human failing heart, and we have uncovered heterogeneous stress response during cardiomyocyte remodeling, showing distinct transcriptional dynamics of the cardiomyocyte gene programs. However, it is difficult to simultaneously analyze transcriptomes of cardiomyocytes and non-cardiomyocytes in the archival heart tissue because of its highly interconnected feature and significant differences in cellular characteristics. We established single-nucleus RNA-seq analysis pipeline to overcome such problems and comprehensively analyze cells consist of heart tissue, to elucidate molecular mechanism in the pathogenesis of heart failure, combining the data with clinical phenotype of the disease. Single-nucleus analysis of the frozen mouse heart successfully reclassified cardiac cells by their transcriptomic features. By comparing single-nucleus RNA-seq profiles with single-cell RNA-seq profiles, we found a significant difference of mRNA localization between the cytosol and nucleus. mRNA of nuclear mitochondrial genes is preferentially localized in the cytosol, whereas that of extracellular matrix genes is in the nucleus. We also conducted single-nucleus RNA-seq analysis of the heart from patients with heart failure, again successfully reclassified cell types, and found disease specific transcriptomic landscape in heart failure. We are going to integrate single-nucleus gene expression profiles with the phenotypic characteristics such as treatment response and clinical prognosis, to reveal the molecular characteristics useful for cardiovascular precision medicine.

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Increased G_{α_o} Expression Plays a Pivotal Role in the Progression of Heart Failure by Impairing Ca^{2+} Handling

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Background: A transcriptional repressor, neuron restrictive silencer factor (NRSF), maintains normal cardiac function and electrical stability. Transgenic mice expressing a dominant-negative mutant of NRSF in their hearts (dnNRSF-Tg) exhibit

systolic dysfunction and premature death due to lethal arrhythmias like human dilated cardiomyopathy(DCM). Underlying mechanisms remain to be elucidated, however.

Purpose: To clarify how NRSF maintains normal cardiac homeostasis.

Methods and Results: cDNA microarray analysis of dnNRSF-Tg and newly generated cardiac-specific NRSF knockout mice (NRSF^{cKO}), which show similar cardiac phenotypes to those of dnNRSF-Tg, revealed that cardiac gene expression of GNAO1 that encodes G α_o , a member of inhibitory G protein G α_i family, is increased in both dnNRSF-Tg and NRSF^{cKO} ventricles.

ChIP-seq analysis, reporter assay and electrophoretic mobility shift assay identified that GNAO1 is a direct target of NRSF. In dnNRSF-Tg, pharmacological inhibition of G α_o by pertussis toxin improved systolic dysfunction and knockdown of G α_o by crossing with GNAO1 knockout mice improved not only systolic function but also frequency of ventricular arrhythmias and survival rates.

Electrophysiological analysis in ventricular myocytes obtained from dnNRSF-Tg demonstrated that genetic reduction of G α_o ameliorated abnormalities in Ca²⁺ handling, which include increased current density in surface sarcolemmal L-type Ca²⁺ channel, reduced content of sarcoplasmic reticulum Ca²⁺ and lowered peak of Ca²⁺ transient.

In addition, genetic reduction of G α_o attenuated increased phosphorylation levels of CAMKII and RyR2 in dnNRSF-Tg ventricles, which presumably underlies the improvement in Ca²⁺ handling.

Furthermore, we identified increased G α_o expression in ventricles of heart failure model mice induced by transverse aortic constriction and cardiac troponin T mutant DCM model mice, in both of which, genetic reduction of G α_o ameliorated cardiac dysfunction.

Conclusions: We found that increased expression of G α_o , induced by attenuation of NRSF-mediated repression, plays a pivotal role in the progression of heart failure by evoking Ca²⁺ handling abnormality. G α_o is a potential therapeutic target for heart failure.

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The Long Noncoding RNA Landscape of the High Altitude Induced Thromboembolic Disorder: Role of Endogenous miRNA Sponge

Prabhash Kumar Jha, Aatira Vijay, Amit Prabhakar, DIPAS, DRDO, Delhi, India; Tathagat Chatterjee, Army Hosp (RR), Delhi, India; Nitin Bajaj, Command Hosp, Chandimandir, Chandigarh, India; Velu Nair, Armed Forces Medical Coll, Pune, India; Bhuvnesh Kumar, Mohammad Zahid Ashraf, Manish Sharma, DIPAS, DRDO, Delhi, India

Background: The pathophysiology of Deep vein thrombosis (DVT) is considered as multifactorial, where thrombus formation is interplay of genetic and acquired risk factors. Lately, different pipelines have been developed to identify novel lncRNAs in different diseases, however a little is known about the expression profile and roles of lncRNAs in human subjects developing DVT at high altitude.

Methods: In the present study, using RNA sequencing, we compared peripheral blood lncRNA expression profile in human High Altitude deep Vein Thrombosis (HA-DVT) patients with high altitude control and sea level control human subjects. We used DESeq to identify differentially expressed (DE) genes. We identified the noncoding RNAs by annotation database that includes lncRNAs from the NONCODE 3.0 human noncoding RNA database. This was followed by the functional characterization of the identified DE lncRNAs by Co-expressed protein-coding genes analysis. We further performed *in silico* putative lncRNA-miRNA association study to unravel the endogenous miRNA sponge associated with our candidate lncRNAs. Finally, to validate the *in silico* findings, siRNA knockdown assay of the candidate lncRNAs was conducted in primary endothelial cells

Results: We identified 973 differentially expressed lncRNAs. Co-expressed protein-coding genes analysis resulted in a list of 722 coexpressed protein-coding genes with a Pearson correlation coefficients >0.7. The functional annotation of co-expressed genes and putative proteins revealed their involvement in the hypoxia, immune response and coagulation cascade. Putative lncRNA-miRNA association analysis revealed several miRNAs associated with cardiovascular disorders. Through its miRNA response elements (MREs) to compete for miR-143 and miR-15, lncRNA-LINC00659 and UXT-AS1 regulates the expression of prothrombotic genes. Furthermore, *in vitro* RNA interference (siRNA) simultaneously suppressed lncRNAs and target gene mRNA level.

Conclusions: This transcriptome profile describes several potential mechanisms of interaction between lncRNAs, the coding genes, miRNAs and regulatory transcription factors that define the thrombotic signature and may be used in establishing lncRNAs as biomarker in HA-DVT.

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Protein Arginine Methyltransferase 6 Controls Cardiac Hypertrophy by Differential Arginine and Lysine Methylation of Histone H3

Vineesh Raveendran, Kamar Al-Haffar, Muhammed Kunhi, King Faisal Specialist Hosp and Res Ctr, Riyadh, Saudi Arabia; K Behlai, Coll of Med, Al-Faisal Univ, Riyadh, Saudi Arabia; Waleed Al-Habib, King Saud Univ, Riyadh, Saudi Arabia; Atli Eyjolsson, Jihad Al-Buraiki, King Faisal Specialist Hosp and Res Ctr, Riyadh, Saudi Arabia; **Coralie I Poizat**, Masonic Medical Res Inst, Utica, NY

Heart failure remains a common cause of hospitalization and death worldwide. Heart failure can be caused by dysregulation of gene expression following abnormal expression of histone modifying enzymes. While lysine acetylation and methylation of histones have been the topic of many investigations, the role of arginine methylation in H3 has been overlooked. In an effort to understand regulatory mechanisms implicated in cardiac hypertrophy and heart failure, we assessed protein arginine methyl transferase (PRMT) members in the left ventricle of failing human hearts and control hearts. Our results show a specific up-regulation of PRMT6 mRNA in failing human hearts (5.95 ± 2.16 fold in failing versus control, $p=0.003$) which also occurs in the compensatory phase of cardiac hypertrophy in mouse hearts subjected to pressure overload hypertrophy, and in neonatal rat ventricular myocytes (NRVM) stimulated with the hypertrophic agonist phenylephrine (PE). These changes are associated with a significant increase in arginine 2 asymmetric methylation of H3 (H3R2me2a) and reduced lysine 3 trimethylation of H3 (H3K4me3) both in NRVM and *in vivo*. Importantly, forced expression of PRMT6 in NRVM stimulated with PE, enhances the expression of atrial natriuretic peptide (ANP). Conversely, silencing of PRMT6 reduces ANP protein expression and cell size, indicating that PRMT6 is critical for PE-mediated cardiac hypertrophy of NRVM. Also, silencing of PRMT6 reduces H3R2me2a, a mark associated with transcriptional repression. To evaluate the role of PRMT6 on cardiac contractility and global ion channel activity, we assessed contractility and global field potentials in live NRVM expressing normal and low level PRMT6 using the RTCA CardioECR system (ACEA Bioscience Inc.). Strikingly, reduced expression of PRMT6 drastically inhibits the contraction rate of NRVM, which is paralleled by a slight increase in the QT interval. All together, our results indicate that PRMT6 is a critical regulator of cardiac hypertrophy, implicating H3R2me2a as an important histone modification. Future studies investigating the specific gene programs regulated by PRMT6 are on their way. This study may help identify novel points of control to design new drugs for the treatment of heart failure.

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Construction and Application of an Epigenetic Atlas of the Human Heart

Dimple Prasher, Univ of Calgary, Calgary, AB, Canada; Hao Zhang, Univ of Alberta, Edmonton, AB, Canada; Patrick M McCarthy, Northwestern Univ, Chicago, IL; Gavin Oudit, Univ of Alberta, Edmonton, AB, Canada; Paul W M Fedak, Cumming Sch of Med, Univ of Calgary, Calgary, AB, Canada; Steven C Greenway, Alberta Children's Hosp, Univ of Calgary, Calgary, AB, Canada

Epigenetic modifications, including DNA methylation, regulate gene expression and contribute to the differentiation of cells. Tissues are characterized by methylation patterns that reflect their specific functions and origin. Currently, there is limited knowledge of the epigenetic patterns of the heart. To address this gap, we generated methylation profiles for all anatomical regions of the human heart. We hypothesize that unique differentially methylated regions (DMRs) of DNA exist for each cardiac region and that these patterns are disrupted in heart failure.

Using non-diseased cardiac tissue and reduced representation bisulfite sequencing on a Illumina platform, we generated genome-wide methylomes for the human right atrium (n=4), left atrium (n=4), right ventricle (n=4), left ventricle (n=4), aorta (n=3), pulmonary artery (n=2), mitral valve (n=3), tricuspid valve (n=3), aortic valve (n=3) and pulmonary valve (n=3). DMRs, defined as regions with significantly different mean methylation differences, were identified using Metilene.

For each tissue we identified between 10-20 million reads covering 8-10 million CpG methylation sites and 4-228 tissue-specific DMRs. There were relatively few (4) different DMRs between the left and right ventricles but 228 unique DMRs were found in the vessels (aorta and pulmonary artery) compared to the ventricles including regions upstream of *BMP3* and *FOXC1*, genes implicated in cardiogenesis. We then applied this approach and normal data to the analysis of disease. We generated additional left ventricular methylomes from adult (n=3) and pediatric (n=3) patients with heart failure.

We identified 19 unique DMRs in the adult group and 107 in the pediatric group. Genes associated with pediatric HF DMRs included *ROCK1* and *FBLN2* that have been previously implicated in contraction and remodelling. We have created an atlas of the human heart based upon differences in DNA methylation between the anatomical regions of the human heart. This data will help increase our understanding of cardiac development, identify new disease biomarkers and have application to a wide range of cardiac diseases

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Long Noncoding RNA Ppp1r1b Regulates Myogenic Differentiation Through Modulating Histone 3 Methylation

Xuedong Kang, Yan Zhao, Joseph Wu, Stanley F Nelson, **Marlin Touma**, UCLA-David Geffen Sch Med, Los Angeles, CA

Introduction: Emerged as critical epigenetic regulators of the transcriptome, the long noncoding RNAs (lncRNAs) play important roles in cardiac development and might be targeted to treat human cardiomyocyte dysfunction in congenital heart disease. In our work, we identified a novel lncRNA that regulates myogenic differentiation. **Methods:** Antisense oligonucleotides (GapmeR) were used to suppress Ppp1r1b-lncRNA. Chromatin immunoprecipitation (CHIP) and chromatin isolation by RNA purification (CHIRP) were used to analyze gene specific histone modification level and lncRNA:chromatin interaction, respectively. RNA pull-down and RNA immunoprecipitation (RIP) were used to identify the protein molecules that interact with Ppp1r1b-lncRNA. **Results:** By silencing Ppp1r1b-lncRNA, C2C12, a skeletal myoblast cell line, and human-induced pluripotent stem cell derived cardiomyocytes failed to develop fully differentiated myotubes. Analysis of GapmeR injected neonatal mouse heart (*in vivo*) and siRNA silenced human myoblasts (*in vitro*) further confirmed the role of Ppp1r1b-lncRNA in normal myogenesis. Members of the MyoD family of muscle-specific transcription factors were not induced during myogenic differentiation in C2C12 cells treated with Ppp1r1b-lncRNA specific GapmeR. Proteins essential for striated muscle development were also suppressed in Ppp1r1b-lncRNA- deficient cells. Histone modification analysis revealed enrichment of histone tri-methylation at Myogenin and MyoD1 promoters in GapmeR treated C2C12 cells. Subsequently, lncRNA- protein complex isolation and lncRNA-DNA interaction assays confirmed Ppp1r1b-lncRNA function. **Conclusions:** Our findings support important role of Ppp1r1b-lncRNA in promoting myogenic differentiation. Ppp1r1b-lncRNA function is mediated by inhibiting histone methylation on promoters of key myogenic genes. Particularly, the identification of EZH2 in pulled Pp1r1b-lncRNA: protein complex supports that Polycomb repressive complex 2 is involved in Ppp1r1b-lncRNA modulated myoblast differentiation. The recovery of MyoD1 promoter DNA in isolated lncRNA: chromatin DNA interactome implies that Ppp1r1b-lncRNA regulates transcription by recruiting PRC2 complex to its target genes.

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RNF20/40 Regulates Cardiomyocyte Maturation

Nathan J Vandusen, Julianna Y Lee, Wei-liang Gu, Isha Sethi, Shengbao Suo, Justin King, Yanjiang Zheng, Yuxuan Guo, William T Pu, Boston Children's Hosp, Boston, MA

Between birth and adulthood, cardiomyocytes (CMs) undergo profound changes in size, ultrastructure, metabolism, and gene expression, a process collectively referred to as CM maturation. Although highly coordinated, the transcriptional network that governs this process is not understood. This lack of understanding is a barrier to cardiac regenerative medicine, where our current inability to mature CMs differentiated from non-myocytes limits their use for disease modeling or replacement therapy. In addition, disruption of maturation by abnormal hemodynamic loads in neonates who have undergone surgery to correct congenital heart defects likely contributes to their high incidence of heart failure in adulthood. A sound understanding of the regulatory network governing CM maturation will inspire hypothesis driven attempts to surmount these challenges.

In mice, a key hallmark of CM maturation is sarcomere isoform switching, including the well documented neonatal switch from Myosin Heavy Chain 7 (Myh7) to Myosin Heavy Chain 6 (Myh6). We have conducted and validated an *in vivo* high throughput CRISPR screen for transcriptional regulators of CM maturation, using the Myh7/6 isoform switch as the readout.

Two top candidates from this screen, Rnf20 and Rnf40, form a complex which deposits the epigenetic mark H2bub1 (histone-2B mono-ubiquitinated on lysine 120). Defects in RNF20/40 and H2Bub1 regulation have been associated with human congenital heart disease, but their mechanistic function in the heart has not been studied. We performed ChIP and RNA-sequencing experiments in control and RNF loss-of-function models to characterize the role of H2Bub1 in transcriptional control of CM maturation. The resulting mechanistic insights into how gene expression is coordinately controlled during maturation will inform efforts to improve CM production protocols and develop targeted therapies.

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Poster Session 3 and Reception

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Recellularization of Acellular Xenogenic Scaffold With Autologous Human Mesenchymal Stem Cells Rescues the Xenoreactive Immune Response

Sabin J Bozso, Jimmy Kang, Benjamin Adam, Michael C Moon, Darren H Freed, Jayan Nagendran, Jeevan Nagendran, Univ of Alberta, Edmonton, AB, Canada

Background: Pre-clinical evidence suggests that structural valve deterioration (SVD) of bioprosthetic xenogenic tissue heart valves (XTHV) is a result of chronic immune rejection. Recellularization of XTHVs with human autologous cells represents one potential strategy to prevent this xenoreactive immune response and mitigate SVD.

Methods: Bone marrow, human pericardium and heparinized whole blood was collected from adult patients undergoing elective cardiac surgery. Decellularized bovine pericardium underwent recellularization with hMSCs, which were isolated and cultured from the bone marrow, via co-incubation to allow for cell seeding. To examine the cell mediated immune response, we performed immunofluorescent staining for CD68+ macrophages and CD3+ T-cells and performed a proliferation assay to quantify T-cell proliferation. To investigate the humoral immune response, we performed an enzyme linked immunosorbent assay for pro-inflammatory cytokines TNF- α and INF- γ . Serum and PBMCs were collected for subsequent biochemical and flow cytometric analysis.

Results: We show that decellularized bovine pericardium, exposed to human serum, had significantly decreased T-cell activation, represented by TNF- α and INF- γ expression, as well as a significant decrease in T-cell proliferation, when compared to wild-type bovine pericardium ($p < 0.01$). Moreover, when decellularized bovine pericardium was recellularized with autologous hMSCs and exposed to human serum, there was an additional decrease in TNF- α , INF- γ expression along with a further significant decrease in T-cell proliferation, when compared to both wild-type and decellularized bovine pericardium ($p < 0.01$). Importantly, recellularized XTHVs exposed to human serum had an equivalent expression of TNF- α , INF- γ and T-cell proliferation when compared to native human pericardium exposed to autologous human serum.

Conclusions: Taken together, our data suggest that autologous human MSC recellularization of decellularized bovine pericardium abrogates the xenoreactive immune response. As such, autologous hMSC recellularization of an acellular xenogenic scaffolds may be an effective approach to decrease the progression of bioprosthetic SVD.

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Mesenchymal Stem Cells Overexpressing FGF21 Improve Functional Recovery After Traumatic Brain Injury

Kai-Yun Chen, Rami Ahmad Shahrour, Chung-Che Wu, Yung-Hsiao Chiang, Taipei Medical Univ, Taipei, Taiwan

Traumatic Brain Injury (TBI) is a progressive and complex brain injury that results in many adverse and long term neurological consequences. Fibroblast growth factor 21 (FGF21) is a novel metabolic regulator that has emerged as a therapeutic agent for the treatment of neurodegenerative diseases and brain injuries, as it has been shown to exhibit neuroprotective effects, promote remyelination, enhances angiogenesis, and to stimulate the neurite growth of glia-like cells. In this study, MSCs were genetically engineered to overexpress fibroblast growth factor 21 (FGF21) in order to improve their efficacy in TBI. MSCs overexpressing FGF21 (MSC-FGF21) were transplanted to mouse brain by intracerebroventricular (ICV) injection 24 hours after TBI. Spatial learning and memory tests were performed to examine the effects of MSC-FGF21

at 24 hours following controlled cortical impact (CCI) insult in TBI mice model. We found the FGF21 levels were reduced in the hippocampus of vehicle- treated group mice and that MSC-FGF21 treatment restored the FGF21 level significantly. MSC-FGF21 treatment significantly reduced spatial learning/memory decline at 21 days post injury as measured in the Morris water maze test. Both MSC-mCherry (vehicle control) and MSC-FGF21 treatments induced a significant improvement in short term memory formation as determined by the novel object recognition test (NOR) after 14 days of injury. In addition, MSC-FGF21 treatment significantly increased the impaired neurogenesis and restored the dendritic process and morphology of immature neurons in the hippocampal dentate gyrus (DG). Taken together, these data provide compelling evidence that MSC-FGF21 treatment promotes neurogenesis and hippocampal neuroplasticity following TBI insult.

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Fusion of Cardiac Progenitor Cell and Cardiomyocyte Induces Cardiomyocyte Division

Zhongming Chen, Ingrid Bender, Jop van Berlo, Univ of Minnesota-Twin Cities, Minneapolis, MN

Recent evidence has shown that cardiac progenitor cells (CPCs) can fuse with existing cardiomyocytes (CMs). The fusion frequency significantly increases during heart injury or drug treatment, but the role of CPC-CM fusion in heart regeneration is largely unknown. In this study, we investigate CM-CPC fusion in vivo and its effects on CM proliferation in vitro using genetic lineage tracing and isolated cardiac cells from adult mice. To distinguish between nuclear fusion and membrane fusion, we utilized double fluorescent nuclear reporter mice. After one month of tamoxifen chow treatment, as many as 78% (n=4) of fused CMs displayed cytoplasm fusion, but not nuclear fusion. To further study the role of cytoplasm fusion in CM proliferation, we designed in vitro experiments where we either induce partial fusion or co-culture freshly isolated CMs with previously isolated c-kit+ cells. We find that 52% (387 of 741 cells, n=10) of the fused CMs maintain rod-shape morphology for over two months during culture, while all CMs ball up and dedifferentiate in co-culture controls (n=10) within 24 hours. Next, EdU was added to the culture media to assess DNA replication. EdU+ CMs were observed in both rod-shape and rounded CMs in 7 days. Finally, we monitored cell division of rod-shaped CMs fused with c-kit+ CPCs using intermittent live-cell imaging. We identified 2 cell division events from 20 randomly monitored rod-shape CMs in two months after fusion. These results demonstrate that fusion of c-kit+ cells with CMs are typically partial fusion events, that may play a role in regulating CM proliferation.

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Poster Session 3 and Reception

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Transplantation of Modified Mesenchymal Stem Cells (MSCs) Helps Reduce Systemic Inflammation in Diet-Induced Obese Mouse Model

Cleyton C Domingues, Nabanita Kundu, Yana Kropotova, Neeki Ahmadi, Sabyasachi Sen, George Washington Univ, Washington, DC

Background: Mesenchymal stromal cells (MSCs) are adult multipotent cells that can home-in to inflamed fat depots. Here we used MSCs to help reduce oxidative stress in the inflamed adipocyte depots in DIO mouse models. We delivered antioxidant upregulated modified MSCs to help reduce systemic inflammation and cardiovascular complications in diet-induced obese (DIO) diabetic mice. **Methods:** Antioxidants Sod2 (mitochondrial) and Catalase (cytosolic) genes were upregulated individually or in combination using adenovirus (Ad) in adipose-derived MSCs. Ad-Null upregulated MSCs were used as control. Modified MSCs were examined invitro in presence of adipogenic media to mimic DIO milieu. Next, modified MSCs were delivered intraperitoneally in mice fed 45% or 60% high-fat diet. **Results:** In-vitro results indicated a reduction in MSCs lipid droplet accumulation when antioxidants were upregulated in comparison to Null-MSCs. Inflammatory marker TNFa mRNA showed a trend for a downregulation in Sod2+Cat combination-MSCs when compared to either Null-MSCs or Sod2 and Cat individually upregulated MSCs. In-vivo results showed an improvement of glucose tolerance in mice that received Sod2-MSCs at week 4 post MSC delivery (p=0.07). Plasma inflammatory marker TNFa was reduced in both Sod2- and Cat-MSC groups (p<0.05). Analysis of omental and pericardial fat showed significant upregulation in mRNA expression of brown fat marker, Ucp1 (~1000 and 100-fold, respectively) and PGC1A mRNA was also upregulated in pericardial fat. A

reduction in liver fat content was observed by histology and it was confirmed by liver triglyceride assay ($p < 0.05$). Analysis of results following Sod2+Cat-MSC delivery is pending. **Conclusion:** In-vitro upregulation of Sod2+Catalase seems to reduce inflammation in adipogenic condition better than Sod2 or Cat individual upregulated MSCs. Whereas in vivo Sod2 and Cat upregulated individually in MSCs reduced systemic inflammation and improved glucose tolerance with concomitant increase of browning of white fat and reduction of liver fat in DIO mice. Therefore, delivery of antioxidant upregulated MSCs could be a safe tool to help reduce oxidative stress and systemic inflammation and reduce CVD risk associated with obesity and diabetes.

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Profiling Differential Response to Myocardial Infarction by Single Cell Analysis of the Cardiac Interstitium

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Myocardial infarction (MI) is one of the main causes of mortality in Western Countries. The non-regenerative adult mammalian heart responds to injury with the formation of a scar. The outcome of repair is highly dependent on interstitial cells and on the delicate interplay between the inflammatory response and fibrosis, which leads to adverse ventricular remodeling and heart failure or cardiac rupture in the early phase post-injury. Our data show that inbred mice strains have different reliance to MI, suggesting differences in the cellular response to the injury. To profile the dynamic response of cardiac interstitial cells to MI and their embryological origin, we performed unbiased scRNAseq analysis of cardiac interstitial cells at homeostasis and 1, 3, 5, 7, 14, 28 days post-injury from transgenic mice on C57bl/6j background (B6), expressing ZsGreen under the control of the epicardial marker *Wt1* (*Wt1^{Cre}; RosaZsGreen^{fl/+}*). About 38,600 cells were captured using the 10xChromium technology and we distinguished 16 main cell populations that evolved over time. By high resolution sub-clustering of stromal cells, 7 main subpopulations were identified. Of these, one was endocardial derived and present at all time points, the others were epicardial derived (ZsGreen+) and included 3 populations of fibroblasts present at different frequencies at all time points, a transient population of immune-response activating fibroblasts (IR) prevailing at day1 and preceding the appearance of myofibroblasts between day 3 and 7 and matrifibrocytes prevalent at day 14 and day 28. To determine the role of cell composition in different reparative outcomes, we compared the data on the C57BL/6J background with 129S1/SvImJ (129), the inbred strain presenting the highest frequency of cardiac rupture (70%) among the 9 tested. We observed increased number of activated IR and myofibroblasts in 129 3 days post-MI. 129 also showed higher level of collagens, chemokines, fibrin deposition and acute phase response genes. The hyperactivation of myofibroblasts was possibly mediated by the enhanced responsiveness to the Renin-Angiotensin System, currently one of the main targets of clinical therapies.

E. Forte: None.

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Synergistic Activation of the Cardiac Enhancer Landscape During Cardiac Reprogramming

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Direct cardiac reprogramming of fibroblasts to cardiomyocytes is an attractive therapeutic strategy to restore cardiac function following injury. The cardiac transcription factors *Gata4*, *Mef2c*, and *Tbx5* are sufficient to directly reprogram fibroblasts to a cardiac fate and their cardiogenic activity is enhanced by the addition of *Hand2* and *Akt1*. However, the mechanisms by which these transcription factors orchestrate this cell fate conversion remains elusive. To understand the mechanistic basis of cardiac reprogramming, we performed a genome-wide analysis of cardiogenic transcription factor binding sites and enhancer activation throughout cardiac reprogramming. We found that cardiogenic transcription factors act cooperatively by co-occupying regulatory elements enriched with *Mef2* binding sites during cardiac reprogramming. Importantly, these transcription factors when overexpressed in isolation are incapable of activating reprogramming enhancer elements. Further, we discovered that the early reprogramming enhancer landscape most closely resembles that of the neonatal heart. Acquisition of enhancers associated with cardiac maturation occurred at later stages in reprogramming, with addition of

Hand2 and Akt1 augmenting activation of this more mature enhancer landscape. Additionally, we constructed a cardiac reprogramming gene regulatory network which demonstrated downregulation of the EGF receptor signaling pathway. We found that inhibition of EGF receptor signaling augments reprogramming toward the cardiac phenotype. Our findings demonstrate that cardiac reprogramming is coordinated by synergistic transcriptional activation across a broad landscape of cardiac enhancers and define the epigenetic landscape of the cardiac phenotype.

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Changes in Translational Efficacy During Zebrafish Heart Regeneration

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Zebrafish are able to regenerate heart muscle after catastrophic injury. Fervent research is underway to discover critical molecules with therapeutic potential, yet many principal biological processes that underlie regeneration likely remain undiscovered. Changes in the abundance of dozens to thousands of mRNA have been reported using both targeted approaches like in-situ hybridization and unbiased approaches like RNA-seq. However, whether the abundance of mRNA represents the entirety of gene expression control remains unknown. Regulation of translation may alter the abundance of a critical protein product without requiring an intervening transcription event. Here, we have taken advantage of ribosome profiling to document the changes in translation efficiency that accompany heart regeneration. Using high-throughput sequencing of the ~28 nucleotide fragments of mRNA protected from enzymatic degradation, we find thousands of transcripts that change in their association with ribosomes. We also find that key components of the translational machinery are transcriptionally induced during regeneration, supporting a general model in which regulation of translation is a fundamental component of zebrafish heart regeneration.

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Cortical-bone Stem Cell Therapy Alters the Inflammatory Response After Myocardial Infarction

Alexander Hobby, Remus Berretta, Giulia Borghetti, Eric Feldsott, Deborah Eaton, Hajime Kubo, Sadia Mohsin, Steven Houser, Temple Univ, Philadelphia, PA

Ischemic heart diseases like myocardial infarction are the largest contributors to cardiovascular disease world-wide. The resulting cardiac cell death impairs function of the heart and can lead to heart failure and death. Re-perfusion of the ischemic tissue is necessary but causes damage to the surrounding tissue by re-perfusion injury and initiates a sterile inflammatory response. Cortical bone stem cells (CBSCs) have been shown to increase pump function and decrease scar size in a large animal swine model of myocardial infarction. To explore the potential cause of these changes, we hypothesized that CBSCs were altering the inflammatory response after re-perfusion thereby changing the wound healing process. To test this, we performed serial immune cell analysis of the blood and tissue from Gottingen mini-swine that underwent 90 minutes of lateral anterior descending coronary artery ischemia followed by 7 days of re-perfusion to assess changes in immune cell recruitment and phenotype. Our findings indicate that CBSCs modify the cytokines released by immune cells in the infarct, decrease macrophage infiltration of the infarct 3 days after MI, and increase the recruitment of CD4+ T-cells to the infarct zone 7 days after MI. In addition, these changes reflect a pro-healing inflammatory environment. From this data, we conclude that CBSCs are influencing immune cell recruitment dynamics and phenotype, and these changes may contribute to the decreased scar size and increased pump function seen in CBSC-treated animals.

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Platelet Derived Growth Factor-Beta Communication Axis is Critical for Mediating Pericyte-Endothelial Cell Interactions and Angiogenic Support in Combination Therapy of Human Umbilical Cord Perivascular Cells and Endothelial Progenitor Cells

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Introduction: First Trimester Human Umbilical Cord Perivascular Cells (FTM HUCPVC) have higher cardiovascular regenerative potential compared to older sources of MSC. We designed a cell combination therapy with FTM HUCPVC and endothelial progenitor cells (EPC) to enhance angiogenesis after MI. However, the mechanism of PVC-EPC interaction is unclear. **Hypothesis:** Platelet derived growth factor beta receptor (PDGFB-R) expression of HUCPVC mediates homing and integration with endothelial networks and required for supporting neovasculature. **Methods:** Expression of pericyte markers PDGFB-R and CD146 of xeno-free expanded (HPL 2.5%) FTM and term HUCPVC was analyzed by flow cytometry. PDGFB-driven trans-ECM migration was assessed by Corning Biocoat Invasion assay with 1-100 ng/ml PDGFB. siRNA for PDGFB-R, CD146 and scrambled control was used with lipofectamine for 72 hours. Silencing was confirmed by qPCR and flow cytometry. 10% HPL was used for PDGFB-R desensitization. HUCPVC of all treatment groups were co-cultured with EPC and rat aortic rings on Matrigel™. Cell migration, endothelial adhesion and network properties were quantified. **Statistics:** one-way ANOVA N=5 **Results:** PDGFB-R and CD146 expression and endothelial network augmenting function of FTM HUCPVC was significantly higher than of term HUCPVC. PDGFB dependent migration of FTM HUCPVC was disabled by both ligand (<10%) and siRNA-based (<5%) downregulation of PDGFB-R. Fluorescence microscopy (24 h) showed limited homing and endothelial interactions of PDGFB-R-reduced FTM HUCPVC. PDGFB-R-reduced FTM HUCPVC had significantly decreased network growth and nodes ($p<0.0001$) compared to control groups (day3). In EPC co-cultures, PDGFB-R-reduced FTM HUCPVC had limited endothelial interactions and coverage compared to control FTM HUCPVC. PDGFB-R-reduced FTM HUCPVC treated networks had significantly thinner ($p<0.001$) and shorter tubules ($p<0.001$) compared to control FTM HUCPVC groups. Down-regulation of C146 did not alter FTM HUCPVC pericyte properties. **Conclusions:** PDGFB-R is crucial for the homing and endothelial support of HUCPVC. This interaction is critical in vascular cell combination therapies and PDGFB-R expression can be a novel criterion for cardiovascular cell therapy.

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DNA Damage-free iPSC-cardiomyocyte Reduces Cardiac Fibrosis Through Downmodulation of Exosomal miR-101

Ramaswamy Kannappan, Jessica M Miller, Vasanthi Rajasekaran, Namakkal Rajasekaran Soorappan, Jianyi Zhang, Univ of Alabama at Birmingham, Birmingham, AL

With the growing field of myocardial repair through transplantation of cardiomyocytes (CM) derived from human induced pluripotent stem cells (iPSC) calls for a method for reducing fibrotic tissue development at the site of cell transplantation. We tested the hypothesis of whether transplanting CMs derived from DNA damage-free (DdF) iPSCs could reduce the recruitment of myofibroblasts and reduce fibrosis at the site of transplantation. Transcription factor p53 functions are DNA damage-dependent: induce apoptosis in DNA damaged cells while promoting cell cycle in normal cells. Utilizing this discriminating nature of p53 we selected iPSCs that are DdF and differentiated them into CMs. Control CMs (Ctrl-CM) and DdF-CMs were tested for transplantation induced fibrosis in a mouse model. The inflammatory markers such as accumulation of ROS, superoxide and DNA damage were significantly reduced in DdF cells. RNA sequencing revealed that, in DdF-CMs, the inflammatory and fibrosis signaling pathways are significantly different compared to Ctrl-CMs. Transplantation of both CMs into the myocardium of ischemia-induced mice resulted in fibrosis around the transplantation site. However, the fibrotic area around transplanted sites and total fibrosis were significantly reduced in mice hearts that received DdF-CMs as evidenced by collagen accumulation and Sirius red staining of fibrotic tissue. Importantly, the number of myofibroblasts at the site of DdF-CM transplantation was significantly reduced compared to Ctrl-CM sites. In an in vitro migration study, fibroblasts preferentially migrated towards the Ctrl-CMs. Human fibroblast treatment with DdF-CM exosomes (DdF-Exo) resulted in reduced expression of fibrotic markers than Ctrl-CM exosomes (Ctrl-Exo) treatment. miRNA analysis in exosomes showed that the anti-inflammatory miR-26a is upregulated and the inflammatory miRNA101 is downregulated in DdF-Exo. Overexpression of miR-101 mimic in DdF-Exo abrogated the anti-fibrotic potential property of DdF-Exo. Our results clearly demonstrate that DdF-CMs has anti-fibrotic potential. Transplanting DdF-CMs attracted

reduced myofibroblast to the engraft site and Ctrl-CMs transplantation induce fibrosis through the expression of exosomal miR-101.

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Quality of Life Assessment in Yucatan Mini Swine after Myocardial Infarction

Sherry L Daugherty, Danielle Spencer-Bearham, Andrew Jauregui, Jeremy Winkelman, Laryenth D Lancaster, Kenneth A Fox, Univ of Arizona, Tucson, AZ; Ryan J Avery, Northwestern Univ, Chicago, IL; Jennifer Koevary, Steven Goldman, **Jordan J Lancaster**, Univ of Arizona, Tucson, AZ

Introduction: Quality of life assessments (QOL) measurements are valuable in assessing functional class in patients with chronic heart failure (CHF). Yet, limited quantifiable QOL measures are available in pre-clinical animal models of CHF. To address this issue, we developed methods of QOL assessments in mini swine after myocardial infarction (MI).

Methods: Quality of life activity was continuously monitored in Yucatan mini-swine using collar-mounted activity monitors (Fitbark 2) and by performing treadmill exercise tests. Activity treadmill data were obtained for 1 month before and 1 month following MI. Myocardial infarction was created by 90-minute occlusion-reperfusion of the left anterior descending coronary artery.

Infarct size and ventricular volumes were measured with Magnetic Resonance Imaging prior to and 1 month after MI.

Results: The swine had an average MI size of $20\pm 3\%$, with increases in LV end-diastolic volume from 62.8 ± 7.8 to 72.1 ± 7.4 ml, LV end-systolic volume from 22.1 ± 2.7 to 36.6 ± 4.8 ml and a decrease in EF from $65\pm 3\%$ to $49\pm 4\%$, $N=5-6$ ($P<0.05$). The 24/7 total FitBark Activity Scores using area under the curve decreased post MI from 184.5 ± 16.8 to 159.4 ± 19.5 , $N=7-8$ ($P<0.01$).

Conclusions: These data demonstrate the feasibility of measuring quality of life in mini swine after myocardial infarction defined by activity level and treadmill exercise testing. This work establishes the potential basis for presenting quality of life measurements in animals as adjunctive assessment of new treatments for chronic heart failure after myocardial infarction.

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Therapeutic Effects of Engineered Human Pluripotent Stem Cell-derived Lymphatic Endothelial Cells on Experimental Lymphedema

Shin-Jeong Lee, Yonsei Univ, Seoul, Korea, Republic of; Young-Doug Sohn, Emory Univ, Atlanta, GA; Eun-Ah Sung, JungWoo Kim, Yonsei Univ, Seoul, Korea, Republic of; Young-sup Yoon, Emory Univ, Atlanta, GA

Current systems generating lymphatic endothelial cell (LEC) from human pluripotent stem cells (hPSCs) have limited value due to low purity, the use of undefined components for differentiation, and poor cell survival *in vivo*. Here, we developed a fully defined system to differentiate hPSCs into LECs and evaluated their therapeutic and engraftment potential when encapsulated in a nanomatrix gel. hPSCs were cultured with GSK3- β inhibitor for 3 days to induce differentiation into the mesodermal lineage. The mesodermal cells were cultured for another 6 days and double-sorted by PDPN and FLT4. These hPSC-PDPN+FLT4⁺ cells showed highly purified and fully functional LEC characteristics *in vitro*. These hPSC-LECs express LEC markers such as PDPN, LYVE1, PROX1, and FLT4 at the mRNA level and the protein level, and formed tube-like structures in Matrigel. We next determined the lymphatic vascular reparative effects of engineered hPSC-LECs. After inducing lymphedema in the tail of mouse, hPSC-LECs, hPSC-LECs/PA-RGDS, human dermal lymphatic endothelial cells (hdLEC), PA-RGDS, or PBS were injected into the tail of mouse. Tail thickness significantly decreased in the groups injected with hPSC-LECs with or without PA-RGDS compared to the other groups at day 28. At day 45, mice injected with PA-RGDS encapsulated hPSC-LECs showed significant decrease in the tail diameter compared to all other groups including those injected with hPSC-LECs. This study demonstrated for the first time that PA-RGDS encapsulation can substantially improve lymphedema repair in mouse tail through enhancement of cell survival and lymphatic neovascularization. This engineered hPSC-LEC therapy represents a novel option for treating lymphedema.

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mRNA Stability in Pluripotency and Differentiation of Induced Pluripotent Stem Cells

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Background: Induced- pluripotent stem cells (iPSC) have distinct transcriptional machinery for the maintenance of pluripotency and achievement of differentiation with the regulation of pluripotency markers, growth factors, and differentiation factors. The Hu family of RNA-binding proteins, HuR (also known as ELAVL1 or human embryonic lethal abnormal vision-like protein), binds to the 3'-untranslated region of mRNAs and regulates transcript stability and translation. However, the role of HuR in pluripotency or differentiation of iPSC to cardiomyocytes (iCM) remains unclear.

Methods: HuR knockdown in human dermal fibroblast-derived iPSCs was achieved by CRISPR/Cas9 or lentiviral shRNA transduction and subsequently differentiated into cardiomyocytes (iCM). Then, the expression of HuR, pluripotency and cardiomyocyte markers were evaluated following the initiation of differentiation.

Results: At basal level, HuR expression was higher in the iPSCs compared to dermal fibroblasts. Upon differentiation of iPSCs into iCM, HuR mRNA expression gradually reduced with significantly lower levels on day 17. As expected, pluripotency markers gradually reduced upon differentiation with significantly lower levels from day 6 onwards. We observed a corresponding increase in ISL1, MESP1 (mesoderm/cardiac progenitor markers) from day 3 through day 8 with a steep fall from day 8 to day 17. This was associated with Myosin light chain-2V and GATA4 expression increases from day 8 through day 17. Interestingly, knockdown of HuR resulted in clumps of colonies with differentiated cells and a corresponding increase in cardiac-troponin positive cells. However, as a general observation, HuR knockdown reduced beating intensity compared to wild type cells.

Conclusions: Based on these data, we could speculate that HuR might be necessary for maintenance of pluripotency and loss of which renders cells to differentiate in culture. HuR knockdown yields higher number of c-troponin positive cells but its effect on functional maturity of iCM needs to be further evaluated.

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Engineering Complex Tissue Mechanical Environment for Cardiac Microtissues Derived From Human Induced Pluripotent Stem Cells

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Previous studies of hiPSC-based cardiac tissue models mainly focused on uniform tissue mechanical environment, which is not sufficient to recapitulate the complex and heterogeneous tissue mechanical environment that would lead to cardiac contractile dysfunction. To create a 3D cardiac tissue model with nonuniform mechanical environment, we have developed state-of-art laser-based 3D bioprinting technology to fabricate "mechanical hybrid" scaffolds comprised of layers of parallel aligned high-aspect-ratio (100:1) fibers. We fabricated the double-hybrid (DH) scaffolds with "1:1 thin-thick" design by positioning the thin fibers (5 μ m, low stiffness) next to the thick fiber (10 μ m, high stiffness) to create a mismatch region in the center (**Fig. A**). The entire microtissue had to self-balance the force generation between high and low stiffness for synchronized contraction (**Fig. B**). This resulted in tissue hypertrophy at the high stiffness side with larger tissue cross-section comparing to low stiffness side (**Fig. C**). We also determined the local contractile velocities from each segment of the microtissues at different spatial locations, which showed a trend of exponential decay on the contractile velocities from edge to center (**Fig. D**). The difference in the decay rate between thick side of the DH scaffolds (DH-K) and thin side of the DH scaffolds (DH-N) indicated the contractile imbalance on the two sides of the entire microtissues. We also calculated the force output of the cardiac microtissues on DH-K side, which showed a HCM-like diastolic dysfunction comparing to the force output from single scaffolds with thick fibers (**Fig. E**).

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Mapping the Transcriptional Dynamics of Pluripotent Stem Cell-derived Endothelial Cell Differentiation by Single Cell RNA Sequencing

Ian R McCracken, Richard S Taylor, Fernando De La Cuesta, Ross Dobie, Beth EP Henderson, Joanne C Mountford, Axelle Caudrillier, Neil C Henderson, Chris P Ponting, Andrew H Baker, Univ Of Edinburgh, Edinburgh, United Kingdom

Objectives: Human embryonic stem cell derived endothelial cell (hESC-EC) differentiation offers a model to study the process of endothelial development, commitment and maturation, thus potentially informing future regeneration strategies. We used high throughput single cell RNA sequencing (scRNA-seq) to characterise the cellular heterogeneity and associated transcriptional signatures throughout hESC-EC differentiation.

Methods and Results: Using an established eight-day hESC-EC (H9) differentiation protocol yielding 66% CD31⁺/CD144⁺ cells, we sampled cells for scRNA-seq (10X Chromium) at pluripotent (day 0), mesodermal (day 4), early (day 6) and late (day 8) endothelial stages (total 21,369 cells). The pluripotent and mesodermal population were largely homogeneous prior to the emergence of distinct endothelial and mesenchymal populations. Pseudotime analysis characterised the transcriptional signatures throughout differentiation, revealing a bifurcation point giving rise to both populations. Repeating the study with a second hESC line (RC11) produced equivalent populations and transcriptional signatures. In addition, scRNA-seq analysis of an alternative hESC-EC differentiation protocol revealed substantially more heterogeneity, including nephrogenic and haemogenic populations. However, pseudotime analysis revealed common expression of known and uncharacterised endothelial transcription factors across both protocols. Comparison of hESC-EC to foetal and mature EC revealed their transcriptional distinction, suggesting their non-specified nature.

Conclusions: We sequenced over 105,000 cells allowing characterisation of hESC-EC differentiation across different hESC lines and protocols. We identified a common bifurcation point and remarkable concordance between the transcriptional signatures across different hESC lines and differentiation protocols. This included identifying novel transcription factors that may drive endothelial commitment and maturation.

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A Metabolic Mechanism for Cardiomyocyte Cell Cycle Arrest

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The mammalian heart undergoes maturation during postnatal life to meet the increased functional requirements of the adult. However, the key drivers of this process remain poorly defined. We are currently unable to recapitulate postnatal maturation in human pluripotent stem cell-derived cardiomyocytes (hPSC-CM), limiting their potential as a model system to discover regenerative therapeutics. Here, we provide a summary of our studies where we developed a 96-well device for functional screening in human pluripotent stem cell-derived cardiac organoids (hCOs). Through interrogation of >10,000 organoids, we systematically optimize parameters, including extracellular matrix, metabolic substrate and growth factor conditions that enhance cardiac tissue viability, function and maturation. Under optimized maturation conditions, functional and molecular characterization revealed that a switch to fatty acid metabolism was a central driver of cardiac maturation. Under these conditions hPSC-CMs were refractory to mitogenic stimuli and we found key proliferation pathways including β -catenin and YAP1 were repressed. This proliferative barrier imposed by fatty acid metabolism in hCOs could be rescued by simultaneous activation of both β -catenin and YAP1 using genetic approaches or a small molecule activating both pathways. These studies highlight that human organoids coupled with higher throughput screening platforms have the potential to rapidly expand our knowledge of human biology and potentially unlock novel therapeutic strategies.

R.J. Mills: None.

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2D and 3D Assessment of Angiogenesis in Cardiac Engineered Tissues Implanted on Infarcted Rat Hearts

Fabiola Munarin, Rajeev J Kant, Cassady E. Rupert, Amelia Khoo, Kareen L.k. Coulombe, Brown Univ, Providence, RI

A lack of efficient vessel perfusion in cardiac engineered tissues affects cell viability and limits the success of implanted cardiac tissues. To address the need for vascular infiltration, we have developed a collagen-based cardiac construct releasing pro-angiogenic growth factors. The constructs were implanted in a rodent model of myocardial infarction four days after ischemia/reperfusion injury. Angiogenesis assessment was performed 4 weeks after implantation by 2D histological analysis with RECA-1 antibody and with Microfil® (Flow Tech, MA) perfusion followed by 3D microCT analysis (voxel dimensions 10x10x10 μ m). Cross-registration of microCT z-stacks with histological images allows segmentation of remote, infarct and implant regions. The heart vasculature was reconstructed in 3D with ImageJ and Imaris software, and Image J volume calculator and skeletonization algorithm have been used to measure vessels volume, length and number of branch points in each region of interest. Results from 2D histological analysis confirmed the development of capillary-like structures in the implants, visible in both cross and longitudinal sections in the histological slides. The density of cross-sectional vessels measured in the cardiac tissues was comparable to the density of vessels present in the infarct region (118 \pm 77 and 93 \pm 74 vessels/mm² counted in infarct and implant regions, respectively). Furthermore, a 1.5-fold increase in vessels density was detected in the infarct zone of the treatment group with respect to the sham rats at 4 weeks. 3D reconstruction of rat heart vasculature showed evidence of the formation of new, perfused vessels in cardiac implants and presumably host-to graft connections. However, perfused vessels volume was not very different in the implants compared to the infarct regions. To the best of our knowledge, this is the first study where a 3D reconstruction of the perfused vasculature of a repaired rat heart has been conducted with a resolution of 10 μ m, and that evidence of host-to-graft connections and vascular integration were shown 4 weeks after implantation. The automated quantification proposed in this study represents a non-biased method that can be further adopted by other groups to assess angiogenesis in cardiac implants.

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CGMP Expanded First Trimester Human Umbilical Cord Perivascular Cells (FTM HUCPVC) Induce Significant Angiogenesis, Myocardial Regeneration and Sustained Functional Recovery Exceeding Older MSC Sources in the Rat Myocardial Infarction (MI) Model

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Introduction: FTM HUCPVC are a novel source of young, immunoprivileged mesenchymal stromal cells (MSC). FTM HUCPVC demonstrate significant homing, angiogenic and tissue remodelling capacities *in vitro*. We aimed to explore the therapeutic potential of cGMP compliant FTM HUCPVC for myocardial infarction (MI). **Methods:** FTM HUCPVC and term HUCPVC were expanded in cGMP compliant xeno-free (5% HPL) multilayer cultures (Corning) and Quantum Bioreactor (Terumo). One week post-MI Fox^{nm} rats (n=6) were intramyocardially injected with 3 million FTM HUCPVC, term HUCPVC, bone marrow MSC (BMSC) or basal media. Cardiac function (echocardiography, pressure/volume catheter and ECG telemetry), infarct scar (Trichrome), ECM remodelling activity (DQ collagenase substrate) vascular density (IB4) and contractile proteins (SarcA) were quantified 4 weeks and 6 months after cell implantation. Myocardial apoptosis (caspase-3) and macrophage infiltration (eNOS, CD68, CD80) were assessed at 5 days after cell treatment. **Results:** FTM HUCPVC significantly reduced apoptosis at 5 days and infarct size at 4 weeks (5.3±1.1, p<0.05) compared to other treatment groups. Histological analysis showed that the infarcted region of FTM HUCPVC treated hearts displayed significantly higher: M2 macrophage ratio, perivascular gelatinase activity, capillary density (543.4±118.6 vs. 276.5±42.69; p<0.05), abundance of contractile sarcomeric actinin (48.9±6.1 vs. 19.5±7.0; p<0.05), and Ca²⁺ pump SERCA (69.9±5.9 vs. 49.8±3.7) over other experimental groups. FTM HUCPVCs induced significant and superior improvements in all measures of contractile function (ESV, EF, dPdt, tau) relative to cell-free media (p<0.001), BMSC (p<0.001) and term HUCPVC (p<0.001). Improvements in inotropy and lusitropy were maintained to 6 months (δ dPdt max 3.8±6.0; δ dPdt min 3.2±8.78) and resulted in near complete abolishment of premature ventricular contractions (<1/min). **Conclusion:** FTM HUCPVC treatment after MI induces superior structural and functional recovery compared to older MSC sources; characteristics that were maintained during xeno-free upscaling and for up to 6 months post injection. FTM HUCPVC are a promising option as a novel cell therapy for optimal cardiovascular regeneration.

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Poster Session 3 and Reception

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Reparative Macrophage Transplantation for Myocardial Repair: A Refinement of Bone Marrow Mononuclear Cell-Based Therapy

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Background. Reparative (alternatively activated or M2-like) macrophages play an important role in post-myocardial infarction (MI) cardiac repair. Transplantation of bone marrow mononuclear cells (BM-MNCs) is an emerging therapy for MI while its therapeutic efficacy in previous clinical trials is not satisfactory. We hypothesized that induced differentiation/polarisation of BM-MNCs to reparative macrophages before transplantation may enhance the effect of BM-MNC transplantation. **Purpose.** This study aimed to develop a robust *in vitro* protocol to produce reparative macrophages from BM-MNCs and to establish the pre-clinical proof of concept data for reparative macrophage transplantation for the treatment of MI. **Methods & Results.** Mouse BM-MNCs were treated with M-CSF plus IL-4, IL-10, TGF-β1 or combinations of these *in vitro*. The concomitant M-CSF+IL-4 protocol produced the highest rate and number of CD11b⁺F4/80⁺CD206⁺ macrophages. Expression and secretion of tissue repair-related factors of the produced cells, including IGF-1, TGF-β1, VEGF and IL1-ra, were more extensive compared to BM-MNCs. Then, reparative macrophages, BM-MNCs or PBS only were intramyocardially injected in a mouse MI model. At 4 weeks after treatment, echocardiography demonstrated that reparative macrophage transplantation markedly improved cardiac function (left ventricular ejection fraction; 57.2±1.6%, n=11) compared to both BM-MNC transplantation (48.4±1.3%, n=9) and control group (44.4±2.0%, n=9). Histological studies showed that infarct size was the smallest after reparative macrophage transplantation in association with the greatest tissue repair in the peri-infarct myocardium, including augmented microvascular formation, reduced cardiomyocyte hypertrophy and reduced pathological interstitial fibrosis. It was also found that reparative macrophage transplantation increased host-derived reparative macrophages through TGF-β1 secretion. **Conclusion.** M-CSF+IL-4 treatment was effective in producing reparative macrophages from BM-MNCs *in vitro*. Addition of this pre-

treatment improved the therapeutic effect of BM-MNC transplantation. Further pre-clinical and clinical development of this advanced cell therapy is warranted.

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CRISPR/Cas9-Based Knockout of the *TLR4* gene Enhances Secretion of Extracellular Vesicles With Anti-Inflammatory Properties From Human Cardiac Mesenchymal Stromal Cells

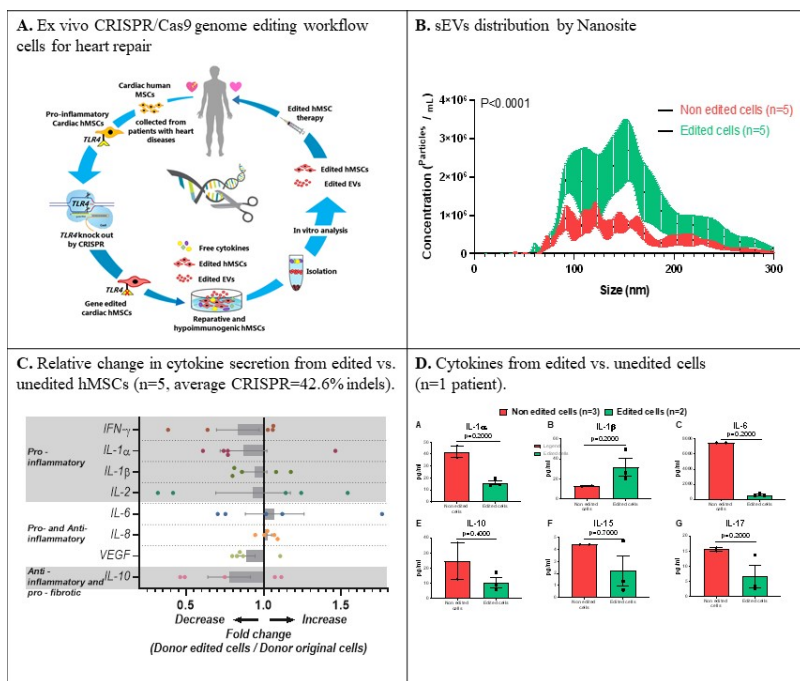
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Background and Aim: The environment of the failing and infarcted myocardium drives resident and transplanted mesenchymal stromal cells (MSCs) toward a pro-inflammatory phenotype and restricts their survival and reparative effects in a mechanism mediated by the toll-like receptor 4 (*TLR4*). CRISPR is a promising tool for genome editing of DNA in cells, which raises hope for therapeutic genome editing in the clinic. **We hypothesize** that *ex-vivo* knockout (KO) of the human *TLR4* gene by CRISPR would switch human cardiac MSCs (hMSCs) to an anti-inflammatory, reparative phenotype that could prevent remodeling of the left ventricle after myocardial infarction (Fig. 1A).

Methods and Results: For gene editing, we electroporated a Cas9 nucleoprotein aimed to KO the *TLR4* gene. To assess the inflammatory response, we analyzed cell secretome.

We achieved up to a 68% (out of 400,000 cells) success rate in editing the genome of hMSCs ($R^2=0.93$). The *TLR4* KO hMSCs secreted smaller extracellular vesicles (sEVs) compared with unedited hMSCs (Fig. 1B $p<0.001$) and decreased the secretion of most pro-inflammatory (e.g. IL-1 α) and pro-fibrotic (e.g. IL-10) cytokines from edited compared with unedited hMSCs (Fig. 1C, D). Additionally, sEVs from *TLR4* KO cells stimulated hMSC migration by "wound healing" scratch assay ($p<0.001$).

Conclusion: Our preliminary results show, for the first time, that CRISPR-based KO of the human *TLR4* gene in hMSCs inhibits inflammatory cytokine secretion and facilitates a reparative response by human-cardiac MSCs *in vitro*. The precise and efficient *ex vivo* gene editing could provide a newly engineered cell line to improve the outcome of hMSC-based cell therapy.



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Poster Session 3 and Reception

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Prenatal Exposure of Cigarette Smoke Impacts Cardiac Regeneration

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Background: Neonatal mammalian hearts have the unique characteristic to fully repair and regenerate following injury. This discovery has led to visionary endeavors to understand and subsequently reawaken regeneration processes in the human adult heart that are lost after birth, contributing to heart failure and death after ischemic insults. *In utero* exposure to tobacco smoke has detrimental effects on fetal development and growth. However, the consequences of prenatal exposure to cigarette smoke on murine neonate's cardiac regeneration have never been explored. **Methods:** To study the impact of cigarette smoke during the entire pregnancy on cardiac regeneration in the offspring, plugged female wild type C57LB/6J mice were exposed either to cigarette smoke (n=6) or filtered air (control, n=6). Two days after birth, 5 pups from each litter were assigned to the two experimental groups: Myocardial infarction (MI) and sham. Detrimental effects of *in utero* exposure to tobacco smoke on the recovery of cardiac function following MI surgery were followed using two-dimensional speckle tracking echocardiography and strain imaging. To investigate the underlying mechanism, cardiac tissue of the infarct and remote zone was collected for non-coding RNA analysis. **Results:** *In utero* exposure to cigarette smoke significantly compromised cardiac regeneration in neonates. At both early and late time points in the phase of cardiac repair, hearts of neonates exposed to cigarette smoke during pregnancy showed a marked impairment of cardiac regenerative potential. In particular, ejection fraction, fractional area change and shortening, as well as left ventricular internal diameter during systole remained pathologically changed following MI insult in the smoked group until the very endpoint. **Conclusions:** Collectively, we here provide evidence that *in utero* exposure to cigarette smoke strongly compromises cardiac regeneration in the newborn offspring. This result reinforces smoking cessation during pregnancy. Additionally, understanding the changes in non-coding RNA expression in a setting of preserved versus disrupted repair of the heart might be an important first step towards the identification of key cellular processes in cardiac regeneration after injury.

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Poster Session 3 and Reception

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Human Neonatal Cardiac Progenitor Cells Derived Exosomes Induce Cardiomyocytes Proliferation

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Background: Human neonatal cardiac progenitor cells (cKit+/CD45-/Lin-, CPCs) improve cardiac function and attenuate adverse left ventricular remodeling after myocardial infarction (MI) through their exosomes (nEXOs). Success of cell therapy to treat injured myocardium depends mainly on reactivation of endogenous quiescent cardiomyocytes to proliferate and recover the lost myocardium. Here, we investigated the mechanism of reactivating quiescent cardiomyocyte proliferation by exosomes. **Hypothesis:** RNAseq analysis of nEXOs identified miRs which may be responsible for modulation of Hippo signaling, thereby promoting cardiomyocytes proliferation. nEXOs effectively stimulate endogenous cardiomyocyte (CM) proliferation by targeting the Hippo pathway to restore cardiac function in an injured heart. **Methods and Results:** nCPCs were conditioned for 48 hours in serum free nutrition mix (Ham'sF12) and nEXOs were purified from supernatant using size exclusion chromatography (SEC; CL2B) coupled with ultracentrifugation. nEXOs were quantified by Nanosight (NS300) and characterized by transmission electron microscopy and flow cytometry for the presence of CD63 and CD9. Our results show recovery of cardiac function (ejection fraction = 63.4% vs 40.5%, n=10, p<0.001) and generation of myocardial mass in a rodent MI model following nEXOs' intra-myocardial injection. We further show that nEXOs are preferentially acquired by CMs in the border zone of the infarction (m-cherry-Alix labeled EXOs). Our *In vitro* experiments show that miR-582-3p and miR-7641 (25nM) are the most effective miRs to induce proliferation of quiescent cardiomyocytes (40.1% and 48.8%, respectively, n=3). Increased miR-7641 expression led to a profound increase in quiescent CMs proliferation, in part through repression of the Hippo signal transduction pathway (increased YAP/pYAP ratio). By immunoblotting we show that LATs1/2 (a protein in the Hippo pathway) is directly targeted by miR-7641. nEXOs enriched with miR 7641 promote CM proliferation

by 3 folds as compared to non-enriched nEXOs. **Conclusion:** Our data for the first time demonstrates the ability of nEXOs derived miRNA based therapeutic approaches to activate cardiomyocyte proliferation.

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Unveiling Ccbe1 Role as a Modulator of Cardiomyocyte Differentiation

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Chronic heart failure is a major unmet clinical need arising from the loss of viable and functional cardiac muscle, representing a major cause of mortality worldwide. There is a need to identify key molecules and signaling pathways acting on the coronary vasculature system towards regenerative therapies. Human induced pluripotent stem cells (hiPSC) are an attractive cell source to understand the regulatory networks involved in cardiac commitment and cardiomyocyte (CM) differentiation. Particularly, CCBE1 (collagen and calcium-EGF binding domain 1) has been studied as a secreted protein critical for lymphatic/cardiac vascular development and recent reports have proposed that CCBE1 may potentially be used to restore cardiac tissue upon heart injury, through CCBE1-mediated cardiac commitment and/or augmentation of lymphangiogenesis. Therefore, the goal of our work is to understand the molecular pathways underlying CCBE1-based cardiac commitment in loss-of-function studies. By exploring gene editing and “omics” tools we aim to unveil the molecular basis of CCBE1-induced cardiogenesis and provide novel insights towards the development of CCBE1-mediated therapeutic strategies for cardiac regenerative medicine. To selectively knock-down (KD) CCBE1 expression along hiPSC cardiac differentiation, we used modified hiPSC line with CRISPR interference technology (CRISPRi-hiPSC). The CCBE1 KD led to a reduction on the expression of cardiac troponin marker *TNNI2* and on the ratios of MYH7:MYH6 and TNNI3:TNNI1. The CCBE1 KD-derived CMs also presented an immature-related ultrastructure, suggesting that CCBE1 may modulate the CM phenotype. On the other hand, the EC differentiation was not impaired by CCBE1 KD. A comprehensive and integrated characterization of the transcriptome and proteome along the differentiation in the presence or absence of CCBE1 is being pursued to identify the key players at cardiac mesoderm and cardiac progenitors stages and their interactions with CCBE1. This work opens new avenues for the identification of CCBE1-modulated proteins/pathways in cardiac commitment, which may contribute for novel cell-based or cell-free approaches towards more efficacious cardiac regenerative therapies.

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Cortical Bone Derived Stem Cells Modulates T Cell Response After Myocardial Injury

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Rationale: The ischemic environment following myocardial infarction induces a harsh, pro-inflammatory state that facilitates the removal of necrotic tissue. Manipulation of T cell responses after cardiac injury directly impacts the outcome of the reparative response. Previous studies have demonstrated Cortical Bone Derived Stem Cells (CBSCs) improve cardiac function after myocardial infarction. CBSCs produce a diverse paracrine profile; whether this can lead to the modulation of T cell response by manipulating T cell subsets and allowing for increased cardiac wound healing post-MI remains unknown. *Objective:* To determine whether CBSCs secrete a paracrine profile that promotes wound healing by modulating T cell response following myocardial injury. *Methods and Results:* CD3+ T-lymphocytes were isolated from the spleen of C57BL/6J male mice ages 8 to 12 weeks via Fluorescent Activated Cell Sorting (FACs). CBSC secretome was introduced to T cell populations via a transwell culture system in the presence of CD3/28 coated beads with IL2. T-regulatory (Treg) populations were quantified via FACs 24 hours after *in vitro* CBSC exposure. Treg populations exposed to CBSC secretome in hypoxic (1% O₂) conditions expanded to 26.9% of the CD4+ compartment, a 4.5 fold increase compared to control conditions. This result was further confirmed via qRT-PCR analysis. T cell culture secretome was quantified via proteome profiling of 111 soluble cytokine proteins 24 hours

post CBSC exposure. CBSCs express a robust chemotaxis signature that promotes immune cell recruitment. T cells cultured in the presence of CBSC (pre-conditioned or co-culture conditions) exhibit increased CCL5, CCL22, CD40, CXCL1, and Lipocalin-2. C-reactive protein, a pro-inflammatory marker, was decreased by ~5.5 fold in the presence of CBSCs, promoting wound healing. **Conclusions:** CBSCs secrete a diverse paracrine profile that promotes Treg expansion during hypoxic conditions. Expansion of Treg populations post-CBSC exposure in the post-IR heart leads to increased inflammation resolution and improved myocardial wound healing, allowing for increased cardiac function and repressed cardiac remodeling.

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Neonatal Heart Regeneration Preserves Native Ventricular Biomechanical Properties After Myocardial Infarction

Hanjay Wang, Ross Bennett-Kennett, Michael J Paulsen, Camille E Hironaka, Akshara D Thakore, Justin M Farry, Anahita Eskandari, Haley J Lucian, Matthew A Wu, Annabel Imbrie-Moore, Amanda N Steele, Lyndsay M Stapleton, Reinhold H Dauskardt, Y. Joseph Woo, Stanford Univ, Stanford, CA

Introduction Neonatal mice exhibit natural heart regeneration after myocardial infarction (MI) until postnatal day 7 (P7). Whether this regenerated muscle is similar biomechanically to native myocardium remains unknown. We hypothesized that neonatal heart regeneration preserves native left ventricular (LV) biomechanical properties after MI. **Methods** C57BL/6J mice (n=58) underwent sham surgery or left anterior descending coronary artery ligation on postnatal day 1 (P1) or P7. After 4 weeks, echocardiography was performed. The explanted anterolateral LV wall was mounted for lenticular hydrostatic deformation testing (Fig 1A-B) and pressurized to 130 mmHg with 37°C saline to impart a homogeneous stress load. Deformation was tracked to calculate a multiaxial composite tissue modulus. Data are expressed as mean±SEM. **Results** P1 MI and sham mice (n=14, each) had similar post-MI LV wall thickness (0.61 ± 0.05 cm vs 0.66 ± 0.03 cm, $p=0.39$), end-diastolic diameter (3.14 ± 0.10 cm vs 3.05 ± 0.12 cm, $p=0.57$), and ejection fraction ($58.2\pm 4.1\%$ vs $55.2\pm 4.4\%$, $p=0.63$). P7 MI mice (n=14) had significant LV wall thinning (0.40 ± 0.04 cm vs 0.71 ± 0.02 cm, $p<0.0001$), end-diastolic LV enlargement (4.24 ± 0.16 cm vs 3.34 ± 0.09 cm, $p<0.0001$), and depressed ejection fraction ($29.7\pm 4.9\%$ vs $61.7\pm 4.0\%$, $p<0.0001$) compared to P7 shams (n=16). While LV tissue modulus for P1 MI and sham mice were similar (460.9 ± 46.4 kPa vs 527.1 ± 61.2 kPa, $p=0.40$, Fig 1C), the modulus for P7 MI mice was significantly elevated compared to that for P7 shams (1099.6 ± 175.1 kPa vs 616.0 ± 95.2 kPa, $p=0.02$, Fig 1D). **Conclusion** In a neonatal mouse MI model, regenerated LV muscle has similar biomechanical properties as native LV muscle.

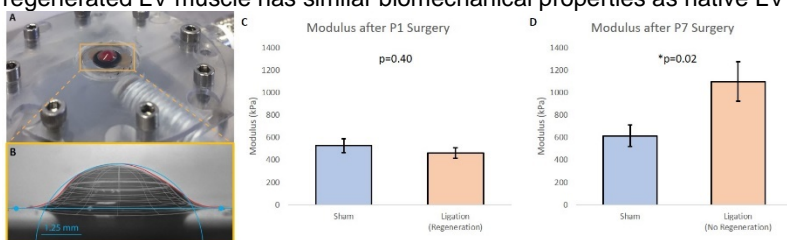


Figure 1. (A-B) Mouse left ventricle mounted over sealed testing chamber and pressurized to 130 mmHg for lenticular hydrostatic deformation surface strain analysis. (C) Heart regeneration after coronary ligation at age P1 resulted in preserved ventricular tissue elasticity. (D) The absence of heart regeneration after coronary ligation at age P7 resulted in significant ventricular tissue stiffening.

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A Transcriptional Basis for Neonatal Heart Regeneration at Single Cell Resolution

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The adult mammalian heart has limited capacity for regeneration following injury, whereas the neonatal heart can readily regenerate within a short period after birth. Deciphering the molecular underpinnings of neonatal heart regeneration and the blockade to regeneration in later life may provide novel insights for heart repair. Neonatal heart regeneration is orchestrated by multiple cell types including cardiac resident cells and immune cells that infiltrate the heart after injury. To systematically elucidate the transcriptional response after injury at cellular level during heart regeneration, we performed single cell RNA-sequencing on hearts at various time points post P1 or P8 myocardial infarction injury, and coupled that with bulk tissue RNA-sequencing data collected at the same time points. This integrated approach provides detailed transcriptome landscape and dynamics during heart regeneration at single cell resolution. We further depict a ligand-receptor interaction network that potentially reveal cellular signaling and cell-cell cross-talks during heart regeneration in cell autonomous and non-autonomous manners. Furthermore, to uncover the transcriptional dynamics in a single cardiomyocyte (CM), which was technically challenging due to incompatibility of CM diameter with Drop-Seq platforms, we performed single nucleus RNA-sequencing for CM population, and identified a subset of CMs in the regenerative hearts with unique molecular properties. Taken together, our data provide , and suggest numerous inroads that might be therapeutically manipulated to enhance cardiac function in response to injury.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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An Improved Cardiac Reprogramming System with the Aid of Two Chemical Compounds

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The direct conversion of cardiac fibroblasts into cardiomyocytes (iCMs) by forced expression of *Gata4*, *Mef2c*, *Tbx5* (GMT) represents a novel and promising approach for cardiomyocyte regeneration. However, the low reprogramming efficiency to obtain functional cardiomyocytes is a major problem which hampers its further application. Here, by chemical screen for about 2,000 selected small molecules and further validation, we identified a combination of two chemical compounds (2C) that greatly boost mouse cardiac reprogramming induced with GMT, and thereby established a robust reprogramming system to generate functional cardiomyocytes from mouse postnatal fibroblasts. Contractile cardiomyocyte-like cells emerge as early as two weeks post transfection, co-expressing cardiomyocyte enriched protein cTnT and α -Actinin, with well-organized sarcomere structure. Importantly, most of the iCMs are contractile and have spontaneous Ca^{2+} waves. 2C also greatly improved *in vivo* cardiac reprogramming in mouse model with myocardium infarction, as indicated by a myofibroblasts-specific Postn lineage tracing system and reduced fibrotic areas. While GMT induced cardiac reprogramming mostly in border zone, GMT+2C enabled cardiomyocyte generation from cardiac myofibroblasts in infarct zone. Furthermore, in the presence of 2C, *Gata4* can be substituted without the reduction of reprogramming efficiency. Only 2C, without transgenes, induced a gene expression profiles closer to iCMs, and enabled *in vivo* cardiac reprogramming. Besides, 2C enhanced human cardiac reprogramming efficiency by 20-fold, in the presence of transgenes. In summary, we identified a chemical combination 2C, that greatly aided cardiac reprogramming both *in vitro* and *in vivo*, even with reduced or none reprogramming genes, providing new opportunities to regenerate injured hearts with the aid of chemical drugs.

Y. Zhao: None.

Poster Session 3 and Reception

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Celltype-Specific Functions in Dilated Cardiomyopathy Caused by the *LMNA* Gene Mutation

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Mutations in the *LMNA* gene, encoding lamins A and C (lamin A/C) cause a diverse group of human diseases termed laminopathies. The most prevalent laminopathy is dilated cardiomyopathy (herein referred to as *LMNA* cardiomyopathy) characterized by variable onset of fibrosis/pathological remodeling that always progresses to heart failure. Despite recent progress, how cell type-specific effects of *LMNA* mutations are integrated at the tissue level to engender complex pathologies in a heterocellular organ such as the heart are not well understood. Using a conditional deletion model that permits cardiomyocyte (CM)-specific *Lmna* deletion coupled with translating ribosome affinity purification, we found that CM-

specific lamin A/C depletion caused rapid fibrosis and severe cardiac dysfunction accompanied by endoplasmic reticulum (ER) stress. At the molecular level, CM-specific translating mRNA profiling identified increased expression of MED25, a member of the Mediator complex implicated in regulating ER stress responses, prior to the onset of cardiac dysfunction. In contrast, lamin A/C depletion selectively in cardiac fibroblasts (CFs) displayed no immediate cardiac pathology whereas a concurrent depletion in both CMs and CFs resulted in lesser fibrosis and pathological remodeling. These results suggest that lamin A/C play an important role in CF biology and that reactive CFs underlie the rapid onset and the exaggerated *LMNA* cardiomyopathy symptoms observed in mice with CM-only *Lmna* deletion. Mechanistically, we found that increased matrix stiffness elevated the expression of MED25 and ER stress markers in lamin A/C-depleted CMs *in vitro*, indicating that the physical component of fibrosis may underlie the observed molecular changes. Taken together, our results suggest lamin A/C-depleted CFs mediate a brake on cardiomyopathy development and interactions between CFs and CMs are important determinants of the rate of progression and the severity of *LMNA* cardiomyopathy. Therefore, strategies targeting lamin A/C function in CFs may hold therapeutic potential for patients with *LMNA* cardiomyopathy as well as for other forms of cardiomyopathy in which fibrosis is integral to disease pathogenesis.

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Poster Session 3 and Reception

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Sorafenib Induces Cardiotoxicity via Damage to Cardiac Endothelial Cells

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Aim: Sorafenib (Nexavar) is a multitargeted tyrosine kinase inhibitor that is used for treatment of hepatocellular carcinoma and renal cell carcinoma. Cancer treatment with sorafenib has been reported to be associated with cardiotoxic side effects such as hypertension, myocardial ischemia, thrombosis and pulmonary embolism, whereas incidence of LV dysfunction is rare. Aim of the current study was to identify the mechanism of sorafenib cardiotoxicity.

Results: Healthy mice were treated with sorafenib (50 mg/kg/d) for 4 weeks. Echocardiography analysis revealed no difference in LV systolic function, but LV posterior wall thickness and relative wall thickness were significantly increased, and LV volume decreased in sorafenib treated mice. Hearts of sorafenib treated mice showed a 10-fold increase in expression of heart failure marker gene ANP and a 3-fold increase in the number of apoptotic cells. Electron microscopy analysis indicated damage to cardiac endothelial cells with no evidence of cardiomyocyte loss and co-staining of cardiac sections with endothelial marker CD31 revealed that ~80% of apoptotic cells in sorafenib treated hearts were endothelial cells (ECs). To assess for the time course for EC damage, we treated mice with sorafenib for 8h, 24h, 48h and 72h. The increase in EC death was already detected after 8h treatment and peaking at 24h-48h. RNA profiling analysis showed that expression of angiopoietin-1 (Angpt1) was reduced, whereas expression of Angpt2 was induced in hearts of mice treated with sorafenib for 4 weeks and in EC fractions from hearts of mice treated with sorafenib for 72h. Angpt-1 overexpression induced protective signaling pathways in hearts of mice treated with sorafenib for 48h, but did not rescue from acute EC injury. Treatment of Angpt1 overexpressing mice with sorafenib for 4 weeks resulted in capillary rarefaction and heart failure, evidenced by a decrease in LV systolic function and a 40-fold increase in ANP expression.

Conclusion: our data shows that sorafenib cardiotoxicity is due to endothelial cell damage in the heart and results in heart failure with preserved ejection fraction. Furthermore, antagonizing the function of Angpt2 by overexpression of Angpt1 is detrimental during sorafenib treatment.

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Attenuation of Tnf- α Induced Injury in Cardiomyocytes by Chlorogenic Acid via Inhibiting Nf- κ B and Jnk Signals

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The traditional Chinese herb *Lonicerae Japonicae Flos* has been used to treat various diseases including heart failure, showing significant benefits in the clinical practice. However, the mechanism of its cardioprotective effects remains unclear. As the main active ingredient found in the plasma after oral administration of *Lonicerae Japonicae Flos*, chlorogenic acid (CGA) has been reported to possess anti-inflammatory, anti-oxidant, anti-apoptosis and analgesic function. To investigate whether the cardioprotective effects of *Lonicerae Japonicae Flos* is through CGA, we applied Transverse Aortic Constriction (TAC)-induced heart failure mouse model, to access the effects of CGA. The results of in vivo experiments show that CGA has cardioprotective effects and could mitigate the TNF- α (Tumor necrosis factor- α) induced toxicity in TAC heart failure mouse model. Therefore, we further used TNF- α -induced cardiac injury in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to explore whether CGA have cardioprotective effects and elucidate the underlying mechanism(s). CGA pretreatment could reverse TNF- α induced cellular injuries, including improved cell viability, increased mitochondrial membrane potential and inhibited cardiomyocytes apoptosis. We then examined the NF- κ B/p65 and major MAPKs signaling pathways involved in TNF- α induced apoptosis of hiPSC-CMs. Importantly, both CGA and NF- κ B inhibitor (QNZ) can reverse the cell viability impairment and apoptosis phenotypes. Moreover CGA can directly inhibit NF- κ B signal by suppressing the phosphorylation of NF- κ B/p65. As for the MAPKs, CGA suppressed the activity of JNK only, but enhanced ERK1/2 and had no effect on p38. In summary, our study revealed that CGA has profound cardioprotective effects through inhibiting the activation of NF- κ B and JNK pathway, providing a novel therapeutic alternative for prevention and treatment of heart failure.

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Poster Session 3 and Reception

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PRMT1 Suppresses Atf4-mediated Endoplasmic Reticulum Response In Cardiomyocytes

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Endoplasmic reticulum (ER) stress signaling plays a critical role in the control of cell survival or death. Upon persistent stress, ER stress activates pro-apoptotic pathway involving the ATF4/CHOP pathway. Although accumulating evidences support its important contribution to cardiovascular diseases such as myocardial infarction and heart failure, the mechanism of ER stress in cardiovascular diseases is not well characterized. In this study, we demonstrate a critical role for Protein Arginine Methyltransferase 1 (PRMT1) in the control of ER stress in cardiomyocytes. Cardiac-specific deletion of PRMT1 causes abnormal cell death during early postnatal days with elevated ER stress response, especially genes related to the ATF4/CHOP pathway. PRMT1 null hearts show starkly exacerbated ER stress and cell death in response to an ER stress inducer, tunicamycin (TN), compared to the wildtype hearts. Consistently, pharmacological or genetic inhibition of PRMT1 augments ER stress response in rat cardiomyocytes while PRMT1 overexpression attenuates TN-induced ER stress. Furthermore, ATF4 depletion attenuates the ER stress response triggered by PRMT1 inhibition. The methylation-deficient mutant of ATF4 with the switch of arginine 239 to lysine exacerbates ER stress response accompanied by enhanced levels of proapoptotic cleaved-Caspase3 and p- γ H2AX in response to TN. The mechanistic study shows that PRMT1 decreases the protein stability of ATF4 through methylation. Taken together, our data suggest that ATF4 methylation on arginine 239 by PRMT1 is a novel regulatory mechanism for protection of cardiomyocytes from ER stress-induced cell death.

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NF- κ B Signaling Regulates Mitochondrial Permeability Transition Pore Opening of Cardiac Myocytes via Cyclophilin D (CypD) Modulation

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Nuclear Factor- κ B (NF- κ B) is ubiquitously present transcription factor that regulates a variety of cellular functions including cell survival. Herein, we show a critical role for NF- κ B signaling in regulation of mitochondrial permeability transition pore opening (mPTP) of cardiac myocytes that involves cyclophilin D (CypD). Cardiac myocytes expressing a kinase defective form of IKK β (IKK β -M), the principle IKK required for NF- κ B activation, displayed impaired NF- κ B gene activity. Defects in NF- κ B signaling coincided with an increase in mPTP opening and cell death. Interestingly, mPTP opening and cell death observed in the NF- κ B defective cardiomyocytes was suppressed by inhibition of mPTP modulator CypD, with cyclosporin A (CSA) or by siRNA knock down (CypDsiRNA), suggesting a link between mPTP regulation and NF- κ B signaling. Earlier, we reported that doxorubicin (Dox) treatment resulted in severe ultra-structural defects including disrupted mitochondrial cristae and impaired respiration, increased mitochondrial calcium overload, mPTP opening and a widespread cell death. Interestingly, we observed a dramatic reduction in NF- κ B signaling in cardiac myocytes treated with doxorubicin (18 Hrs), coupled with mitochondrial dysfunction including impaired respiration. Inhibition of CypD suppressed doxorubicin induced cell death of cardiac myocytes. Finally restoration of NF- κ B signaling in cardiac myocytes treated with doxorubicin by IKK β , active kinase, suppressed mitochondrial calcium overload, mitochondrial perturbations, respiration and cell death. The data herein, provides the first direct evidence that impaired NF- κ B signaling predispose Dox treated cardiac myocytes to cell death. Hence, interventions that preserve NF- κ B survival signaling pathways in the heart may prove beneficial in reducing cardiac dysfunction and heart failure in cancer patients undergoing doxorubicin chemotherapy.

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A Novel Class of Sphingolipids Mediate Autophagy and Apoptosis in Models of Ischemia

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Rationale: Heart failure caused by ischemic cardiomyopathy is the leading cause of death and disability in the USA and world, accounting for 10 million deaths in 2016. Sphingolipids including ceramides and sphingosine-1-phosphate have been demonstrated to play roles in myocardial injury. In general, these lipids are synthesized from serine palmitoyltransferase (SPT) derived using serine and palmitoyl-CoA. While the standard SPT enzyme exists as a heterodimer of two subunits, Sptlc1 and Sptlc2, a new subunit was recently discovered, Sptlc3, which enables the SPT complex to use myristoyl-CoA, thereby synthesizing a previously underappreciated group of atypical sphingolipids. We previously demonstrated that these atypical lipids are a major component of myocardial sphingolipid pools. Furthermore, in contrast to canonical sphingolipids, the atypical lipids promote cardiomyocyte apoptosis. Therefore, the goal of this research is to determine the contribution of these novel lipids to cardiomyocyte apoptosis and therefore whether they may contribute to ischemic injury.

Objectives: To determine the contribution of Sptlc3 and atypical sphingolipids to cardiomyocyte apoptosis and ischemic injury and to assess the potential relevance of Sptlc3 to human HF.

Methods: Murine primary cardiac fibroblasts and cardiomyocytes were isolated and subject to ischemic induction. Following this, cells were analyzed with TUNEL staining, lipidomic analysis or treatment with a Sptlc3 mimetic.

Results: Ischemic cells show significantly increased Sptlc3 expression, non-canonical sphingolipids in the sphingolipid pool and apoptotic cells as compared to their controls. The cells treated with the Sptlc3 mimetic show apoptotic and a non-canonical autophagic pathway being induced in cells.

Conclusions: We have identified a novel class of sphingolipids enriched in myocardium of mice with cardiomyopathies. Furthermore, the enzyme that produces these, Sptlc3, is robustly increased in human heart failure. Because the Sptlc3-derived lipids promoted cardiomyocyte apoptosis, we propose that Sptlc3 may mediate myocardial injury in ischemia.

A. Kovilakath: None. **A. Cowart:** None.

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Increased Cell Death in Regions of Elevated Aortic Wall Shear Stress in Bicuspid Valve Aortopathy

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Bicuspid aortic valve (BAV) is a common congenital malformation associated with aortopathy and potential rupture. Identifying those patients at greatest risk for dissection is difficult at present. Abnormal hemodynamics in the ascending aorta creates regions of increased wall shear stress (WSS) which causes extracellular matrix dysregulation and may affect cell death. Dying cells release fragmented DNA into the blood and levels of aorta-specific cell-free DNA (cfDNA), identified by the presence of tissue-specific differentially methylated regions (DMRs), could be leveraged as a biomarker. Our objective was to determine if a relationship between elevated WSS and apoptosis in the ascending aorta existed and identify aorta-specific DMRs in the cfDNA of BAV patients. We hypothesized that areas of increased WSS would demonstrate increased cell death that would correlate with increases in aorta-specific cfDNA from patient blood.

BAV patients undergoing 4D-flow cardiac magnetic resonance imaging (CMR) and surgery for ascending aorta dilation (range 36-63 mm) were recruited. Blood was collected from 23 patients at the time of CMR and used for the isolation of plasma cfDNA. Aortic wall samples (n = 30) corresponding to regions of high and low WSS were collected at surgery from 15 patients and stained for cell death using the TUNEL assay. Publicly-available methylomes and Metilene were used to identify aorta-specific DMRs *in silico*.

Regions of elevated WSS were seen in all individuals regardless of absolute aortic dimension. These regions of elevated WSS by CMR showed significantly greater cell death when compared to the region of normal WSS in the ascending aorta (0.14 ± 0.05 vs. 0.08 ± 0.06 , $p=0.00006$). Levels of cell death did not correlate with maximal aortic diameter. Twenty-three aorta-specific DMRs were identified. Of the four that were tested, three were found to be hypomethylated in genomic DNA from the aorta compared to 14 other tissues. Levels of aorta-specific cfDNA based on our DMRs did not correlate with maximal aortic diameter.

Increased regional cell death corresponding to elevated WSS may implicate abnormal hemodynamic flow in the progression of aortopathy and provides a biological rationale for the use of cfDNA as a biomarker for aortopathy.

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N-ethylmaleimide sensitive factor (NSF) is Essential in Necrotic Cell Death

Emma K Murray, Alyssa A Lombardi, Jonathan P Lambert, John W Elrod, Temple Univ Medical Sch, Philadelphia, PA

Necrotic cell death is the main way in which cells die during myocardial infarction (MI) and heart failure (HF), yet the molecular mechanisms regulating necrotic cell death are poorly defined. To elucidate the key regulators of plasma membrane rupture during necrotic cell death a genome-wide shRNA loss-of-function screen was performed which identified components of SNARE-mediated membrane fusion as potential facilitators of Ca^{2+} and ROS-induced necrosis. To examine if the SNARE machinery is involved in cellular necrosis we targeted N-ethylmaleimide sensitive Factor (NSF) due to its requirement in SNARE recycling, lack of gene homologs, and redox sensitivity. Deletion of NSF from 3T3 fibroblasts by CRISPR-Cas9n (*Nsf*^{-/-}), inhibited membrane rupture and improved cell viability following Ca^{2+} overload (ionomycin), ROS (H_2O_2), and necroptotic (TNF α , CHX, zVAD)-induced cell death but did not alter apoptotic (staurosporine) cellular demise. We next created a cardiac-specific conditional *Nsf* knockout mouse model to determine if NSF contributes to myocyte death during IR injury. Loss of NSF in cardiomyocytes did not alter baseline cardiac function or structure. Currently, studies are underway to determine if NSF contributes to necrotic cell as occurs in IR injury and heart failure. In summary, our results suggest that NSF is an important molecular component of membrane rupture and pathogenic cell death.

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Comparative Cardiotoxicity of Tyrosine Kinase Inhibitors Ponatinib and PF114

Anand Prakash Singh, Prachi Umbarkar, Michael S Glennon, Qinkun Zhang, Vanderbilt Univ Med. Ctr, Nashville, TN; Ghermes Chilov, Fusion Pharma, LLC, Moscow, Russian Federation; Jason R Becker, Hind Lal, Vanderbilt Univ Med. Ctr, Nashville, TN

Background: The advent of tyrosine kinase inhibitor (TKI) targeted therapy revolutionized the treatment of chronic myelogenous leukemia (CML) patients, leading to a significant impact on remission rates and overall survival. However, cardiotoxicity associated with these targeted therapies put the cancer survivors at greater risk. Ponatinib is an example shown unexpected wide spectrum of cardiotoxic complications which limits its clinical applications. A newly designed 4th-generation tyrosine kinase inhibitor PF114 having better target spectra in CML, could be a potential alternative to ponatinib. But the cardiotoxic potential of PF114 is not yet fully understood. **Objectives:** The objectives of the present study were to compare cardiotoxicity of ponatinib with PF114. **Methods:** In this study, we employed zebrafish transgenic BNP reporter line that expresses luciferase under control of the *nppb* promoter (*nppb*: F-Luciferase) to compare cardiotoxic potential of TKIs. We also exploited NRVMs to explore cardiotoxic mechanism associated with ponatinib and PF114. **Results:** We observed that increasing dose of PF114 showed minimal induction of the BNP reporter compared to ponatinib. PF114 did not reduce the ventricular fractional shortening in zebrafish as compared to ponatinib. Consistently, in cultured rat cardiomyocytes, PF114 could not reiterate the same cardiotoxic effect of ponatinib as depicted by cell viability and TUNEL assay. Mechanistically, PF114 has shown no effects on essential prosurvival AKT and ERK signaling pathway while ponatinib is known to exerts its cardiotoxic effects by their inhibition. Additionally, the allosteric tyrosine kinase inhibitor ABL001(Asciminib) in combination with PF114 depicts less pronounced cardiotoxic effects than ponatinib in similar settings. **Conclusions:** This study provides further rationale to utilize zebrafish for the prediction of cardiotoxicity of anticancer drugs. The comparative cardiotoxicity of ponatinib and PF114 suggest that PF114 is a significantly less cardiotoxic analogue and safer treatment option than ponatinib for CML patients harboring BCR/ABL-T315I "gatekeeper" mutation.

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Voluntary Late-life Exercise Attenuates Frailty and Improves Maladaptive Changes Associated with Cardiac Aging in Female Mice

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Voluntary aerobic exercise has many positive health benefits and may actually attenuate frailty. Exercise may also improve cardiac function in the setting of aging, although links between exercise, frailty and age-dependent cardiac remodeling are unclear. To investigate age-related effects of exercise, 21-23 month old female C57BL/6 mice were divided into two groups, an exercise group (n=9) and a sedentary group (n=9). Frailty was measured with a clinical frailty index (FI) tool, then mice were randomly assigned to either exercise or sedentary groups matched by their initial FI scores. The exercise group was allowed constant access to a running wheel for 13 weeks, while sedentary animals had no wheel access. Cardiac function was measured with echocardiography at baseline, midpoint (6 weeks) and endpoint (13 weeks) in both groups. FI scores increased with age in sedentary mice (0.18 ± 0.02 to 0.34 ± 0.05 ; baseline vs endpoint) but not in the exercise group (0.18 ± 0.02 to 0.20 ± 0.01) and this difference was significant (at endpoint; $p < 0.05$). Fractional shortening (FS) decreased with age in sedentary mice ($34.81 \pm 3.3\%$ to $22.86 \pm 5.5\%$; $p < 0.05$; at endpoint) but exercise protected hearts against this decline ($34.64 \pm 2.3\%$ to $36.16 \pm 3.1\%$). A similar pattern was seen with ejection fraction (EF) where EF deteriorated with age ($63.28 \pm 4.2\%$ to $44.70 \pm 9.2\%$; $p < 0.05$ at endpoint) but stayed similar in exercised animals ($63.79 \pm 3.6\%$ to $65.74 \pm 4.1\%$). Isovolumic relaxation time decreased with age in sedentary mice (20.43 ± 1.1 ms to 16.50 ± 1.7 ms; $p < 0.05$) but not in exercised mice (19.32 ± 1.1 ms to 16.80 ± 1.1 ms). In addition, left ventricular (LV) mass was actually smaller in the exercise group compared to the sedentary group at endpoint (138.4 ± 19 mg vs 211.6 ± 34 mg; $p < 0.05$). Interestingly, age-associated changes in EF, FS and LV mass at the endpoint were correlated with and closely graded by FI score in all mice (e.g. EF: $r = 0.67$, $p < 0.05$, FS: $r = 0.68$, $p < 0.05$; LV mass: $r = 0.68$, $p < 0.05$). These results show that voluntary exercise attenuates frailty and prevents negative signs of cardiac aging in older female mice. These data suggest that cardiac function is closely linked to overall health, quantified as frailty, and that late-life interventions to reduce frailty can improve heart health.

E.S. Bisset: None. **S.E. Howlett:** None.

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The Effect of Aging-related Metabolic Disorder on Resistin Expression and Valvular Heart Disorder

Rihab Bouchareb, Najla Saadallah, Roger Hajjar, Djamel Lebeche, CVRC, New York, NY

Calcific aortic valve stenosis (AS) is the most prevalent valvular heart disease and the third most frequent cardiovascular disease after coronary artery disease and hypertension. AS is characterized by a period of asymptomatic progressive valve calcification, which eventually leads to the development of a severe stenosis and to the onset of symptoms. Visceral obesity is a strong predictor of type-2 diabetes and is associated with insulin resistance. In addition, increased visceral adiposity leads to an increase in systemic release in resistin (Retn) and possibly interleukins. Elevation of circulating adipokines such as Retn plays a role in the development of muscle insulin resistance. Moreover, higher Retn levels were associated with increased valvular inflammation and calcification in the elderly patients, whereas this association was not observed among middle-aged patients. In the current study, we explored the potential role of Retn in ectopic valvular calcification. Analyses of scanning electron microscopy micrographs of calcified aortic valve showed that Retn is more abundant next to calcified nodes. These data corroborate with increased levels of circulating Retn in apoE mice that were fed a high fat high sucrose diet for 24 weeks. Moreover, apoE^{-/-}Retn^{-/-} double KO mutant mice have lower plasma cholesterol level compared to apoE KO mice. In addition, knocking down Retn decreased valve lipid infiltration, lowered glycaemia, calcium nodes and reduced fibrosis. In conclusion, Retn may be a new pharmacological target to stop the progression of aortic stenosis in patients with visceral obesity.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

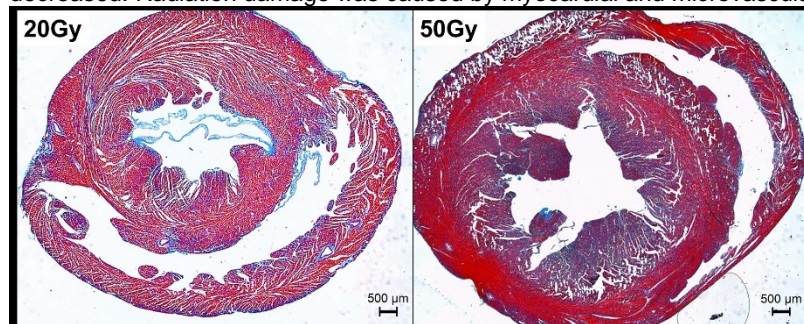
747

Structural and Functional Changes of Rat Heart After High-dose Irradiation

Myung-jin Cha, Seoul Natl Univ Hosp, Seoul, Korea, Republic of; Ji Hyun Chang, Boramae Hosp, Seoul, Korea, Republic of; Hak Jae Kim, Seil Oh, Seoul Natl Univ Hosp, Seoul, Korea, Republic of

Objective: It is known that radiation can cause cardiac damage, and researches are being conducted to use this radiation effect for noninvasive treatment for cardiac arrhythmias. We irradiated the whole heart of rats to observe dose-dependent cardiac structural, functional or histological changes. **Methods:** A total of 33 Lewis rats were divided into 5 groups according to the radiation dose and harvested at 2 or 4 weeks after irradiation. Radiation dose groups were divided into 5 groups of 20, 25, 30, 40, and 50 Gy, and three rats were assigned to each group. To confirm the irradiation effect, body weight, electrocardiography were measured before and after irradiation, The heart size and function were measured by echocardiography. Harvested hearts sectioned by transverse and coronal axes were observed by light microscopy. **Findings:** There was no change in body weight of 20, 25, and 30 Gy group, but 3% of 40 Gy and 10% weight loss of 50 Gy rat. There was no change in ejection fraction in all 5 groups and conduction block or heart rate decrease was not observed. Pericardial effusion was not observed in any rat. The left ventricle end-diastolic dimension was reduced by 19% and the total volume was reduced by 45% in 50Gy group rats. As a result, stroke volume was reduced by 43% in 50 Gy rats. When observed with a light microscope, myocardial degeneration and fibrotic changes, microvascular damage was observed in only 50 Gy rat group (Figure). **Conclusion:** There was no definite change in the cardiac conduction or contractility, even with a high dose radiation in the whole heart. However, whole heart volume and stroke volume were

decreased. Radiation damage was caused by myocardial and microvascular damage.



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Circulating Extracellular Vesicles as Novel Biomarkers of Cardiovascular Risk in Older Women With Different Sitting Time Patterns

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Background and Hypothesis Extracellular vesicles (EVs) are membrane-bound particles shed from a variety of cell types. They contain molecular cargo from the parent cell including metabolites, proteins, and nucleic acids. EVs are present in biofluids and could be promising biomarkers for monitoring wellness. Total sedentary time is linearly associated with cardiovascular and coronary heart disease mortality risk among older women, indicating the intensive impact of sedentary behavior on the circulatory system. Endothelial cells (ECs) provide a barrier and maintain circulatory homeostasis in response to physical and biochemical stimuli. We hypothesize that EC-derived EV count and content changes with EC health and that EC-EVs play a role in bridging sedentary lifestyle with cardiovascular disease risk. **Materials and Results** Archival parent study data and plasma samples from a combined cohort of 518 women aged ≥ 55 years and BMI $\geq 25 \text{ kg/m}^2$ enrolled in the Metabolic, Exercise, and Nutrition at UCSD, Reach for Health, and Community of Mine studies were available to examine EV levels and contents associated with sitting time. Physical activity was measured objectively using accelerometers. Women in the lowest quartile of moderate-vigorous physical activity and who had the highest and lowest mean sitting bout duration across quartiles were indicated as Super Sitters and Interrupted Sitters, respectively. For EC-EV characterization, CD144 was shown to be a specific marker of EC-derived EVs and an anti-CD144 antibody specifically recognized EC-derived EVs from human plasma. EV biochemical markers (e.g., Hsp70, LAMP1, and CD63) were detected on the CD144⁺EVs. Immune-gold staining identified CD81, CD63, LAMP1, and CD144 on individual EVs with a typical toroidal shape featuring phospholipid-bilayers using transmission electron microscopy. A protection assay showed CD144⁺EVs protect miR-126, an EC-enriched miRNA, from RNase degradation. Study of EV levels and contents in plasma from Super Sitter and Interrupted Sitter groups is underway. **Conclusion** CD144⁺EVs carrying molecular cargo from ECs. Hence, EVs and their cargo (e.g., miRNAs) are valuable for examination as novel biomarkers associated with sedentary behavior-induced cardiovascular risk.

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Circulating miRNAs and Apoptosis Genes With Hyperglycemia in Ischemic Stroke Patient

Yi-Chen Hsieh, Nai-Fang Chi, Hung-Yi Chiou, Taipei Medical Univ, Taipei, Taiwan

Stroke is the third cause of death and the leading cause of adult disability in Taiwan. More than half of acute ischemic stroke patients had hyperglycemia, which is one of the major reason for unfavorable outcomes. However, the physiopathology is

remain unclear and better knowledge of underlying genetic mechanisms is needed to improve prognosis. This study aimed to identify possible genes and its regulated miRNA involving in hyperglycemia-induced unfavorable stroke outcomes. This is a two-stage study design. In the first screening stage, 3 hyperglycemia ischemic stroke patients and 3 normal-glycemia cases were recruited. MiRNA sequencing and PCR profile arrays were conducted. Messenger RNA with significant differences and miRNA were analyzed using miRSystem database and Cytoscape. The results showed that apoptosis regulatory pathway genes (FAS and BCL2L1) and its related microRNAs (has-let-7i-5p, has-let-7b-5p, has-let-7a-5p, has-let-7f-5p, has-let-7g-5p, has-let-7e-5p, has-miR-98-5p) were highly associated with acute hyperglycemia ischemic stroke. In the second validation stage, we then verified the above mentioned apoptosis regulatory pathway genes and its related miRNAs using middle cerebral artery occlusion (MCAO) mice model and validate in 143 ischemic stroke patients. From the animal model, the results showed that the FAS gene of hyperglycemic rats exhibited a higher performance than that of normal glycemic rats. The results of miRNAs indicated that the expression of has-let-7a-5p, has-let-7b-5p, has-let-7e-5p, and has-let-7i-5p was lower in hyperglycemia rats than those belonged to normal glycemic rats. For ischemic stroke patients, unfavorable outcome patients had higher FAS expression. Furthermore, there is a significant group-by-time interactions in FAS expression, with unfavorable outcome patients having increasing expression in FAS gene ($p=0.0208$). A remarkable group-by-time interaction in has-let-7e-5p expression was also observed, with unfavorable outcome patients having decreasing expression in has-let-7e-5p ($p=0.0128$). Our study uncovers FAS gene play an important role in hyperglycemia resulting in poor outcomes in ischemic stroke patients, and might provide new direction for treatment in the future.

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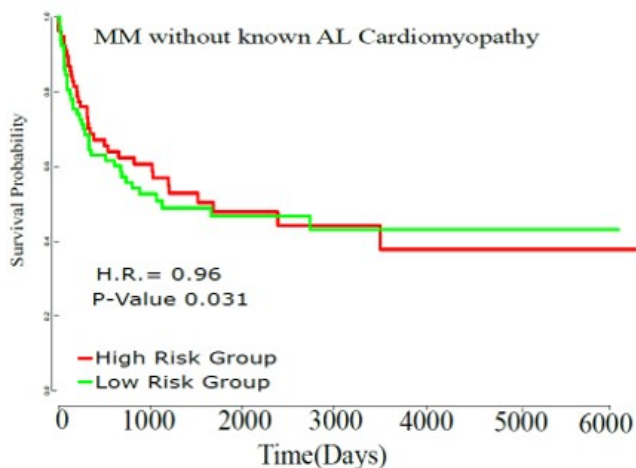
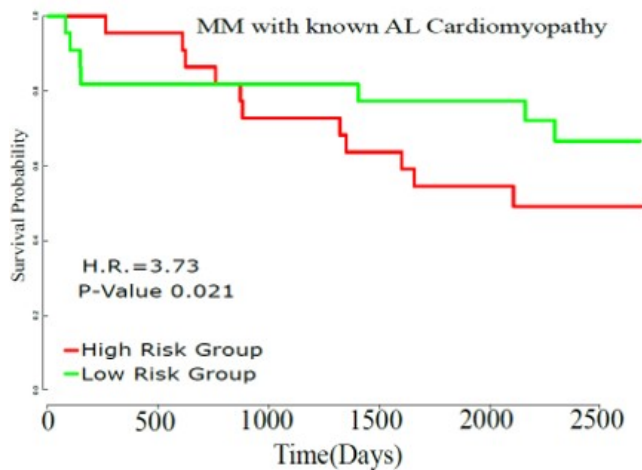
Gene Signatures to Distinguish Amyloid Cardiomyopathy Risk in Multiple Myeloma Patients

Alokkumar Jha, Stanford Sch of Med, Stanford, CA; Isabel Morgado, Dian Jang Lee, Kevin Alexander, CVI, Stanford Sch of Med, Palo Alto, CA; Cheng-Yu Tsai, Stanford Sch of Med, Palo Alto, CA; Katharina Schimmel, CVI, Stanford Sch of Med, Palo Alto, CA; Jennifer Ward, Brigham & Woman Hosp, Boston, MA; R Witteles, Michaela Liedtke, Stanford Amyloid Ctr, Palo Alto, CA; Ronglih Liao, CVI, Stanford Sch of Med, Palo Alto, CA; Seema Dangwal, CVI, Stanford Sch of Med, Stanford, CA

Amyloid light chain (AL) amyloidosis results from tissue deposition of clonal light chains, most commonly produced by clonal plasma cells. AL amyloidosis is closely associated with multiple myeloma (MM), another disease which arises from clonal plasma cell proliferation. Here we aim to identify gene signatures to distinguish AL cardiomyopathy (AL-CM) risk in MM patients.

We utilized publicly available data sets and applied Graph Cluster Perturbation approach to cluster the co-expression networks based on pathways in MM and AL-CM utilizing 225 samples from four datasets: GSE42955 study (12 dilated CM, 12 ischemic CM and 5 control LV human heart tissues), GSE95077 study (16 amiloride drug treated/untreated myeloma cell line samples), GSE24128 study (16 newly diagnosed AL with monoclonal plasma cell samples over- or under-expressing cyclin D1), and GSE6477 study (76 primary bone marrow samples from hyper-diploid myeloma patients and 80 non-hyperdiploid MM patients). From these data sets, the networks for extracellular matrix organization, immune system, innate immune system, metabolism, and neutrophil degranulation pathways for MM and amyloid CM were extracted.

Summary: Ranking of the perturbed genes based on pathways similarity index in MM and AL resulted in a panel of genes: CD44, NRAS, GRAP2, CTLA4, GSN, CCND1, NFKB1, and IRF1 ($p < 0.01$). As shown in **Figure**, the high hazard ratio of this gene cluster in MM with AL-CM (3.76; $p=0.021$) compared to MM without CM (0.96; $p=0.032$) suggests the potential of this gene panel to distinguish high or low risk groups in MM based on survival.



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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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The Inhibition of PARP Provides Greater cardioprotection in Mice with Heart Failure

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The most cause of death in Japan is cancer and the second is heart disease. It is expected that the number of patients with both diseases will continue to increase in the increasingly aging society. Cardiotoxicity by anticancer agents has been recognized for a long time, for example alkylating agents, antimetabolites, proteasome inhibitors, and anthracycline anticancer agents. As cancer patients and heart failure patients increase, the number of patients suffering both diseases at the same time will naturally increase. In recent years, many new anticancer drugs have been developed and are on the market in succession, and the research on cardiotoxicity in each drug has not kept up. In 2017, from our group, there was a report that inflammation was caused by an increase in cardiomyocyte single strand breaks in pressure-overload heart failure model mice, and that inflammation contributes to declining cardiac function. DNA repair related protein PARP1 plays an important role in the repair mechanism of SSBs. PARP inhibitor disturbs its mechanism and exerts antitumor effect by causing tumor cells to become apoptosis. Therefore, we hypothesize that orally administered PARP inhibitors in heart failure patients will inhibit DNA repair by PARP, thus exacerbate inflammation and impair cardiac function more. To examine this

hypothesis, we administered a representative PARP inhibitor, olaparib to pressure-overload heart failure model mice. Surprisingly, the cardiac function was improved in the olaparib administrated group. PARP is known to recruit DNA repair related proteins through synthesis of PAR (Poly-ADP-Ribose) on the DNA damage sites. In recent reports, several studies have shown that the accumulation of PAR would be involved in the worsening of the disease state in cerebellar degenerative ataxia and Parkinson's disease. In our immunostaining study of PAR using the heart tissue specimens from 58 patients with dilated cardiomyopathy, PAR was stained much more in patients with poor outcomes. I believe that PARP inhibitors may have a cardioprotective effect by preventing the accumulation of PAR, and we are now conducting further analyzes.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

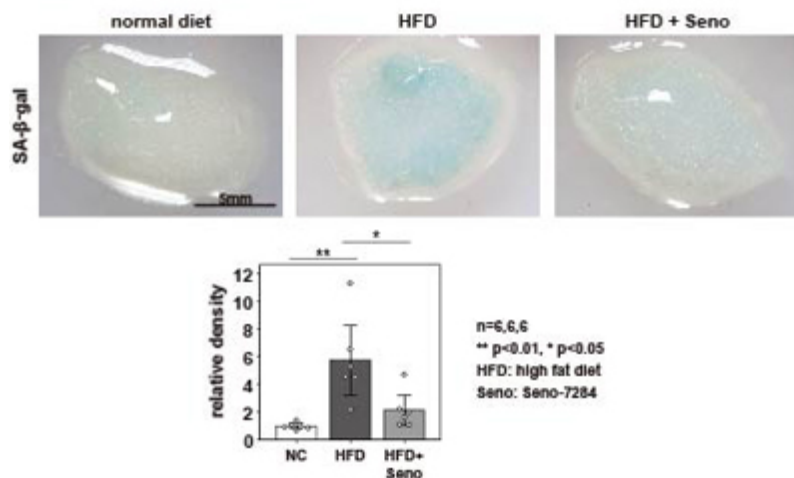
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A Novel Senolytic Drug, Seno-7284 Ameliorates Aging and Age-related Cardiometabolic Disorders

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Accumulation of senescent cells is promoted in various organs as aging, and it also contributes to the progression of age-related disorders. Recent reports have demonstrated the elimination of senescent cells, so-called "senolysis" ameliorated various age-related disorders including cardiovascular diseases. However, there is currently no senolytic drug available in clinical settings. Here, we found a novel senolytic drug (termed "seno-7284") from those already used in clinical setting and it exhibited senolytic effect in murine models of type 2 diabetes, atherosclerosis and progeroid aging. Conducting senescence-associated beta-galactosidase staining (SA- β gal), we found that administrating seno-7284 for one week significantly reduced the accumulation of senescent cells in visceral adipose tissue of diabetic mice induced by fed high-fat diet (Figure). This drug also ameliorated systemic glucose metabolism and adipose tissue inflammation without a reduction of body weight. Further analysis including RNA-seq analysis suggested seno-7284 stimulates the endogenous senolytic function of NK cells and CD8+ T cells via the Cxcl9-Cxcr3 axis. We also found administrating seno-7284 for two weeks also reduced the accumulation of senescent cells and atherosclerotic lesions in the aorta of western-diet-fed ApoE knock out mice. Surprisingly, this drug significantly improved the lifespan of Zmpste24 KO progeroid aging mice. Correctively, our results indicate that seno-7284 mediates its senolytic effect through the recruitment of lymphocytes. Senolytics would become a promising therapy for aging and age-related cardiometabolic disorders.

Figure. Seno-7284 reduced accumulation of senescent cells in visceral white adipose tissue.



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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Exercise Capacity, Cardiac fibrosis and Left Ventricular Remodeling in a Rat Model of Chronic Pressure Overload: Serial Echocardiography, Pressure-Volume Analysis and Gene Expression Profiling

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Background: Pressure overload (PO) left ventricular (LV) hypertrophy is a known precursor of heart failure with preserved ejection fraction (EF). The aims of this study were to establish a reliable model of chronic PO in rats and verify the pathophysiological features of this model by evaluating cardiac function using serial echocardiography and a pressure-volume analysis. Methods: Constriction of the abdominal aorta was used to induce PO LV hypertrophy (n = 40) in rats. Twenty sham rats were compared with PO rats. Serial echo studies and exercise were performed at 2-week intervals, and invasive hemodynamic examination by a pressure-volume catheter system was performed 12 weeks after the procedure. The gene expression profiles of the left ventricle (LV) 12 weeks after the procedure were analyzed by DNA chip technology. Results: Serial echo revealed that the LV wall thickness began to increase after the PO and showed progressive increasing until the 8th week (LV septal wall thickness at 8 weeks 1.4mm±0.1mm for PO vs 0.6mm ±0.05mm for Sham, p <0.01). LV dimension was comparable increased until 14 th week (LV end-systolic dimension at 14 weeks, 4.91±0.50 mm 4.71±0.25 mm for PO vs Sham; LV end-diastolic dimension, 9.02±0.42 mm vs. 8.71±0.47 mm for PO vs Sham, p>0.05). The LV ejection fraction showed similar between groups until the 14th week (70.0±2.2% vs. 69.1±3.1% for PO vs. Sham). In hemodynamic analyses, the LV end-diastolic pressure and the end-diastolic pressure-volume relationship slope were greater in the PO group than sham group. When we compared LV remodeling and exercise capacity, cardiac fibrosis and exercise intolerance developed in the PO group but not in the sham group (exercise duration, 234.0 ± 80.3 vs. 597.8 ± 49.0 seconds, p < 0.05, respectively). Transcriptional profiling of cardiac apical tissues revealed that gene expression related to the cardiac fibrosis, cytoskeletal pathway and G-protein signaling genes were enriched in the PO group. Conclusions: We established a small animal model of chronic PO and verified its pathophysiological features. Cardiac fibrosis and cytoskeletal pathway were important pathways in the PO group and influenced exercise capacity. This model may provide a useful tool for future research on PO heart failure.

K. Kim: None. **Y. Park:** None. **J. Byun:** None. **S. Rha:** None.

Poster Session 1 and Reception

Monday, July 29, 2019, 4:40 pm - 7:00 pm

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The Relationship Between Obstructive Sleep Apnea and Coronary Plaque Instability: An Optical Frequency Domain Imaging Study

Takao Konishi, Hokkaido Univ, Graduate Sch of Med, Sapporo, Japan

Background and purpose: Obstructive sleep apnea (OSA) is associated with coronary artery disease (CAD) and with an increased risk of myocardial infarction, stroke or death due to cardiovascular disease. Optical frequency-domain imaging (OFDI) is a useful modality for evaluating the characteristics of atherosclerotic plaque. The purpose of the study was to use OFDI to investigate the association of OSA with coronary plaque characteristics in patients undergoing percutaneous coronary intervention (PCI). **Methods:** We retrospectively analyzed OFDI data for coronary artery plaques from 15 patients with OSA and 35 non-OSA patients treated between October 2015 and October 2018. Plaque morphology was evaluated for 70 lesions, including 21 from patients with OSA and 49 from non-OSA patients. **Results:** Compared with the non-OSA group, patients with OSA had significantly higher prevalences of thinned cap fibroatheroma (TCFA) (67% vs. 35%, P=0.014) and microchannels (86% vs. 55%, P=0.014); a significantly higher mean lipid index (1,392±982 vs. 817±699, P=0.021), macrophage grade (8.4±6.4 vs. 4.8±4.5, P=0.030), and maximum number of microchannels (1.5±1.0 vs. 0.7±0.7, P=0.001); and a significantly lower mean minimum fibrous cap thickness (69.4±28.7 vs. 96.1±51.8 µm, P=0.008). **Conclusions:** This OFDI analysis suggests that OSA is associated with unstable plaque characteristics in patients with CAD. More intensive medical management for stabilization of coronary atherosclerotic plaque is required in patients with OSA.

T. Konishi: None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Fas Ligand- and Nitric Oxide-Releasing Stent

Mehmet Hamdi Kural, Juan Wang, Liqiong Gui, Guangxin Li, Yifan Yuan, Katherine L Leiby, Elias Quijano, Geroge Tellides, W. Mark Saltzman, Laura E Niklason, Yale Univ, New Haven, CT

To restore blood flow in stenotic vessels such as coronary arteries, metallic vascular stents are often used. However, stent deployment under high pressure leads to vascular wall and smooth muscle (SMC) damage, with compensatory thrombosis, inflammation, and neointimal proliferation of SMCs. These events result in vessel re-occlusion and treatment failure. In an effort to prevent intimal hyperplasia and treatment failure, drug-eluting stents were developed roughly 15 years ago. These stents release global inhibitors of proliferation (eg. "mTOR inhibitors") which stop the growth of SMC as well as vascular endothelial cells (EC). Despite being widely used, drug-eluting stents mandate long-term treatment with potent anticoagulants. This is because mTOR inhibitors prevent re-endothelialization of the stent surface, leaving the clot-inducing metal exposed to the bloodstream. Yet, long-term dual antiplatelet therapy leads to increased risk of bleeding/stroke and myocardial infarction. Here, we leveraged the fact that nitric oxide (NO) increases Fas receptors on the SMC surface. Fas forms a death-inducing complex upon binding to Fas ligand (FasL), while ECs are relatively resistant to this pathway (Figure 1). Selected doses of FasL and NO donor synergistically increased SMC apoptosis and inhibited SMC growth more potently than did everolimus or sirolimus, while having no significant effect on EC viability and proliferation. We verified this differential effect in an ex vivo pig coronary artery model, where the intimal thickening was inhibited by the drug combination, but endothelial viability was retained. We also deployed FasL and NO donor-releasing ethylene-vinyl acetate copolymer (EVAc)-coated stents into pig coronary arteries, and cultured them in perfusion bioreactors for one week. FasL and NO donor, released from the stent coating, killed SMCs in the proximity of the stent struts, even in the presence of flow rates mimicking those of native arteries. Therefore, the FasL-NO donor-combination has the potential to prevent intimal hyperplasia and in-stent restenosis, without harming endothelial restoration, and hence may be a superior drug delivery strategy for drug-eluting stents.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Sunitinib-Induced Cardiotoxicity in an Engineered Cardiac Microtissue Model With Dynamically Tunable Afterload

Carissa Livingston, Abhinay Ramachandran, Univ of Pennsylvania, Philadelphia, PA; Elise Corbin, Univ of Delaware, Newark, DE; Alexia Vite, Alexander Bennett, Kenneth B. Margulies, Univ of Pennsylvania, Philadelphia, PA

Introduction: Anti-angiogenic tyrosine kinase inhibitors (TKIs) have efficacy against many solid neoplasms. Unfortunately, their on- and off-target effects frequently cause hypertension (HTN) and heart failure. Nearly all cases of cardiac dysfunction are associated with HTN, but the mechanism for this association is unclear. **Aims:** We have developed a novel in vitro 3D tissue engineering platform that uses a magnetically tunable elastomer to create dynamic alterations in afterload felt by cardiac microtissues. We are using this platform to examine the impact of increased in vitro afterload (a proxy for HTN) on cardiotoxicity of the TKI sunitinib. **Hypotheses:** 1) Increased afterload augments sunitinib-induced cardiotoxicity 2) Repeat exposures to sunitinib and increased afterload will further augment this toxicity. **Methods:** Human induced pluripotent stem cell cardiomyocytes (96%) and human cardiac fibroblasts (4%) from a commercial vendor (NCardia, Inc.) were allowed to self-assemble and form cardiac microtissues. Microtissues were exposed to sunitinib (0 μ M-10 μ M) at differing levels of afterload for a minimum of 8 hours. After a washout period, tissues were either harvested for analysis or exposures to sunitinib and afterload were repeated. Outcomes measured after each drug exposure included caspase activation, contractile ability, and force generation. **Results:** The applied magnetic field produced increases in afterload from 0.509 \pm 0.032 to 0.698 \pm 0.031 μ N/ μ m. At 36 hours after exposure to supratherapeutic concentrations of sunitinib, recovery of

contractile function as measured by force generation of microtissues was greater under soft afterload conditions compared with stiff afterload conditions (2.52 μ N soft vs 1.16 μ N stiff). **Conclusion:** Our platform creates dynamic alterations in afterload in engineered cardiac microtissues. Use of this versatile platform to examine the cardiotoxic effects of sunitinib has given promising initial results. Future studies examining removal/application of afterload, repeat exposures to sunitinib, and exposure to cardioprotective drugs will allow for insight into the mechanisms, treatment, and prevention of sunitinib-induced cardiotoxicity.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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β -arrestin-Biased β 2-Adrenergic Receptor Signaling Mediates Novel Mechanisms of Cardiomyocyte Contractility

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During heart failure, chronically decreased cardiac output can be treated with positive inotropes, but classic inotropes such as β -adrenergic receptor (β AR) agonists that increase cAMP-dependent Ca^{2+} mobilization and contractility ultimately enhance patient mortality. Thus, an alternate approach would be to enhance cardiomyocyte contractility without alterations in cAMP and Ca^{2+} levels, such as regulation of sarcomeric proteins. Recently, we demonstrated that a small lipidated pepducin designed from the 1st intracellular loop of β 2AR (ICL1-9) enhanced cardiomyocyte contractility in a Ca^{2+} -independent, β -arrestin-dependent manner, yet the complete mechanisms remained unclear. We also showed that β 2AR stimulation in hearts in vivo or neonatal rat ventricular myocytes (NRVM) in vitro activated RhoA in a β arr-dependent manner. Therefore, we sought to determine both the proximal and distal mechanisms by which ICL1-9 enhances cardiomyocyte contractility. Using adult murine cardiomyocytes isolated from wild-type C57Bl/6J mice, we measured basal, ICL1-9- and isoproterenol (ISO, as a positive control)-promoted contractility either alone or in the presence of inhibitors of $G\alpha_i$ activity (Pertussis toxin), ROCK1 (Y-27632), myosin light chain kinase (ML7), and protein kinase D (CID755673). Inhibition of $G\alpha_i$ activity prior to ICL1-9 stimulation led to a decreased contractile response. Consistent with RhoA activation by ICL1-9, ROCK1 inhibition was able to attenuate ICL1-9-mediated contractility, as was inhibition of MLCK. Interestingly, we observed that inhibition of PKD also attenuated ICL1-9-mediated contractility. These data suggest that ICL1-9 acts proximally to engage a β 2AR/ $G\alpha_i$ / β arr signaling axis, which may distally increase the activation of kinases including PKD, MLCK, and ROCK to alter the regulation of sarcomeric proteins.

A. Okyere: None. **C. de Lucia:** None. **J. Song:** None. **R.L. Carter:** None. **J.L. Benovic:** None. **W.J. Koch:** None. **J.Y. Cheung:** None. **D.G. Tilley:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Circulating Preoperative microRNA-29, Biomarkers of Collagen Synthesis, and Age Predictive of Postoperative Atrial Fibrillation After Coronary Artery Bypass Grafting

Farhan Rizvi, Mahek Mirza, CIRCA, Milwaukee, WI; Susan Olet, Aurora Res Inst, Milwaukee, WI; Melissa Albrecht, Stacie Edwards, Aurora Sinai/Aurora St. Luke's Medical Ctrs, Milwaukee, WI; Larisa Emelyanova, CIRCA, Milwaukee, WI; David Kress, Aurora Cardiovascular Services, Aurora Sinai/Aurora St. Luke's Medical Ctrs, Univ of Wisconsin Sch of Med and Public Health, Milwaukee, WI; Gracious R Ross, Ekhsan Holmuhamedov, CIRCA, Milwaukee, WI; A. Jamil Tajik, Bijoy K Khanderia, Arshad Jahangir, Aurora Sinai/Aurora St. Luke's Medical Ctrs, Univ of Wisconsin Sch of Med and Public Health, Milwaukee, WI

Background: Postoperative atrial fibrillation (PoAF) is a common complication occurring in 35-50% patients within 2-3 days after cardiac surgery. Identification of patients, especially those with no prior history of atrial fibrillation before surgery, is a challenge. A pre-existing atrial substrate appears to be an important factor in the development of PoAF. The aim of this study was to assess the role of biomarkers in identifying patients at risk of PoAF in a pathophysiology-based risk predictive model by combining clinical and biochemical risk factors.

Methods: Preoperative blood from patients undergoing cardiac surgery with no previous history of AF was assessed for circulating levels of biomarkers reflecting collagen synthesis/degradation and extracellular matrix remodeling using ELISA. Reverse transcriptase polymerase chain reaction assessed microRNA29 in the serum and correlated to extent of atrial fibrosis. Echocardiographic evaluation of LA mechanics was performed preoperatively using M-mode, 2D Doppler, and 3D Speckle tracking.

Results: Out of 55 patients, 31 patients (56.4%) who developed PoAF after surgery during their hospital stay were older in age (70.0 ± 4.0 years vs. 63.4 ± 9.9 ; $p < 0.01$) with abnormal global longitudinal strain (6.9 ± 0.69 vs. 10.9 ± 0.93 , $p = 0.007$), higher amino-terminal peptide procollagen III (PIIINP) levels (101.1 ± 42.7 vs. 36.6 ± 20.0 ; $p = 0.043$) with increased collagen to myocardial ratio (0.20 ± 0.09 vs. 0.09 ± 0.01 , $p = 0.026$), and reduced preoperative circulating microRNA-29a, -29b and -29c levels. By combining the clinical risk factors, circulating biochemical and molecular biomarkers, we developed a model that identified PoAF patients (AUC=0.7987; 95% CI, 0.6174—0.98) with reduced preoperative atrial ejection fraction ($32 \pm 2\%$ vs. $42 \pm 2\%$; $p = 0.01$) as an independent risk factor for PoAF.

Conclusion: Our study developed a noninvasive tool to identify those who are at risk for new-onset PoAF in patients with no previous history of AF when combining age, biomarkers of collagen synthesis and microRNA-29a.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Metabolic Syndrome Impairs Cardiac Remodeling During Pregnancy in Mice

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Rationale: Due to the change of life style in the past decades, the incidence of metabolic syndrome (MetS) and obesity in women of childbearing age has been increased. Evidences have shown that complications during pregnancy, such as obesity and gestational diabetes, strongly link to future heart diseases. During normal pregnancy, the heart undergoes a physiological remodeling with mild ventricular dilation and hypertrophy. However, the effects of MetS on cardiac phenotype during pregnancy are not well understood. Moreover, animal models for MetS so far are not well established, which are usually species limited, gender limited and lack of translational significance. Thus, it's important to develop an animal model which resembles the etiology of MetS in human beings.

Objective: Establish a model of MetS in female C57BL/6J mice and determine the effects of MetS on cardiac remodeling during pregnancy.

Methods and Results: To establish a mouse model of MetS, high fat (49% kcal)/high carbohydrates (HF-HC) diet were given to C57BL/6J female mice at the age of 8-week. Mice fed with normal diet (10% kcal fat) served as control. After 14 weeks feeding, mice fed with HF-HC diet showed significantly higher body weight and gonadal fat weight compared with control mice. Moreover, mice fed with HF-HC diet showed impaired glucose tolerance and higher serum total cholesterol level. The symptoms of obesity, impaired glucose tolerance and dyslipidemia suggest the development of MetS. Female mice after 14-week special diet feeding were subjected to breeding. Cardiac function was evaluated by echocardiography during pregnancy and 1-day post-partum, then the mice were terminated 1-day post-partum. Female mice that developed MetS showed impaired cardiac function and pathological remodeling during pregnancy. Moreover, mice fed with HF-HC diet showed more myocardium interstitial fibrosis accompanied with upregulation of fibrosis related genes in the heart compared with Ctrl diet.

Conclusion: We've developed a mouse model of MetS in C57BL/6J mice strain with 14 weeks feeding of HF-HC diet, which resemble the western diet that affects human beings nowadays. Females that have MetS showed impaired cardiac function and pathological remodeling during pregnancy.

Word Count 1935

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Sex Difference in Cardiomyocyte Functional Etiology in Heart Failure with Preserved Ejection Fraction

Claire L Curl, Antonia Ja Raaijmakers, James R Bell, Tristan W Harding, Stephen B Harrap, **Lea M Delbridge**, Univ Melbourne, Melbourne, Australia

Heart failure with preserved ejection fraction (HFpEF) accounts for >50% of heart failure patients and is particularly prevalent in women. A mechanistic understanding of the intrinsic cardiomyocyte pathology in HFpEF is limited with no selective treatments identified. Gender-specific aspects of HFpEF etiology have not been well characterized. The aim of this study was to experimentally evaluate sex difference in cardiac and cardiomyocyte performance in HFpEF, using a novel recently developed rodent model of HFpEF - the 'Hypertrophic Heart Rat'.

Echocardiography was performed in 50 week male and female HHR/NHR (Normal Heart Rat) using a GE Vivid 9. Fibrosis measurements employed picosirius red staining. Single myocyte force, shortening and intracellular Ca^{2+} measurements were obtained (Myostretcher, Ionoptix).

Male and female HHR hearts were hypertrophic (M: 4.9 ± 0.3 vs 3.7 ± 0.1 ; F: 6.6 ± 0.5 vs 4.4 ± 0.1 mg/g CWI) with significant diastolic dysfunction (M: 27.8 ± 1.4 vs 18.0 ± 1.7 ; F: 28.9 ± 1.2 vs 18.9 ± 1.0 E/E') and preserved systolic function (M: 78.4 ± 1.9 vs 74.5 ± 1.3 ; F: 80.9 ± 1.3 vs 78.1 ± 0.8 EF). Female HHR, but not males, exhibited diffuse interstitial fibrosis (M: 3.33 ± 0.1 vs 3.51 ± 0.1 ; F: 11.45 ± 0.4 vs $8.44 \pm 0.2\%$). In male, but not female, cardiomyocytes, hypercontractility and high cytosolic operational Ca^{2+} levels were observed (64% and 116% increase respectively). The force-length gradient produced by serial stretches of isolated cardiomyocytes was increased in male and female HHR (M: 6.8 ± 0.4 vs 4.3 ± 0.8 ; F: 7.4 ± 0.5 vs 3.0 ± 0.4 nN/%SL stretch) indicating stiffer cardiomyocytes in the HFpEF hearts. The increase in stiffness was significantly accentuated in females.

This study indicates that in HFpEF the cardiomyocyte functional etiology is different in males and females. Whilst in males the relationship between cardiomyocyte hypercontractility is the most prominent characteristic of HFpEF, in females the remodelling of the extracellular matrix exacerbates intrinsic myocyte stiffness. Further investigation of the molecular pathways involved in these processes is required for identification of potential therapeutic targets.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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SGLT-Mediated Na^+ Overload Results in Oxidative Stress and Abnormal SR Ca^{2+} Release in Diabetic Hearts

Sathya Velmurugan, Amanda Hoskins, Florin Despa, **Sanda Despa**, Univ of Kentucky, Lexington, KY

Intracellular Na^+ concentration ($[Na^+]_i$) regulates Ca^{2+} cycling, oxidative state and electrical stability of the heart. $[Na^+]_i$ is linked to glucose uptake through the Na^+ -glucose cotransporter (SGLT). The SGLT1 isoform is present in the heart and its overexpression causes hypertrophy and left-ventricular dysfunction. Here, we hypothesized that cardiac SGLT activity is increased in type-2 diabetes (T2D), which causes myocyte Na^+ overload and results in oxidative stress and exacerbated sarcoplasmic reticulum (SR) Ca^{2+} leak. To test this hypothesis, we analyzed myocardial tissue from humans with/without T2D and compared rats with late-onset T2D that display diabetic cardiomyopathy similar to that seen in humans (HIP rats) with their non-diabetic littermates (WT). SGLT1 expression was increased in hearts from T2D patients compared to non-diabetic individuals and in hearts from HIP vs. WT rats. SGLT inhibition significantly decreased the uptake of both glucose and Na^+ in myocytes from HIP rats but not in WT, which indicates that SGLT function is increased in T2D hearts. While SGLT1 upregulation may partially compensate for the reduced insulin-dependent glucose uptake, the ensuing raise in Na^+ influx resulted in elevated $[Na^+]_i$ in HIP myocytes (by ~ 3 mM compared to WT). Higher $[Na^+]_i$ causes oxidative stress by activating the *mitochondrial* Na^+/Ca^{2+} exchanger (mitoNCX), which lowers mitochondrial $[Ca^{2+}]$ and thus slows down regeneration of the antioxidant NADPH. In agreement with a role for elevated $[Na^+]_i$ in causing oxidative stress in T2D hearts, H_2O_2 production was increased in HIP myocytes vs. WT and mitoNCX inhibition, which uncouples mitochondria from $[Na^+]_i$, significantly reduced H_2O_2 production in HIP hearts. Oxidative stress enhances the SR Ca^{2+} leak directly through oxidation of ryanodine receptors (RyRs) and indirectly via CaMKII activation and consequent RyR phosphorylation. SR Ca^{2+} leak was augmented in myocytes from HIP vs. WT rats and mitoNCX inhibition significantly reduced the leak in HIP myocytes, but not in the WT. Thus, our data indicate that SGLT activity is enhanced in myocytes from T2D rats, which increases Na^+ influx and causes Na^+ overload. Elevated $[Na^+]_i$ contributes to oxidative stress and abnormal SR Ca^{2+} leak in diabetic hearts.

S. Velmurugan: None. **A. Hoskins:** None. **F. Despa:** None. **S. Despa:** None.

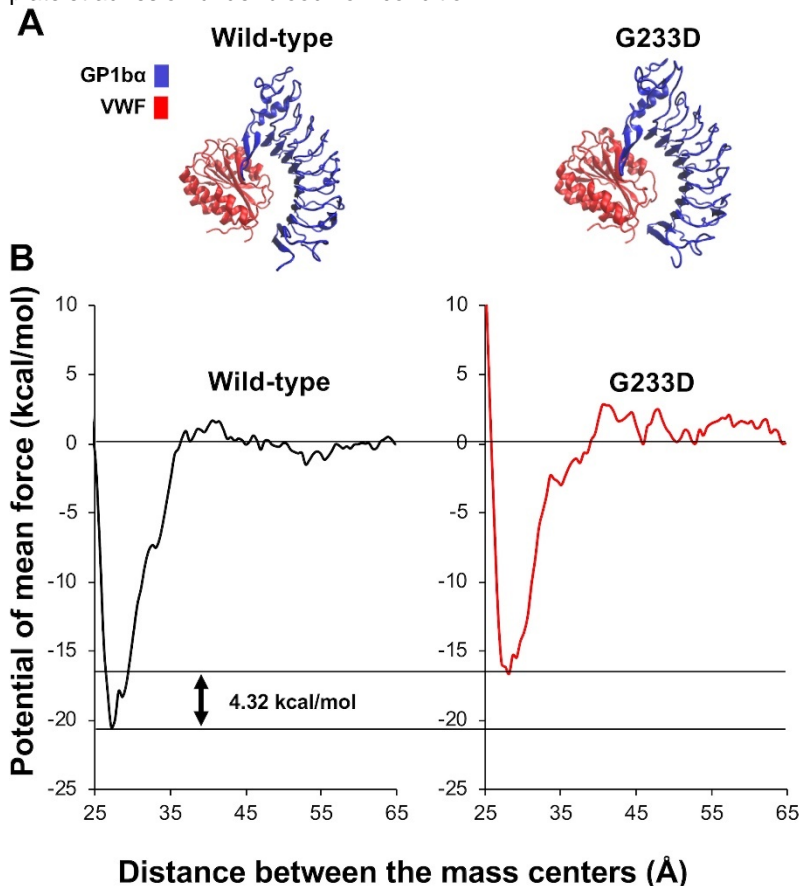
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Potential Explanation of the Mechanism for Loss of Function with G233D Mutation in Platelet Glycoprotein Iba: Results from Molecular Dynamics Simulation

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Background: Platelet play crucial role for the onset of acute coronary syndrome. The initial binding following vessel injury is exclusively mediated by the binding of von Willebrand factor (VWF) and platelet glycoprotein (GP) Iba. Previous biological experiments revealed loss of function of platelet expressing GPIba with G233D mutation. **Objective.** To elucidate the mechanism underlining the loss of function in G233D mutant. **Methods.** Dynamic fluctuating three-dimensional structures and the Potential of Mean Force (PMF) were calculated for the binding of VWF and platelet GPIba in wild-type or G233D mutant. PMF were calculated at each 0.5Å for 25 Å to 65 Å of mass center distance between GPIba and VWF. The energy required to dissociate the bond between GPIba and VWF was calculated by subtracting the lowest PMF from the PMF at 65 Å mass center distance. Chemistry at HARvard Molecular Mechanics (CHARMM) force field with NANoscale Molecular Dynamics (NAMD) was used for calculation. The initial structure of each mutant was obtained by inducing single amino-acid substitution with Visual Molecular Dynamics (VMD) to the stable water-soluble binding structure of wild-type VWF and GPIba. **Results.** The energetically most stable binding structure of VWF and GPIba in wild-type and G233D did not differ substantially (Figure panel A). However, The energy required to dissociate the bond between GPIba and VWF was 4.32 kcal/mol (19.5 %) lower for G233D mutant compared to wild-type (Figure panel B). **Conclusions.** Our results suggest that the reduction of dissociation energy of single molecule of G233D GPIba mutant as a possible explanation for the reduced platelet adhesion under blood flow condition.



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Identification of Pathological Pathways in Hypertrophic Cardiomyopathy via Single Nuclei RNA-Sequencing

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Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease, is characterized by left ventricular hypertrophy, is often asymmetric, and is unexplained by secondary causes. The molecular and cellular basis of this asymmetric hypertrophy is unknown. HCM has been thought of as a disease of the sarcomere, with mutations in more than a dozen sarcomere-associated proteins correlated with HCM. These mutations, however, exhibit large phenotypic heterogeneity with little correlation between mutation and severity. Treatment of HCM is limited and thus, understanding what drives the disease at the molecular and cellular level could lead to novel treatment strategies. Obstructive HCM is a severe form of the disease where the left ventricular outflow tract is obstructed. Patients with obstructive HCM often undergo septal myectomy surgery to remove the obstructive lesion. To understand the underlying mechanisms of obstructive HCM, we performed single nuclei RNA-sequencing on thousands of nuclei from 11 patients' myectomy samples to determine the gene expression patterns in individual cells and identify the cell populations in those lesions. We used rejected donor hearts that do not exhibit any LV hypertrophy or have any major cardiovascular defects as control samples to compare the gene expression patterns in those cardiomyocytes to those in obstructive HCM lesions. We performed principal component analysis and hierarchical clustering on the RNA expression counts to identify the different cell populations in each dataset, and looked for cell populations that were present across all HCM datasets. This analysis revealed distinct cardiomyocyte populations in obstructive lesions. Differential gene expression analysis among these clusters revealed a minority cardiomyocyte population in the obstructive lesions that exhibited a relatively normal gene expression pattern, while the majority cardiomyocyte cluster exhibited a shift in gene expression to genes involved in growth pathways. This abnormal cardiomyocyte population was absent in the control datasets. These findings imply that obstructive lesions seen in HCM may arise from abnormal growth of a subset of cardiomyocytes that could possibly be targeted by novel therapeutics.

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The Highly Prevalent 25bp Intronic Deletion in *MYBPC3* is Benign Under Baseline Conditions

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Background: Hypertrophic cardiomyopathy (HCM) affects at least 1 in 500 people worldwide, and results in the thickening of the ventricular walls and reduced cardiac function. Mutations in *MYBPC3*, encoding cardiac myosin binding protein-C, are the most common cause of HCM. Previously, a highly prevalent 25bp deletion within intron 32 of *MYBPC3* was described in the South Asian population. The *MYBPC3*^{d25bp} variant is present in approximately 100 million people, and encompasses a splicing branch point predicted to result in abnormal splicing of exon 33. Thus, there is a critical need to understand the mechanism by which *MYBPC3*^{d25bp} may cause cardiomyopathy.

Methods: To determine the role of the 25bp deletion *in vivo*, knock-in humanized mice were created in which intron 32 was replaced with the human intron 32, with or without the *MYBPC3*^{d25bp} mutation. Mice were characterized at 3- and 6-months of age by echocardiography, histological, and protein analysis. The presence of aberrant exon splicing was also determined in mice carrying the *MYBPC3*^{d25bp} variant through RT-PCR and mini-gene assays. Finally, exon trapping experiments were performed to understand the mechanism behind exon skipping.

Results: Under baseline conditions, *MYBPC3*^{d25bp} displayed no changes in cardiac function or morphology as measured by echocardiography (FS (%): NTG 35.3%, WT 32.8%, Het 33.7%), heart weight to body weight ratio, or histology. While exon 33 skipping was not detected by RT-PCR, the presence of an alternative splice site within exon 33 was identified in *MYBPC3*^{d25bp} mice. However, this did not affect the protein levels of cMyBP-C. Furthermore, mini-gene experiments demonstrated that the *MYBPC3*^{d25bp} mutation significantly reduced the percentage of correctly spliced transcripts (86.2% vs.

77.5%).

Conclusions: These data demonstrate that the presence of the highly prevalent 25bp deletion is not sufficient to cause disease under baseline conditions. However, it is possible that the increased levels of aberrant splicing may increase the risk for developing HCM.

J.W. McNamara: 2. Research Grant; Significant; 17POST33630095. **J.A. Schwanekamp:** None. **P.N. Patel:** None. **S.K. Viswanathan:** None. **M. Bohlooly:** None. **K. Madeyski-Bengtson:** None. **R. Knöll:** 1. Employment; Significant; AstraZeneca. **J.G. Seidman:** 7. Ownership Interest; Significant; Myokardia. **C.E. Seidman:** 7. Ownership Interest; Significant; Myokardia. **S. Sadayappan:** 2. Research Grant; Significant; 17CCRG33671128, R01HL130356, R01HL105826, R01AR067279, R01/R56HL139680.

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Neonatal and Adult Cardiac Transcriptome Analysis Reveals Distinct Mechanisms in Noncompaction Dilated Cardiomyopathy Development Induced by p.K69R-MLP (Muscle Lim Protein) Mutation *in vivo*

Enkhsaikhan Purevjav, Fuyi Xu, Undral Munkhsaikhan, Neely Alberson, Ramona Sabau, Lu Lu, Jeffrey A. Towbin, Univ of Tennessee Health Scie, Memphis, TN

Introduction: Pediatric and adult dilated cardiomyopathy (DCM) represent distinct pathological entities, however, the basis of this phenomena remains obscure. The pathogenic variant p.K69R in MLP/CSRP3 induced DCM in adult Mlp^{K69R} knock-in mice. A noncompacted ventricular wall and dilated ventricles were seen in embryonic and neonatal Mlp^{K69R} hearts, reminiscent of that seen in infant variant carriers. **Hypothesis:** Mlp-K69R associated genetic modifiers govern DCM phenotypes in neonatal and adult heart. The study was to find genetic modifiers associated with divergent neonatal and adult phenotypes induced by Mlp-K69R. **Methods:** RNA extracted from neonatal and adult Mlp^{K69R} and Mlp^{WT} mouse myocardium was applied to Affymetrix microarray and QPCR analysis. Data were normalized, subjected to differentially expression genes and gene set enrichment analysis. **Results:** We found 1024 genes in adult and 252 genes in neonatal heart that were significantly altered in Mlp^{K69R} vs WT littermates. 23 of these genes were mutually altered in adult and neonatal mutants. In adult mutants, 15 sarcomeric genes were significantly over-expressed compared to WT littermates. The mutation altered expression of genes involved in sarcolemmal and desmosomes unity. Calcium handling, integrin-associated networks, and heart failure-associated genes were upregulated in mutants vs WT littermates. In neonates, genes involved in cardiac and vascular development (*Rhox2c*, *Tbx3*, *Mir23a*, *Tbc1d7*), cell adhesion and migration (*Tff2*), and apoptosis (*Mir297a-2*) were significantly altered. Two cardio-protective miRNAs, *Mir98* and *Mir451a*, were highly reciprocally altered in both, adult and neonatal, mutant vs WT hearts. Their expression was increased in mutant adult hearts, but decreased in neonatal mutant hearts, suggesting novel candidates associated with K69R-MLP-induced DCM and noncompaction phenotypes. **Conclusions:** Differential transcriptomes in neonatal and adult hearts suggest that pathogenic K69R-MLP variant induces DCM and noncompaction phenotypes *via* distinct underlying genetic modifiers. Contrary to mutant adult mice, mutant newborn hearts show significant decrease in *Mir98* and *Mir451a*, which may predispose them to developing myocardial noncompaction.

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Gene-Environment Regulatory Circuit of Right Ventricular Pathology in Cyanotic Congenital Heart Defects

Zhao Yan, Xuedong Kang, Reshma Biniwale, Fadi Shahoud, Van Arsdell Glen, Fabiola Quintero, Nelson Stanley F, **Marlin Touma**, UCLA-David Geffen Sch Med, Los Angeles, CA

Introduction: The phenotypic spectrum of congenital heart defects (CHDs) is contributed by both genetic and environmental factors. Their contributions are profoundly heterogeneous but may operate on common pathways that are not well understood. Elucidating the gene-environment interplays in CHDs pathogenesis is essential to identify chamber specific therapies. **Methods:** Following the UCLA CHD-BioCore approved protocol, right ventricle outflow tracts (RVOTs) samples were collected from infants with tetralogy of fallot (TOF) or Ventricular Septal Defect (VSD). Total RNA was used for RNA-

seq. Systemic hypoxia was induced using hypoxia chamber. Weighted gene network co-expression analysis was performed using R package. **Results:** Genome-wide transcriptome analysis of RVOT samples from cyanotic and non-cyanotic CHDs uncovers disease-associated differences in gene expression. Co-expression network analysis reveals that individuals with CHDs show diagnosis-specific and environment-specific gene modules. In particular, hypoxia-dependent induction of proliferation and E2F1 dependent cell cycle reprogramming are inversely associated with WNT11 signaling in the cyanotic TOF. In addition, epithelial mesenchymal transition (EMT), fibrosis and apoptosis are repressed. Importantly, perinatal hypoxia attenuates the baseline differences between ventricular chambers in neonatal mouse heart. Remarkably, the observed attenuation of ventricular patterning (AVP) genes is more robust in the right ventricle and highly concordant in hypoxemic hearts in mouse and human. Transcriptional dysregulation of the P53 network is a shared hallmark among AVP genes and cyanotic TOF modules. Importantly, P53 network analysis suggests CREB1, a hypoxia sensor, and LEF1, an EMT regulator during cardiogenesis, as potential mediators of hypoxia-Wnt11 circuit. Finally, the data demonstrate that genetic subtypes of TOF may influence hypoxia-induced regulation leading to phenotypic heterogeneity at molecular and cellular levels in cyanotic TOF hearts. **Conclusions:** Gene-environment interrelations may influence changes in regional gene expression leading to phenotypic heterogeneity at the molecular cellular level in complex cyanotic CHDs.

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Interaction of Phosphate Transport and Calcification in Placenta Vasculature

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Phosphate transport has been associated with pathological vascular calcification, but this relationship remains largely unexplored in placenta tissue. In this study we investigated the interaction of maternal-fetal phosphate transport and placental calcification using in vitro and in vivo models. Sodium-dependent phosphate transporter transcriptomics validated by quantitative RT-PCR revealed that the type III sodium-dependent phosphate transporter family members Slc20a1 and Slc20a2 are the predominant sodium-dependent symporters in both mouse and human placenta. Global loss of Slc20a2 generates multiple maternal and fetal phenotypes. We examined Slc20a2 promoter activity and protein localization of Slc20a2 throughout placentation. Slc20a2 was detected in embryonic structures that give rise to the maternal-fetal interface and in regions of the placenta that are susceptible to vascular calcification in the absence of Slc20a2. Pathologically, vascular malformations were found to precede calcium deposition in the absence of Slc20a2. Activation of osteocalcin in the placenta suggested that physiological calcification mechanisms may potentiate the mineral deposition observed with Slc20a2 loss. Histological analyses of human placenta revealed focal localization of Slc20a2 protein and distinct calcification deposition patterns. Lastly, the BeWo trophoblast model confirmed cellular competency for active, sodium-dependent phosphate symport as well as extracellular matrix calcification. Taken together, our findings support that normal growth and maintenance of the placenta requires Slc20a2. Moving forward, we now propose the working hypothesis that Slc20a2-mediated placental phosphate transport contributes to the energy requirements for rapid placental vascular development, enabling vascular patterning that maximizes surface area and minimizes tissue damage caused by aberrant flow, thereby promoting organ health and transport of nutrients, including phosphorus, across the placenta.

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MYBPC3 Mutations in Indian Patients with Hypertrophic Cardiomyopathy

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Introduction: Hypertrophic cardiomyopathy (HCM), the most common inherited heart disease, is characterized by thickening of the ventricular wall, myocyte disarray, and fibrosis, resulting in impaired cardiac function and leading to adverse ventricular remodeling, heart failure and increased risk for sudden cardiac death. Importantly, mutations in the gene encoding cardiac myosin binding protein-C (*MYBPC3*), a muscle contractile protein, account for approximately 50% of the

reported HCM cases. However, the prevalence of penetrance of *MYBPC3* mutations in Indian patients with HCM is yet to be systematically studied.

Methods: The Institutional Human Ethics Committee at the University of Pune approved the present study. HCM patients undergoing treatment in the Cardiology Unit of Bharti Hospital, Pune, were identified. With the approval of written informed consent, blood samples were obtained from all patients and their relatives and screened by single-strand conformation polymorphism (SSCP) and direct DNA sequencing, followed by correlation with clinical outcome.

Results: Of the 23 probands screened in this study, one, #16, showed a mutation in exon 07 (A/G) at chromosomal position 47370041, causing a protein change at position NP_000247.2:p.S236G, and reported as SNP status ID rs386584787. The mutation was localized to the immunoglobulin domain of cardiac myosin binding protein-C, causing an amino acid change from serine to glycine with a score of 0.00, as shown by the PolyPhen-2 (Polymorphism Phenotyping v2) tool. G>C transversion of intron variant in exon 21 of *MYBPC3* results in a new exon splicing enhancer (ESE) site which may alter splicing. G>A transition at g. 20167 is an intron variant of a previously reported status (rs373904644). This polymorphism results in the creation of an intronic splicing enhancer (ISE) site. The proband had left ventricular wall thickness of 24 mm, ejection fraction of 60 (%), and left ventricular internal diameter of 30 mm, suggesting early onset of HCM.

Conclusion: The *MYBPC3* mutations might play a key role in the development of HCM in the Indian population, indicating the soundness of establishing a systematic screening of all HCM patients.

V.W. Wankhade: None.

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Dual-Specificity Phosphatase 6 Deficiency Protects Against Intimal Hyperplasia After Arterial Injury

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Dysregulation of MAPK activation cascades has been implicated in various diseases. Dual-specificity phosphatases (DUSPs) are a subfamily of protein tyrosine phosphatases that dephosphorylate MAPKs and modulate critical signaling pathways. Studies have shown that DUSP6 plays an important role in the heart and diet-induced obesity. However, the role of DUSP6 in vascular disease is largely unclear. Our goal is to investigate whether DUSP6 has a role in occlusive vascular disease. To this end, we used a neointima formation model by denuding endothelium of mouse femoral arteries with a guide wire. We showed that DUSP6 was expressed at very low levels in the normal uninjured mouse arteries; however, DUSP6 expression was increased in the arterial wall of injured blood vessels, suggesting a potential role of DUSP6 in vascular disease. Compared with wild-type mice, DUSP6 deficiency reduced neointima formation 4 weeks after arterial injury in mice. BrdU incorporation assays revealed that lack of DUSP6 resulted in decreased smooth muscle cell (SMC) proliferation in the neointima and media 2 weeks after injury, indicating DUSP6 may promote SMC proliferation in response to injury. Indeed, overexpressing DUSP6 in primary SMCs increased cellular proliferation, similar to that exerted by inflammatory cytokine IL-1 β ; while knocking down DUSP6 expression with siRNA abrogated IL-1 β -induced SMC proliferation. Pathway analysis showed that IL-1 β -induced ERK1/2 activation preceded DUSP6 induction in SMCs. Intriguingly, ERK1/2 inhibitor U0126 abolished IL-1 β -mediated ERK1/2 activation, DUSP6 induction, and SMC proliferation. Taken together, our results indicate that DUSP6 induction promotes vascular remodeling under pathological conditions. DUSP6 might be a therapeutic target for preventing/treating occlusive vascular disease.

S. Yet: None.

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Duchenne Muscular Dystrophy Cardiac Exosomes Contribute to the Pathogenesis of Dystrophin-deficient Cardiomyopathy

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Introduction: Cardiomyopathy is a devastating consequence of Duchenne muscular dystrophy (DMD) and its pathogenesis is not well known. Exosomes (exos) are small, secreted vesicles with the ability to exert paracrine effects in target cells by transfer of their molecular cargo. This cargo can be dysregulated in disease and may contribute to pathogenesis. Duchenne muscular dystrophy exos in skeletal muscle promoted pathology, but whether exos are pathological in the DMD heart is unclear. We hypothesize that exposure to DMD cardiac exos promotes pathogenesis and their inhibition will protect against

cardiomyocyte injury. To investigate, we used DMD iPSC-derived cardiomyocytes (iCM) and *mdx* mouse models of DMD.

Methods/Results: *In vitro*, 2 non-affected (N), 2 patient-derived DMD and 1 gene-edited DMD iCMs were exposed to either exos isolated from N- or DMD-iCMs for 48 hr, or to GW4869, an exo inhibitor for 24 hr, followed by 1 hr stress (100 μ M H₂O₂ in 10 mM deoxyglucose in RPMI - glucose) and 4 hr recovery (RPMI + glucose) and stained with either DHE, TMRE and propidium iodide to assess ROS levels, mitochondrial membrane potential ($\Delta\psi$ m) and cell death, respectively. Stress increased ROS levels in all 3 DMD-iCMs from 413-1102AU to 955-2008AU, which was diminished with N-exo (346-1061AU) but not DMD-exo (882-1995AU). Stress caused $\Delta\psi$ m loss in DMD-iCM from 4049-4082AU to 439-1315AU, and N-exo (1917-2867AU) but not DMD-exo (870-1586AU) were protective. Stress increased cell death in DMD-iCM from 3-10% to 30-45% which was decreased with N-exo (10-21%) but not DMD-exo (26-46%). GW4869 decreased ROS levels from 955-2008AU to 344-528AU, cell death from 30-45% to 6-28% and partially rescued $\Delta\psi$ m from 439-1315AU to 1947-3842AU in DMD-iCM.

In *mdx* mice, GW4869 reduced isoproterenol induced cardiac injury (*GW+Iso*: 3 \pm 2% vs. *Veh+Iso*: 13 \pm 3%).

Conclusions: *In vitro*, unlike N-exo, DMD-exo exposure failed to protect DMD-iCM from stress. Inhibiting DMD-exo with GW4869 protected DMD-iCM against cell stress and reduced *mdx* cardiac isoproterenol-induced injury. Together, these data suggest that DMD-exo may dysregulate the DMD cardiomyocyte response to stress thereby contributing to injury. Furthermore, exosome reduction may be a strategy for the treatment of DMD cardiomyopathy.

M. Gartz: None. **Z. Afzal:** None. **J.L. Strande:** None.

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Doxorubicin-Induced Cardiotoxicity and Novel GATA4-Targeted Compounds

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Doxorubicin (DOX) is a widely used anticancer drug which, unfortunately, induces dose-related cardiotoxicity. A deep understanding of DOX's toxicity is still unclear, partly because most *in vitro* studies have evaluated the effects of short-term high-dose DOX treatments. Thus, a more precise model of DOX cardiotoxicity is needed to more accurately simulate clinical scenario.

The first aim of this study was to establish an *in vitro* model of long-term low-dose administration of DOX utilizing human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The second aim was to investigate if novel GATA4-targeted compounds can protect CMs from DOX toxicity.

Acute toxicity was studied in both primary neonatal rat ventricular cardiomyocytes (NRVCs) and hiPSC-CMs. The cells were exposed to 0.1-3 μ M DOX for 48 h, after which the lactate dehydrogenase and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were carried out. To study long-term toxicity, hiPSC-CMs were exposed to 100 nM DOX for up to 14 days. MTT assay and high-content analysis were carried out after 4, 7 and 14-day exposures. The effect of the test compound was studied by exposing the cells simultaneously to DOX and the compound.

A 48-hour exposure to DOX at 1 and 3 μ M concentrations induced more than 62% reductions in both NRVC and hiPSC-CM viability, whereas a 14-day exposure to 100 nM DOX induced a 26% reduction in hiPSC-CM viability. A 4-day exposure to 100 nM DOX induced a 3.1-fold increase in the percentage of cells positive for pro-B-type natriuretic peptide (proBNP) compared to control. When the cells were exposed simultaneously to 100 nM DOX and the test compound (10 μ M), the percentage of proBNP+ cells increased only 1.4-fold compared to control. However, over 14-day exposure the compound at 10 μ M concentration reduced hiPSC-CM viability 50% compared to control.

A long-term exposure of hiPSC-CMs can be utilized as an *in vitro* model to investigate the mechanisms of delayed DOX-induced cardiotoxicity. The GATA4-targeted test compound exhibited cardioprotective potential against subacute DOX toxicity. Over chronic exposure the compound was, however, toxic to CMs, indicating that further structural optimization is required to develop non-toxic derivatives.

T. Karhu: None. **S. Kinnunen:** None. **V. Talman:** None. **H. Ruskoaho:** None.

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Human-induced Pluripotent Stem Cell-derived Cardiomyocytes as a Model for Trastuzumab-Induced Cardiac Dysfunction

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Background and Objective: Trastuzumab (Herceptin)-based chemotherapy regimens play a prominent role in treatments of breast cancer, but a significant number of patients develop cardiac dysfunction during the therapy. In this study, we examined whether human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) could serve as an effective platform to study this particular cardiotoxicity. **Methods and Results:** We assessed the effects of trastuzumab on structural and functional properties in three control iPSC-CMs from healthy individuals and found that clinically relevant doses of trastuzumab (1 μ M) significantly reduced the contraction velocity (-7.4%, $P < 0.01$) and calcium amplitude (-14.1%, $P < 0.01$) in iPSC-CMs without inducing cardiomyocyte death or sarcomeric structural abnormalities. RNA-sequencing analysis revealed mitochondrial dysfunction and altered cardiac energy metabolism pathway as primary causes of this cardiotoxicity. Subsequently, we found that significant reductions in basal, maximal, and ATP-linked oxygen consumption (-19.8%, -10.7%, and -19.0%, respectively, $P < 0.01$ for all) and cellular ATP content (-10.3%, $P < 0.01$) in iPSC-CMs after trastuzumab treatment. Next, we recruited seven breast cancer patients with trastuzumab therapy, including five patients diagnosed with trastuzumab-induced cardiac dysfunction and generated iPSCs from these patients. We found that contraction velocity and maximal oxygen consumption were more severely reduced by trastuzumab in iPSC-CMs generated from patients who experienced severe cardiac dysfunction from trastuzumab therapy, compared to iPSC-CMs generated from patients who did not experience cardiac dysfunction (-16.6% vs. -1.4%, and -29.4% vs. -3.7%, respectively, $P < 0.01$ for all). Lastly, we demonstrated that metabolic modulation with AMPK activators, such as metformin, could avert the detrimental effects of trastuzumab in iPSC-CMs. **Conclusion:** Our results indicate that human iPSC-CM model can recapitulate the clinical phenotype of trastuzumab-induced cardiac dysfunction, and therefore targeting altered metabolism may be a promising therapeutic approach for treating this cardiotoxicity.

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Epicardial Contribution to Arrhythmogenic Cardiomyopathy

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Arrhythmogenic cardiomyopathy (ACM) is an inherited disease mainly caused by desmosomal gene mutations and characterized by myocardial loss, replacement with fibro-fatty tissue, arrhythmias and sudden cardiac death. To date, it is unclear which cell type and molecular mechanisms contribute to the fibro-fatty phenotype. The epicardium is the outer mesothelial layer of the heart which has the capacity to undergo epithelial-to-mesenchymal transition (EMT) and differentiate into various cardiac cell types. The aim of this study is to investigate whether epicardial cells contribute to the excess fibro-fatty infiltration seen in ACM patients. To this end, we differentiated induced pluripotent stem cells (iPSCs) from an ACM patient with a haploinsufficiency-causing mutation in the desmosomal gene plakophilin-2 (PKP2) (c.2013delC), and an isogenic control into epicardial cells. While Western blot, flow cytometry, qPCR and immunofluorescence imaging indicated comparable epicardial differentiation efficiencies, RNA-sequencing revealed a profound increased expression of fibroblast and lipid markers only in mutant epicardial cells. These data were corroborated by the spontaneous accumulation of lipid droplets and adipogenic markers in the PKP2 mutant epicardial cells. Single cell sequencing analysis revealed a significant induction of Activating Enhancer-Binding Protein 2 (AP2) family of transcription factors in a subset of PKP2 mutant epicardial cells, which are known to play roles in EMT and lipid biogenesis. siRNA-mediated knock down of AP2 members in mutant epicardial cells significantly reduced the expression of fat and fibroblast markers, suggesting an AP2-mediated fibro-fatty signalling in epicardial cells. The PKP2-dependence of these findings was further validated in healthy iPSC-epicardial cells treated with siRNAs targeting PKP2 which recapitulated the observations made in the mutant cells. Using this human *in vitro* model system, we were able to show the epicardial role during fibro-fatty tissue replacement upon PKP2 suppression. Ongoing experiments, including studies on explanted ACM hearts and a PKP2 c.2013delC knock-in mouse model, aim at further elucidating the molecular mechanisms of ACM pathogenesis.

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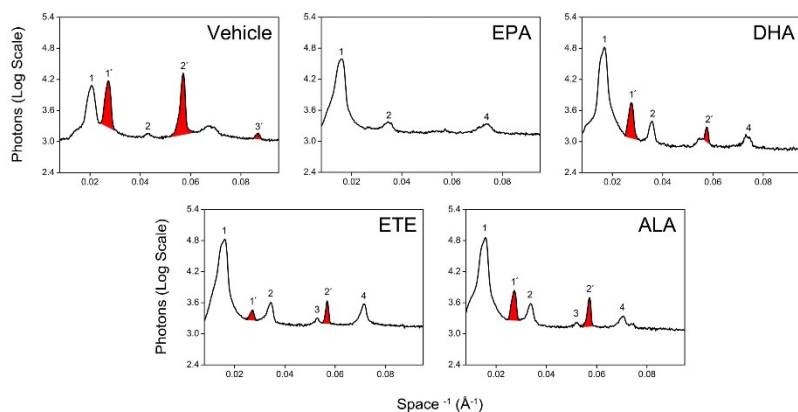
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Eicosapentaenoic Acid, Unlike Other Omega-3 Fatty Acids, Inhibits Membrane Cholesterol Crystalline Domains Under Conditions of Hyperglycemia

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Prescription eicosapentaenoic acid (EPA) reduced cardiovascular events in the REDUCE-IT study population, including in patients with diabetes. During hyperglycemia, there is abnormal membrane cholesterol aggregation which has been linked to oxidative stress and plaque instability. EPA at a pharmacologic dose may preserve normal cholesterol distribution as compared to other omega-3 fatty acids (FAs), including docosahexaenoic acid (DHA), eicosatriaenoic acid (ETE) and α -linolenic acid (ALA). Membrane vesicles were prepared from dilinoleoylphosphatidylcholine (DLPC) at a cholesterol-to-phospholipid mole ratio of 0.6:1 and treated with the omega-3 FAs under conditions of hyperglycemia (200 mg/dL). Changes in membrane lipid organization and width were measured using small angle X-ray diffraction approaches. Cholesterol domains were identified by the presence of diffraction peaks corresponding to a unit cell periodicity or width of 34 Å. Results showed that only EPA was able to prevent the formation of cholesterol domains and had a membrane structure characterized by a normal width (54 Å) and cholesterol distribution despite hyperglycemia. But samples containing the other FAs had a biphasic membrane structure containing prominent cholesterol crystalline domains. The relative size of the cholesterol domains was greatest in the order of ALA>DHA>ETE. Thus, EPA preserved normal cholesterol distribution under conditions of hyperglycemia in a manner that was not reproduced with other omega-3 FAs. These data indicate differences between these long chain FAs and support a potential benefit for EPA in reducing atherosclerotic disease.



Comparative effects of EPA, DHA, ETE, and ALA on cholesterol crystalline domain formation in membranes under conditions of hyperglycemia. Peaks corresponding to cholesterol domains are highlighted in red. Each omega-3 FA was tested at 1:30 drug-to-phospholipid mole ratio. Measurements were taken following exposure to oxidative conditions for 96 hr.

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Modeling Congenital Heart Disease-Associated Variants in *GATA6* Using CRISPR/Cas9 and Human Induced Pluripotent Stem Cells

Arun Sharma, Lauren Wasson, Jon Willcox, Sarah U Morton, Joshua M Gorham, Daniel M DeLaughter, Meraj Neyazi, Manuel Schmid, Radhika Agarwal, Megan Jang, Christopher N Toepfer, Tarsha Ward, Yuri Kim, Alexandre C Pereira, Steven R DePalma, Angela Tai, Seongwon Kim, David Conner, Harvard Medical Sch, Cambridge, MA; Benoit Bruneau, Gladstone Insts, San Francisco, CA; Jon G Seidman, Christine E Seidman, Harvard Medical Sch, Cambridge, MA

The discovery of damaging gene mutations in congenital heart disease (CHD) patients enables identification of regulators of cardiac development. Exome sequencing identified *de novo* heterozygous loss-of-function (LoF) and missense variants in *GATA6* among CHD probands, most with outflow tract malformations. Other subjects with *GATA6* LoF mutations developed pancreatic agenesis. To elucidate the molecular basis for the predominance of this heart defect, we modeled *GATA6* mutations in cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs). *GATA6* variants were introduced into isogenic hiPSCs using CRISPR/Cas9 genome editing. Genome-wide molecular profiles including chromatin accessibility (ATAC-Seq) and gene expression (single cell and bulk RNA-Seq) were evaluated during hiPSC-CM differentiation. Analyses of *GATA6* mutant hiPSC-CMs showed deficits in hiPSC-CM differentiation, chromatin accessibility and transcriptional profiles. Heterozygous *GATA6* LoF hiPSCs made hiPSC-CMs but exhibited reduced expression of second heart field genes. Homozygous *GATA6* LoF hiPSCs failed to differentiate and adopted fibroblast expression profiles. hiPSCs carrying a homozygous *GATA6* missense variant, R456G, which altered a DNA-binding domain residue, showed enhanced capacity to differentiate into neuroepithelial-like cells. Chromatin-accessibility studies confirmed that *GATA6* normally binds to genes in the promoter region and other genes at distal enhancers. Human *GATA6* haploinsufficiency disrupts developmental transcriptional responses driving cardiac morphogenesis. The *HAND2*-dependent genetic program, operant during outflow tract development, is particularly sensitive to *GATA6* dosage. The mixed differentiation patterns observed in mutation-carrying hiPSCs likely contributes to vascular phenotypes observed in CHD patients. *GATA6* haploinsufficiency preferentially alters binding of distal enhancers to promoters in genes where *GATA6* normally binds the enhancer rather than the promoter. We speculate that pathogenicity of *GATA6* haploinsufficiency is mediated by weaker binding of *GATA6* to distal enhancers than to promoter elements, altering expression of these genes in *GATA6* haploinsufficient patients.

A. Sharma: None. **L. Wasson:** None. **J. Willcox:** None. **S.U. Morton:** None. **J.M. Gorham:** None. **D.M. DeLaughter:** None. **M. Neyazi:** None. **M. Schmid:** None. **R. Agarwal:** None. **M. Jang:** None. **C.N. Toepfer:** None. **T. Ward:** None. **Y. Kim:** None. **A.C. Pereira:** None. **S.R. DePalma:** None. **A. Tai:** None. **S. Kim:** None. **D. Conner:** None. **B. Bruneau:** None. **J.G. Seidman:** None. **C.E. Seidman:** None.

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A Novel Desmoplakin Mutation Contributes to Arrhythmogenic Cardiomyopathy

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Genetic alterations in desmosomal genes are associated with arrhythmogenic cardiomyopathy (ACM). This condition is characterized by sudden cardiac death, severe arrhythmias, and the presence of fibro-fatty patches within the myocardium. Despite the fact that the pathophysiology of ACM is well described, the molecular mechanisms underlying disease onset and progression are poorly understood.

In an effort to identify disease-driving mechanisms we obtained induced pluripotent stem cells (iPSC) from a patient harboring a novel heterozygous mutation in desmoplakin (*DSP*^{p.Tyr1188His/WT}), which we corrected to yield an isogenic control. iPSC cells carrying a known pathogenic mutation in desmoplakin (*DSP*^{p.Arg1113X/WT}) served as a positive control. Gene expression analyses of *DSP*^{p.Tyr1188His/WT} iPSC-derived cardiomyocytes revealed a dysregulation of desmosomal and ion channel-related genes. At the functional level we observed a prolonged action potential duration in *DSP*^{p.Tyr1188His/WT} cardiomyocytes. Importantly, we validated these findings in an independent knock-in line carrying the same heterozygous (*DSP*^{p.Tyr1188His/WT}) mutation. To study the *in vivo* relevance we additionally generated two corresponding mouse models harboring the mouse equivalent of the human desmoplakin mutations in the endogenous locus (*Dsp*^{p.Tyr1200His/WT} and *Dsp*^{p.Arg1125X/WT}). Both lines show altered connexin 43, phospholamban and Nav1.5 expression levels under baseline conditions, recapitulating the changes observed in the iPSC-derived cardiomyocytes. Moreover, Fos- and RhoA-signaling pathways are enriched in these mice compared with wildtype littermates. Since exercise can be a trigger for the development of ACM, we are currently in the process of examining the effect of intense swimming exercise in the models.

We show that the *DSP*^{p.Tyr1188His/WT} mutation causes cellular changes linked to ACM pathogenesis, indicating this mutation to be disease-driving. Moreover, our novel mouse models of ACM recapitulate the findings observed in our human models and show key hallmarks of ACM disease progression. Future studies will focus on the underlying pathogenic mechanisms in the presented models.

S.J. van Kampen: None. **E. Kyriakopoulou:** None. **S. Han:** None. **B. Goversen:** None. **J. Monshouwer-Kloots:** None. **J. Eding:** None. **T. de Boer:** None. **T. van Veen:** None. **E. van Rooij:** None.

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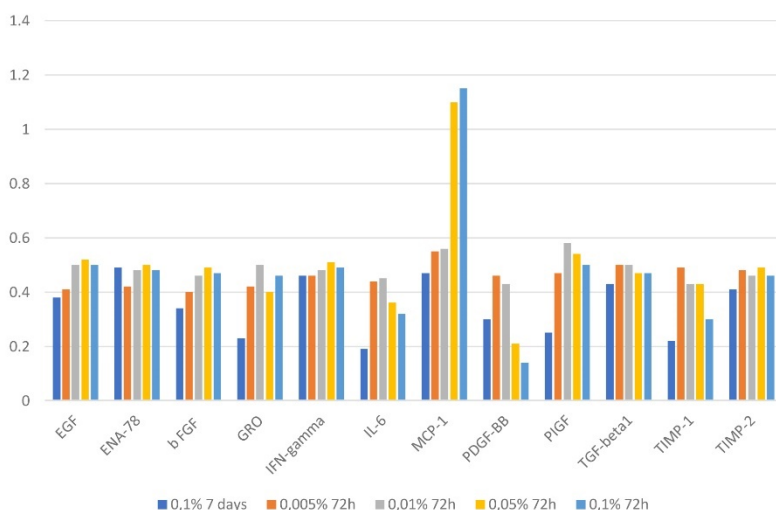
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Rose - A Novel Vascular Anti-inflammatory Agent

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INTRODUCTION: Inflammation is common in clinical practice and often results in significant complications. The study was performed in search of a novel anti-inflammatory agent for inflammation modulation which would be useful for cardiovascular disorders and various clinical scenarios. **Methods:** A crushed red rose extract was prepared from the petals, and it was processed for analysis. The extract was tested on HUVEC cells at various concentrations. By microscopic analysis of cells, a safe concentration was identified, and the levels below the safe limit were tested at 72 hours and seven days for selected cytokines secretion. **Results:** Majority of the tested Inflammatory cytokine secretion was reduced by the treatment of red rose extract on the cells. VEGF and angiogenic cytokine levels were reduced, but VEGF-R levels were maintained after the cell treatment. Below the safe concentration limit of the extract there were only minimal changes in the cytokines levels tested at various dilutions of the extract. The study results show a significant reduction in the secretion of anti-inflammatory cytokines by red rose extract. The effects of rose extract inhibited major inflammatory cytokines like IL-1, IL-2, RANTES, IGF-1, IL-8 etc. MCP 3 (CCL7) and PDGF BB levels were less than 10% of the baseline values. There was also a tendency in fall at 72 hours, and thereafter a rise at 7 day levels observed in the values of some cytokines. **Conclusion:** There is potential for a red rose extract treatment in the regulation of inflammatory cytokines secretion. Further studies need to be performed to identify the benefits and side effects profile.

Cytokine levels with fold changes <0.6



M.C. Arokiaraj: None. **E. Menesson:** None.

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Neutrophil Specialization in Blood and Heart After MI

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Myeloid cells play a central role in the orchestrating the response to myocardial infarction (MI) and the progression to heart failure. While monocytes and macrophage have been studied extensively in ischemic models and credited with both proinflammatory and healing phenotypes, neutrophils, the first responders to MI, remain poorly understood and largely considered homogenous immune effector cells. Here, we use single cell RNA-Seq to map transcriptional states of

neutrophils, from their origins in the blood and bone marrow to the injured and healing heart. We FACS sorted live, single cells isolated from bone marrow, blood and infarcted heart tissue collected D1, D2 and D4 post MI and used inDrop and 10X genomics droplet-based single cell barcoding and single cell RNA-sequencing (scRNA-seq). Cell transcriptomes were analyzed by unbiased and supervised clustering as well as pseudotime trajectory analysis. Neutrophils were bioinformatically isolated and subclustered to reveal transcriptional states in blood and heart. Our results show that blood neutrophils travel as two subtypes that further specialize within the infarcted heart in a time-dependent manner into two distinct subsets, characterized by expression of SiglecF and a transcriptional program regulated by HIF1alpha. Taken together, our data define the transcriptional changes that occur in neutrophils as they traverse the blood and enter and evolve within the injured heart after MI. Work is ongoing to define the functional significance of these newly identified neutrophil subsets.

D.M. Calcagno: None. **R.P. Ng Jr:** None. **A. Toomu:** None. **K.M. Huang:** None. **Z. Fu:** None. **A.D. Aguire:** None. **K.R. King:** None.

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Genetic and Pharmacological Disruption of Epsins Attenuates Atherosclerosis

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Atherosclerosis, the cause of heart attack, stroke and cardiovascular death, remains an unmet challenge for seeking elective therapeutic targets despite current pharmaceutical interventions. Myeloid epsins regulate atherosclerosis, but whether endothelial epsins contribute to atherosclerosis is unknown. Here, we show that loss of endothelial epsins greatly attenuates atherosclerosis. In response to inflammation, epsin binds and destabilizes the endoplasmic reticulum (ER)-resident protein IP3R1, which perturbs intracellular calcium homeostasis, augments ER stress, and promotes atherosclerosis. In atherosclerotic patients and animals, levels of endothelial IP3R1 is reduced, correlating with epsins upregulation. Conversely, loss of epsins stabilizes IP3R1, mitigates ER stress, and halts atherosclerosis. The Ubiquitin Interacting Motif (UIM) in epsins mediates the proteasomal degradation of ubiquitinated IP3R1. Accordingly, an epsin UIM mimetic peptide abolishes epsin:IP3R1 interaction and diminishes atherosclerosis. These findings reveal an unexpected role for epsins in atherogenesis, provide mechanistic insight into epsin-mediated IP3R1 regulation, and uncover a potential targeted therapy for atherosclerosis.

H. Chen: None.

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Core Cell Survival Requirements of Hypoxia Inducible Factors in Macrophages Dominate Isoform-Specific Function during Cardiac Repair

Matthew Deberge, Connor Lantz, Lisa Wilsbacher, Edward B Thorp, Northwestern Univ, Chicago, IL

Objective: Hypoxia inducible factors (HIF) regulate the adaptive response to ischemic injury and enhancement of HIF levels has been proposed as a therapeutic target to improve tissue repair after acute myocardial infarction (MI). HIFs also regulate the inflammatory function of macrophages, the main protagonists of tissue repair after MI, but it is unknown whether changes in macrophage HIF levels will affect outcomes after MI. Using a murine model of acute MI and conditional knockouts, we investigated a role for macrophage HIFs in tissue repair after MI.

Methods and Results: In mice subjected to permanent occlusion MI, we observed an accumulation of hypoxic cardiac macrophages and sequential stabilization of HIF-2 α followed by HIF-1 α , which persisted into the development of heart failure. In patients with ischemic cardiomyopathy, flow cytometry and single-cell transcriptomics revealed a parallel stabilization of HIFs and hypoxia signaling in cardiac macrophages. Since HIFs were stabilized early after MI in cardiac macrophages, we selectively targeted macrophage HIFs using conditional knockouts. Selective deletion of macrophage HIF-2 α promoted cardiac macrophage production of IL-10, activating fibroblasts and stimulating collagen deposition, culminating in improved ventricular remodeling and preservation of systolic function. Similarly, selective deletion of macrophage HIF-1 α improved cardiac repair; however, this was mechanistically distinct from HIF-2 α , inhibiting HIF-1 α -dependent proteolytic destruction of MerTK and leading to improved efferocytosis by cardiac macrophages. Contrary to our expectations, loss of both HIF-1 α and HIF-2 α in hypoxic macrophages enhanced mitochondrial reactive oxygen species and activated

necroptosis, leading to increased cell death and cardiac rupture after acute MI. Inhibition of necroptosis with necrostatin-1 attenuated hypoxic macrophage cell death and significantly improved survival after acute MI.

Conclusions: These data imply strategies that broadly enhance or inhibit HIFs will lead to catastrophic outcomes after MI, while selective inhibition of macrophage HIF-1 α or HIF-2 α may enhance tissue repair, preserve systolic function, and limit progression to heart failure.

M. Deberge: None. **C. Lantz:** None. **L. Wilsbacher:** None. **E.B. Thorp:** None.

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Macrophage Transcription Factor EB Attenuates Left Ventricular Remodeling Via Lysosomal Lipolysis

Ali Javaheri, Geetika Bajpai, Antonino Picataggi, Smrithi Mani, Layla Foroughi, Hosannah Evie, Attila Kovacs, Carla Weinheimer, Krzysztof Hyrz, Trent Evans, Qingli Xiao, Andrea Ballabio, Jin-Moo Lee, Scot Matkovich, Babak Razani, Joel Schilling, Kory J Lavine, Abhinav Diwan, Washington Univ in St Louis, Saint Louis, MO

Background: Autophagy, lipid metabolism, and inflammation are interrelated cellular processes that implicate lysosomes in human disease. After ischemia reperfusion (IR) injury, inflammasome activation and interleukin 1-beta (IL1-beta) secretion promote heart failure progression. Whether macrophage autophagy and lysosomal biogenesis can attenuate post-IR remodeling and inflammation is unknown. We hypothesized that macrophages exhibit lysosome dysfunction and autophagic impairment after IR injury, and that augmentation of macrophage lysosomal biogenesis via macrophage-specific overexpression of transcription factor EB (Mf-TFEB), a master regulator of autophagy and lysosomal biogenesis, would attenuate myocardial remodeling and inflammation in ischemic cardiomyopathy. **Methods and Results:** In mice subject to IR injury and humans with ischemic cardiomyopathy, we observed evidence of lysosomal and autophagic impairment. To ameliorate post-IR macrophage lysosomal injury, we expressed Mf-TFEB in a closed-chest IR murine model using a tamoxifen-inducible CX3CR1erCre and TFEB overexpression cassette bearing a Cre-excisable STOP codon. Compared to Cre-only controls, Mf-TFEB mice had significantly increased left ventricular (LV) ejection fraction 28-days post-IR (40% relative increase, $p=0.002$, $n=15-17$ per group), decreased abundance of pro-inflammatory macrophages, and reduced levels of IL1-beta in myocardial tissue. Surprisingly, neither inflammasome suppression nor TFEB-mediated attenuation of post-IR remodeling required intact macro-autophagy as evidenced by Mf-TFEB-mediated rescue of post-IR LV dysfunction in mice with concomitant inducible ATG5 ablation. RNA sequencing of flow-sorted macrophages from post-IR mice identified lysosomal acid lipase amongst other lipases regulated by TFEB. Mechanistically, pharmacologic inhibition of lysosomal acid lipase specifically abrogated the in vivo effects of TFEB on post-IR remodeling. **Conclusions:** Our findings suggest that macrophage TFEB regulates lysosomal lipolysis to attenuate inflammasome activity and protect against post-IR LV dysfunction, suggesting an alternative paradigm for how lysosome function may impact acute inflammation in vivo.

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Endothelial Specific Ablation of Estrogen Receptor Alpha Rapid Signaling Revealed Exacerbated Vascular Remodeling Response

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Background Estrogen exerts complex physiological effects via its rapid (non-genomic) and genomic actions. In particular, rapid signaling of estrogen receptor alpha (ER α) has been implicated in the vasculo-protective effects, in which both endothelial and smooth muscle cells might be involved. However, no prior studies have determined the role of ER α rapid signaling in the endothelium. This study aims to clarify the impact of ER α rapid signaling in the vasculo-protection, using a novel mouse model lacking endothelial-specific ER α rapid signaling. **Methods and Results** In immunoblotting, p85 α and

pGSK3 β , non-genomic pathway downstream signals, were reduced in ER α mutants RR259/260AA with estradiol (E2) stimulation at concentrations of 10^{-11} and 10^{-10} M. ERE-luciferase assay demonstrated E2 induced genomic pathway activity was preserved even in ER α mutants RR259/260AA. We generated a mouse model in which rapid signaling of ER α was ablated in the endothelium by crossing Tie2-Cre transgenic mice with floxed ER α mutants (RR 259/260 AA) in which p85 α and ER α interaction was disrupted. In endothelial cells isolated from ER $\alpha^{KI/KI}$ Tie2^{cre/+} animals, E2 at a concentration of 10^{-8} M failed to induce phosphorylation of Akt, confirming the absence of ER α rapid signaling. Baseline characteristics at 8 to 12 weeks of age were undistinguishable between the genotypes, including body weight (KI:19.2 \pm 0.366 g vs WT: 18.9 \pm 0.356 g) systolic blood pressure (KI:106.1 \pm 3.336 vs WT: 103.5 \pm 2.133 mmHg) and echocardiographic fractional shortening (KI:54.4 \pm 0.751 vs WT: 55.19 \pm 0.947 %). We then assessed how vascular remodeling process was impacted in a carotid artery wire injury model. Histological analyses with Elastica van Gieson staining two weeks after induction of injury revealed that wall thickness and area of medial layer composed mainly of smooth muscle cells were significantly increased in ER $\alpha^{KI/KI}$ Tie2^{cre/+} mouse, as compared to wild types (KI:44.85 \pm 1.948 μ m vs WT: 29.32 \pm 1.753 μ m, KI:42,047 \pm 1,560 μ m² vs WT: 32,815 \pm 1,211 μ m², respectively). **Conclusions** Our results demonstrate that the rapid signaling of ER α in the endothelium critically regulates vascular smooth muscle cell growth after vascular injury, suggesting the essential role to vascular remodeling.

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TRAF2-GRK2 Interaction Mediates Non-Canonical Desensitization of Beta-Adrenergic Receptors to TNF- α

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β - Adrenergic receptor (β AR), a prototypical G-protein coupled receptor (GPCR) is a key regulator of cardiac function. Impaired G-protein coupling (desensitization) of β ARs is a key hallmark of heart failure. Desensitization occurs by phosphorylation of β ARs by GRK2 to sympathetic overdrive leading to β -arrestin binding and endocytosis. Our studies show that TNF α -induces β AR phosphorylation/desensitization via GRK2 recruitment that is independent of the classical G $\beta\gamma$ mechanism suggesting a non-canonical pathway of GRK2 recruitment. Comorbid conditions like obesity/ hypertension have a higher risk of heart failure and is associated with increase in TNF α , a known cardio-depressant. β -blockers are known to be contra-indicative in obesity which we believe is due to β AR desensitization through non-canonical GRK2 recruitment to the β AR in response to elevated TNF α . However the underlying mechanisms for β AR desensitization are not known and as TNF α recruits TRAF2 to mediate downstream signaling, we hypothesized that TRAF2 recruits GRK2 to the plasma membrane causing receptor phosphorylation and desensitization. Our study shows TNF α drives β AR dysfunction by recruiting GRK2 to β AR complex through TRAF2. Our studies indicate that TRAF2 and GRK2 form a cytosolic complex and is recruited to the receptor complex post-TNF α treatment. Pharmacologic inhibition of GRK2 results in decreased β AR phosphorylation after TNF α . Furthermore, siRNA knockdown of TRAF2 results in reduction of β AR phosphorylation indicating the key role for TRAF2 in TNF α -mediated desensitization. Given that nothing is known about TRAF2 and GRK2 interaction we performed molecular docking using Haddock software to identify that the N-terminal region of GRK2 may interact with TRAF2. Overexpression of GRK2 N-terminal domain as dominant negative strategy resulted in reduced β AR phosphorylation in response to TNF α , suggesting the requirement of GRK2 N-terminal in non-canonical β AR desensitization. We are currently using cardiac specific GRK2 and TRAF2 knockout mice to assess their role in cardiac function in response to TNF α showing the role of GRK2-TRAF2-TNFR axis in mediating agonist-independent β AR desensitization/dysfunction.

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Dysfunctional Immune Cell Clock Contributes to Heart Failure Progression

Samir Rana, Guihua Zhou, Tariq Hamid, Gregg Rokosh, M. Ameen Ismahil, Univ of Alabama at Birmingham, Birmingham, AL; Martin E. Young, UNIVERSITY OF ALABAMA AT BIRMINGHAM, Birmingham, AL; Sumanth D. Prabhu, Univ of Alabama at Birmingham, Birmingham, AL

Objective: Inappropriately sustained activation of monocytes/macrophages contributes to heart failure (HF) progression. Time-of-day greatly influences cardiovascular and immune systems, in part through cell autonomous circadian clocks. Here, we propose that disruption of monocyte/macrophage circadian clocks contributes to pathologic inflammation and HF progression following myocardial infarction (MI).

Methods: MI-induced HF was achieved by permanent ligation of coronary artery in myeloid-specific *Bmal1* knockout (MBK) mice and wild-type (WT) mice. Left ventricular (LV) function was assessed echocardiography. Flow cytometry was utilized for immune cell profiling. RNAseq and qRT-PCR was utilized for gene-expression studies. Tissue fibrosis was quantified using Masson-trichrome staining.

Results: WT (C57Bl/6) HF mice exhibited altered Ly6C^{hi} monocyte circadian clock gene expression as compared with sham-operated mice, with significantly reduced expression of the key clock effector *Bmal1*. Genetic disruption of the monocyte clock in MBK mice had no effect on macrophage inflammatory markers during unstressed, baseline conditions. However, MBK-HF mice (4 w post-MI) exhibited increased infiltrating CCR2⁺ heart macrophages (2642±803 vs. 937±190 per heart, p<0.05), augmented cardiac fibrosis (22±1 vs. 14±3 %, p<0.05), greater LV dysfunction [ejection fraction 19.7±2 vs. 28.4±4 %, p<0.05], increased LV end-diastolic and end-systolic volume (EDV 140.4±10 vs. 100±8 µl, p<0.01; ESV 115±10 vs. 74±9 µl, p<0.01) and cardiac hypertrophy (heart weight/tibia length 12.1±0.6 vs. 10.5±0.4 mg/mm, p<0.05) as compared with WT HF littermate control mice. Moreover, circulating monocyte levels in MBK-HF mice were significantly increased at zeitgeber time 6 versus WT HF littermate controls (296±58 vs. 131±17 cells/µl, p<0.05).

Conclusion/Broader Impact: Monocyte/macrophage circadian gene dysregulation resulting from *Bmal1* loss-of-function exacerbates pathological LV remodeling, systemic and cardiac inflammation, and tissue fibrosis in chronic ischemic HF. Restoration of circadian clock function in innate immune cells may represent a potential therapeutic approach to immunomodulation in chronic HF.

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HECT Domain Containing-3 (HECTD3) is a Novel Regulator of Cardiac Hypertrophy and Inflammation

Ashraf Y Rangrez, Ankush Borlepawar, Nesrin Schmiedel, Lynn Christen, Anushka Deshpande, Alexander Bernt, Manju Kumari, Anca Remes, Univ Medical Ctr Kiel, Kiel, Germany; Andreas Helbig, Christian-Albrechts Univ Kiel, Kiel, Germany; Andreas Jungmann, Univ Medical Ctr Heidelberg, Heidelberg, Germany; Samuel Sossalla, Univ Medical Ctr Regensburg, Regensburg, Germany; Andreas Tholey, Christian-Albrechts Univ Kiel, Kiel, Germany; Oliver Mueller, Derk Frank, Norbert Frey, Univ Medical Ctr Kiel, Kiel, Germany

Objective: In this study, using a Yeast two-hybrid screen, we identified the HECT domain-containing E3 ubiquitin ligase 3 (HECTD3) as one of the novel cardiac binding partners of SUMO2. Present study thus focusses on deciphering the cardiac function of HECTD3. **Methods and Results:** HECTD3-SUMO2 interaction was further validated by co-immunoprecipitation analysis. Interesting, adenovirus-mediated overexpression of HECTD3 in neonatal rat ventricular cardiomyocytes (NRVCMs) resulted in downregulation of endogenous as well as overexpressed SUMO2. Proteasome inhibitor MG132 treatment however inhibited HECTD3-mediated SUMO2 downregulation, suggesting SUMO2 as a likely substrate of HECTD3 in ubiquitin-proteasome system dependent protein degradation. To get further insights into cardiac substrates of HECTD3, we performed mass-spectrometry analysis of NRVCm protein lysate. Interestingly, several proteins from interferon signaling, including signal transducer and activator of transcription-1 (STAT1), were significantly downregulated by HECTD3 overexpression. Functional dissection of HECTD3-SUMO2-STAT1 interaction revealed attenuation of cellular hypertrophy and LPS mediated activation of inflammatory cascade in NRVCms. Importantly, *in vitro* findings were consistent *in vivo*, where, AAV-9 mediated overexpression of HECTD3 substantially reduced cardiac hypertrophy and fibrosis, inhibited activation of STAT1-mediated downstream signaling, and attenuated infiltration of inflammatory cells to the heart after transverse-aortic constriction or Angiotensin II treatment.

Conclusions: In conclusion, we describe here a novel cardioprotective mechanism involving the ubiquitin ligase HECTD3, which exerts anti-hypertrophic and anti-inflammatory effects via dual regulation of SUMO2 and its sumoylation target STAT1. This "dual pathway" inhibition may be exploited for the prevention and/or therapy of cardiac hypertrophy and heart failure.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

Characterization of Cardiac Myeloid Cells in Aged Mice and Their Role in Cardiac Dysfunction During Bacterial Infection

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Biological aging is a risk factor for the development of cardiovascular disease. The changes in the normal immune cell phenotype and function in the myocardium due to immunosenescence is the major source for cardiac distress. Cardiac resident macrophages in the heart play a critical role in the maintenance of homeostatic functions and tissue repair during myocarditis. Recently, we have demonstrated that non-tuberculosis *Mycobacterium avium* (NTM- common in lung infections in the elderly population in the U.S.) infection predisposes the aged mice to cardiac dysfunction; however the mechanism is not known. Here, we investigated the various subsets of myeloid cells and their contribution to the heart function in old mice. We infected young (10 weeks) and old mice (18 months) with NTM and characterized the resident and infiltrated myeloid cells. Our results revealed that before infection old mice showed elevated numbers of myeloid cells in heart tissue compared to young mice. In contrast, NTM infection (30 day post infection) significantly increased the frequency of different myeloid cell subsets in the young mice. Although, the number of cardiac macrophages in young and old mice are not significantly different in infected and uninfected mice, we found that MHCII^{low} macrophages (cardiac resident) are significantly higher in the heart tissue of old mice than young mice before infection which significantly decreased after infection. Interestingly, we observed that MHCII^{high} and MerTK⁺ macrophage (infiltrated macrophages) populations are increased in young and old mice but with a higher rate in young mice. Notably, we found that the number of myeloid-derived suppressor cells (MDSCs) in the heart of aged mice is decreased by infection, which was increased in young mice. In conclusion our data indicates that the NTM infection alters the myeloid cell population and function in the aged heart and failed to maintain the normal heart function, where as in young mice, infection leads to the recruitment of myeloid cells which help to confront the infection and maintain the normal heart function. Further MDSCs accumulation in the heart of young mice after infection is responsible for anti-inflammatory effects to control the hypertrophy, fibrosis and inflammation.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

Cardiomyocyte-derived Exosomal MicroRNAs Regulates Post-Infarction Inflammation and Myofibroblast Phenoconversion

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Background: The inclusion of microRNAs (miRNAs) in extracellular microvesicles/exosomes (named cardiosomes when deriving from cardiomyocytes) allows their active transportation and ensures cell-cell communication.

Aim: We hypothesize that cardiosomal miRNAs play a pivotal role in the activation of myofibroblasts following ischemic injury.

Methods: Using bioinformatic approaches and an established murine model of myocardial infarction (MI, achieved via ligation of the left anterior descending coronary artery), we tested our hypothesis by measuring in isolated fibroblasts and cardiosomes the expression levels of a set of miRNAs, upregulated in cardiomyocytes post-MI and involved in myofibroblast phenoconversion.

Results: We found that miR-92a and miR-195 were significantly upregulated (absolute values) in cardiosomes as well as in fibroblasts isolated at different time points after MI compared with SHAM conditions. These miRs were demonstrated to be both predicted (bioinformatics) and biologically validated (luciferase assays) targets of SMAD7. Moreover, primary isolated cardiac fibroblasts were activated both when incubated with cardiosomes isolated from ischemic cardiomyocytes and when cultured in conditioned medium of post-MI cardiomyocytes, whereas no significant effects were observed following incubation with cardiosomes or medium from sham cardiomyocytes. Myofibroblast activation was assessed measuring the expression of α SMA, periostin, collagen I/III, FAP, fibronectin ED-A. Both miRs also regulated the inflammatory response (evaluated in terms of IL-6 and CXCL1). The addition of an inhibitor of exosome release (GW4869 10 μ M for 12 h) significantly attenuated all these responses. The mechanistic contribution of miR-92a and miR-195 was further confirmed using specific miR mimics and inhibitors.

Conclusions: Taken together, our findings indicate for the first time that two cardiomyocyte-specific miRNAs (miR-92a and miR-195), transferred to fibroblasts in form of exosomal cargo, are essential in the activation of myofibroblasts in MI.

G. Santulli: None. **C. Sardu:** None. **J. Shu:** None. **A. Matarese:** None. **X. Wang:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Sepsis-induced Acute Heart Failure is Driven by MEF2D-dependent Transcription

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Rationale: Our recent work unmasked an unexpected link between inflammation and adverse cardiac remodeling via activation of the transcription factor myocyte enhancer factor 2 (MEF2). We identified a signaling pathway consisting of PGE2, EP3-receptors, PKD and Rac1 leading to a nucleo-cytoplasmic shuttling of HDAC5 with subsequent activation of MEF2. Here, we aimed to elucidate the in vivo role of MEF2 in a model of acute inflammatory cardiomyopathy. Results: Exposure of BALB/c-mice to 7 mg/kg from E. coli derived lipopolysaccharide (LPS) led to severe depression of left-ventricular function and was accompanied by considerably increased levels of PGE2. Strikingly, cardiac myocyte-specific MEF2D deficient mice (MEF2D-KO) were largely protected from LPS-induced acute heart failure, as documented by preserved ejection fraction (LVEF after 24 hours: 49% in contrast to 31% in LPS-treated littermates). Moreover, expression of typical target genes of MEF2 (e.g. Myomaxin) were attenuated in MEF2D-KO, confirming that the loss of MEF2D contributed to reduced MEF2 activity. A cardiomyocyte-specific unbiased transcriptome-sequencing in the early stage of inflammation-induced acute heart failure revealed new MEF2D-dependent genes. Conclusion: Thus, we provide new conceptual insights into inflammation-induced acute heart failure and provide evidence that the transcription factor MEF2D mediates acute contractile dysfunction. We therefore propose that transcriptional therapies might represent a novel approach to combat not only chronic but also acute heart failure.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Elimination of Cells Expressing Senescence Associated Glycoprotein Attenuates Theatherosclerotic Diseases

Masayoshi Suda, Ipei Shimizu, Yohko Yoshida, Goro Katsuumi, Yuka Hayashi, Ryutaro Ikegami, Ayako Nagasawa, Masaaki Nakao, Ryo Fruuchi, Takuya Ozawa, Tohru Minamino, Niigata Univ, Niigata, Japan

Although accumulation of senescent vascular endothelial cells impairs the vessel homeostasis and promote atherosclerotic diseases, underlying mechanisms are largely unknown. We found that Senescence associated glycoprotein (SAGP) expression was significantly increased in human endothelial cells undergoing replicative senescence. SAGP expression in aorta was significantly increased in Apo-E knockout mice. Furthermore, mean SAGP expression was significantly higher in the leukocytes of the patients with atherosclerotic diseases. These data suggest that SAGP would become the novel cellular senescence and/or atherosclerotic disease marker. The genetic deletion of SAGP resulted in high level of mitochondrial reactive oxygen species (ROS) and promoted premature senescence in human endothelial cells. And this associated with suppression of mitochondrial autophagy, mitophagy, suggesting that SAGP protect cells from ROS by inducing mitophagy. Thus, we generated ApoE-KO / SAGP gain-of-function transgenic mice and found that atherosclerotic plaque burden was attenuated in these transgenic mice. In contrast, in SAGP / ApoE double knockout mice atherosclerosis were impaired. These data suggest that modulation of SAGP would become a new therapeutic target for atherosclerotic diseases. Recently, it is reported that elimination of senescent cells (senolysis) reversibly improved pathological aging phenotypes. We generated SAGP-DTR (diphtheria toxin receptor) transgenic mice, inducible elimination of SAGP positive senescent cells upon administration of diphtheria toxin. Elimination of SAGP expressing cells significantly reduced the atherosclerotic plaque burden. Furthermore, we developed a cytotoxic vaccine targeting SAGP. Administration of SAGP vaccine to ApoE-KO mice significantly reduced atherogenesis. These data indicate that targeting SAGP-positive cells could be a strategy for senolytic therapy.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

A Toll-like Receptor 9 Inhibitor Prevents the Development and Progression of Sterile Inflammatory Heart Failure Induced by Mitochondrial DNA Accumulation

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Mitochondrial DNA contains unmethylated cytidine-phosphate-guanosine motif, and is recognized by Toll-like receptor (TLR) 9, inducing sterile inflammation. We previously reported that the accumulation of mitochondrial DNA in cardiomyocytes induces myocardial inflammation and heart failure using an animal model. (Nature 2012) In this study, to investigate the effect of inhibition of the inflammation via TLR9 induced by mitochondrial DNA accumulation on heart failure, we examined whether a TLR9 inhibitor, E6446 (6-[3-(pyrrolidin-1-yl)propoxy]-2-(4-(3-(pyrrolidin-1-yl)propoxy)phenyl]benzo[d]oxazole), prevents the development and progression of heart failure in mice. In in vitro study, isolated cardiomyocytes were stimulated by a TLR9 ligand (ODN1668) in the presence of E6446. ODN1668 significantly increased the expression levels of inflammatory cytokine mRNAs in the cells. Incubation of cardiomyocytes with E6446 significantly reduced the level of those mRNAs induced by ODN1668. CCCP increased the number of autolysosomes with DNA accumulation. Although E6446 had no effect on the number of the autolysosomes, it significantly reduced the production of inflammatory cytokine mRNAs induced by CCCP in cardiomyocytes. In in vivo study, mice orally received E6446 or saline 2 days before transverse aortic constriction (TAC) and every two days for 4 weeks thereafter. Four weeks after TAC, chamber size and fractional shortening (FS) of left ventricle (LV) and lung weight were significantly reduced in E6446 group compared to saline group. Furthermore, the infiltration of inflammatory cells including macrophages in the TAC-operated heart was inhibited by E6446. Next, mice with LV dysfunction at 2 weeks after TAC were subjected to the oral administration with E6446 or saline every two days for 4 weeks. The LV chamber size was significantly smaller and FS was higher in E6446 group than those in saline group 6 weeks after TAC. Our study showed that E6446 prevented the development of LV dilatation and dysfunction with inflammation and slowed progression of cardiac remodeling induced by pressure overload. E6446 might be a novel therapeutic to treat patients with heart failure.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

PD-1 in Cardiac Immune Responses After Ischemic Injury or Transverse Aortic Constriction

Daniel A Zlotoff, Chun Yang Xiao, Robert M. H. Grange, Yoshiko Iwamoto, Massachusetts General Hosp, Boston, MA; Gordon Freeman, Dana Farber Cancer Inst, Boston, MA; Anthony Rosenzweig, Massachusetts General Hosp, Boston, MA

Background: Heart failure is associated with inflammation, but the precise immune mechanisms that contribute to cardiomyopathy remain elusive. Myocarditis is a rare toxicity of checkpoint inhibitors, which are oncologic therapies that block signaling through the immunoinhibitory molecule PD-1. We investigated whether PD-1 attenuates cardiac immune responses.

Methods: Expression of PD-1 and its ligand PD-L1 on cardiac cell populations were assessed by flow cytometry. The functional implications of PD-1 signaling were determined using PD-1 blocking antibodies (Abs) in two surgical models of cardiac injury: ischemia/reperfusion and transverse aortic constriction (TAC). Ischemic injury was measured by troponin release and histology. Systolic function was assayed by echocardiography, diastolic function by invasive pressure-volume loops, and fibrosis by histology.

Results: PD-L1 is expressed on most cardiac endothelial cells and a small fraction of cardiomyocytes. While intracardiac T cells do not express PD-1 under baseline conditions, 15% of both CD4 and CD8 T cells express PD-1 after TAC. Blocking PD-1 signaling after ischemic injury did not affect troponin levels (5.8 ng/ml in mice receiving isotype control (IC) Ab vs 7.8 ng/ml in mice receiving anti-PD-1 Ab, $p=0.26$), size of infarct (1.6% of ventricular cross-sectional area for IC Ab vs 2.0% for anti-PD-1 Ab, $p=0.27$), or systolic function (fractional shortening 52.6% for IC Ab vs 53.2% for anti-PD-1 Ab, $p=0.92$). Similarly, abrogation of PD-1 signaling after TAC did not worsen systolic dysfunction (fractional shortening 36.8% for IC Ab vs 32.4% for anti-PD-1 Ab, $p=0.38$), diastolic function (dP/dT minimum -9445 mmHg/s vs -10950 mmHg/s, $p=0.44$; Tau 6.8 ms for IC Ab vs 6.0 ms for anti-PD-1 Ab, $p=0.45$), or the extent of cardiac fibrosis (1.4% of ventricular cross-sectional area for IC vs 1.7% for anti-PD-1 Ab, $p=0.41$).

Conclusion: Collectively, these data do not implicate PD-1 as an important regulator of intracardiac immune responses in the injury models tested. This suggests that additional pathways may contribute to myocarditis occurring in cancer patients treated with anti-PD-1 Ab, the identification of which could provide insight into immune mechanisms active in heart failure.

D.A. Zlotoff: None. **C. Xiao:** None. **R.M. Grange:** None. **Y. Iwamoto:** None. **G. Freeman:** 7. Ownership Interest; Significant; Roche, Merck, Bristol-Myers-Squibb, EMD-Serono, Boehringer-Ingelheim, AstraZeneca, Dako, Novartis. 8. Consultant/Advisory Board; Modest; Roche, Bristol-Myers-Squibb, Xios, Origimed. **A. Rosenzweig:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Rad Ablation as a Treatment to Target Cardiac Inotropy via L-type Calcium Channel Function

Brooke Ahern, Bryana Levitan, Sudhakar Veeranki, Mihir Shah, Nemat Ali, Andrea Sebastian, Univ of Kentucky, Lexington, KY; Jiayang Li, Julian E Stelzer, Case Western Reserve Univ, Cleveland, OH; Doug Andres, Jonathan Satin, Univ of Kentucky, Lexington, KY

The L-type Ca^{2+} channel current ($I_{Ca,L}$) provides trigger Ca^{2+} to contribute to cardiac contraction. Rad GTPase associates with the L-type Ca^{2+} channel (LTCC) and serves as an endogenous inhibitor of LTCC activity. Overexpression of Rad blocks $I_{Ca,L}$; absence of Rad increases $I_{Ca,L}$. Rad attenuates β -adrenergic receptor (β -AR) signaling. Chronic β -AR stimulation associates with Ca^{2+} mishandling and can promote signaling that progresses towards heart failure. Early studies of global, constitutive Rad-knockout mice (gRadKO) suggested that elevated Ca^{2+} dynamics leads to pathological cardiac hypertrophy; however, Rad is also expressed in non-cardiac tissues.

Our objective is to test the hypothesis that increased myocardial $I_{Ca,L}$ via Rad deletion safely enhances cardiac function without driving pathological remodeling.

We created a cardiac-restricted inducible Rad knockout mouse (Rad $^{\Delta/\Delta}$). *In vivo* function was measured with echocardiography. We examined $I_{Ca,L}$ through whole cell configuration of the patch clamp technique, assessed Ca^{2+} handling, and sarcomere dynamics. .

Unlike gRadKO, Rad $^{\Delta/\Delta}$ showed no elevation of fetal gene program, nor fibrosis, and no change to aortic pressure. Rad $^{\Delta/\Delta}$ had a sustained increase of inotropy without structural or functional remodeling (EF: Rad $^{\Delta/\Delta}$ = 76 \pm 2%, n=16; Rad $^{fl/fl}$ = 59 \pm 4%, n=7; p=0.001.) $I_{Ca,L}$ was significantly increased, with Rad loss mirroring a β -AR modulated phenotype on basal $I_{Ca,L}$ (max. conductance: Rad $^{\Delta/\Delta}$ = 254 \pm 19 pS/pF, n=15; Rad $^{fl/fl}$ = 144 \pm 12 pS/pF, n=18; p<10 $^{-4}$). Contrary to models of chronic β -AR stimulation, Rad $^{\Delta/\Delta}$ retained β -AR signaling shown *in vivo* using isoproterenol, and by preserved phosphorylation of protein regulators of Ca^{2+} reuptake and contractility. Rad $^{\Delta/\Delta}$ cardiomyocytes show enhanced cytosolic Ca^{2+} handling (Decay of Ca^{2+} transient: Rad $^{\Delta/\Delta}$ = 0.07 \pm 0.003 (F₃₄₀/F₃₈₀)/s, n=67, Rad $^{fl/fl}$ = 0.10 \pm 0.005, n=69; p<10 $^{-4}$), increased contractile function, and elevated SERCA2a expression.

These new findings challenge the canonical assumption that increased myocardial Ca^{2+} necessarily promotes pathology. We conclude that cardiac hypertrophy in gRadKO was caused by non-cardiac tissue effects, and myocardial Rad deletion is a promising cardiac inotropic therapeutic direction.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Functional Characterization of a Novel Scn5a Mutation Associated With the Brugada Syndrome

Anthony Frosio, David Molla, Giorgia Bertoli, Claudia Bazzini, Raffaella Milanese, Univ degli Studi di Milano, Milano, Italy; Francesca Gennaro, Mazzoni Hosp, Dept of Cardiology, Ascoli Piceno, Italy; Andrea Barbuti, Annalisa Bucchi, Univ degli Studi di Milano, Milano, Italy; Luciano Moretti, Procolo Marchese, Mazzoni Hosp, Dept of Cardiology, Ascoli Piceno, Italy; Dario DiFrancesco, Mirko Baruscotti, Univ degli Studi di Milano, Milano, Italy

Background: Brugada syndrome (BrS) is a cardiac disorder characterized by conduction abnormalities that can lead to sudden death; syncope and cardiac arrest are clinical manifestations which are often associated with an enhancement of the vagal activity. Mutations in the SCN5A gene (Na_v1.5 channel) are the most common cause of the inherited forms of BrS. **Objective:** To characterize the functional behavior of mutant Na_v1.5 channels expressing a novel heterozygous mutation (S805L) recently identified in an Italian family affected by the BrS. **Methods:** HEK cells were used as experimental model to express both the wild-type (WT) and the mutated S805L channels (alone, Homo or in combination, Hetero) and the accessory β -subunit (SCN1B). Patch-clamp and western blot experiments were carried out to assess the dysfunctional role

of the mutation. **Results:**When compared to the WT current, the S508L mutation significantly ($P<0.05$) decreases the peak current density by about 65% for the Homo condition (WT: -120.2 ± 10.2 , $n=28$); Homo: -40.3 ± 4.2 , $n=16$) and by 35% for the Hetero condition (Hetero: -78.2 ± 8.3 , $n=27$). Densitometric analysis carried out on western blot data further support the conclusion that S805L channels are less abundant in the plasma membrane. We also observed that the S805L mutation positively shifts the $V_{1/2}$ values of the voltage dependence of the inactivation of both Homo and Hetero currents ($V_{1/2}$: WT - 85.5 ± 0.2 mV, $n=55$; Homo -80.9 ± 0.3 mV, $n=22$; Hetero -81.9 ± 0.2 mV, $n=25$; $P<0.05$); a positive shift of the $V_{1/2}$ of the activation was also observed but only in the Homo condition ($V_{1/2}$: WT -33.0 ± 0.4 mV, $n=28$; Homo -30.0 ± 0.5 , $n=16$, $P<0.05$). The kinetics of recovery from inactivation and the amplitude of the late sodium current were also evaluated but they were unaffected by the mutation. **Conclusion:** When expressed in the Hetero condition, the S805L mutation causes a reduction in the channel expression, however, the positive shift of the inactivation curve suggests an increase in Na channel availability. We thus believe that the precise quantitative balance between these two phenomena and their relation with vagal activity may underlie the clinical manifestation of the disease.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Chronic Testosterone Deficiency Increases Late Inward Sodium Current in Ventricular Myocytes from Aging Male C57BL/6 Mice

Stefan Heinze-milne, Shubham Banga, Omar Ayaz, Susan Howlett, Dalhousie Univ, Halifax, NS, Canada

Clinical studies suggest that testosterone modulates the electrical activity of the heart and that low testosterone promotes cardiac arrhythmias. Here we investigated cellular mechanisms involved in the electrophysiological effects of chronic testosterone deficiency on the heart in aging male mice. Male C57BL/6 mice were subjected to either a gonadectomy (GDX) or a sham surgery at 1 month of age and then aged to 16-18 months. Ventricular myocytes were isolated and transmembrane voltage plus ionic currents were recorded with microelectrodes (20-25 M Ω ; current clamp & discontinuous single electrode voltage clamp; 37°C). Cells were paced at 2 and 4 Hz. Action potential duration at 90% repolarization (APD₉₀) was prolonged by GDX (57.9 ± 1.7 vs 108.2 ± 14.1 msec; $p<0.05$), while APD₅₀ and resting membrane potential were unchanged. We next determined whether an increase in late inward sodium current (I_{Na-L}) contributed to the increase in APD₉₀ in GDX cells. Voltage clamp studies showed that I_{Na-L} was significantly larger in GDX cells compared to sham cells (-0.70 ± 0.16 vs -1.53 ± 0.27 pA/pF; $p<0.05$). When myocytes were superfused with the I_{Na-L} antagonist ranolazine (10 μ M), the increase in APD₉₀ was blocked in cells from GDX mice, so that APD₉₀ no longer differed between the two groups. By contrast, ranolazine had no effect on either APD₅₀ or resting membrane potential. Ranolazine also blocked the increase in I_{Na-L} in cells from GDX mice. Prolongation of the action potential in GDX myocytes was associated with a significant increase in the incidence of early afterdepolarizations (EADs) when compared to sham controls. EADs were also inhibited by ranolazine. These data demonstrate that long-term GDX prolongs APD₉₀ at least in part by increasing the magnitude of I_{Na-L} . These findings also suggest that an increase in I_{Na-L} may promote arrhythmias in older men with low circulating testosterone levels.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Structural and Electrophysiological Maturation of Human iPSC-Derived Atrial Cardiomyocytes to Serve as a Platform to Model Atrial Fibrillation

Olivia T Ly, Seock Won Youn, Grace Brown, Liang Hong, Arvind Sridhar, Meihong Zhang, Erin Lambers, Salman Khetani, Dawood Darbar, Univ of Illinois COM, Chicago, Chicago, IL

Background: Atrial fibrillation (AF), the most globally common arrhythmia requiring treatment, is associated with significant morbidity and mortality. Although antiarrhythmic drugs (AADs) are most commonly used to treat symptomatic AF, AADs are incompletely and unpredictably effective, and fail to target underlying cellular mechanisms of AF. Previously, pluripotent stem cell derived immature atrial cardiomyocytes (iPSC-aCMs) were shown to recapitulate the structural and electrophysiologic

(EP) phenotype of AF-linked mutations. However, there are gaps in structural and EP phenotype between immature and mature iPSC-aCMs to successfully evaluate cellular mechanisms.

Objective: The goal is to determine optimal condition(s) to enhance maturity of iPSC-aCMs, to establish iPSC-aCMs as a novel platform to model AF in a dish, elucidate cellular mechanisms and molecular pathogenesis, and identify and assess novel, personalized mechanism based therapies.

Methods: Conditions used to enhance iPSC-aCMs maturity are 1) different ECMs to culture iPSC-aCMs, 2) patterned ECM to improve alignment and formation of intercellular gap junctions, and 3) treatment with postnatal factors (T3, IGF-1 and dexamethasone; TID). Maturity was assessed with immunofluorescence and EP measurement with patch-clamping and optical voltage analysis. Results were compared to equivalent parameters in adult atrial cardiomyocytes (aCM) and untreated atrial iPSC-CMs.

Results: Fibronectin and laminin were ECMs found suitable for long time culture of iPSC-aCMs. Structural maturity (α -actinin and cardiac troponin T) and EP maturity (resting membrane potential [$83 \pm 3\%$], APD₉₀ [$67 \pm 2.5\%$], and conduction velocity [$59.6 \pm 3\%$]) with patterned ECM and TID treatment were significantly improved compared to untreated iPSC-aCMs, and closer to those of adult aCMs.

Conclusion: Structural and EP maturity of iPSC-aCMs are significantly enhanced by precise microenvironmental engineering of in-vivo relevant cell-cell, cell-ECM, and cell-soluble factor interactions. Thus, our findings will provide a platform to investigate cellular mechanisms of AF.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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The Role of Mitochondrial Stress and the Late Sodium Current in Ibrutinib-Mediated Atrial Fibrillation

Ambili Menon, Brandon Chalzan, Liang Hong, Arvind sridhar, Meihong Zhang, Dawood Darbar, Univ of Illinois at Chicago, Chicago, IL

Background: Ibrutinib (a Bruton's tyrosine kinase inhibitor, BTKI) is rapidly becoming the treatment of choice for many B-cell malignancies, but its proarrhythmic effects may limit its widespread use. GWAS have consistently identified an AF risk locus on chromosome (chr) 4q25, and the nearest gene *Pitx2c* has been implicated in AF development as evidenced by increased AF in *Pitx2c*^{+/-} mice which serve as an experimental model for clinical AF.

Objective: To assess the role of the late cardiac Na current (I_{NaL}) and oxidative stress in mediating increased susceptibility to AF in *Pitx2c*^{+/-} mice which are genetically prone to developing AF as well as in atrial human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) exposed to ibrutinib.

Method: *Pitx2c*^{+/-} mice were administered ibrutinib (30mg/kg/day IP) for 14 days while atrial hiPSC-CMs were exposed to ibrutinib for 48 hrs.

Results: There was a graded increase in AF episodes and burden with the number of days the *Pitx2c*^{+/-} mice were exposed to ibrutinib versus controls (**Fig. 1A**). The action potential duration at 90% repolarization and the I_{NaL} were markedly prolonged (**Fig. 1B-C**). Furthermore, we observed a significant increase in mitochondrial fragmentation (**Fig. 1D-E**) and superoxide production (**Fig. 1F-G**) in ibrutinib treated atrial hiPSC-CMs versus controls.

Conclusion: We showed in *Pitx2c*^{+/-} mice and atrial hiPSC-CMs that ibrutinib-mediated AF may in part be related to enhanced I_{NaL} and mitochondrial dysfunction leading to increased oxidative stress. Patients undergoing ibrutinib treatment may benefit from novel therapies which target mitochondrial dysfunction or the late cardiac sodium current.

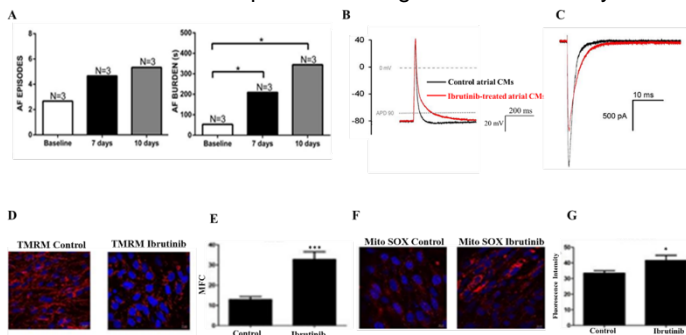


Fig. 1. A: *Pitx2c*^{+/-} mice treated with ibrutinib for 7 and 10 days show increased AF burden; **B:** Ibrutinib prolongs the APD₉₀ of atrial hiPSC-CMs by prolonging the late Na current (I_{NaL}); **C:** **D:** Red fluorescent mitochondrial potential dye (TMRM) staining is significantly increased (E) in atrial hiPSC-CMs treated with ibrutinib for 48 hrs suggesting increased mitochondrial fragmentation; **F:** Ibrutinib treated atrial hiPSC-CMs release more mitochondrial oxidant quantified with superoxide sensitive dye (MitoSOX).

A. Menon: None. B. Chalzan: None. L. Hong: None. A. sridhar: None. M. Zhang: None. D. Darbar: None.

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Mechanisms of Sinus Node Dysfunction and Chronotropic Incompetence in Rats with Heart Failure and Preserved Ejection Fraction

Thassio Mesquita, Jae Hyung Cho, Rui Zhang, Joshua I. Goldhaber, Eduardo Marbán, Eugenio Cingolani, Cedars-Sinai Heart Inst, Los Angeles, CA

Introduction Chronotropic incompetence is a common feature in patients afflicted by heart failure with preserved ejection fraction (HFpEF). However, the specific mechanisms remain unknown. **Aim** To probe the mechanisms of chronotropic incompetence in HFpEF. **Methods** Dahl salt-sensitive rats were fed high-salt diet (8% NaCl). Rats fed normal-salt diet (0.3% NaCl) served as controls. Echocardiography was used to confirm the development of diastolic dysfunction and preserved EF. Telemetry devices were implanted and treadmill exercise tests were performed to assess heart rate response to exercise. Sinus node recovery time (SNRT) was measured *in vivo*, and *ex vivo* optical mapping was performed in isolated sinoatrial node (SAN) tissue to measure heart rate response to isoproterenol. **Results** HFpEF rats showed decreased E/A ratio (17%, $p < 0.05$) and increased E/E' ratio (40%, $p < 0.05$) compared to controls, indicative of diastolic dysfunction, while EF remained unchanged in both groups. Resting mean blood pressure was elevated in HFpEF rats compared to controls (75%, $p < 0.05$). HFpEF rats exhibited low chronotropic response to maximal exercise, in association with prolonged cSNRT (46%, $p < 0.05$), indicative of abnormal SAN function. Despite unchanged baseline heart rate, *ex vivo* high-resolution optical mapping in isolated SAN tissue revealed delayed slow diastolic depolarization of spontaneous action potentials underlying the lower β -AR responsiveness, as well as slowed conduction (in association with histologically-evident increases in SA fibrosis). Next-generation RNA sequencing revealed that SAN from HFpEF rats shows numerous changes in transcripts associated with ion channels, protein kinases, and tissue remodeling. At the single SAN cell level, HFpEF rats showed decreased abundance of L-type Ca^{2+} channels ($Ca_v1.3$, 55%) and hyperpolarization-activated "pacemaker" channels (HCN4, 67%) by western blot; such protein changes were associated with remarkable reductions of both currents. **Conclusions** Chronotropic incompetence in HFpEF is due, in large measure, to intrinsic abnormalities of the SAN. Among these, a depressed membrane "clock" features prominently in the underlying mechanism.

T. Mesquita: None. J.H. Cho: None. R. Zhang: None. J.I. Goldhaber: None. E. Marbán: None. E. Cingolani: None.

Poster Session 3 and Reception

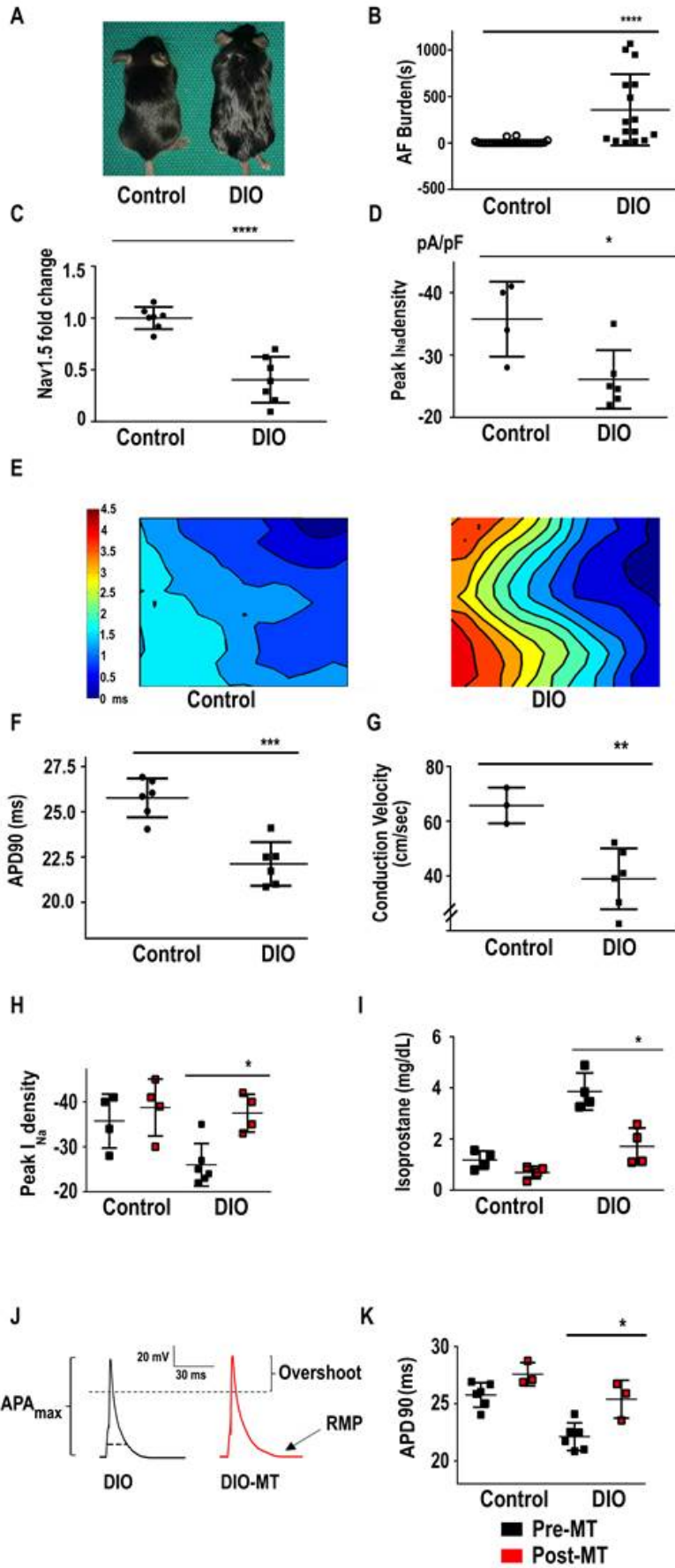
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Downregulation of the Cardiac Sodium Channel Nav1.5 Mediates Obesity-Induced Atrial Fibrillation

Arvind Sridhar, Mark McCauley, Liang Hong, Ambili Menon, Srikanth Perike, Meihong Zhang, Univ of Illinois, Chicago, Chicago, IL; Xun Ai, JiaJie Yan, Rush Univ, Chicago, IL; Seock-Won Youn, Ivson Bezerra da Silva, Marcelo Bonini, Jalees Rehman, Dawood Darbar, Univ of Illinois, Chicago, Chicago, IL

Background: Obesity increases atrial fibrillation (AF) risk, but the underlying mechanisms are unclear. Emerging evidence supports reduced cardiac Na^+ channel (Nav1.5) expression and current (I_{Na}) as one potential mechanism. **Objective:** We used an acquired (diet-induced obese, DIO) obese mouse model to test the hypothesis that obesity increases AF risk by downregulating Nav1.5. **Methods:** Weight, BP, plasma glucose, and F_2 -isoprostanes were measured in DIO mice and compared to controls. Echocardiography, EP studies, immunohistochemistry, Western blotting, cellular patch clamping and optical mapping studies were performed. **Results:** DIO (37.5 ± 3.8) mice were heavier than controls (24.3 ± 4.1 ; $P < 0.001$). AF burden was 355 ± 383 sec in DIO mice vs. 8.3 ± 21.4 sec in controls ($P < 0.0001$). Nav1.5 expression, I_{Na} , atrial action potential duration (APD) and conduction velocity (CV) were reduced, and F_2 -IsoPs were increased. AF burden was reduced in the DIO model after chronic treatment with MitoTEMPO antioxidant, with partial restoration of the APD90, I_{Na} and AP upstroke and reduction in F_2 -IsoPs. **Conclusions:** Mice with either acquired or genetic susceptibility to obesity are more prone to develop AF. And this is partially mediated by increased oxidative stress leading to downregulation of Nav1.5, shortened atrial APD and reduced atrial conduction velocity suggesting a possible reentrant mechanism of AF.



A. Sridhar: None. **M. McCauley:** None. **L. Hong:** None. **A. Menon:** None. **S. Perike:** None. **M. Zhang:** None. **X. Ai:** None. **J. Yan:** None. **S. Youn:** None. **I. da Silva:** None. **M. Bonini:** None. **J. Rehman:** None. **D. Darbar:** None.

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Trpv4 Deletion in Endothelium Protects Heart Against Pressure Overload Induced Hypertrophy

Ravi K Adapala, Anantha K Kanugula, Vahagn Ohanyan, Nina Lenkey, North East Ohio Medical Univ, Rootstown, OH; Sailaja Paruchuri, Univ of Akron, Akron, OH; William M Chilian, **Charles K Thodeti**, North East Ohio Medical Univ, Rootstown, OH

Coronary microvascular dysfunction has been identified as one of the underlying causes for progression of heart failure following hypertrophy or myocardial infarction. However, the mechanisms underlying microvascular dysfunction during the progression of heart failure are unknown. Recently, we found that transient receptor potential vanilloid 4 (TRPV4) channel acts as mechanosensor in endothelial cells and negatively regulates angiogenesis. Therefore, to determine the role of TRPV4 in coronary microvascular function during pathological hypertrophy, we have induced pressure-overload in WT and TRPV4KO mice via transverse aortic constriction (TAC). We found that TAC-induced cardiomyocyte hypertrophy and reduced cardiac function in WT mice, after 28 days. In contrast, both myocyte structure and cardiac function were preserved in TRPV4KO-TAC compared to WT-TAC mice. Notably, WT-TAC hearts exhibited increased ECM deposition and reduced microvascular density compared to TRPV4KO-TAC hearts, suggesting that the absence of TRPV4 may protect myocardium from pressure-overload-induced stress. To evaluate the specific role of endothelial TRPV4 in coronary microvascular function, endothelial specific TRPV4KO (TRPV4^{ECKO}) mice were generated by crossing TRPV4^{lox/lox} mice with Tie2-Cre mice. After confirming endothelial deletion of TRPV4 through RT-PCR and immunostaining, we have subjected TRPV4^{lox/lox} and TRPV4^{ECKO} mice to TAC. Immuno-histological analysis revealed that TRPV4^{ECKO} hearts exhibited increased microvascular density compared to TRPV4^{lox/lox} mice, 28 days post TAC. Further, we found preserved cardiac structure (myocyte cross sectional area) and cardiac function (% ejection fraction and fractional shortening) with reduced cardiac fibrosis in TRPV4^{ECKO} mice compared to TRPV4^{lox/lox}, post TAC. Thus, our results suggest that endothelial TRPV4 channels are key regulators of coronary microvasculature function and deletion of endothelial TRPV4 offers cardio-protection via increased coronary angiogenesis following pressure overload-induced by TAC.

R.K. Adapala: None. **A.K. Kanugula:** None. **V. Ohanyan:** None. **N. Lenkey:** None. **S. Paruchuri:** None. **W.M. Chilian:** None. **C.K. Thodeti:** None.

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Reduced Cardiac Transmembrane Protein 65 Resulted in Dilated Cardiomyopathy and Progressive Cardiac Fibrosis *in vivo*

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Intercalated discs (ICDs) are unique and functionally indispensable to the heart, but its structural organization remains less understood. Previously, we showed that an ICD-bound transmembrane protein 65 (Tmem65) was required for Connexin 43 (Cx43) localization in cultured mouse neonatal cardiomyocytes, and that reduced Tmem65 was associated with a decrease and internalization of Cx43, and impaired electrical conduction between neighboring cardiomyocytes. Here, we investigated the role of Tmem65 *in vivo* by injecting CD1 mice with recombinant adeno-associated virus 9 (rAAV9) harboring Tmem65 (or scrambled) shRNA. Quantitative polymerase chain reactions and immunoblots confirmed greater than 90% reduction in Tmem65 expression in mouse ventricles compared to control samples. Immunoblots and immunofluorescence showed reduced and internalized Cx43 in Tmem65 knockdown (KD) hearts compared to controls, respectively. Kaplan-Meier survival plot showed that all Tmem65 KD mice died within 7 weeks (> 50% death 3 weeks post viral injection), whereas no death was seen in control mice. Tmem65 KD mice developed eccentric hypertrophic cardiomyopathy in 3 weeks and dilated cardiomyopathy with severe cardiac fibrosis in 7 weeks as confirmed by H&E and Masson's Trichrome staining. Echocardiography also confirmed ventricular dilatation and showed a 60% reduction in cardiac output (19.27±1.46 mL/min in control vs. 6.63±0.52 mL/min in Tmem65 KD mice, p<0.01, n = 6 per group) at 7 weeks. Transmission electron microscopy

showed an altered ICD pattern and disorganized myofibril structure in Tmem65 KD ventricles while control tissues had smooth ICDs and organized myofibers. Together, these findings suggest a critical role of Tmem65 in maintaining cardiac ICD and myofibers and loss of Tmem65 leads to cardiomyopathy *in vivo*.

A.C. Teng: None. **L. Gu:** None. **M. Di Paola:** None. **M. Fatah:** None. **D. Chatterjee:** None. **T. Kislinger:** None. **R. Hamilton:** None. **A.O. Gramolini:** None.

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Surgical Bilateral Stellate Ganglionectomy Reduces Mitochondrial Reactive Oxygen Species (mROS) and Prevents Sudden Cardiac Death (SCD) in Non-ischemic Heart Failure (HF)

Swati Dey, Brian O'Rourke, Johns Hopkins Univ, Baltimore, MD; Deepankar DeMazumder, Div of Cardiology and of Pharmacology and Systems Physiology, Univ of Cincinnati Coll of Med, Cincinnati, OH

INTRODUCTION: Current therapies for SCD are limited. Bilateral sympathetic stellate ganglionectomy (**BSG**) is a promising new adjunctive therapy in patients with ischemic HF who have incessant ventricular tachyarrhythmias (**VT/VF**) despite optimal medical management. The underlying mechanisms are unknown. The effect of BSG in non-ischemic HF is unclear. **HYPOTHESIS:** BSG prevents VT/VF and SCD in non-ischemic HF by restoring sympathovagal balance and reducing mROS. **METHODS:** We used a unique pressure-overload guinea pig model that closely mimics human non-ischemic HF, including a high incidence of spontaneous VT/VF/SCD. We randomized the animals to non-failing controls, HF+Sham, or HF+BSG surgery. We used intention to treat analysis to evaluate VT/VF-free survival. We analyzed continuous ECG (VT/VF burden, heart rate variability, QT variability) and echo (cardiac function). In isolated left ventricular (LV) myocytes, we measured Ca^{2+} transients and mitochondrial reactive oxygen species (mROS) using targeted ratiometric probes. **RESULTS:** Half of the HF+Sham animals experienced SCD in 4 weeks. In contrast, BSG abolished SCD, reduced VT/VF, and increased parasympathetic tone (beyond corresponding reductions in sympathetic tone). Fractional shortening of the LV was markedly reduced in HF+Sham (0.28 ± 0.02) vs. control (0.50 ± 0.01), but preserved by BSG (0.45 ± 0.02). Whereas HF+Sham increased mROS levels in LV myocytes (fractional oxidation, F_{ox} : 0.53 ± 0.015) compared to control (0.29 ± 0.020), BSG reduced mROS (0.38 ± 0.035). Mitochondrial antioxidant capacity, as indexed by H_2O_2 challenge of LV myocytes, was compromised in HF+Sham (F_{ox} : 0.81 ± 0.040) compared to control (0.44 ± 0.061), but preserved by BSG (0.48 ± 0.023). **CONCLUSIONS:** Surgical BSG prevents SCD in non-ischemic HF by reducing mROS, decreasing sympathetic tone, and by increasing parasympathetic tone (possibly by facilitating cholinergic transdifferentiation). This promising new minimally-invasive surgical therapy may be particularly useful in patients with non-ischemic HF on optimal medical therapy who require recurrent ICD shocks to prevent SCD. Patients in the early stages of HF who are not eligible for ICDs but remain at higher SCD risk may also benefit from BSG surgery.

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Enoxaparin Reverses Ventricular Tachycardia and Torsades de Pointes in Rat Isolated Heart Model

Enio R Vasques, **Enio R Vasques**, Insto Mogiano De Cardiologia, Mogi Das Cruzes, Brazil; Ivarne Ls Tersariol, Helena B Nader, UNIFESP, Sao Paulo, Brazil; Carlos Mg Godoy, UNIFESP, Sao Jose Dos Campos, Brazil

Introduction: Enoxaparin (Enox) is used in cardiovascular emergency and coronary angiography due to its effects on coagulation mechanisms. However, its effect on changing the structure of the exchange-inhibiting peptide present in the sodium-calcium exchanger, leading to the acceleration of its function and withdrawing calcium from the cell in overload situations could lead to a new therapeutic function, where the intracellular calcium overload would be deleterious, as in cardiac arrhythmias and myocardial infarction. Objective: To analyze the action of Enox on cardiac arrhythmias induced by ischemia and reperfusion (IR) in isolated rat hearts. Materials and method. Adult Wistar rats were sacrificed, hearts isolated and placed under perfusion in Langhendorf Model with Krebs solution (KS) and monitoring of the electrocardiogram. After 30 minutes in sinus rhythm, ischemia was performed with discontinuation of the KS for 5 min and then reconnected. They were divided into 2 groups: Control (n = 10), with IR without medication and Enox Group, with infusion of 2mg / kg, in the cannula

that perfuses the heart, soon after initiation and support for 30 seconds of Ventricular Tachycardia (VT) or Torsade de Pointes (TP). In case of reversal of the arrhythmia, a new arrhythmia induction will be attempted with the same protocol. Monitoring is continued until asystole or completion scheduled for 60 minutes of experimentation. **RESULTS:** The control group presented VT in 70% of hearts and total atrioventricular block (TAVB) in 30% of hearts, followed by asystole after 7 minutes in 100% of hearts, and no further attempt of ischemia was possible. In the Enox group, 60% of VT, 30% of TP and 10% of TAVB were observed. All arrhythmias were reversed between 10 and 15 seconds after infusion of Enox, with return and maintenance of sinus rhythm after medication. In this group, a new attempt was made to induce arrhythmia due to IR without success after 10 minutes of interruption of the arrhythmia by Enox and sinus rhythm was maintained until the interruption of the experimental protocol. **Conclusion:** Enoxaparin reverses arrhythmias triggered by IR, with its effect prolonging and protecting new events after infusion, as well as decreasing organ mortality in isolated hearts of rats.

E.R. Vasques: None. **E.R. Vasques:** None. **I.L. Tersariol:** None. **H.B. Nader:** None. **C.M. Godoy:** None.

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Experimental Verification of Value of $T_{peak-T_{end}}$ Interval in Ventricular Arrhythmia Inducibility in Early Repolarization Syndrome Model

Namsik Yoon, Seo Na Hong, Hyung Ki Jeong, Ki Hong Lee, Hyung Wook Park, Jeong Gwan Cho, Chonnam Natl Univ Hosp, Gwangju, Korea, Republic of

Background: In patients with early repolarization (ER) pattern on ECG, many researchers have studied to find predictors of fatal arrhythmia, nevertheless no satisfying predictor exists. **Objective:** We evaluated the value of $T_{peak-T_{end}}$ interval on pseudo-ECG in canine myocardial wedge preparation models of early repolarization syndrome (ERS).

Methods: Transmural pseudo-ECG and endocardial/epicardial action potentials were recorded from coronary-perfused canine left ventricular wedge preparations ($n = 34$). The I_{to} agonist NS5806 (7-10 μM) and Ca^{2+} channel blocker verapamil (3 μM) were used to pharmacologically mimic ERS genotypes. Ventricular arrhythmia induction test was done. QT_{peak} , QT_{end} , corrected QT, $T_{peak-T_{end}}$, corrected $T_{peak-T_{end}}$ interval and $T_{peak-T_{end}}/QT_{end}$ were measured at 15-20 min after the provocative agents infusion. **Results:** Polymorphic ventricular tachycardias (pVT) developed in 23 of 34 preparations (67%). In preparations of induced pVT, $T_{peak-T_{end}}$ and $T_{peak-T_{end}}/QT_{end}$ increased gradually as the infusion time of provocative agents went by. Maximal value was recorded just before pVT induction. In the baseline state without the provocative agent, $T_{peak-T_{end}}$ and $T_{peak-T_{end}}/QT_{end}$ were not different between pVT induction and non-induction preparations. $T_{peak-T_{end}}$ of the pVT induction preparations was longer than that of non-induction preparation (58 ± 26.8 msec vs. 33 ± 6.8 msec, $p < 0.001$). $T_{peak-T_{end}}/QT_{end}$ of pVT induction preparations was larger than that of non-induction preparations (0.220 ± 0.1017 vs. 0.128 ± 0.0312 , $p < 0.001$). Transmural dispersion of repolarization (TDR) and epicardial dispersion of repolarization (EDR) of pVT induction preparations were larger than those of pVT non-induction preparations. TDR and EDR had a positive correlation with $T_{peak-T_{end}}$. **Conclusion:** $T_{peak-T_{end}}$ was able to predict malignant ventricular arrhythmias in ERS. $T_{peak-T_{end}}$ reflects repolarization heterogeneity of ventricular myocardium. Therefore, $T_{peak-T_{end}}$ is expected to be helpful to make a risk-stratification in ER pattern.

N. Yoon: None. **S. Hong:** None. **H. Jeong:** None. **K. Lee:** None. **H. Park:** None. **J. Cho:** None.

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Enhanced Left Ventricle TGF β 2 Signaling in a Model of Dilated Cardiomyopathy

Andrew Antolic, Ahmed B Alsalem, Da Young Lee, Zhe Jiao, Michael A Burke, Div of Cardiology, Dept of Med, Emory Univ Sch of Med, Atlanta, GA

Pathological gene transcription and induction of proinflammatory signaling is a hallmark of dilated cardiomyopathy (DCM) and HF. Previous studies from our lab identified TGF β 2 as a putative driver of inflammation during DCM progression. To study the role of TGF β 2 in DCM hearts, we analyzed TGF β signaling networks, as well as TGF β 2-induced gene expression changes from mice carrying a human DCM mutation in phospholamban (PLN^{R9C}). ELISA was used to confirm TGF β isoform levels in PLN and wild type (WT) hearts: only TGF β 2 was significantly increased in PLN^{R9C} mice (TGF β 1: 1.6-fold, $p=0.15$; TGF β 2: 9.6-fold, $p=0.01$; TGF β 3: 1.0-fold, $p=0.88$) (Figure). Levels of total SMAD2 (1.5-fold, $p=0.002$), phosphorylated

SMAD2 (P-SMAD2; 3.9-fold, $p=0.0003$), and the P-SMAD2/SMAD2 ratio (2.6-fold, $p=0.0004$) were greater in PLN^{R9C} mice. Treatment with exogenous TGF β 2 results in activation of cultured cardiac fibroblasts from WT hearts (*Col1a1* (3.3-fold), *Col4a1* (2.7-fold), *Ctgf* (6.4-fold), *Eng* (2.6-fold), *Fn1* (2.8-fold), *Lox* (2.6-fold), *Mmp2* (2.8-fold), *Postn* (4.1-fold), *Rhob* (3.6-fold), *Tgfb1* (3.6-fold), *Tgfb2* (2.9-fold), *Tgfb3* (3.0-fold), *Tie1* (2.4-fold), *Thsb1* (4.3-fold)). Our results demonstrate that TGF β 2 levels are increased and that TGF β signaling is activated in PLN^{R9C} hearts with DCM. These data suggest that TGF β 2 is a specific and potent inflammatory mediator in the heart and mark this isoform as a potential therapeutic target in DCM.

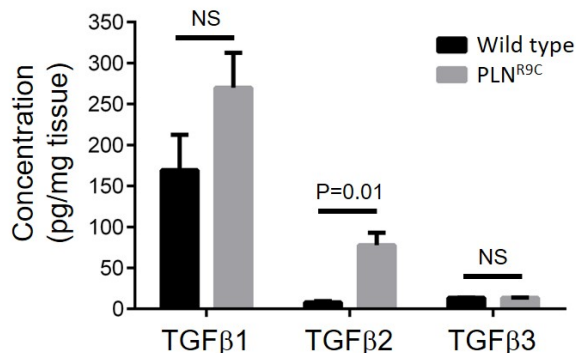


Figure: TGF β 2 is specifically and highly upregulated in PLN^{R9C} mice with DCM.

A. Antolic: None. A.B. Alsalem: None. D. Lee: None. Z. Jiao: None. M.A. Burke: None.

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The Extracellular Matrix Component, Hyaluronan, Provokes a Pro-Inflammatory Phenotype in Macrophages

Timothy Ndagi Audam, Sujith Dassanayaka, Andrea Jurkovic, Bethany Long, Kenneth R Brittan, Marcin Wysoczynski, Steven P Jones, Univ of Louisville, Louisville, KY

Background: The extracellular matrix (ECM) provides structural and functional support for the myocardium, but myocardial infarction (MI) changes the composition of the ECM. One of the chief components of the ECM, hyaluronan (HA), is elevated after MI; however, specific biological actions of HA—particularly at the level of infiltrating immune cells and implications of such interactions on ventricular remodeling—have not been explored. **Goals:** Because upregulation of HA coincides with macrophage infiltration after MI, we determined whether hyaluronan interacts with macrophages and investigated the implication of such interactions on macrophage function. **Methods:** WT mice were subjected to non-reperfused MI to determine changes in hyaluronan synthases (HAS), hyaluronidases (HYAL), and HA levels in the heart. Interaction of HA with macrophages was studied by polarizing bone marrow derived macrophages and analyzing cells by flow cytometry. Next, we characterized the ability of macrophages to metabolize HA by profiling polarized macrophages for HA-metabolizing enzymes, HA receptors, HA-binding proteins, hyaluronidase activity, and phagocytosis. **Results:** Compared to Sham hearts, MI (n=5/group) augmented the expression of HAS-2 (10-fold, $p=0.002$) and HYAL-2 (2 to 4-fold, $p=0.0004$) in the infarct and remote regions of the heart at 5 d post-MI. HA levels (n=8/group) were elevated in the infarct (1 to 2-fold, $p=0.0200$) and remote (2 to 3-fold, $p=0.0007$) regions of the heart compared to sham hearts. Polarizing macrophages (n=3/group) in the presence fluorescein-conjugated HA (HA-FL) showed that naïve (M0), pro-inflammatory (M1), and pro-resolving (M2) macrophages interact with HA-FL; M1 showed the highest FITC intensity. Interestingly, exposing macrophages (n=5/group) to HA provoked an inflammatory phenotype, as reflected by enhanced expression of TNF α (4-fold, $p=0.0001$) and IL-1 β (7-fold, $p=0.0094$) mRNA; HA also enhanced macrophage phagocytosis (0.5-fold, $p=0.0476$). **Conclusion:** Hyaluronan is elevated following MI and can influence macrophage function. Because of the accumulation of hyaluronan and macrophages in the post-MI heart, macrophage-hyaluronan interactions may be a nexus regulating ventricular remodeling.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Thromboxane/Prostanoid Receptor Activation Increases Calpain-Mediated Proteolysis and Alters Calcium Handling and Fibrosis Following Right Ventricular Pressure Overload

James D West, Kyungsoo Kim, Toshio Suzuki, Christy Moore, Bjorn C Knollmann, **Erica J. Carrier**, Vanderbilt Univ Medical Ctr, Nashville, TN

In pulmonary arterial hypertension (PAH), the right ventricle undergoes remodeling and fibrosis as it struggles to adapt to the increased pressure overload. RV dysfunction and failure is the primary cause of death in PAH patients. The G protein-coupled thromboxane/prostanoid receptor (TPr) is expressed in vascular smooth muscle, myofibroblasts, and immune cells, and is upregulated in cardiomyocytes following PAH. Activation of the cardiomyocyte TPr increases intracellular calcium via G_{α_q}/IP_3 ; activation of the receptor in other cells leads to fibrosis and vasoconstriction. The TPr is activated by isoprostane as well as thromboxane, which suggests that the receptor could contribute to deleterious remodeling during cardiac stress. Our previous studies demonstrate that TPr antagonism prevents RV fibrosis and dilatation in murine models of PAH, without affecting pressures. Because the TPr can signal through Gq, we hypothesized that its activation in PAH causes changes in cardiomyocyte calcium-handling proteins which contribute to remodeling and failure. In this study, we used pulmonary arterial banding (PAB) to induce fixed pressure overload of the RV. Mice were treated for 4 weeks past PAB with normal drinking water or water containing 25 mg/kg/day of the TPr antagonist ifetroban, and either underwent pressure-volume catheterization and whole RV evaluation, or cardiomyocytes were isolated for calcium handling and protein. PAB caused an increase in cardiomyocyte resting (end-diastolic) intracellular calcium, which was ameliorated in mice given TPr antagonist. The increased intracellular calcium following PAB was associated with increased activity of the calcium-activated protease calpain, also blocked with TPr antagonism. There was no decrease in caffeine-mediated release of calcium from the sarcoplasmic reticulum (SR) at 4 weeks past PAB, and phosphorylation of phospholamban was increased, suggesting compensation to drive calcium into the SR. Our findings suggest that TPr activation produces alterations in RV calcium handling, signaling, and calpain activity that contribute to deleterious remodeling and early failure in pressure overload. Therapeutic TPr antagonism may help preserve RV function in patients with PAH.

J.D. West: None. **K. Kim:** None. **T. Suzuki:** None. **C. Moore:** None. **B.C. Knollmann:** None. **E.J. Carrier:** 3. Other Research Support; Modest; Study drug provided by Cumberland Pharmaceuticals.

Poster Session 3 and Reception

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Attenuation of Cardiac Fibrosis by Scleraxis Gene Deletion Improves Pressure Overload-Induced Cardiac Remodeling

Raghu S Nagalingam, Univ of Manitoba, Winnipeg, MB, Canada; David Y Cheung, Nina Aroutiounova, St Boniface Hosp Res Ctr, Winnipeg, MB, Canada; Davinder S Jassal, **Michael P Czubyrt**, Univ of Manitoba, Winnipeg, MB, Canada

Cardiac fibrosis is a significant independent risk factor for heart failure with increasing incidence. The fibrotic myocardium shows increased arrhythmogenesis, and poor pumping and relaxation due to greater tissue stiffness. A critical step in this process is the conversion of fibroblasts to myofibroblasts, which are responsible for excessive extracellular matrix (ECM) production; limiting this conversion may reduce fibrosis and restore cardiac function. We previously reported that the transcription factor scleraxis, following mechanical stretch or TGF β signaling, is both sufficient and necessary to convert fibroblasts to myofibroblasts by direct transcriptional control of myofibroblast genes including collagens, α -smooth muscle actin and fibronectin. In a pressure overload transverse aorta constriction (TAC) mouse model analyzed by echocardiography, we found that fibroblast-specific scleraxis gene deletion prior to TAC using a tamoxifen-inducible TCF21-Cre/loxP approach attenuated both systolic (LV ejection fraction, fractional shortening) and diastolic (early and late filling velocity) dysfunction, as well as chamber dilation, despite persistent hypertrophy. Functional improvement was matched by an almost complete attenuation of cardiac fibrosis (Masson's trichrome; qPCR and western blots for fibrillar collagens and ED-A fibronectin). Scleraxis deletion also prevented induction of the myofibroblast marker periostin, suggesting a failure of scleraxis-null fibroblasts to convert to myofibroblasts. We next tested if scleraxis deletion 4 weeks post-TAC could reverse subsequent remodeling at 8 weeks post-TAC. Adverse remodeling occurred in all animals 4 weeks post-TAC (prior to scleraxis deletion), but cardiac function and chamber dimensions subsequently declined further in scleraxis-intact animals, while scleraxis-deleted animals showed preserved or improved cardiac function and morphology. Our results demonstrate that scleraxis is required for the initiation and progression of cardiac fibrosis, and that reducing fibrosis alone improves

cardiac performance and morphology even in the presence of persistent pressure overload. Scleraxis is thus an important target for anti-fibrotic therapy development.

R.S. Nagalingam: None. **D.Y. Cheung:** None. **N. Aroutiounova:** None. **D.S. Jassal:** None. **M.P. Czubyrt:** None.

Poster Session 3 and Reception

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Histone Deacetylase Inhibition Improves Heart Failure With Preserved Ejection Fraction Cardiopulmonary Phenotype and Induces Metabolomic Switch

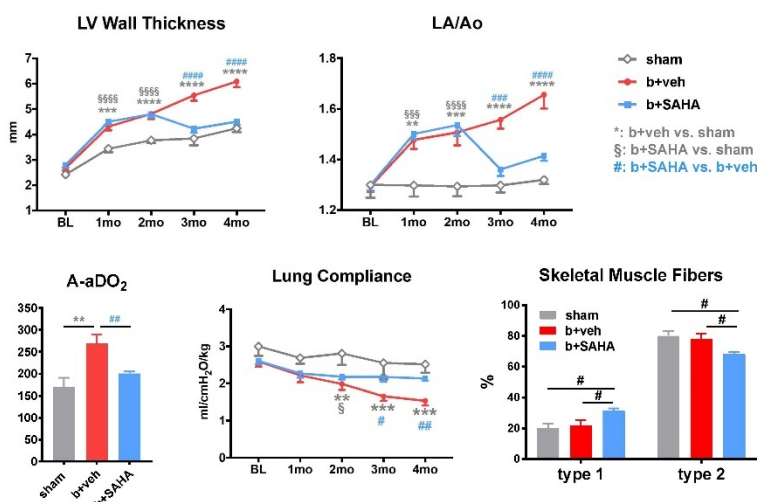
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Rationale: Approximately 50% of heart failure patients are diagnosed with Heart Failure with preserved Ejection Fraction (HFpEF), but there are currently no effective treatments.

Hypothesis: Treatment of a feline HFpEF animal model with a pan HDAC inhibitor, SAHA, will improve the cardiopulmonary phenotype and mediate changes in the metabolome and skeletal muscle composition.

Methods and results: Male domestic short hair cats (n=21, age 2mo) underwent either a sham procedure (n=5) or aortic constriction (n=16) using a customized pre-shaped band causing slow progressive pressure overload during maturation. At 2-months post-banding, banded animals received either daily treatment with 10mg/kg SAHA (b+SAHA) (n=8) or vehicle (b+veh) (n=8) for 2 months. At 4 months post-banding, b+ SAHA animals had significantly reduced LV wall thickness, LA size (LA/Ao), and improved LA function (LA EF) vs. b+ veh animals (fig). Invasive hemodynamics and lung mechanics were performed after 2 months of treatment. Banded animals had significantly increased filling pressures (LVEDP), increased pulmonary arterial pressures (mPAP), decreased lung compliance and arterial oxygenation (A- aDO_2). SAHA significantly reduced LVEDP, mPAP, and A- aDO_2 and increased lung compliance (fig). b+SAHA animals had an increase in the percentage of type 1 muscle fibers (increased oxidative capacity) compared to type 2 (fig). Blood-based metabolomics revealed SAHA-induced a metabolic shift towards increased lipolysis and mitochondrial oxidation.

Conclusion: Treatment with SAHA improved cardiopulmonary structure and function in banded animals and caused changes in the metabolome and skeletal muscle.



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Single-cell Transcriptomic Profiling Provides Insights Into Disease-related Processes During Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a genetic disease usually caused by a heterozygous mutation in a sarcomeric gene. Histologically, HCM is characterized by a heterogeneous population of hypertrophic, disorganized cardiomyocytes and fibrosis of heart tissue. This hypertrophic growth can lead to outflow tract obstruction which can ultimately lead to progressive heart failure due to cardiac overload.

Here we use single-cell RNA sequencing (scRNA-Seq) on septal myectomy samples from patients suffering from HCM to interrogate cellular heterogeneity in disease context. Using our previously optimized digestion and sorting protocol we were able to obtain good quality RNA for single cell analysis. Bioinformatic clustering analysis of single cell transcriptomes revealed 8 transcriptomically distinct subpopulations of cardiomyocytes. Our data indicate an NPPA/NPPB enriched cluster of cells and show TTN to function as an important determinant of the cellular heterogeneity. Correlation analysis links TTN expression to the expression of, among others, cardiomyopathy-associated protein (CMYA) 5 and 3, while there appears to be a negative correlation with, among others, TMEM212. We were able to confirm these correlations by RT-PCR on bulk RNA in an additional cohort of 97 HCM myectomy samples. Additionally, immuno-histochemical staining on myectomy samples and explanted HCM hearts confirmed the transcriptional heterogeneity among cardiomyocytes. A unique advantage of FACS-based single cell sorting cardiomyocytes is the availability of index data for the sorted cells. Correlation analysis of forward scatter, as a proxy for cell size, confirmed both well-known stress markers MYL2 and MYL7 and additional genes to be upregulated in the larger cells. In summary, we show that FACS-based sorting of HCM cardiomyocytes allows for scRNA-Seq of intact cardiomyocytes. Initial analyses of the data could be validated in a wider cohort of comparable samples, showing reliability of the data. Finally, incorporation of index data reveals genes correlated with cell size. Further interrogation of this data has the potential to reveal novel insights into the pathogenesis of HCM.

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Poster Session 3 and Reception

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The Functional Role of Myofibroblast-Expressed Smooth Muscle Alpha Actin in Post-Myocardial Infarction Tissue Healing

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After myocardial infarction (MI), dead cardiomyocytes are permanently replaced by scar tissue due to the lack of cardiomyocyte regeneration. In response to MI, cardiac fibroblasts (CFs) differentiate into myofibroblasts which possess highly organized smooth muscle α -actin (α SMA) stress fibers and secrete a large amount of extracellular matrix (ECM) proteins, essential for the post-MI infarct scar formation. It is well known that the accumulated ECM in the infarct scar stabilize the scar and prevents cardiac rupture. However, the functional role of α SMA stress fiber itself in CF-derived myofibroblasts is still largely unknown besides the wide use of it as a marker of myofibroblast differentiation. Our recent work demonstrated that during myofibroblast differentiation of CFs post-MI, the expression of α SMA reaches a peak a few days earlier than genes encoding ECM proteins. Myofibroblasts then lose α SMA expression and differentiate to the newly identified matrifibrocytes as the infarct scar becomes increasingly stable. These facts suggest a more important role of α SMA stress fibers than ECM proteins in the tissue healing process in early phase post-MI, possibly through providing a support lattice which in turn strengthens the infarcted myocardium. To test our hypothesis, we generated a mouse line with *Acta2* (the gene encoding α SMA) flanked by LoxP sites (*Acta2^{fl/fl}*) to enable cre-mediated *Acta2* knockout. Here we show that the loss of *Acta2* in CFs leads to enhanced proliferation and reduced contractility upon TGF β -induced myofibroblast differentiation *in vitro*. By using a mouse line with tamoxifen-inducible CF-specific *Acta2* knockout and CF lineage tracing (*Tcf21^{MerCreMer};Acta2^{fl/fl};R26^{GFP}*) and a WT control mouse line (*Tcf21^{MerCreMer};R26^{GFP}*), we identified that CF-specific *Acta2* knockout results in significantly exacerbated post-MI cardiac function, increased cardiac rupture incidence, and enhanced fibrosis. More lineage tracing experiments and transcriptome analyses are being conducted to better decipher the impact of *Acta2* knockout on CF physiology and post-MI tissue healing.

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Endothelial Estrogen Non-Nuclear Signaling Plays a Key Role in Anti-remodeling Effects via cGMP Signaling in Failing Heart

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Background

Estrogen plays a significant role in cardiovascular benefits. As one of its beneficial pathways, ER α non-nuclear signaling is known to be protective for the vascular system, but its role for cardiac remodeling is unknown thus far. We previously reported cGMP pathways are one of the critical therapeutic targets for heart failure and ER α non-nuclear signaling played a pivotal role in cardioprotection and was indispensable to the therapeutic efficacy of cGMP-PDE5 inhibition. However, it is still remaining undetermined which cells are playing the key role in cardioprotective effects via ER α non-nuclear signaling.

Methods

We generated genetically modified mouse lacking ER α in cardiomyocytes (ER $\alpha^{fl/fl}/\alpha MHC^{Cre/+}$) or endothelial cells (ER $\alpha^{fl/fl}/Tie2^{Cre/+}$) and assessed the effects of chronic pressure-overload (TAC) and the efficacy of PDE5 inhibitor (PDE5i) sildenafil after TAC. In addition, for the first time we generated endothelial cell specific genet-modified mouse lacking interactions between p85 α and ER α which are critical for non-nuclear signaling (ER $\alpha^{Kl/Kl}/Tie2^{Cre/+}$). Cardiac functions were assessed for evaluating the effects of endothelial ER α non-nuclear signaling to failing hearts.

Results

Under physiological E2 status, PDE5i's anti-remodeling effects after TAC were abrogated in ER $\alpha^{fl/fl}/Tie2^{Cre/+}$ (FS(%) \pm SEM with vs without PDE5i 28.16 \pm 3.43 vs 31.87 \pm 2.30) but not in ER $\alpha^{fl/fl}/\alpha MHC^{Cre/+}$ (FS(%) \pm SEM with PDE5i vs without PDE5i 54.57 \pm 3.418 vs 43.3 \pm 1.21) consistent with hemodynamic analysis. Assessment with ER $\alpha^{Kl/Kl}/Tie2^{Cre/+}$ showed decreased cardiac functions even with physiological E2. Hemodynamic analysis also supported the cardioprotective effects of ER α non-nuclear signaling in endothelial cells.

Conclusion

ER α non-nuclear reaction in endothelial cells plays an important role in cardio-protective mechanism, and endothelial estrogen signals are indispensable to the therapeutic efficacy of cGMP-PDE5 inhibition. Our results clarified one of the cardioprotective signals of estrogen and highlighted the new strategy for heart failure treatment.

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Development of Molecular Targeted Therapy Against Right Ventricular Failure: Evaluation by Transcriptome and *in vivo* Analysis

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Backgrounds: Right ventricular (RV) failure is a final common pathway in heart failure. But there is no specific therapy for RV failure. Moreover, it remains unclear how RV failure is developed. To develop a novel therapy for RV failure, we focused on the RV specific character and elucidate the function in RV failure. **Methods:** Microarray analysis using several parts of adult murine heart was conducted and differentially expressed genes (DEGs) were applied to pathway analysis. Molecular mechanism was examined by using rat cardiomyocytes *in vitro*. To understand the function of target molecule *in vivo*, we induced RV failure by pulmonary artery constriction (PAC) in mice and the pathway was specifically blocked in the RV failure model. **Results:** In microarray analysis for upper RV, RV free wall, LV and ventricular septum, 995 genes were statistically extracted as DEG in upper RV. An alternative complement pathway was significantly activated in upper RV and complement factor, and C3a was a potential upstream factor attributable to unique feature of upper RV. Because C3a plays a central role

in alternative complement pathway, we examined the direct role of C3a in cardiomyocytes and RV function. Administration of C3a recombinant protein to primary-cultured cardiomyocytes activated the several MAP kinases including ERK, p38 and JNK, via C3a receptor. Mice developed severe RV failure around 14 days after PAC. Surprisingly, administration of C3a receptor antagonist dramatically improved right ventricular contractile dysfunction in PAC mice. C3 (substrate of C3a) deficient PAC mice also attenuated RV contractile dysfunction, fibrotic change and fetal gene expressions. These results indicated that complement factor C3a regulates the unique phenotypes in RV. Chemical or genetical blockade of C3a ameliorates RV dysfunction in PAC mice. **Conclusion:** We revealed that alternative complement pathway was activated in RV and C3a had a crucial role in the pathogenesis of RV failure. Accordingly, the blockade of C3a pathway would be a potential therapeutic target for RV disorders.

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Regulation of *Bmp7* and *Ctgf* Suppresses Dilated Cardiomyopathy and Cardiac Fibrosis

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Introduction: Cardiac *Lmna* insufficiency causes dilated cardiomyopathy (DCM) coupled with cardiac fibrosis and inflammation. TGF β family members are key mediators for cardiac performance, cardiac fibrosis and inflammation. However, how TGF β family members are regulated in heart is unknown.

Hypothesis: Modulation of downstream pathways could be served as a therapeutic strategy.

Methods and Results: We demonstrate that regulation of *Bmp7* and *Ctgf* suppresses *Lmna* DCM and cardiac fibrosis. Upregulation of *Bmp7* alone was not sufficient to suppress DCM and cardiac fibrosis. Importantly, upregulation of *Bmp7* together with *Ctgf* silencing significantly suppressed DCM and cardiac fibrosis. Mechanistically, downregulation of *Ctgf* inhibited TGF β /Smad signaling pathway in DCM hearts. Co-modulation of *Bmp7* and *Ctgf* reduced infiltration of T cells in hearts. A screen of cardiac related factors identified *Yy1* enhanced *Bmp7* promoter activity and prevent the upregulation of *Ctgf* promoter activity. *Yy1* suppressed *Lmna* DCM by inducing *Bmp7* expression and reduced *Ctgf* upregulation in cardiomyocytes. Importantly, knockdown of upregulated *Bmp7* attenuated the suppressive effect of *Yy1* on DCM and cardiac fibrosis.

Conclusions: Our findings demonstrate that DCM caused by *LMNA* insufficiency can be treated with co-modulation of *Bmp7* and *Ctgf*.

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Poster Session 3 and Reception

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Different Disease States in Heart Have Distinct Cardiac Interstitial Cells Contributing to Fibrosis

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Cardiac fibrosis is a grim consequence for almost all myocardial injuries. In myocardial infarction (MI), what starts as a protective scarring process to prevent ventricular wall rupture becomes a pathological remodeling of the tissue with the accumulation of excess extracellular matrix (ECM) proteins. Eventually, this adaptation impedes the mechanical and electrical properties of the myocardium resulting in heart failure. Recently, we showed that periostin (Postn) expressing resident cardiac fibroblasts (CFs) are a potential therapeutic target since they differentiate into the scar associated, matrix-producing myofibroblasts (MFs) after injury. In fact, deletion of these cells after an acute injury eliminates interstitial fibrosis but results in ventricular rupture which is a hallmark outcome of impaired ECM deposition during the acute phase of MI. On the other hand, ablation of these cells during a chronic injury such as pressure overload-induced cardiac fibrosis model, we observe sustained perivascular fibrosis. Previous studies report heterogeneity of origin and function for ECM producing cells associated with different cardiac diseases. Here we utilized several novel mouse models that permit lineage tracing of all activated MFs as well as perivascular mural cells in the heart to elucidate the role and fate of these distinct cardiac interstitial

cells during fibrogenesis. Cells were lineage traced with a tamoxifen-inducible cre recombinase cDNA knock-in alleles (PostnMCM and Gli1CreER) in combination with a Rosa26-eGFP cre-dependent reporter. Hearts subjected to MI, TAC or Angiotensin injury were processed for extensive histological and RNAseq analyses. Results show that interstitial fibrosis in acute MI injury is a result of Postn+ MFs activity, whereas a subset of Gli1+ mural cells are responsible for the perivascular fibrosis observed after pressure overload models. Therefore, we concluded that pathological ECM deposition resulting in fibrosis comes from disease-specific specialized sub-populations of interstitial cells of the heart with distinct gene expressions and require manipulation of alternative cell- and state-specific therapeutic targets.

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High Molecular Weight FGF2 Contributes to Pressure Overload Induced Systolic Dysfunction by a Mechanism Associated With Modulation of the NR1D1 Orphan Nuclear Receptor Expression

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Fibroblast growth factor 2 (FGF2) is implicated in normal cardiac development as well as cardiac pathophysiology; however, FGF2 exist as multiple high and low molecular weight isoforms. While endogenous low molecular weight FGF2 (Lo-FGF2) is cardioprotective during chronic stress, the more prevalent endogenous high molecular weight FGF2 (Hi-FGF2) is proposed to promote maladaptive cardiac remodeling. We have investigated the hypothesis that genetic elimination of Hi-FGF2 attenuates cardiac dysfunction in mice that have been subjected to pressure overload by transverse aortic constriction (TAC).

Two groups of male C57BL/6mice were compared: (1) Wild type (WT) mice, expressing Hi- and Lo-FGF2 (FGF[WT] mice); and (2) Hi-FGF2 knock-out mice, expressing only Lo-FGF2 (FGF[Lo] mice). Echocardiographic assessment of heart function and dimensions was done at baseline and then 4 and 8 weeks after TAC or sham surgery. FGF[WT] mice displayed a decline in systolic function compared to their corresponding sham animals at 4- and 8-weeks post-TAC, which was absent in the FGF[Lo] mice. Relative levels of B-type natriuretic peptide, a marker of cardiac pathology severity, were elevated in FGF[WT] but not FGF[Lo] mice compared to shams. Increased accumulation of the pro-cell death protein BCL2/adenovirus E1B 19 kDa protein-interacting protein-3 was more pronounced in the FGF(WT) compared to FGF(Lo) mice, post TAC. Microarray analysis of the whole transcriptome of hearts in FGF2[WT] and FGF2[Lo] mice indicated the pathway linked to circadian rhythm as a candidate for the most significant differentially regulated. Specifically, upregulation of the circadian rhythm master regulator, Nuclear Receptor Subfamily 1 Group D Member 1 (NR1D1), was validated by qPCR and protein immunoblotting in FGF[Lo] mice versus downregulation of NR1D1 in FGF[WT] mice post-TAC, when compared to their sham operated littermates.

Taken together these studies suggest that expression of Hi-FGF2 contributes to cardiac systolic dysfunction in left ventricular pressure overloaded WT mice by downregulation of Nr1D1, post-TAC.

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Poster Session 3 and Reception

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Sphingosine 1-Phosphate Receptor 1 in Cardiomyocytes Protects Against Cardiac Fibrosis

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Sphingosine 1-phosphate receptor 1 (S1P₁, encoded by *S1pr1*) is a G protein-coupled receptor that binds the ligand S1P and signals in multiple cell types including endothelium and cardiomyocytes. We previously demonstrated that excision of *S1pr1* in cardiomyocytes during embryonic development leads to ventricular noncompaction and perinatal death in most but not all mutant mice. To investigate roles for S1P₁ in adult cardiomyocytes, we used three conditional *S1pr1* deletion strategies. Mice that survived early embryonic deletion of *S1pr1* in cardiomyocytes using *Mlc2a-Cre* demonstrated interstitial cardiac fibrosis by 4 months of age. Animals with later embryonic deletion of cardiomyocyte *S1pr1* using α MHC-Cre escaped

the ventricular noncompaction phenotype, but they displayed significantly increased interstitial fibrosis after isoproterenol infusion. Likewise, *S1pr1* excision from cardiomyocytes in adult mice using α MHC-MerCreMer led to increased cardiac fibrosis after angiotensin II infusion. In all conditional *S1pr1* excision strategies, systolic function in mutant mice remained similar to control mice. Finally, we conditionally deleted the sphingosine kinases *Sphk1* and *Sphk2* in developing cardiomyocytes and found no reduction in survival for cardiomyocyte *Sphk1/2* mutant mice. These results suggest that S1P from non-cardiomyocyte sources support cardiac development, and that cardiomyocyte S1P₁ activity protects against cardiac fibrosis.

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Poster Session 3 and Reception

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Epicardial Prestrained Confinement and Residual Stresses: A Newly Observed Heart Ventricle Confinement Interface

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The heart epicardial layer, with elastin as the dominant component, has not been well investigated, specifically on how it contributes to ventricular biomechanics. In this study, we revealed and quantitatively assessed the overall status of prestraining and residual stresses exerted by the epicardial layer on the heart left ventricle (LV). During porcine heart wall dissection, we discovered that bi-layered LV surface strips, consisting of an epicardial layer and cardiac muscle, always curled towards the epicardial side due to epicardial residual stresses. We hence developed a curling angle characterization technique to intuitively and qualitatively reveal the location-dependency and direction-dependency of epicardial residual stresses. Moreover, by combining prestrain measurement and biaxial mechanical testing, we were able to quantify the epicardial prestrains and residual stresses on the unpressurized intact LV. To investigate the potential mechanical effect of epicardial prestraining, a finite-element (FE) model has been constructed, and we demonstrate that it is the prestraining of the epicardial layer, not the epicardial layer alone, providing an additional resistance mechanism during LV diastolic expansion and ventricular wall protection by reducing myocardial stress. In short, our study on healthy, native porcine hearts has revealed an important phenomenon—the epicardial layer, rich in elastin, acts like a prestrained 'balloon' that wraps around the heart and functions as an extra confinement and protection interface. The obtained knowledge fills a gap in ventricular biomechanics and will help design novel biomimicking materials or prosthetic devices to target the maintenance/recreation of this ventricle confinement interface.

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Poster Session 3 and Reception

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Loss of S-nitrosylation of G Protein-Coupled Receptor Kinase 2 Leads to Cardiovascular Dysfunction With Aging

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Nitric oxide (NO) and S-nitrosothiol (SNO) have cardiovascular-protective properties and we have shown that one mechanism is through SNO-modification of G protein-coupled receptor (GPCR) kinase 2 (GRK2). S-nitrosylation at Cys340 (C340S) of GRK2 inhibits its activity on GPCRs such as B-adrenergic receptors in the heart and other GRK2 dependent processes. S-nitrosylation is believed to act as a brake on this pathological kinase. We generated global knock-in (KI) mice that express endogenous levels of GRK2, but with C340 mutated to Ser (GRK2-C340S). The C340 site is the primary site of S-nitrosylation and is largely responsible for eNOS-mediated cardioprotection after acute ischemic injury of the heart. In this study we observed that the loss of SNO-mediated inhibition of GRK2 leads to cardiovascular dysfunction during normal aging. GRK2 has been shown to be up-regulated during several cardiovascular (CV) pathologies including hypertension and heart failure, and aging is a major risk factor of CV disease (CVD). GRK2-C340S KI mice compared to their wild-type (WT)

controls showed significant pathology as early as 52 weeks of age and was maintained up to 82 weeks. Maladaptive cardiac hypertrophy was present in aged GRK2-C340S mice with significant perivascular myocardial fibrosis. Fibrosis was prevalent in the kidneys as well. These histological changes were accompanied by reduced systolic function and pulse pressure compared to WT mice. Within the vasculature, aged GRK2-C340S aortas had reduced medial thickness and had reduced contraction in response to PE, KCl, or L-NAME. Acetylcholine-induced relaxation responses remained intact. Consistent with enhanced GRK2 activity *in vivo* in the heart, we found increased circulating levels of serum catecholamines. Many of these phenotypes we observed in GRK2-C340S mice are also observed in human aging and CVD. These phenotypes predispose patients to developing other diseases. As the US population becomes increasingly older, understanding the dynamic between aging and CVD is important in disease management and therapy. The aged GRK2-C340S mouse model represents a model of higher CVD risk and provides insights into consequences of chronic GRK2 global over-activity when the regulation of this kinase by NO is lost.

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Poster Session 3 and Reception

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Protein kinase G - Regulator of G protein Signaling 2 Axis of Cardiac Myocytes Critically Determines Cardiac Performance in Early Cardiac Remodeling

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Introduction: Regulator of G protein signaling 2 (RGS2) is a negative regulator of G protein-coupled signaling, and is potentiated by cGMP-PKG. Our prior work using a global deletion model demonstrated the protective role for RGS2 against mal-adaptive cardiac remodeling, which is essential to the cGMP-PDE5 inhibitor efficacy; however, cell type-specific contribution remains to be determined, given the expression of RGS2 in myocytes, fibroblasts and vascular smooth muscle cells. We hypothesized that myocyte RGS2 might serve as a primary contributor to the cardio-protection and mediates cGMP-PKG benefits. **Method:** We generated cardiomyocyte-specific RGS2 knockout mice (cKO) on a C57BL/6 background. Pressure overload was produced by transverse aortic constriction (TAC) in male RGS2-cKO mice (RGS2cKO) and their littermate controls (WT). To investigate cGMP-PKG enhancing effects, a soluble guanylyl cyclase stimulator (riociguat) was orally administered daily (3mg/kg/day). Invasive hemodynamics, anatomy, histology and molecular signaling were assessed at 1wk after TAC.

Results: RGS2-cKO revealed exacerbated dilative cardiac remodeling (LVDD: cKO 3.57±0.36 vs WT 2.90±0.16) and severely impaired cardiac function (dPdt/IP: cKO 166±3.49 vs WT 195±7.40), while their basal phenotype was undistinguishable from WT. cKO-TAC hearts showed larger cardiomyocyte size and more fibrosis than WT-TAC hearts, associated with aberrant gene expression induction including *Myh6*, *Myh7*, *Col1a2*, and *TGFβ*. Intriguingly, concomitant riociguat treatment reduced LV chamber size and fibrosis but failed to improve cardiac performance in cKO-TAC hearts, while riociguat ameliorated all these in WT-TAC hearts.

Conclusion: These results suggest that RGS2-PKG axis in cardiac myocytes critically contributes to cardiac functional performance and that cGMP-PKG exerts anti-fibrotic effects independently of myocyte conditions.

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Non-oxidized Protein Kinase G 1 Alpha Antagonizes mTORC1 Signaling in a Tuberin-dependent Manner to Ameliorate Cardiac Disease

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Rationale: Protein kinase G 1 α (PKG1 α) confers anti-hypertrophic effects in hearts subjected to mechanical and neurohumoral stress. Human heart failure with a reduced ejection fraction (HF α EF) and mouse pressure overloaded hearts present with increased mechanistic target of rapamycin complex 1 (mTORC1) activity, protein aggregation, oxidative stress, and, as we previously described, increased PKG1 α disulfide dimer formation indicative of PKG1 α oxidation. Recently we demonstrated that stimulation of PKG1 α phosphorylates tuberin (TSC2) at one specific serine, S1365, to inhibit mTORC1 signaling and attenuate pathological hypertrophy. **Objective:** To determine if the redox state of PKG1 α impacts its ability to target TSC2 signaling in a chronic pressure-overload mouse model exhibiting pathologic hypertrophy. We hypothesize non-oxidized PKG1 α will increase TSC2 S1365 phosphorylation to antagonize mTORC1 signaling, thereby enhancing autophagic flux to clear protein aggregates, culminating in ameliorated cardiac disease. **Methods and Results:** Mice expressing a non-oxidizable (redox-dead) PKG1 α (cysteine 42 serine, CS) knock-in mutation and wild type (WT) littermate controls were subjected cardiac pressure overload stress via transaortic constriction (TAC) or sham surgeries. Following TAC, PKG1 α CS mice exhibited reduced mTORC1 activation leading to increased autophagic flux and preventing protein aggregation, compared to WT mice. PKG1 α CS TAC mice had decreased expression of the hypertrophic genes, attenuated cardiac hypertrophy ($p < 0.0001$), and improve fractional shortening compared to WT TAC mice (28.14% \pm 10.82 in WT vs. 47.42% \pm 15.62 in CS; $p < 0.01$). Treating WT TAC mice with an mTORC1 inhibitor (everolimus) abrogated mTORC1 hyperactivation, which lead to enhanced autophagic flux, attenuated hypertrophy, and improved cardiac function. Crossing PKG1 α CS mice with TSC2 S1365 phospho-null mice resulted in increased cardiac hypertrophy and reduced lifespan ($p < 0.05$). **Conclusions:** Preventing PKG1 α oxidation attenuates mTORC1 activation to enhance autophagic flux, prevent protein aggregation, and ameliorate pathological hypertrophy in following cardiac pressure overload, dependent on TSC2 S1365 phosphorylation.

C.U. Oeing: None. **M.J. Ranek:** None. **B.L. Dunkerly-Eyring:** None. **D.A. Kass:** None.

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β_{IV} -spectrin Regulates Cardiac Fibroblast Phenotype, Fibrosis, and Cardiac Function

Nehal J. Patel, Drew M. Nassal, Benjamin W. Scandling, Amara D. Greer-Short, Sathya D. Unudurthi, Xianyao Xu, Peter J. Mohler, Keith J. Gooch, Thomas J. Hund, The Ohio State Univ, Columbus, OH

Increased fibrosis is associated with cardiac dysfunction and arrhythmias. Activation of quiescent cardiac fibroblasts (CFs) occurs in response to injury although the underlying mechanistic pathways remain unclear. The cytoskeletal protein β_{IV} -spectrin has been shown to control targeting and activity of the multifunctional transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) for maladaptive remodeling. The mechanism linking spectrin dysfunction to altered STAT3 signaling, fibrosis, and function remains unknown. The objective of this study was to investigate the role of stress-induced disruption of β_{IV} -spectrin/STAT3 complex in modulating CF phenotype, fibrosis, and cardiac function. Cardiac function (echocardiography) and fibrosis (Masson's trichrome) were evaluated in adult wildtype (WT) and qv^{AJ} mice expressing truncated β_{IV} -spectrin lacking STAT3 interaction. A subset of WT mice were subjected to 6 wks of transaortic constriction (TAC) to induce heart failure. From these groups, CFs were isolated from ventricles and analyzed for STAT3 localization, gene expression, and activity. CFs were treated with selective STAT3 inhibitor S31-201 (100 μ M) or transfected for 72 hrs with β_{IV} -spectrin fragment from repeat 10 to C-terminus ($\beta_{IV,10-C}$, contains STAT3 binding motif). Ejection fraction was decreased in qv^{AJ} and WT TAC relative to WT baseline (49.5 \pm 0.85% vs 34.9 \pm 2.2% vs 63.2 \pm 0.60% respectively, $p < 0.05$, N=5). Ventricular fibrosis was augmented in qv^{AJ} and WT TAC compared to WT baseline (2.4 \pm 0.09% vs 3.2 \pm 0.25% vs 0.48 \pm 0.01% respectively, $p < 0.05$, N=5). qv^{AJ} CFs showed STAT3 mislocalization (49.7 \pm 10.7% nuclear accumulation vs 5.3 \pm 1.8% in WT, $p < 0.05$, N=5) with higher STAT3 transcriptional activity (2.6 \pm 0.17-fold increase over WT, $p < 0.05$, N=9). In parallel, qv^{AJ} CFs displayed elevated expression of fibrotic genes associated with CF activation. At the functional level, qv^{AJ} CFs showed increased proliferation (1.9 \pm 0.19-fold increase over WT at 24 hrs, $p < 0.05$, N=4). STAT3 inhibition with S31-201 or transfection with $\beta_{IV,10-C}$ normalized gene expression and proliferation in qv^{AJ} CFs compared to WT. These studies identify a requirement of β_{IV} -spectrin for normal STAT3 signaling and quiescent phenotype in CFs.

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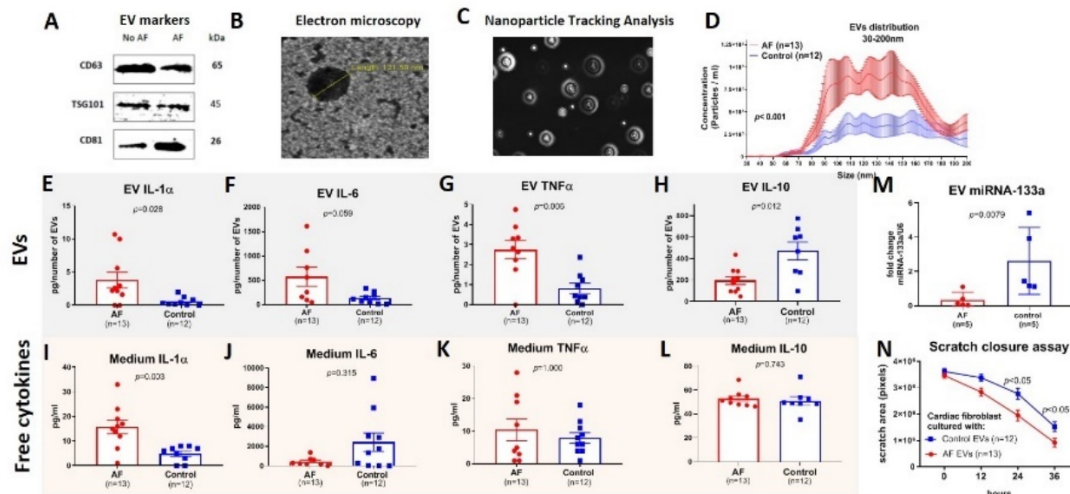
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Distinctive Inflammatory and Fibrotic Signature of Extracellular Vesicles from Epicardial Fat of Patients with Atrial Fibrillation

Background and Aim: Epicardial fat (eFat) has been linked to atrial remodeling and fibrillation (AF). We aimed to determine whether extracellular vesicles (EVs) derived from eFat play a role in the pathogenesis of AF. **Methods and Results:** We collected small specimens of eFat from patients (pts) with and without (w/o) AF undergoing heart surgery. eFat specimens were incubated as organ cultures and EVs were isolated from the culture medium by ultra-centrifugation. We used immunoblotting, electron microscopy, and nanoparticle tracking analysis to characterize the EVs (**Fig. A-C**). Significantly, eFat specimens from AF pts secreted greater amounts of EVs compared with patients w/o AF (**Fig. D**). Moreover, eFat EVs from AF pts secreted a higher concentration of inflammatory and fibrotic cytokines but less anti-inflammatory cytokines, compared with patients w/o AF (**Fig. E-H**). Notably, EV cytokines reflected the inflammatory and fibrotic status of eFat in AF pts better than free cytokines (**Fig. I-L**). Next, we tested several miRNA that could influence cardiac fibrosis. For example, miR-133 inhibits TGF- β , decreases collagen content and inhibits atrial remodeling. Expression of EV miR-133 was lower in eFat of AF pts than in patients w/o AF (**Fig. M**). Eventually, "wound healing" scratch assay show that fibroblast migration was greater after incubation with eFat EVs from AF patients, compared with pts w/o AF. (**Fig. N**). **Conclusions:** eFat from AF patients secretes a higher number of EVs with an inflammatory and fibrotic profile. Our findings suggest that eFat EVs contribute to inflammation and fibrosis, both of which contribute to the pathogenesis of atrial remodeling and fibrillation.



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The Effects and Mechanisms of Pyridostigmine on Cell Proliferation and Fibrosis of Human Cardiac Fibroblasts

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Background Heart failure (HF) is a common, costly, and potentially fatal condition. Cardiac fibrosis plays a key role in the process of HF. Pyridostigmine (PYR) could improve the cardiac function by inhibiting collagen deposition in cardiac tissue, but the molecular mechanisms are unknown.

Objective To investigate the effects and mechanisms of Pyridostigmine on cell proliferation and fibrosis in human cardiac fibroblasts (HCFs).

Methods The model of fibrosis in HCFs was induced by transforming growth factor beta. After the intervention of PYR, the content of collagen I (COL1) was observed by measuring the hydroxyproline concentration. The cell cycle of HCFs was screened by Flow cytometry. The mRNA levels of Connective tissue growth factor (CTGF) and Smad3 were examined by real-time qPCR analysis. The expressions of CTGF, Smad3, pSmad3 were examined by Western blot.

Results Compared with the TGF- β group, the TGF- β +PYR group had significantly lower proportion of S/G2 phased cells [(30.8 \pm 1.4) % vs (48.6 \pm 3.5)%], less synthesis of COL1 [(0.95 \pm 0.15) vs (1.25 \pm 0.18)] and fewer expressions of CTGF mRNA [(1.73 \pm 0.10) vs (2.92 \pm 0.12)] and CTGF protein [(1.23 \pm 0.42) vs (2.36 \pm 0.62)]. However, the expressions of Smad3 mRNA [(1.50 \pm 0.06) vs (1.55 \pm 0.08)], Smad3 protein [(2.85 \pm 0.45) vs (2.98 \pm 0.47)] and pSmad3 protein [(2.39 \pm 0.32) vs (2.51 \pm 0.52)] between these two groups showed no difference (P > 0.05).

Conclusion Pyridostigmine significantly alleviated cell proliferation and collagen synthesis in HCFs induced by TGF- β , and its mechanism was related to the inhibition of CTGF expression rather than blocking the TGF- β /Smads signaling pathway.

Table 1. Effect of PYR on cell cycle of HCFs induced by TGF- β 1

Group	G1(%)	S(%)	G2(%)	S/G2(%)
Control	65.3 \pm 3.5	20.6 \pm 1.8	10.2 \pm 1.1	30.8 \pm 1.6
PYR	64.2 \pm 1.6	18.1 \pm 2.1	11.4 \pm 1.1	29.5 \pm 1.9 *
TGF- β 1	43.2 \pm 2.6	33.0 \pm 4.1	15.6 \pm 2.2	48.6 \pm 3.5 #
10ng/ml TGF- β 1+50uM PYR	58.3 \pm 3.7	25.0 \pm 4.2	9.6 \pm 1.5	34.6 \pm 3.4 Δ \blacktriangle
10ng/ml TGF- β 1+100uM PYR	61.3 \pm 4.1	20.8 \pm 2.0	10.0 \pm 1.2	30.8 \pm 1.4 Δ
10ng/ml TGF- β 1+200uM PYR	58.0 \pm 6.8	24.1 \pm 3.6	11.9 \pm 2.1	36.0 \pm 2.8 Δ \blacktriangle
10ng/ml TGF- β 1+400uM PYR	58.7 \pm 2.9	23.2 \pm 3.3	10.3 \pm 1.7	33.5 \pm 2.1 Δ \blacktriangle

The data were expressed as the mean \pm SEM (n=6). *P>0.05 versus Control group, # P<0.05 versus Control group; Δ P<0.05 versus TGF- β 1 group; \blacktriangle P<0.05 versus 10ng/ml TGF- β 1+100uM PYR group.

L. Shen: None.

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Adipocyte-specific Pharmacological Inhibition of Adipose Triglyceride Lipase (ATGL) Ameliorates Cardiac Fibrosis in Heart Failure

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Objectives. ATGL is a key enzyme that regulates triglyceride lipolysis in most cells, including the adipocyte and the cardiomyocyte. Recent studies indicate that accelerated adipose tissue lipolysis contributes to worsening heart failure (HF), suggesting that ATGL inhibition specifically in the adipocyte may prevent worsening HF. Indeed, we have shown that the ATGL inhibitor, Atglistatin, which appears to be largely adipocyte-specific, ameliorates cardiac dysfunction and hypertrophy in HF induced by transverse aortic constriction (TAC). However, the mechanisms by which Atglistatin treatment prevents worsening HF in mice remains unknown. **Methods.** Eight week-old C57BL/6N mice underwent TAC or sham surgery. After 1 week, mice with HF were assigned into 4 groups: 1) vehicle (standard chow)-sham, 2) Atglistatin-sham, 3) vehicle-TAC and 4) Atglistatin-TAC. Atglistatin was administered in food pellets containing 2mmol Atglistatin/kg for 2 weeks. Thereafter, mice were euthanized and hearts were used for subsequent histological and quantitative PCR analysis. **Results.** Picro-sirius red staining demonstrated that hearts from the Atglistatin TAC mice had significantly less cardiac fibrosis than the vehicle TAC mice (1.6 \pm 0.4 vs 5.3 \pm 1.1%, in Atglistatin TAC vs vehicle TAC; p<0.01). Consistent with this finding, Atglistatin treatment of the TAC group reduced transcript levels of fibrosis-related genes, including *Col1a1* (-58.7%, p=0.03) and *Postn* (-54.7%, p<0.01), compared to vehicle-treated hearts. Furthermore, Atglistatin decreased the transcript levels of the macrophage polarization-related genes, including *Lgals3* (-55.3%, p<0.01) and *Spp1* (-38.7%, p=0.03), in the hearts from TAC mice compared to controls. Moreover, immunofluorescent analysis showed that Atglistatin reduced infiltration of Galectin-3 positive macrophages in the heart compared to vehicle-TAC mice, suggesting reduced macrophage infiltration and activation and less cardiac fibrosis. **Conclusion.** Our data indicate that reducing triglyceride lipolysis in the adipose tissue via ATGL inhibition may ameliorate HF via modulation of HF-induced macrophage and fibroblast activation in the heart.

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The Iron Chelator Deferiprone Clears Hemorrhagic Byproducts Following Acute Myocardial Infarction in a Swine Model of Ischemia-Reperfusion

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Current treatments for myocardial infarction (MI) aim to remove the vascular occlusion and reperfuse the infarcted tissue. While reperfusion can salvage myocardium, it can also increase damage to the unsalvageable tissue, one mechanism of which is hemorrhage; the resulting blood and iron deposits increase edema and inflammation. We hypothesize that removing these iron deposits with the iron chelating drug deferiprone (DFP) will reduce inflammation and lead to faster healing post-MI.

Using a swine model of ischemia-reperfusion, pigs were administered either DFP or saline for four weeks post-MI. Cardiac MRI tracked heart function, infarct progression (via late gadolinium enhancement), hemorrhage (via T2* imaging), and edema (via T2 imaging) at baseline and 1 day, 1 week, and 4 weeks post-MI. Animals were then sacrificed and hearts were processed for histology.

Treatment with DFP decreased the presence of iron in the infarct compared to saline by one week post-MI, as shown by increased T2* values. DFP treatment also decreased end diastolic wall swelling, suggesting less inflammation post-MI. To support this, T2 values and histology both revealed trends toward less edema with DFP. Histology also showed trended increases in fibroblast proliferation and collagen deposition at four weeks post-MI in the treatment group, consistent with a faster resolution to scar. Ventricular remodeling (by ventricular volumes), infarct size (by MRI, histology and blood Troponin I levels), and infarct transmuralty were all unaffected, but contractile function improved with DFP treatment: ejection fraction and wall thickening both improved faster.

In conclusion, DFP treatment successfully reduced hemorrhagic iron within the infarct as compared to saline, indicating a clearing of the iron byproducts. Treatment also decreased wall swelling, suggesting reduced inflammation, and improved contractile function. In the infarct, reduced swelling could indicate faster remodeling and a faster resolution to scar, and histology confirms a more progressed scar structure with DFP treatment. Overall, iron chelation appears to be a promising therapy that could reduce the negative effects of hemorrhage and speed the functional recovery of patients post-MI.

J.J. Weyers: 3. Other Research Support; Modest; We received the drug deferiprone and funding support from Apopharma, Inc. **R. Thomas:** 3. Other Research Support; Modest; We received the drug deferiprone and funding support from Apopharma, Inc.. **X. Qi:** None. **J. Barry:** None. **V. Rabadia:** 1. Employment; Significant; Employed by Aphopharma. **D. Manca:** 1. Employment; Significant; Employed by Aphopharma. **J. Connelly:** 1. Employment; Significant; Employed by Aphopharma. 7. Ownership Interest; Modest; A joint patent application was filed by ApoPharma and Sunnybrook Research Institute. **M. Spino:** 1. Employment; Significant; Employed by Aphopharma. 7. Ownership Interest; Modest; A joint patent application was filed by ApoPharma and Sunnybrook Research Institute. **B.H. Strauss:** None. **G.A. Wright:** 2. Research Grant; Significant; A research grant from the Ontario Research Fund: Research Excellence Program supports this work. 7. Ownership Interest; Modest; A joint patent application was filed by ApoPharma and Sunnybrook Research Institute. **N.R. Ghugre:** 2. Research Grant; Significant; A research grant from the Ontario Research Fund: Research Excellence Program supports this work. 3. Other Research Support; Modest; We received the drug deferiprone and funding support from Apopharma, Inc.. 7. Ownership Interest; Modest; A joint patent application was filed by ApoPharma and Sunnybrook Research Institute.

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Drp1-dependent Altered Mitochondrial Dynamics Contribute to Protein Aggregation and Mitochondrial Dysfunction in R120G α B-crystallin-induced Proteotoxicity

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Introduction: α B-crystallin (CryAB) is a chaperone protein that plays a pivotal role in the maintenance of the structural and mechanical integrity of the sarcomere. Mutation in α B-crystallin (CryAB R120G) causes sarcomere disorganization leading to protein aggregation. Accumulation of toxic protein aggregates causes cardiac contractile dysfunction and perturbed mitochondrial spatial organization. However, the role of mitochondria in the pathogenesis of protein aggregation-induced cardiomyopathies remain obscure. **Objective:** In this study, we investigated the role of mitochondrial dynamics and function in the development of R120G α B-crystallin induced cardiac proteotoxicity. **Methods and Results:** CryAB R120G Tg hearts showed accumulation of toxic protein aggregates resulting in disruption of the sarcomere structure, development of cardiomyocyte hypertrophy, and contractile dysfunction. Hearts obtained from CryAB R120G Tg mice showed increased expression and localization of Drp1 on the mitochondrial membrane. Alteration in the expression of mitochondrial dynamics regulatory proteins was observed at two months age when these mice exhibited normal cardiac morphometry and systolic function. Adenoviral-mediated expression of CryAB R120G in cardiomyocytes also showed increased mitochondrial fission, expression dependent inhibition of mitochondrial respiration and activation of cellular toxicity. Inhibition mitochondrial fission by Drp1 partial knockdown in CryABR120G expressing cardiomyocytes significantly decreased protein aggregation and improved mitochondrial respiration. **Conclusion:** Drp1-dependent excessive mitochondrial fission results in defects in mitochondrial respiration and accumulation of protein aggregates in CryABR120G expressing cardiomyocytes. Therefore, our study shows that maladaptive aberrant mitochondrial fission causes CryABR120G -induced mitochondrial dysfunction and cardiac proteotoxicity-associated cellular dysfunction.

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Metabolic Landscape of Human Heart Failure

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Heart failure is characterized by insufficient pumping to match the needs of the body. Heart failure is thought to be associated with dysfunctional mitochondria and metabolic alterations, but there is little to no direct evidence in humans. The heart metabolizes a variety of substrates for energy production, including fatty acids, glucose, ketones, and branch chain amino acids (BCAAs). In the healthy heart, fatty acids are the main fuel source, and oxidation is balanced with energy need. In heart failure, it is asserted that fatty acid oxidation is suppressed, and glucose utilization is increased, but this has not been directly measured in humans. If true, this metabolic switch toward glucose may contribute to mechanical insufficiency by disrupting the balance between metabolism and energetic need, thus representing a potential area for therapeutic intervention in heart failure. Prior studies were largely performed in animal models, and results are often inconsistent across models and species. Challenges have constrained the use of human myocardium, including costly and cumbersome techniques for in vivo assessment; limited availability of high-quality human myocardial tissue; and low yields and short durability of isolated human cardiomyocytes. We addressed these challenges by taking advantage of human explanted hearts available via the Penn Heart Transplant program to investigate the metabolic differences between failing and healthy human hearts.

We performed metabolomics analysis using mass spectrometry on myocardium from 18 failing and 20 nonfailing human hearts. Of the 334 metabolites measured, 99 were significantly different between groups by Wilcoxon test and FDR correction. Metabolite set enrichment analysis revealed that the most enriched pathways included the glycerol phosphate shuttle, ammonia recycling, methylhistidine metabolism, and glutathione metabolism. The mitochondrial electron transport chain, BCAA catabolism, purine metabolism, the urea cycle, gluconeogenesis, glycolysis, ketone body metabolism, and the pentose phosphate pathway were also included in the top 50 most enriched pathways. These data will guide future targeted metabolic studies in human heart failure.

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NCLX Expression Attenuates Pathological Remodeling in Experimental Cardiac Hypertrophy and Non-ischemic Heart Failure

Joanne F. Garbincius, Timothy S. Luongo, Devin W. Kolmetzky, Alycia N. Hildebrand, John W. Elrod, Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA

Mitochondrial calcium (mCa^{2+}) uptake couples acute changes in cardiomyocyte bioenergetic demand to ATP production, but in excess triggers mitochondrial permeability transition and cardiomyocyte necrosis, as occurs during cardiac injury. Despite established roles for mCa^{2+} flux in response to acute stress, the role of mCa^{2+} signaling in chronic stress is poorly defined. As mCa^{2+} regulates the TCA cycle with the potential to affect mitochondrial signaling and/or metabolite pools required for biosynthesis, we reasoned that altered mCa^{2+} homeostasis may be an essential mechanism underlying hypertrophic growth and remodeling of the myocardium. Here, we used mice with cardiac-specific overexpression (OE) of the mitochondrial Na^+/Ca^{2+} exchanger (NCLX), the primary mediator of mCa^{2+} efflux in the heart, to test the hypothesis that mCa^{2+} signaling contributes to cardiac remodeling during sustained hemodynamic stress. We subjected NCLX OE (TRE-NCLX x α -MHC-tTA) and control (α MHC-tTA) mice to 12-wk transverse aortic constriction (TAC) or 4-wk infusion with angiotensin II + phenylephrine (AngII/PE) as experimental models of cardiac hypertrophy and non-ischemic heart failure. Cardiac function of NCLX OE and control mice was monitored throughout these studies via echocardiography and remodeling was assessed via tissue gravimetrics, histology, and qPCR gene expression analysis. Cardiac NCLX OE preserved ejection fraction, prevented afterload-induced hypertrophy and fibrosis, and attenuated the induction of both hypertrophic (*Nppa*, *Nppb*, *Acta1*) and fibrotic (*Postn1*, *Spp1*) gene programs in mice subjected to 12-wk TAC. Examination at 2-wk post-TAC revealed attenuated hypertrophy and blunted hypertrophic and fibrotic gene expression in NCLX OE mice. These data indicate that increased capacity for mCa^{2+} efflux mitigates TAC-induced remodeling, prior to the development of contractile dysfunction. NCLX OE similarly attenuated atrial hypertrophy and the induction of hypertrophic and fibrotic gene programs in mice infused with AngII/PE. Together, these findings support a critical role for mCa^{2+} in driving pathological remodeling in non-ischemic heart disease and point to NCLX as a potent therapeutic target for cardiovascular disease.

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The Pathological Role of Coagulation Factors in Promoting Brown Adipose Tissue Dysfunction and Systemic Metabolic Disorder in Obesity

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Obese individuals are predisposed to cardio-metabolic disorders. Brown adipose tissue (BAT) is an active metabolic organ abundant with mitochondria, and studies suggest a potential role of BAT in the maintenance of metabolic health in rodents and humans. Metabolic stress causes BAT dysfunction, but the underlying mechanisms are largely unknown. Coagulation factor Xa (FXa) is critically involved in a coagulation cascade, and it is also known to mediate biological effects by the activation of protease-activated receptor (PAR)-signaling. Accumulating evidence shows that PAR1 contributes to tissue remodeling in cardiovascular system. Here we show a previously unknown role of FXa-PAR signaling in promoting BAT dysfunction and systemic metabolic disorder in a murine dietary obese model. Imposing a high fat diet (HFD) on C57BL/6Ncr mice led to a marked increase in tissue factor (TF), coagulation factor VII and FXa in BAT. TF-FVIIa (activated form of FVII)-FXa complex is known to activate PAR1, and we found a significant increase in PAR1 expression in BAT upon metabolic stress. Administration of a FXa inhibitor ameliorated BAT whitening, improved thermogenic response and systemic glucose intolerance upon dietary obesity. In contrast, administration of warfarin did not show any phenotype in BAT. BAT specific TF and PAR1 over-expression model showed significant whitening of this tissue, which was associated with systemic glucose intolerance. BAT specific PAR1 KO mice improved glucose intolerance and thermogenic response under a metabolically stressed condition. In differentiated brown adipocytes, FXa markedly increased mitochondrial reactive oxygen species (ROS) and reduced mitochondrial membrane potential. Inhibition of PAR1 ameliorated FXa-induced mitochondrial ROS production and reduction in membrane potential. We also found that plasma FXa level did not increase in obese mice as well as in obese individuals. These results suggest the previously unknown role of coagulation systems in promoting BAT dysfunction, leading to systemic metabolic disorders. Maintenance of BAT homeostasis through the suppression of FXa-PAR1 signaling would become a new therapeutic target for obesity and diabetes

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GPX4 Gene Expression is Dose-responsive to Doxorubicin Exposure in iPSC-Cardiomyocytes and Correlated With Mitochondrial Function

Monica E. Reyes, Rashida A. Callender, UT MD Anderson Cancer Ctr, Houston, TX; Jianzhong Ma, Megan L. Grove, Alanna C. Morrison, UT Health Science Ctr at Houston, Houston, TX; **Michelle A.t. Hildebrandt**, UT MD Anderson Cancer Ctr, Houston, TX

Impaired mitochondrial function has been implicated as a mechanism of doxorubicin-induced cardiotoxicity, however the precise genes that regulate this process in human cardiomyocytes remain to be elucidated. We hypothesized that doxorubicin significantly alters expression of genes involved in mitochondrial function in human cardiomyocytes, which in turn impairs mitochondrial respiration. Towards this, we treated human inducible pluripotent stem cell (iPSC)-cardiomyocytes with doxorubicin at 15 different time and dose conditions. Gene expression was assessed by RNAseq for each condition and 169 genes involved in mitochondrial function were analyzed for differential expression between control and treated conditions. Mitochondrial respiration (basal respiration, ATP production, maximal respiration, and spare respiratory capacity) was measured using the Seahorse Bioscience XFe96 Cell Mito Stress Test kit and correlated to gene expression levels. Of the 169 genes analyzed, 25 were significantly differentially expressed ($P < 0.05$) including *GPX4* ($P = 5.70 \times 10^{-3}$). Expression of *GPX4* remained significant in pairwise comparisons by dose for day 2 and qRT-PCR validation confirmed a dose-dependent decrease in *GPX4* expression. Maximal respiration ($r = -0.62$; $P = 0.031$) and spare respiratory capacity ($r = -0.67$; $P = 0.017$) correlated with *GPX4* expression in doxorubicin-treated iPSC-cardiomyocytes. *GPX4* encodes a glutathione peroxidase that is responsible for protecting the cell against oxidative damage. Damage due to reactive oxygen species (ROS) is one of the established mechanisms of anthracycline-induced cardiac damage. Our findings underscore a role for mitochondrial function and ROS in the development of doxorubicin-induced cardiotoxicity and implicates *GPX4* in this process. The assessment of doxorubicin-altered gene expression in iPSC-cardiomyocytes may provide insight into how impaired mitochondrial respiration leads to cardiotoxicity and heart failure in cancer survivors treated with doxorubicin and other anthracyclines.

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The Role of Mitochondrial Permeability Transition in the Regulation of ETC Supercomplexes Assembly by OPA1

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Optic atrophy type 1 (OPA1), a dynamin-related GTPase is the inner mitochondrial membrane (IMM) protein, which, in addition to mitochondrial fusion, participates in maintaining the structural integrity of the IMM. The function and topography of the IMM is also regulated by changes in the matrix volume. Opening of the mitochondrial permeability transition pores (PTP) is the leading cause of mitochondrial matrix swelling. However, the cause-and-effect relationship between OPA1 proteolytic cleavage and PTP induction remains unknown. Here, we elucidated the role of PTP in OPA1-mediated regulation of electron transport chain (ETC) supercomplexes (SCs) assembly. First, intact mitochondria isolated from the rat heart were used to analyze the effect of PTP induction on the cleavage of long (L)-OPA1. Calcium-induced mitochondrial swelling increased L-OPA1 cleavage (66%, $P < 0.001$) associated with accumulation of short (S)-OPA1. Inhibition of swelling by sanglifehrin A (a PTP inhibitor) prevented L-OPA cleavage whereas tert-butyl hydroperoxide (TBH) had no effect on L-OPA levels. Second, OPA1 knockdown (KD) by siRNA in HEK cells resulted in a 74% decrease ($P < 0.001$) in OPA1 protein expression but did not change the proportion of L-OPA1 to total OPA. Mitochondria isolated from OPA1 KD cells showed reduced calcium-stimulated swelling with no changes in protein levels of cyclophilin D, a major PTP regulator. OPA1 KD cells demonstrated a 20% increase ($P < 0.05$) in calcium-induced ROS generation. Analysis of individual ETC complexes revealed significant reduction of protein levels of the complexes I, III, and IV in OPA1 KD cells. Likewise, activities of complexes I and IV were reduced (48% and 37%, respectively, $P < 0.01$ for both). OPA1 KD cells contained 38% ($P < 0.05$) less ATP whereas the citrate synthase activity reflecting mitochondria mass was not affected. Blue native PAGE analysis demonstrated a 35% decrease ($P < 0.01$) of high molecular weight proteins (>100 kDa) and a 24% decrease ($P < 0.05$) of the respirasome, the main SC, levels in OPA1 KD mitochondria. We conclude that mitochondrial swelling by PTP opening stimulates OPA1 cleavage,

and reduced OPA1 levels result in cristae malformation including reduced respirasome levels and the ETC complexes activity.

S. Jang: None.

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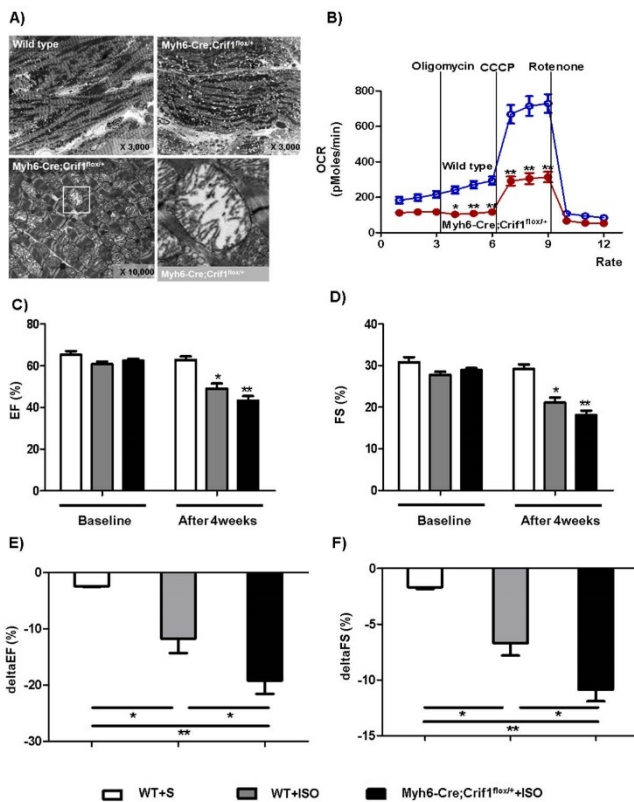
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Crif1 Haploinsufficiency in Heart Impairs Mitochondrial Oxidative Capacity and Leads to Aggravated Cardiac Dysfunction in Isoproterenol Infusion Model

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Heart requires high energy metabolism to maintain contractile activity and pump sufficient blood flow to body. The optimal supply of energy from the mitochondria is very important for maintaining normal cardiac function. Crif1 is a critical protein for the synthesis and insertion of the OxPhos complex in mitochondria. Recent studies reported that conditional knockout of Crif1 in specific tissues is associated with mitochondrial dysfunction. To investigate the role of Crif1 and mitochondrial dysfunction in heart, we generated cardiac-specific, Crif1 haploinsufficient mice. Haploinsufficient Crif1 in heart resulted in abnormal structure of mitochondria and decreased maximal values of oxygen consumption rates in cardiomyocytes. Although cardiac specific, haploinsufficient Crif1 results in mitochondrial dysfunction, cardiac phenotype was still normal. However, their cardiac function was aggravated by stress condition, showing more decreased EF and FS after 4 weeks isoproterenol infusion. Cardiac hypertrophy, which is considered as normal compensatory mechanism, was not occurred during isoproterenol infusion. These indicate that Crif1 plays a critical role in the maintenance of mitochondrial structure and function of cardiomyocyte. Also, mitochondrial abnormalities in heart inhibit the compensatory mechanisms for stress and aggravate cardiac function.



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Mitochondrial Protein Kinase B (akt) Translocation Mediates Insulin-stimulated Cardiac Glucose Oxidation

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Introduction Insulin stimulates glucose oxidation, an effect which is associated with stimulating pyruvate dehydrogenase (PDH). However, how the insulin signal is transduced from the cell membrane to the mitochondria to stimulate PDH is not known. Protein kinase B (Akt), protein kinase C-delta (PKC δ) and glycogen synthase kinase-3beta (GSK-3 β) are main components of the cytosolic insulin signalling pathway and it has been suggested that they can be translocated to the mitochondria following insulin receptor activation in noncardiac tissue. Therefore, we investigated whether any of these kinases has a role mediated cardiac insulin-stimulated glucose oxidation in the heart. **Methods and Results** Male and female C57BL/6 mice were anesthetized and hearts were collected and perfused in the isolated working heart mode. Hearts were perfused with [5-³H] glucose and [U-¹⁴C] glucose to simultaneously measure glycolysis and glucose oxidation rates, respectively, in the presence and absence of insulin. Insulin enhanced the phosphorylation and translocation of Akt^{Ser473}, PKC δ ^{Tyr311} and GSK-3 β ^{Ser9} to the cardiac mitochondria along with enhancing PDH activity by decreasing its phosphorylation. Pharmacological inhibition of Akt using AktiVIII completely abolish the stimulatory effect of insulin on cardiac glucose oxidation rates (354 \pm 145 vs 2307 \pm 185 nmol. g dry wt⁻¹. min⁻¹ in vehicle-treated hearts, p<0.05). However, pharmacological inhibition of either PKC δ or GSK-3 β using Bisindolylmaleimide I or 3F8, respectively, did not have a significant effect on insulin-stimulated glucose oxidation rates. None of the pharmacological inhibitors had any significant effect on cardiac glycolysis. **Conclusion** Insulin mediates its stimulatory effect on cardiac glucose oxidation via a mechanism which involve the activation and translocation of Akt to the mitochondria.

Q.G. Karwi: None. **C.S. Wagg:** None. **T.R. Altamimi:** None. **G.D. Lopaschuk:** None.

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MCUB Regulates the Molecular Composition of the Mitochondrial Calcium Uniporter Channel During Cardiac Stress to Limit Mitochondrial Calcium Overload

Jonathan P Lambert, Timothy S Luongo, Dhanendra Tomar, Pooja Jadiya, Erhe Gao, Xueqian Zhang, Anna M Lucchese, Devin W Kolmetzky, Neil Shah, John W Elrod, TEMPLE UNIVERSITY, Philadelphia, PA

The mitochondrial calcium uniporter (mtCU) is a ~700 kD multi-subunit channel residing in the inner mitochondrial membrane required for mitochondrial Ca²⁺ (mCa²⁺) uptake. Mitochondrial Calcium Uniporter B (*MCUB*) is reported to negatively regulate mCa²⁺ uptake, but its precise functional role and contribution to cardiac physiology remain unresolved. Size exclusion chromatography of ventricular mitochondria revealed MCUB was absent from high-molecular weight (MW) mtCU complexes in sham animals, but present 24 hours following myocardial ischemia-reperfusion injury (IR). To investigate *MCUB*'s contribution to mtCU regulation we created a *MCUB*^{-/-} cell line by CRISPR-Cas9n. *MCUB* deletion increased histamine-mediated [mCa²⁺] transient amplitude by ~50% vs. WT controls (mito-R-GECO1). *MCUB* deletion increased mtCU capacitance (mitoplast patch-clamp) and rate of [mCa²⁺] uptake. Size-exclusion chromatography revealed loss of MCUB increased MCU incorporation into high-MW mtCU, suggesting stoichiometric replacement and overall more functional mtCU's. To examine MCUB's role in cardiac physiology we generated a cardiac-specific, tamoxifen-inducible MCUB mouse model (CAG-CAT-MCUB x MCM; MCUB-Tg). FPLC revealed MCUB was undetected in high-MW mtCU complexes of Cre controls, but enriched in MCUB-Tg hearts. MCUB incorporation decreased the presence of channel gatekeepers, MICU1/2, and decreased the MW of the mtCU complex. Immunoprecipitations suggest MCUB interacts with MCU but not MICU1/2. MCUB-Tg adult cardiomyocytes (ACMs) expressing AAV9-mitycam (mCa²⁺ reporter) were paced and displayed a ~30% decrease in mCa²⁺ transient peak amplitude with significantly reduced mCa²⁺ uptake rates vs controls. A reduction in OxPhos reserve capacity correlated with a severe impairment in cardiac contractile reserve (LV invasive hemodynamics during isoproterenol infusion). MCUB-Tg cardiac mitochondria were resistant to Ca²⁺-induced permeability transition and MCUB-Tg mice displayed ~50% decrease in infarct size per area-at-risk after in vivo IR-injury. These data suggest MCUB regulation of the mtCU is an endogenous compensatory mechanism to decrease mCa²⁺ overload during ischemic injury, but maladaptive to cardiac energetic responsiveness.

J.P. Lambert: 2. Research Grant; Significant; Held an AHA Predoctoral fellowship. **T.S. Luongo:** None. **D. Tomar:** None. **P. Jadiya:** None. **E. Gao:** None. **X. Zhang:** None. **A.M. Lucchese:** None. **D.W. Kolmetzky:** None. **N. Shah:** None. **J.W. Elrod:** None.

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NAD(H) Redox Imbalance in the Heart Accelerates Diabetic Cardiomyopathy

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Diabetes is linked to NAD(H) redox imbalance and lowered NAD⁺ levels, but the roles of NAD⁺ metabolism in diabetic cardiomyopathy are not established. We induced diabetes in C57BL6 wild type mice (WT) by streptozotocin (STZ) injections to cause depletion in serum insulin levels and elevation in fasting glucose levels. Hyperglycemic, male WT mice showed gradual declines in systolic and diastolic functions (FS, 50% vs 30%; E'/A' ratio, 1.5 vs 1.0; 16-week after STZ), without causing cardiac hypertrophy. Dysfunctions in diabetic hearts were associated with lowered NAD⁺/NADH ratio and protein hyperacetylation, suggesting the roles of NAD(H) redox imbalance in diabetic cardiomyopathy. To test if NAD(H) redox imbalance promotes dysfunctions of diabetic hearts, we employed cardiac-specific Ndufs4-KO mice (cKO), which exhibit lowered NAD⁺/NADH ratio without overt cardiac dysfunction at baseline. Changes in cardiac functions at 2-, 4-, and 8-week after STZ injections, insulin and fasting glucose levels of control and cKO male mice were measured. Similar insulin depletion and hyperglycemia (~450 mg/dL) were observed in diabetic control and cKO mice. Systolic and diastolic dysfunctions were worsened in diabetic cKO mice (control vs cKO: FS 37% vs 22%; E'/A' 1.25 vs 0.95, 8-week after STZ). Cardiac fibrosis levels were slightly elevated in diabetic control and cKO hearts, but were not different between the two groups (~4% in both group of mice). The results suggested that the accelerated declines in cardiac functions contributed by NAD(H) redox imbalance are not due to the altered extracellular matrix environment. To restore the NAD(H) redox balance in diabetic hearts, we elevated NAD⁺ levels in male control and cKO mice with cardiac-specific NAMPT overexpression. Elevations in cardiac NAD⁺ levels alleviated systolic and diastolic dysfunctions in diabetic control and cKO mice, despite similar levels of hyperglycemia among groups. Similar exacerbation of cardiac dysfunction was observed in female diabetic cKO mice, while NAMPT overexpression similarly improved cardiac function in female diabetic control and cKO mice. Our findings showed that NAD(H) redox imbalance promotes the progression of diabetic cardiomyopathy, via a fibrosis-independent mechanism.

Y. Chiao: None. **C. Light:** None. **R. Tian:** None. **J. Sadoshima:** None. **C. Lee:** None.

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Sepsis-Induced Cardiomyopathy is Caused by Mitochondrial Dysfunction Due to Protein Kinase C (PKC) Delta Activation

John P Morrow, Leroy C Joseph, Michael V Reyes, Konstaninos Drosatos, Columbia Univ, New York, NY

Objective: Sepsis-induced cardiomyopathy (SIC) is a frequent complication of sepsis that is associated with increased mortality. There are no effective therapies. Oxidative stress and mitochondrial dysfunction are part of the pathophysiology of SIC but the upstream molecular pathways involved are unclear. Methods: We used PKCdelta knock-out (KO) mice and WT littermates for surgically-induced polymicrobial sepsis from cecal ligation and puncture (CLP). Isolated cardiomyocytes were used to evaluate oxidative stress, mitochondrial function, contractility, and calcium transients. Results: PKCdelta is activated in cardiomyocytes in sepsis models. In vivo, PKCdelta KO mice do not have decreased systolic function after sepsis, unlike WT littermates. WT cardiomyocytes show increased mitochondrial oxidative stress, partial depolarization of the mitochondrial inner membrane, decreased contractility, and decreased calcium transient amplitude. PKCdelta KO cardiomyocytes are protected from all of these abnormalities. Further, mitotempo improves contractility and calcium transients in WT cardiomyocytes, supported the role of mitochondrial dysfunction as a mechanism of SIC. Conclusion: PKCdelta KO mice are protected from SIC. Cardiomyocyte experiments indicate that mitochondrial dysfunction promotes oxidative stress and a

decrease in contractility during sepsis. Translational impact: PKCdelta inhibition could be effective treatment for SIC.

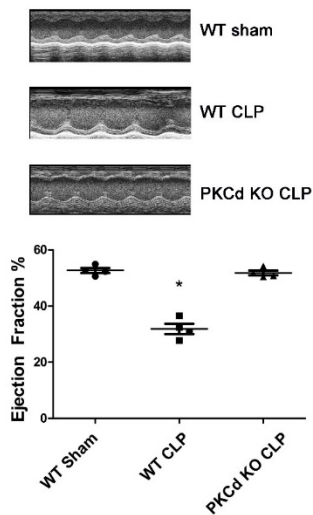


Figure: PKCdelta KO mice do not develop sepsis-induced cardiomyopathy. Representative echocardiography images from WT littermates and PKCd KO mice and graph of ejection fraction (EF). * indicates sig different from control. CLP = cecal ligation and puncture

J.P. Morrow: None. **L.C. Joseph:** None. **M.V. Reyes:** None. **K. Drosatos:** None.

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Functional Characterization of a Novel Human Heart-specific Microprotein With a Potential Mitochondrial Localization and Role in Sarcomere Dynamics

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Cardiac microproteins are an emerging class of important regulators of cellular homeostasis, often encoded by presumed long noncoding RNAs. With only few described so far, their prevalence and biological significance largely remains unclear. To create an atlas of cardiac-expressed microproteins, we performed ribosome profiling on 80 human heart samples. This revealed the translation of hundreds of previously undetected microproteins, which we validated *in vivo* by targeted mass spectrometry. A single heart-specific microprotein caught our attention as it showed strong expression coregulation with components of mitochondrial OXPHOS pathway. We next deleted this microprotein's lncRNA host gene in hiPSCs using CRISPR/Cas9, and, upon differentiation into 30-d old cardiomyocytes, find a strong expression upregulation of genes involved in oxidative phosphorylation and calcium handling. To investigate the impact of the microprotein KO on cardiomyocyte mechanical function, we assessed sarcomere dynamics using an endogenous TTN-GFP tag, enabling live cell imaging analysis with the SarcTrack algorithm. Preliminary analysis shows a putative increase in sarcomere contraction and relaxation times, suggesting a repressive regulatory function of the eliminated microprotein encoding lncRNA.

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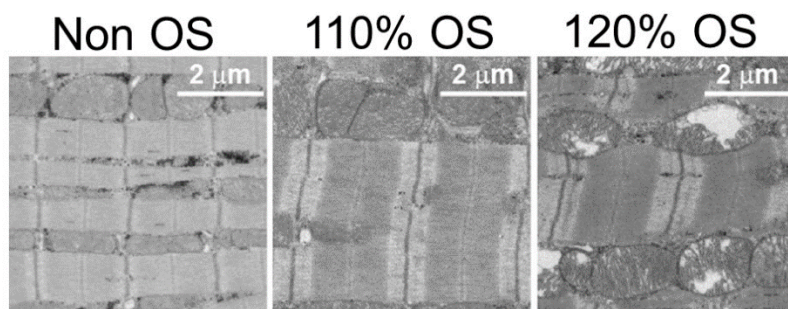
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Inner Mitochondrial Collapsing in Response to Acute Overstretch of Rat Ventricular Papillary Muscles

Naritomo Nishioka, Yoichiro Kusakari, Jun Tanihata, Susumu Minamisawa, The Jikei Univ Sch of Med, Tokyo, Japan

Introduction: The effect of acute ventricular volume overload on organelles such as myofibrils and mitochondria are incompletely understood. **Purpose:** We performed acute overstretch on cardiac papillary muscle to investigate the functional and morphological changes. **Methods:** We used male SD-rats (10-12 weeks old) and dissected papillary muscles from the right ventricle. A papillary muscle preparation was stretched to L_{max}. We overstretched it within 2 seconds up to 110% of L_{max} (110%Overstretch; 110%OS) or 120% of L_{max} (120%OS), and stimulated it (1Hz, 36°C) with tension measurement, compared with L_{max} group. After the measurement of tension, we analyzed the morphological changes in muscle fibers and organelles using a Transmission electron microscopy (TEM). **Results:** The active tension after overstretch was immediately decreased to 67.8±3.8% in 110%OS, and to 17.3±4.0% in 120%OS. In TEM study, muscle fibers kept their well-organized structures and the sarcomere length was stretched to 2.4 μm in 110%OS, and to 2.6 μm in 120%OS. In contrast, in 110%OS, inner mitochondrial density was lower than that in L_{max}, indicating swelled mitochondria. In 120%OS, inner mitochondrial empty space with vacuolation was found in the large area of myocardium, indicating that inner mitochondrial cristae were susceptible to mechanical stress-induced deterioration. **Conclusions:** Acute overstretch of rat papillary muscles causes inner mitochondrial collapsing with preserved the sarcomere structure. It could account for the mechanisms of acute volume-overloaded heart failure.



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SGK-1 Metabolically Reprograms VSMC Energy Metabolism by Modulation of Substrate Utilization and Mitochondrial Structure

Krystal Roggerson, Wei Zhong, Oguljahan Babayewa, Sharon Francis, Morehouse Sch of Med, Atlanta, GA

Obesity is associated with vascular remodeling which increases the risk of developing heart disease; the leading cause of death world-wide. The mechanisms underlying vascular remodeling during obesity are not completely understood, but impaired cellular energy metabolism is thought to play an important role. Oxidative phosphorylation (OXPHOS) and glycolysis are the cell's two main energy generating pathways that rely on energy substrate availability. Glucose is the primary substrate for glycolysis, whereas, pyruvate which is metabolized from glucose, long chain fatty acids (LCFAs) and glutamine are the substrates metabolized for OXPHOS. Previously, we reported that serum and glucocorticoid-inducible kinase 1 (SGK-1) is up-regulated in the vasculature of diet-induced obese mice, implicating a role for this serine/threonine kinase in obesity-related vascular remodeling. Indeed, our current data demonstrate that deletion of serum and glucocorticoid-inducible kinase 1 (SGK-1) specifically in vascular smooth muscle cells (VSMC) protects against obesity-induced vascular remodeling. Further, protection from remodeling was concomitant with metabolic reprogramming of VSMCs due to a metabolic shift from glycolysis towards OXPHOS in SGK-1 knockout (KO) VSMCs compared to wildtype (WT) VSMCs. Interestingly, metabolic reprogramming was also evident in SGK-1 KO VSMC under basal conditions, suggesting a novel role for SGK-1 in cellular energy metabolism. Inhibition of glycolysis was due to an overall reduction in glucose uptake,

whereas, an increase in OXPPOS was associated with redistribution of mitochondria and altered levels of the mitochondrial fusion and fission regulatory proteins, suggesting that SGK-1 reprograms energy metabolism through modulation of energy substrate utilization and mitochondrial structure. Altogether, our data point to SGK-1 inhibition as a novel therapy for obesity-related vascular pathology.

K. Roggeron: None. **W. Zhong:** None. **O. Babayewa:** None. **S. Francis:** None.

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Pathological Roles of Senometabolites in Cardiovascular Disorders

Ipppei Shimizu, Yohko Yoshida, Ryutaro Ikegami, Tohru Minamino, Niigata Univ, Niigata, Japan

Mechanisms contributing for the synchronization of aging are yet to be defined. Here, we define “senometabolite” as circulating metabolites having causal roles for the synchronization and progression of aging. Analyzing metabolomic studies in coronary plaque corrected with directional coronary atherectomy (DCA), we found that hydroxyl-metabolite significantly increased in patients with unstable angina compared with stable angina. We generated murine diet induced obese model and found this metabolite increased in aorta and plasma. Administration of this metabolite into human umbilical vein endothelial cells (HUVECs) induced cellular senescence, and immunofluorescence study showed that putative transhydrogenase increased in patients with atherosclerotic disorders. We did metabolomics studies in aged individuals or patients with heart failure and identified another metabolite, oxidized choline, increased under these conditions compared to respective controls. We generated murine left ventricular (LV) pressure overload model and found that oxidized choline increased both in plasma and failing heart. Administration of oxidized choline deteriorated cardiac function, in contrast, genetic model showed suppression of this metabolite ameliorated systolic dysfunction in LV pressure overload model. Proteomic study showed that oxidized choline reduced the expression of cytochrome c oxidase subunit1, and metabolomics study showed that both ATP and phosphocreatine level significantly reduced in cardiac tissues of wild type mice administrated with this metabolite. Administration of oxidized choline also reduced muscle strength, induced fibrosis in skeletal muscle, and electron microscopy showed an increase in dysfunctional mitochondria both in the heart and skeletal muscle. In aged wild type mice, metabolomic study showed oxidized choline increased in plasma. Aging process is still mysterious and continues to be an interesting topic to be explored. Our findings indicate that circulating senometabolites contribute for the progression of pathologies in age related disorders.

I. Shimizu: 2. Research Grant; Significant; AMED, The Naito Foundation, Astellas Foundation for Research on Metabolic Disorders, Takeda Science Foundation, JSPS KAKENHI, Uehara Memorial Foundation, Daiichi Sankyo Foundation of Life Science, TERUMO FOUNDATION for LIFE SCIENCES and ARTS, Japan Diabetes Foundation, Grant From Japan Cardiovascular Research Foundation, MSD Life Science Foundation, Public Interest Incorporated Foundation, Tsukada grant for Niigata University Medical Research, Japan Heart Foundation Research Grant, ONO Medical Research Foundation, The Senri Life Science Foundation, The Nakajima Foundation, Suzuken Memorial Foundation, Mochida Memorial Foundation for Medical & Pharmaceutical Research, Manpei Suzuki Diabetes Foundation. **Y. Yoshida:** None. **R. Ikegami:** None. **T. Minamino:**None.

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Mitochondrial Morphology During Stress is Regulated by GJA1-20k Interaction With Actin

Daisuke Shimura, Rachel Baum, Shaohua Xiao, TingTing Hong, Robin Mark Shaw, Cedars-Sinai Medical Ctr, Los Angeles, CA

Connexin (Cx) 43 is encoded by the GJA1 gene and GJA1-20k has been identified as an N-terminal truncation of Cx43 generated endogenously by internal translation. Previously we have found GJA1-20k increases with ischemic stress and induces actin organization and, in mice, mitochondrial biogenesis. In this study we explored the acute effect of GJA1-20k on mitochondria. We transfected GFP-tagged GJA1-20k plasmid into HEK293T cells and analyzed mitochondrial morphology by fluorescent microscopy. We found GJA1-20k results in mitochondria that are associated with actin and also are smaller and more circular than in GST-transfected control cells. In addition, quantitative analysis indicated preservation of mitochondrial area and thus enhanced mitochondrial fission in the presence of GJA1-20k. Disruption of actin polymerization

inhibited the effect of GJA1-20k on mitochondrial fission as did use of the actin stabilizer phalloidin, indicating the need for robust actin dynamics for GJA1-20k to be effective. These data suggest that the interaction between GJA1-20k and actin cytoskeleton is necessary for stress related mitochondrial fission. The results indicate that, during stress, upregulated GJA1-20k recruits the actin cytoskeleton to directly affect mitochondrial dynamics and cellular metabolic function.

D. Shimura: None. **R. Baum:** None. **S. Xiao:** None. **T. Hong:** None. **R.M. Shaw:** None.

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The E3 Ubiquitin Ligase Parkin Regulates Metabolism From the Nucleus

Sarah E Shires, Rita H Najor, Leonardo J Leon, Melissa Q Cortez, Åsa B Gustafsson, Univ of California San Diego, San Diego, CA

Parkin facilitates mitophagy by ubiquitinating depolarized mitochondria to label them for degradation. We have previously shown that Parkin is important for adaptation to myocardial infarction (MI) and that loss of Parkin leads to accumulation of dysfunctional mitochondria. Although Parkin is mainly studied for its role at the mitochondria, it is unclear whether Parkin also functions in other subcellular compartments. Here, we used immunofluorescence and subcellular fractionation to find that a portion of Parkin localizes to the nucleus under basal conditions *in vitro* and *in vivo*. Pathogenic Parkin mutants, however, are selectively excluded from the nucleus. To further examine conditions that induce Parkin nuclear enrichment, we subjected HeLa cells stably expressing YFP-Parkin to starvation or hypoxia. During nutrient-deprivation, Parkin rapidly exits the nucleus and accumulates in the cytosol. Conversely, exposure to hypoxia causes Parkin enrichment in the nucleus and depletion from the cytosol. To separate the roles of nuclear versus mitochondrial Parkin, we generated nuclear- and mitochondrial- targeted constructs: NLS-Parkin and Mito-Parkin. RNA-sequencing analysis revealed widespread transcriptional changes in response to targeting Parkin to the nucleus, particularly in metabolic processes. Through a non-biased proteomics screen, we identified the transcription factor estrogen related receptor α (ERR α) as a potential Parkin target. ERR α is highly expressed in the heart and promotes transcription of genes involved in mitochondrial biogenesis and metabolism. We therefore used co-immunoprecipitation and qPCR to determine whether Parkin regulates cellular energetics through ERR α . Indeed, we found that NLS-Parkin interacts with and ubiquitinates ERR α . Additionally, expressing NLS-Parkin increases protein levels of both endogenous and overexpressed ERR α and promotes transcription of ERR α -targets. These data indicate that Parkin-mediated ubiquitination of ERR α leads to its increased stability and activity resulting in increased transcription of genes involved in mitochondrial biogenesis and metabolism. This study provides insight into new ways Parkin may be targeted to enhance energetic function in the heart.

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Cardiac-specific Deletion of General Control of Amino-Acid Synthesis 5-like 1 Regulates Fatty Acid Oxidation in Diet Induced Obesity

Dharendra Thapa, Janet R Manning, Michael W Stoner, Manling Zhang, Iain Scott, Univ of Pittsburgh, Pittsburgh, PA

Background: Healthy hearts use several fuel substrates for energy metabolism, with fatty acid oxidation (FAO) accounting for ~70% of total ATP production. Lack of flexibility to utilize different substrates, and a shift towards increased fatty acid utilization, is a key feature of obesity and diabetes. We previously reported that increased acetylation of several mitochondrial FAO enzymes, regulated in part via increased abundance of the mitochondrial acetyltransferase GCN5L1, correlated with increased enzymatic activity and FAO in the heart. The focus of the current study was to investigate whether decreased acetylation, via cardiac depletion of GCN5L1, regulates cardiac energy metabolism following exposure to a high fat diet (HFD). **Hypothesis:** We hypothesize that decreased acetylation of metabolic enzymes in GCN5L1 cardiac KO (cKO) mice may limit excessive cardiac FAO activity and improve glucose utilization under HFD conditions. Furthermore, we postulate that this metabolic phenotype would result in improved mitochondrial bioenergetic output and cardiac function. **Methods:** To examine the role of GCN5L1 in regulating mitochondrial protein acetylation, immunoprecipitation with acetylated lysine antibodies was performed to assess the acetylation status of metabolic proteins. Western blots and qPCR were performed to quantitate protein and mRNA expression levels. Further, metabolic enzyme activities were analyzed *in*

vitro. Finally, cardiac workload was measured *ex vivo* to assess changes in cardiac function. **Results:** We found that increased mitochondrial protein acetylation under HFD conditions was significantly decreased in GCN5L1 cKO mice relative to WT. This decrease correlated with reduced acetylation of the FAO enzymes SCAD and HADHA in GCN5L1 cKO mice. HFD exposure led to increased cardiac FAO enzyme activity in WT animals, which was reduced in GCN5L1 cKO animals. Finally, a significant decrease in cardiac workload observed in WT animals was not present in GCN5L1 cKO animals under HFD conditions. **Conclusions:** These findings indicate that decreased acetylation of FAO proteins via cardiac depletion of GCN5L1 results in decreased cardiac FAO utilization and enzymatic activity under HFD conditions.

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NAD⁺ Repletion Reverses HFpEF by Attenuating Myocardial Metabolic Dysfunction

Dan Tong, Gabriele G Schiattarella, Nan Jiang, Francisco Altamirano, Pamela A Szweda, Abdallah Elnwasany, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Dong Ik Lee, Johns Hopkins Sch of Med, Baltimore, MD; Luke I Szweda, Univ of Texas Southwestern Medical Ctr, Dallas, TX; David A Kass, Johns Hopkins Sch of Med, Baltimore, MD; Thomas G Gillette, Joseph A Hill, Univ of Texas Southwestern Medical Ctr, Dallas, TX

Background: Heart failure with preserved ejection fraction (HFpEF) is a highly prevalent clinical condition associated with significant morbidity, mortality and health care expenses. Yet, no effective treatment has been identified. We recently demonstrated that concomitant metabolic and hypertensive stress in mice elicited by a combination of high fat diet (HFD) and constitutive nitric oxide synthase inhibition by N^ω-nitro-L-arginine methyl ester (L-NAME) faithfully recapitulates the numerous and myriad features of human HFpEF (paper in press at *Nature*).

Methods: Wild type C57Bl6 mice were fed with HFD and L-NAME via drinking water for 5-8 weeks. Myocardial mitochondrial morphology was assessed by electron microscopy. Mitochondrial function was assessed by measuring oxygen consumption rates using an oxygen electrode. Protein expression and modification were assessed by Western blotting and immunoprecipitation.

Results: Using this novel mouse model, we discovered significant impairment of mitochondrial fatty acid oxidation (FAO) associated with hyperacetylation of key FAO enzymes in HFpEF myocardium. Mechanistically, downregulation of Sirtuin3, the major mitochondrial deacetylase, and deficiency of its co-substrate nicotinamide adenine dinucleotide (NAD⁺), culminated in mitochondrial protein hyperacetylation. Strikingly, supplementation with nicotinamide riboside (NR), a NAD⁺ precursor, led to dramatic improvement of mitochondrial function, and importantly, amelioration of the HFpEF phenotype.

Conclusion: In summary, we have unveiled that protein hyperacetylation-mediated mitochondrial dysfunction is a crucial mechanism of HFpEF pathogenesis. This is, to our knowledge, the first study identifying a specific signature of metabolic remodeling in HFpEF heart. We also demonstrated the therapeutic effect of NAD⁺ repletion in a preclinical HFpEF model. In next steps, confirming this benefit in a clinical trial is warranted.

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A Cardiac Specific Branched Chain Aminotransferase Deletion Increases Insulin Stimulated Glucose Oxidation in the Mouse Heart

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Impaired branched chain amino acid (BCAA) oxidation at the level of downstream BCAA catabolic enzymes cause cardiac insulin resistance and dysfunction. However, it is not known whether it is the accumulation of BCAAs or branched chain keto acids (BCKAs) due to impaired cardiac BCAA oxidation that result in these cardiac metabolic detrimental effects. Our objective was to inhibit cardiac branched chain aminotransferase (BCATm), the first enzyme in the BCAA oxidative pathway, which functions to produce BCKAs. We anticipate deletion of upstream BCAA catabolic enzyme will result in reduced cardiac

BCAA oxidation and increase BCAAs but decrease BCKAs. Ten-week-old BCATm cardiac specific knockout (BCATm^{-/-}) male mice and their α -MHC-Cre expressing wildtype littermates (WT-Cre^{+/+}) received 6 intraperitoneal injections of tamoxifen (50 mg/kg). All mice were allowed a 6-week washout post-tamoxifen, following which 16-week-old mice were used to assess cardiac energy metabolism. Isolated working hearts were perfused with oxygenated Krebs–Henseleit solution, consisting of either 5mM [5-³H/U-¹⁴C] glucose, 0.8mM palmitate, 3%BSA, 0.5mM BCAA for glycolysis and glucose oxidation measurements, or 0.5mM glucose, 0.8mM [9,10-³H] palmitate, 3%BSA and 0.5mM [U-¹⁴C] BCAA from combination of 0.15mM leucine/isoleucine and 0.2mM valine for fatty acid oxidation and BCAA oxidation measurements respectively. There was no body weight, glucose tolerance or cardiac function differences observed in BCATm^{-/-} mice compared to WT-Cre^{+/+} mice (control). As expected, cardiac BCAA oxidation was significantly reduced in BCATm^{-/-} mice compared to control, however, adding insulin after 30 min of perfusion did not change BCAA oxidation rates. Interestingly, glucose oxidation was significantly higher in BCATm^{-/-} mice. Adding insulin further increased glucose oxidation in BCATm^{-/-} mice. Additionally, the contribution of glucose oxidation to ATP production was significantly higher in the BCATm^{-/-} mice compared to control. We conclude that impaired BCAA oxidation due to the upstream catabolic enzyme deletion increases cardiac insulin sensitivity. This also indicates that accumulation of BCKAs, not BCAAs, may be primarily contributing to cardiac insulin resistance.

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Cysteine-based Redox Regulation of Soluble Guanylyl Cyclase

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Soluble guanylyl cyclase (GC1) is the major receptor of nitric oxide (NO) and a key regulator for cardiovascular physiology. NO binding to the heme of GC1 catalyzes conversion of GTP to cGMP, which in turn activates protein kinase G pathway, thus leading to vasodilation. However, the mechanism by which NO signaling is propagated within the GC1 molecule for its stimulation is still poorly understood. The high frequency of solvent-exposed cysteines (Cys) of GC1 suggests a potential role of Cys posttranslational modifications (PTMs) in regulating GC1 activity. Our previous studies show that the disulfide reductase Thioredoxin 1 (Trx1) can interact with GC1 and affect its activity in cells. Together with studies showing that GC1 activity is modulated by thiol redox, we hypothesize that thiol/disulfide switch might be a mechanism that regulates the activity of GC1. Here we show that dithiol oxidant diamide can dose-dependently affect the activity of GC1. PEG-switch assay, LC/MS/MS and mutational analysis identified Cys involved in redox regulation of GC1. In addition, we show that Trx1 modulates Cys-based redox PTMs of GC1 in cells and blocking the interaction between Trx1 and GC1 affects GC1 activity in a purified system. Taken together, these results suggest that the thiol/disulfide switch of GC1 and its interaction with Trx1 could be an underlying mechanism for NO stimulation of GC1.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Cardiac Gene Therapy With Relaxin Receptor 1 Overexpression Protects Against Acute Myocardial Infarction and Associated Adverse Remodeling

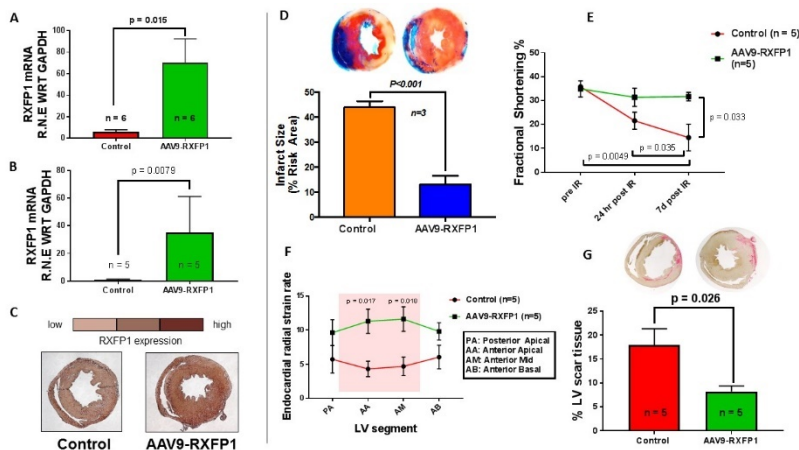
Teja Devarakonda, Virginia Commonwealth Univ, Richmond, VA; Erik Kohlbrenner, Icahn Sch of Med at Mount Sinai, New York City, NY; Adolfo G Mauro, Chad Cain, Anindita Das, Virginia Commonwealth Univ, Richmond, VA; Roger J Hajjar, Icahn Sch of Med at Mount Sinai, New York City, NY; Fadi N Salloum, Virginia Commonwealth Univ, Richmond, VA

Background: Relaxin signaling confers cardioprotection against acute myocardial infarction (MI) and mitigates adverse cardiac remodeling. We investigated the role of relaxin receptor (RXFP1) overexpression via cardiotropic AAV9 vectors in attenuating MI-associated adverse remodeling.

Methods and Results: Murine RXFP1 cDNA with CMV promoter was introduced into AAV cis plasmids for generation of AAV9-RXFP1 viral vectors. 1.0×10^{11} viral genomes in 100 μ L saline were administered i.v. to 8-week-old CD1 male mice.

Four weeks later, overexpression was confirmed via qPCR of RXFP1 mRNA in whole cardiac tissue lysates (**Fig A**) and primary cardiomyocytes (**Fig B**) versus control. Overexpressing (AAV9-RXFP1) mice were subjected to MI (30 minutes of coronary artery ligation followed by reperfusion for 24h or 7d). Immunohistochemical staining of cardiac sections 7d post-MI shows increased RXFP1 expression in AAV9-RXFP1 mice, as shown in **Fig C**. Infarct size was reduced at 24h post-MI (TTC staining, **Fig D**) and fractional shortening (M mode echo) was preserved at 24h and 7d post-MI in AAV9-RXFP1 mice (**Fig E**); endocardial strain rate assessment (speckle tracking analysis) showed preserved function in the affected ventricular segments of AAV9-RXFP1 mice 7d post-MI (**Fig F**). Picrosirius staining showed reduced LV fibrosis at 7d post-MI in AAV9-RXFP1 mice (**Fig G**).

Conclusion: Administration of AAV9-RXFP1 successfully upregulates RXFP1 in cardiac tissue and primary cardiomyocytes, reduces infarct size and fibrosis, and preserves cardiac function post MI. Targeting cardiac RXFP1 via gene therapy could be a novel strategy to prevent MI-related adverse remodeling.



T. Devarakonda: None. E. Kohlbrenner: None. A.G. Mauro: None. C. Cain: None. A. Das: None. R.J. Hajjar: None. F.N. Salloum: None.

Poster Session 3 and Reception

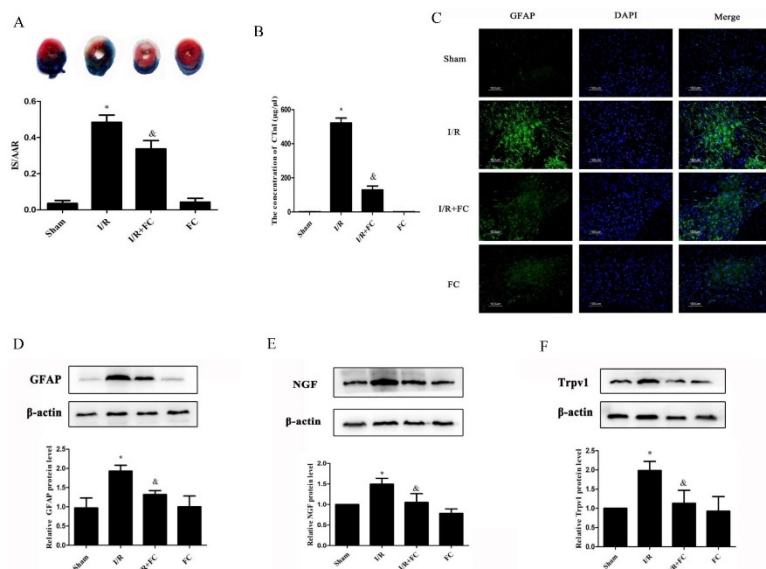
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Inhibition of Spinal Astrocytes Activation Attenuates Myocardial Ischemia-Reperfusion Injury by Regulating NGF/TRPV1 Nociceptive Signaling

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Background and Aim Spinal astrocytes activation are implicated in nociception and pain processing after noxious stimuli, while its role in cardiac ischemic nociception is unknown. We recently reported spinal nerve growth factor (NGF)-sensitized transient receptor potential vanilloid 1 (TRPV1) nociceptive signaling following cardiac ischemia and reperfusion (I/R). This study was design to investigate whether spinal astrocytes are activated following myocardial I/R and its effects on spinal NGF-TRPV1 signaling. **Methods and Results** Myocardial I/R injury was induced by 30 min occlusion of the left coronary artery followed by 120 min of reperfusion in rats. Glial fibrillary acidic protein (GFAP), an astrocyte marker, was found up-regulated in thoracic spinal cord during ischemia and reperfusion. Intrathecally pre-injection of fluorocitrate (FC), an astrocyte inhibitor, reduced infarct size (IS/AAR, area at risk), from 48.4 ± 2.67 to 29.7 ± 3.13 ($P < 0.05$, Fig.1 A). The high level of cardiac troponin T caused by I/R was decreased by FC injection as well (from 530 ± 65 to 129 ± 50 $\mu\text{g}/\mu\text{l}$, $P < 0.01$, Fig.1 B). Upon immunofluorescence staining, GFAP immunopositive cells in spinal cord were found markedly increased after I/R, while the staining was suppressed by intrathecal FC administration (Fig.1 C). Moreover, the up-regulation of NGF and TRPV1 protein levels in spinal cord were both inhibited by FC pretreatment (Fig.1 D-F). **Conclusion** Myocardial I/R induced the activation of spinal astrocytes, inhibition of which reduces infarct size and cTnT level, as well as NGF-TRPV1 signaling, which suggests a novel therapeutic target for preventing myocardial ischemic injury.



S. He: None. R. Liu: None. L. zhang: None. Y. Zhang: None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Acetate Protects the Heart Against Ischemic Injury by Alternating TCA Cycle-related Metabolites and AMP-activated Protein Kinase

Genki Ichihara, Yoshinori Katsumata, Shinichi Goto, Seien Ko, Takahiro Hiraide, Hiroki Kitakata, Hidenori Moriyama, Kohsuke Shirakawa, Atsushi Anzai, Jin Endo, Masaharu Kataoka, Fukuda Keiichi, Motoaki Sano, Keio Univ Sch of Med, Tokyo, Japan

Introduction: Acetate is preferentially taken up in the heart, and converted into acetyl-CoA by acetyl-CoA synthetase2 (AceCS2), which is abundant in the mitochondria of cardiomyocytes, resulting in activation of AMPK through an elevation of AMP/ATP ratio. In this context, we investigated the effects of acetate on myocardial ischemic injury in isolated cardiomyocytes and *in vivo* mouse hearts. **Methods and Results:** In isolated cardiomyocytes, ¹³C-labelled metabolites were analyzed using capillary electrophoresis-electrospray ionization mass spectrometry (CE-ESI-MS) in order to determine the fate of exogenously administered ¹³C-acetate. The oxygen consumption rate (OCR) was evaluated using a flux analyzer. A variety of ¹³C-labelled intermediates in the TCA cycle increased 10 minutes after administration of ¹³C-acetate followed by a subsequent increase in OCR. The OCR elevation was sustained more than 2 hours after acetate injection. The acetate-induced OCR elevation was partially blocked by the glycolysis inhibitor 2-Deoxyglucose (2-DG), carnitine palmitoyl transferase1A (CPT-1A) inhibitor (etomoxir), and mitochondrial pyruvate carrier (MPC) inhibitor (UK5099). The acetate-induced OCR elevation was also blocked by AMP-activated protein kinase (AMPK) inhibitor (compound C). These findings indicated that acetate caused an elevation in mitochondrial function through activation of various metabolic pathways, which may be related to activation of the AMPK pathway. Next, left anterior descending artery-ligated hearts were processed with focused microwave and analyzed by CE-MS and matrix-assisted laser desorption/ionization imaging mass spectrometry in order to examine the region-specific metabolite changes at the early phase of ischemia. Nicotinamide adenine dinucleotide (NADH) elevation in the ischemic core region was suppressed by acetate administration. Furthermore, acetate inhibited cardiac remodeling in a protective manner several weeks after myocardial ischemia when viewed by cardiac ultrasound. **Conclusion:** Acetate caused an increase in OCR via both a significant elevation in TCA cycle metabolites and activation of AMPK pathway, and may have a protective effect on myocardial ischemic injury.

G. Ichihara: None. Y. Katsumata: None. S. Goto: None. S. Ko: None. T. Hiraide: None. H. Kitakata: None. H. Moriyama: None. K. Shirakawa: None. A. Anzai: None. J. Endo: None. M. Kataoka: None. F. Keiichi: None. M. Sano: None.

Poster Session 3 and Reception

Role of Ginsenoside Rb1 in Resistin-Induced Vascular Smooth Muscle Cell Dysfunction

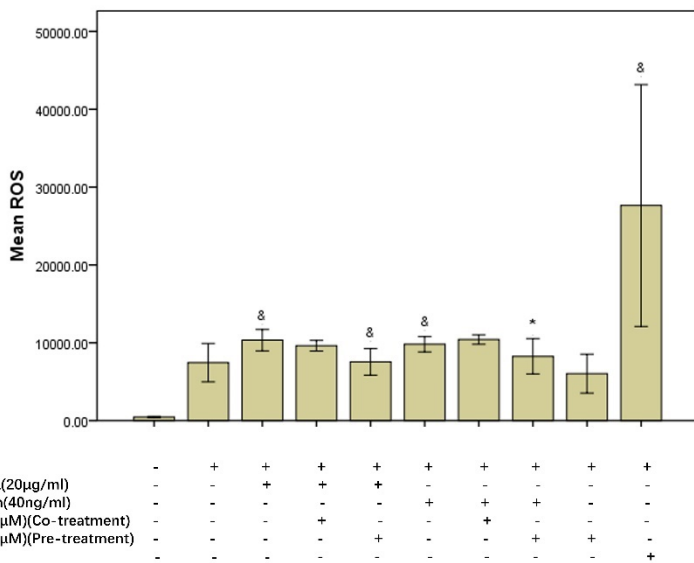
Weifeng Lu, Nezam Haider, Pricila Moly, Wei Zhou, Div of Vascular Surgery, Dept of Surgery, Univ of Arizona, Tucson, AZ

Background: Acetyl-LDL and resistin have been proved to play a critical role in inducing VSMC malfunction in the process of atherosclerosis. Ginsenoside Rb1, a major constituent of Ginseng that has been used for thousands of years, was reported to have powerful vascular-protective effect. Here we aim to explore its protective effect on Acetyl-LDL and resistin induced dysfunction in VSMCs.

Methods: Human coronary artery smooth muscle cells (HCASMC) were used at passage 5 to 8 for experiments. Cells were treated for various time points with or without resistin at 40 ng/mL and Acetyl-LDL in the presence or absence of Rb1. TBHP was also used as a positive control. Cell migration was analyzed using scratch wound assay, and cytosolic ROS (H2DCFDA as a dye probe) was measured by microplate reader and compared among groups.

Results: Resistin time-dependently increased HCASMC migration, which could be significantly attenuated by Rb1 at 20µM. Resistin and Act-LDL increased ROS production in HCASMCs to a similar level, while pretreatment with Rb1 reversed the effects by resistin and acetyl-LDL. In addition, we demonstrated that both resistin and Rb1 had dose-dependent effect on ROS production in HCSMCs and that Rb1 at 40µM pre-treatment significantly reduced resistin (40ng/ml) induced cytosolic ROS in VSMCs.

Conclusion: We demonstrated the protective effect of Rb1 on HCASMC and showed, for the first time, that Rb1 pre-treatment being more effective than co-treatment, suggesting that ginsenoside Rb1 has the potential of protecting against atherosclerosis. More in-dept study is needed.



W. Lu: None. **N. Haider:** None. **P. Moly:** None. **W. Zhou:** None.

Poster Session 3 and Reception

Cardiomyocyte Derived DDT Protects Against Myocardial Infarction Induced Heart Failure

Yina Ma, Xiaoyue Hu, Xiaohong Wu, Daniel Pfau, Lin Leng, Yale Univ, New Haven, CT; Kenneth Bedi, Kenneth Margulies, Univ of Pennsylvania, Philadelphia, PA; Richard Bucala, Lawrence Young, Yale Univ, New Haven, CT

Background: D-dopachrome tautomerase (DDT), the only homolog of macrophage migration inhibitory factor (MIF), is a cytokine highly expressed in cardiomyocytes and has autocrine-paracrine effects, signaling through the CD74 receptor. Endogenous DDT and MIF prevent ischemia-reperfusion injury in mice. In this study, we investigated whether endogenous cardiomyocyte DDT protects against myocardial remodeling and heart failure after myocardial infarction. **Methods:** Cardiomyocyte-specific DDT knockout (cKO) and littermate control (CON) mice underwent myocardial

infarction (MI) or sham surgery. Serial echocardiography was performed at baseline, 1 day before permanent coronary ligation and 1 day, 1 week, and 4 weeks after MI or sham surgery. Hearts were analyzed at 3 days after MI or sham surgery for histology and molecular studies. **Results:** No baseline differences in left ventricular (LV) function were observed in cKO and CON mice at baseline or after sham surgery. The initial infarction sizes were comparable at 1 day after coronary ligation in cKO and CON. Compared to CON, cKO mice developed more rapid LV dilatation, and decreased LV ejection fraction (EF) and stroke volume (SV) as early as 1 week after MI. At 4 weeks after MI, LVEF was 33% lower and SV lower by 43% in cKO vs. CON (n=4-5/group, all P<0.01). Hemodynamic analysis showed that LV end diastolic pressure in the cKO mice was 38% higher in cKO vs. CON after 4 weeks MI (p=0.052). Immunoblots showed diminished phosphorylation of AMPK and downstream ACC in the cKO mice 3 days after MI. Immunohistochemistry staining with CD3 and F4/80 staining showed increased T cells and macrophage cells in the cKO mice 3 days after MI (p<0.05). We further assessed the regulation of cardiac DDT expression in chronic human heart failure. In hearts explanted from patients with advanced heart failure during transplantation for ischemic cardiomyopathy (n=8), DDT mRNA and protein expression were also reduced by 40% and 58% compared to controls (P<0.01). **Conclusion:** Cardiomyocyte-derived DDT prevents adverse cardiac remodeling after MI, potentially through AMPK signaling and anti-inflammatory effects. Down-regulation of cardiac DDT may contribute to pathogenesis of advanced heart failure.

Y. Ma: None. **X. Hu:** None. **X. Wu:** None. **D. Pfau:** None. **L. Leng:** None. **K. Bedi:** None. **K. Margulies:** None. **R. Bucala:** None. **L. Young:** None.

Poster Session 3 and Reception

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Chronic GPER1 Cardioprotection in H9c2 Myoblasts is Mediated by an MST1-YAP Pathway and Mitochondrial Function and Dynamics

Ngonidzashe Madungwe, Yansheng Feng, Jean C Bopassa, UT Health Science Ctr, San Antonio, TX

Introduction: We recently showed that cardiac tissue treated acutely with estrogen (17 β -estradiol; E2), pre- and post-ischemia, exhibited reduced myocardial infarct size and better functional recovery after ischemia /reperfusion via the G protein-coupled estrogen receptor 1 (GPER1). These E2-GPER1 cardioprotective effects have been found to involve the preservation of mitochondrial structure and function. Here, we investigate the impact and mechanisms underlying chronic GPER1 activation in H9c2 cardiac myoblasts treated with a cytotoxic agent, H₂O₂. **Methods:** H9c2 cells were treated with 200 μ M H₂O₂, for 24 h and reoxygenated in no-serum conditions for 4 and 8 days with the addition of E2 (80nM) and GPER1 agonist (G1; 100nM) and GPER1 antagonist (G15; 1 μ M). We measured cell death and proliferation, as well as gene expression, mitochondrial morphology and function using flow cytometry, cell assays, qRT-PCR, along with fluorescence and electron microscopy. We also analyzed cell lysates by Western blot to assess the mechanisms involved. **Results:** We found that chronic treatment of cardiac myoblasts with E2 and G1 results in reduced cell death. The surviving cells showed improved mitochondrial function as evidenced by better cristae morphology, increased ATP production and increased resistance to opening of the mitochondrial permeability transition pore. Particularly chronic GPER1 activation led to reduced cell cycle activity along with changes in mitochondrial dynamics of fusion and fission. Additionally, we found that chronic GPER1 activation signaling involves decreased phosphorylation of mammalian sterile-20-like kinase (MST1) and increased translocation of the transcription coactivator, yes-associated protein (YAP), to the nucleus for upregulation of pro-survival genes. These effects were mainly prevented by the G15, suggesting a GPER1-dominant mechanism. **Conclusion:** Chronic GPER1 activation in H9c2 cardiac myoblasts treated with oxidative stress results in reduced cell death by preserving mitochondrial structural integrity and function by increasing mitochondrial dynamics. This chronic GPER1 cardioprotection involves activation of MST1/YAP signaling pathways as well as upregulation of pro-survival gene.

N. Madungwe: None. **Y. Feng:** None. **J.C. Bopassa:** None.

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Thioredoxin-1 Maintains Cardiac Function and Metabolic Gene Expression via mTOR Signaling

Shinichi Oka, Wataru Mizushima, Chun-yang Huan, Junichi Sadoshima, Rutgers New Jersey Medical Sch, Newark, NJ

Thioredoxin-1 (Trx1) is a 12 kDa oxidoreductase that reduces proteins with disulfide bonds through thiol disulfide exchange reactions. Several lines of evidence show that Trx1 protects cardiomyocytes against stress, such as ischemia and pressure overload. However, due to the embryonic lethality of systemic Trx1 knockout mice, the role of endogenous Trx1 in cardiac function has not been tested with loss-of-function mice. In this study, we generated cardiac-specific Trx1 knockout (Trx1cKO) mice, using Trx1floxflox and α MHC-Cre transgenic mice, to elucidate the physiological role of Trx1 in the postnatal heart. The Trx1cKO mice were viable but started dying at 13 days of age. The median survival age was 27 days. Echocardiographic analyses indicated cardiac dysfunction in Trx1cKO mice, evidenced by reduced ejection fraction (WT: 0.78; Trx1cKO: 0.50*; $p < 0.05$ vs. WT). RNA-sequencing and pathway analyses revealed that metabolic genes involved in energy production were most prominently downregulated in Trx1cKO mice. Mitochondrial morphological abnormality and increased oxidative stress were evident in Trx1cKO mice. Among Trx1 substrates possibly involved in metabolic gene expression, mechanistic target of rapamycin (mTOR) was oxidized and inhibited in Trx1cKO mice. In cultured cardiomyocytes, Trx1 knockdown inhibited mTOR signaling, as evidenced by reduced phosphorylation of mTOR substrates, such as S6K and 4EBP1. Trx1 knockdown in cardiomyocytes inhibited mitochondrial protein expression, respiration and ATP production (relative ATP production in isolated mitochondria; control: 1; Trx1 knockdown: 0.69*; $p < 0.05$ vs. control), suggesting that Trx1 maintains mitochondrial function in a cell autonomous manner. Importantly, mTOR-C1483F mutant is resistant against oxidation and inactivation induced by downregulation of Trx1. Trx1 knockdown suppressed reporter gene activity driven by the promoter sequences of endogenous metabolic genes, such as Ndufa1 and Ndufs1, which was rescued by mTOR-C1483F. These results suggest that Trx1 is essential for cardiac function and metabolism, possibly through reduction of mTOR at Cys1483.

S. Oka: None. **W. Mizushima:** None. **C. Huan:** None. **J. Sadoshima:** None.

Poster Session 3 and Reception

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Aldehyde Dehydrogenase Activator 1 (Alda-1) Attenuates Coronary Endothelial Dysfunction-Mediated Cardiac Damage in ALDH2*2 Diabetic Mice

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Background: Endothelial dysfunction plays a critical role in the pathogenesis of diabetic complications. Diabetes-mediated reactive aldehydes like 4-hydroxy-2-nonenal (4HNE) are associated with endothelial cell impairment. Aldehyde dehydrogenase (ALDH) 2, a mitochondrial enzyme which detoxifies 4HNE, protects heart function against diabetic cardiomyopathy. Nearly 600 million East Asians have a point mutation of ALDH2, termed as ALDH2*2, which causes an intrinsic low ALDH2 activity. Previously, we reported an aggravated coronary endothelial dysfunction in ALDH2*2 mutant knock-in mice (ALDH2*2 mice) with diabetes. Aldehyde dehydrogenase activator 1 (Alda-1) is the only known activator for both normal ALDH2 and mutant ALDH2*2. We hypothesized that Alda-1 could protect endothelial cells (ECs) from hyperglycemia induced coronary endothelial dysfunction in diabetic ALDH2*2 mice. **Methods and results:** Diabetes was induced by streptozotocin injection (150 mg/kg) to ALDH2*2 mice. Alda-1 (10 mg/kg/d, DM-A) or vehicle (DM-V) was delivered through mini-pumps for 3 weeks after diabetes. We found an increased left ventricular pressure (59.9±18.7 vs 66.4±20.1 mmHg) and a decreased perfusion pressure (89.4±8.1 vs 83.6±12.1 mmHg) in Langendorff hearts from DM-A (DM-V vs DM-A, $p < 0.05$, respectively). In heart sections, CD31+ (127.2±22.3 vs 270.5±53.7 /mm²) and eNOS+ cells (136.2±22.8 vs 366.7±33.6 /mm²) were increased while apoptosis (1117.5±303.6 vs 727.1±254.8 /mm²) and 4HNE+ (515.8±8.3 vs 181.7±72.5 /mm²) cells were reduced in DM-A (DM-V vs DM-A, $p < 0.05$, respectively). Direct perfusion of 4HNE to Langendorff hearts from control ALDH2*2 mice (4HNE) increased perfusion pressure (Δ 3.18±0.5 vs Δ 36.6±7.7 mmHg), arteriole closure (8.3±0.4 vs 39.8±3.7 /mm²), and decreased eNOS+ cells (2877.1±117.3 vs 37.5±5.6 /mm²) (Sham vs 4HNE, $p < 0.05$). One-day Alda-1 (10 mg/kg) pre-treatment before 4HNE perfusion (A-4HNE) decreased perfusion pressure (Δ 7.1±1.7 mmHg), number of closed arterioles (22.4±4.8 /mm²), and increased eNOS+ (878.0±121.3 /mm²) cells (4HNE vs A-4HNE, $p < 0.05$, respectively). **Conclusion:** Alda-1 protects hyperglycemia mediated 4HNE induced coronary endothelial dysfunction and thereby cardiac tissue in ALDH2*2 diabetic mice.

G. Pan: None. **H. Pang:** None. **M. Deshpande:** None. **S. Palaniyandi:** None.

Poster Session 3 and Reception

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The Role of Sialidase Neu3 in the Cardiac Response to Ischemia and Reperfusion Injury

Marco Piccoli, IRCCS Policlinico San Donato, San Donato Milanese, Italy; **Maria Elena Canali**, Univ of Milan, Milan, Italy; **Andrea Ghiroldi**, **Federica Cirillo**, **Luigi Anastasia**, IRCCS Policlinico San Donato, San Donato Milanese, Italy

Reperfusion strategies are life-saving approaches to restore the blood flow in the cardiac tissue after acute myocardial infarction (AMI). However, they come with the drawback that they inevitably induce ischemia/reperfusion injury (IRI), resulting in increased cardiomyocytes damage and heart failure. In this context, the physiological activation of several pro-survival kinases, such as Akt and Erk, as well as of the hypoxia inducible factor (HIF) has been recognized to be critical during IRI. Along with this line, we recently discovered a novel, PHDs independent, mechanism of HIF-1 α ; activation mediated by sialidase Neu3. Interestingly, Neu3 is upregulated under chronic hypoxia in cyanotic congenital cardiac patients. Moreover, induced activation of Neu3 increased myoblast resistance to hypoxic stress. Thus, in this study, we further investigated the possible role of Neu3 in protecting cardiac myoblasts during IRI. In particular, we set-up an in-vitro model of IRI on H9C2 rat cardiomyoblasts. Results showed a modulation of Neu3 during IRI, with a progressive down-regulation during the ischemic phase, followed by a reactivation during the reperfusion phase. Remarkably, overexpression of Neu3 significantly increased cardiomyoblasts resistance to IRI, both in terms of cell proliferation and resistance to apoptosis. Treatment with Akt and Erk inhibitors, as well as with a Neu3 specific inhibitor completely reverted the beneficial effects mediated by Neu3 upregulation. In conclusion, our results show that Neu3 activation has a cardioprotective effect during IRI, calling for further studies to unveil its full potential as a therapeutic target to treat cardiac ischemia and reperfusion injury and to improve patients recover after AMI.

M. Piccoli: None. **M. Canali:** None. **A. Ghiroldi:** None. **F. Cirillo:** None. **L. Anastasia:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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The Valosin-containing Protein Resists Pathological Cardiac Calcium Overload via Inhibiting Mitochondrial Calcium Uptake

Shaunrick Stoll, Ben Ma, Erick J. Behringer, **Hongyu Qiu**, Loma Linda Univ, Loma Linda, CA

Stress-induced mitochondrial calcium (Ca²⁺) overload is a trigger of cardiomyocyte death through the opening of mitochondrial permeability transition pore (mPTP) during cardiac ischemic injury. We previously found that the valosin-containing protein (VCP), an ATPase-associated protein, protects cardiomyocytes against stress-induced death and also inhibits mPTP opening in vitro. However, the underlying molecular mechanisms are not fully understood. Here, we tested our hypothesis that VCP protects cardiomyocytes against stress-induced mPTP opening through resisting excessive mitochondrial Ca²⁺ uptake. By using a cardiac-specific transgenic (TG) mouse model in which VCP is overexpressed by 3.5 folds in the hearts, we found that, under the pathological extra-mitochondrial Ca²⁺ overload (10-70 μ M), Ca²⁺ entered into cardiac mitochondria was much less in VCP TG mice compared to little-matched WT mice, subsequently preventing mPTP opening and ATP depletion under the Ca²⁺ challenge. Mechanistically, overexpression of VCP in the heart results in post-translational protein degradation of the mitochondrial Ca²⁺ uptake protein 1 (MICU1), an activator of the mitochondria Ca²⁺ uniporter (MCU) which is responsible for mitochondrial calcium uptake. Together, our results reveal a new regulatory role of VCP in cardiac mitochondrial Ca²⁺ homeostasis and unlocks the potential mechanism by which VCP confers its cardioprotection.

S. Stoll: None. **B. Ma:** None. **E. Behringer:** None. **H. Qiu:** None.

Poster Session 3 and Reception

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Nadph Oxidase Nox1-dependent Endocytosis and Activation is Regulated by a Conserved Trafficking Motif in Vascular Smooth Muscle Cells

Brandon M Schickling, Duke Univ, Durham, NC; **Jennifer Streeter**, Univ of Iowa, Iowa City, IA; **Francis J Miller Jr.**, Duke Univ, Durham, NC

We previously reported that TNF- α -mediated Nox1-dependent redox signaling of vascular smooth muscle cells (SMC) requires endocytosis. However, the molecular mechanisms regulating Nox1 endocytosis are not known. The goal of this study was to identify motifs in the Nox1 protein sequence necessary for Nox1 endocytosis and reactive oxygen species (ROS) generation. Immunoprecipitation of Nox1, after treatment of SMC with TNF- α , showed it to be associated with Early

Endosome Antigen 1 (EEA1) and Ras-related protein Rab-5A (Rab5), markers of early endosomes. Analysis of the Nox1 protein sequence identified two conserved putative trafficking motifs, Leucine-Leucine (LL), and Valine-Leucine-Valine (VLV). Biotinylation of SMC plasma membrane proteins under non-stimulated conditions suggested increased levels of Nox1 VLV mutant in the plasma membrane, whereas levels of Nox1 LL mutant were comparable to wildtype (WT) Nox1. Furthermore, cells expressing the VLV mutant showed impaired endocytosis of Nox1 following TNF- α as compared to cells expressing WT Nox1 or the LL mutant. SMC were treated with TNF- α in the presence of Oxyburst, a non-cell permeable redox-sensitive fluorophore whose internalization requires endocytosis. Cells expressing WT or Nox1 LL, but not Nox1 VLV, exhibited punctate intracellular fluorescence, indicating endosomal ROS production. Next, we examined whether the VLV motif is required for Nox1-dependent SMC migration. Transwell migration of SMC was similar in cells expressing WT or the LL Nox1 mutant; however, migration was decreased in cells expressing the Nox1 VLV mutant. Finally, we identified proteins whose association with Nox1 during endocytosis were dependent on the VLV motif. Nox1 WT and VLV mutant were cloned in frame with the biotin ligase BioID2. Following stimulation with TNF- α , Western blotting for anti-biotin, and subsequent mass spectrometry, identified several proteins directly associated with WT Nox1 but not Nox1 VLV. These findings suggest that endosomal compartmentalization of Nox1 and its redox signaling is regulated by a specific motif on Nox1 that is necessary for protein interactions. Targeted inhibition of Nox1 activation is a novel therapeutic strategy in the prevention of vascular disease.

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Expression of Anti-mir-33a-5p in Cultured Endothelial Cells Increases Macrophage and Smooth Muscle Cell Cholesterol Efflux by Exosome-mediated Transfer of Anti-mir-33a-5p

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Background: The microRNA miR-33a-5p impairs apoAI-mediated cholesterol efflux by silencing ABCA1 expression. Systemic injection of antagomirs to miR-33a-5p reduces atherosclerosis but can cause hepatic steatosis and hypertriglyceridemia. In contrast, local delivery of miR-33a-5p antagomirs to lipid-laden intimal cells [smooth muscle cells (SMC) and macrophages (M ϕ)] could potentially treat atherosclerosis without causing metabolic abnormalities. Helper-dependent adenoviral vectors (HDAd) provide durable transgene expression in endothelial cells (EC) in vivo; however, HDAd does not cross an intact large vessel EC layer and therefore cannot deliver antagomirs directly to SMC and M ϕ . We hypothesized that transducing EC with an HDAd expressing anti-miR-33a-5p (HDAdAnti) could result in packaging of anti-miR-33a-5p into EC-derived exosomes that, in turn, deliver anti-miR-33a-5p to neighboring SMC and M ϕ . Inhibition of miR-33a-5p in SMC and M ϕ would upregulate ABCA1, and enhance apoAI-mediated cholesterol efflux.

Methods: We transduced cultured EC with either HDAdAnti or a control HDAd expressing a "scrambled" shRNA (HDAdScr), harvested conditioned medium (CM) from the EC, and treated SMC and M ϕ (some of which were cholesterol-loaded) with the CM. We measured levels of anti-miR-33a-5p and miR-33a-5p, ABCA1 protein, and apoAI-mediated cholesterol efflux in the treated SMC and M ϕ .

Results: Exosomes containing anti-miR-33a-5p were present only in CM from HDAdAnti-transduced EC. Compared to M ϕ incubated with CM from EC transduced with HDAdScr, M ϕ incubated with CM from HDAdAnti-transduced EC had 80% lower levels of miR-33a-5p, a 2.2-fold increase in ABCA1 protein, and a 1.6-fold increase in apoAI-mediated cholesterol efflux (all $P < 0.001$). Similar results were obtained with SMC: 65% lower levels of miR-33a-5p expression, 1.6-fold increase in ABCA1 protein, and 1.4-fold increase in apoAI-mediated cholesterol efflux (all $P \leq 0.01$).

Conclusions: EC transduced with HDAdAnti release exosomes containing anti-miR-33a-5p. These exosomes transfer anti-miR-33a-5p to SMC and M ϕ , increasing cholesterol efflux. Application of this approach in vivo may accelerate vessel wall cholesterol efflux, preventing and reversing atherosclerosis.

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Poster Session 3 and Reception

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The Role of Paraoxonase 2 (PON2) in Mitochondrial Membrane Phospholipid Composition and Lipid Peroxidation

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Introduction: We have recently reported that PON2 plays a protective role against myocardial ischemia-reperfusion injury (IRI) by reducing oxidative stress and mitochondrial dysfunction, in the form of improved calcium retention capacity and integrity of the mitochondrial membrane potential. It is known that mitochondrial phospholipids (PL) play critical roles in mitochondrial homeostasis via maintenance of normal bioenergetics and membrane function. These PLs undergo oxidative stress during IRI resulting in an increase in lipid peroxidation, damage of the respiratory complexes and loss of mitochondrial membrane potential.

Hypothesis: PON2 protects cardiomyocytes against myocardial IRI by modulating the mitochondrial membrane phospholipid composition and lipid peroxidation.

Methods: Two methods were developed to measure 1) various oxidized lipid species (i.e. 5, 12, and 15 HETE, 9, 13 HODE, and 5-oxoETE) and inflammatory markers (lipid panel) and 2) a panel of phospholipid species (i.e. PC, PE, PS) (PLP) via mass spectrometry (ESI LC-MS/MS). Mitochondria were isolated from male C57BL6/J (WT) and PON2 deficient (PON2-def) mice. PLs were then extracted using a modified Bligh and Dyer method with the respective internal standards, and run on a SCIEX 5500 QTrap run in negative ion mode and controlled by Analyst 1.6.2 software. Data is expressed as mean±SEM. Student's T-test is used for statistical analysis, with p<0.05 considered statistically significant.

Results: We determined that under baseline conditions, PON2-deficient mice have altered mitochondrial membrane PL composition and lipid peroxidation. We observe that PON2-def mitochondria have a significant decrease in various phosphatidylcholine species and an increase in phosphatidylethanolamine (42:9). In addition, there were variations in the composition of phosphatidylserine and phosphatidylinositol. The alteration of mitochondrial PL were accompanied by significant increases in various oxidized lipids including 9, 13-HODE, 14S, 17S-HDHA, 5, 15-HETE, and 5-oxoETE.

Conclusion: Our preliminary studies point to the important role that PON2 plays in modulating mitochondrial PL composition and lipid peroxidation, and warrant further investigation under myocardial IRI.

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Mitochondrial Stress Response by LonP1 in Cardiac Function and Protection

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LonP1 is a mitochondrial protease that is crucial for maintaining cardiac mitochondrial homeostasis. We recently showed that LonP1 mediates cardioprotection during cardiac ischemia-reperfusion (I/R) by reducing oxidative stress and infarct size. We hypothesize that LonP1 promotes mitochondrial biogenesis during cardiac differentiation and development and protects the heart from oxidative injury by recalibrating bioenergetics. Here, we employ human induced pluripotent stem cells differentiated into cardiomyocytes (iPSC-CMs), rat neonatal ventricular myocytes (rNVMs), and mouse models with cardiac-specific LonP1 overexpression (LonTg) and heterozygous LonP1 knockout (*LONP1^{+/-}*). In iPSC-CMs, LonP1 is induced 4.8-fold at 39 vs. 12 days after differentiation. During the maturation of murine hearts, LonP1 is increased 4.6-fold at 3 months vs. during 1 day. Upregulation was also observed in rNVMs in which LonP1 is induced 5-fold after 5 days culturing, compared to 0 days. LonP1 induction in rNVMs is accompanied by substantial 2-fold upregulation of mitochondrial transcripts, MT-CYB and MT-COI. However, the levels of another mitochondrial matrix protease ClpP was not altered, suggesting that LonP1 specifically may play an important role during mitochondrial biogenesis and proteostasis in the heart. In vivo, LonTg protected the myocardium against ischemia-reperfusion (I/R) injury by preventing oxidative damage. In LonTg hearts, basal complex I activity was significantly (p<0.05) reduced (Ntg-0.01 ± 0.001 vs. LonTg-0.0078 ± 0.001 mOD/min), as well as during I/R (Ntg-0.0139 ± 0.0005 vs LonTg-0.0102 ± 0.001 mOD/min) (n=3 each). However, no difference was observed in the complex I activity in *LONP1^{+/-}*(0.0118 ± 0.0 mOD/min) and *LONP1^{+/-}* (0.0121 ± 0.0 mOD/min) mice (n=3 each). In addition, our preliminary native gel data of LonTg hearts shows a 40% reduction (n=1) in the levels of mitochondrial supercomplexes, compared to NTg hearts (n=4). Further studies are underway to explore the role of LonP1 during differentiation, and in response to mitochondrial cardiac stress. Together, our findings show a novel role of LonP1 in regulating cardiac function during mitochondrial biogenesis and in mitigating cardiac stress-induced injury.

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Activation of Autophagic Flux Rescues Mitochondrial Homeostasis During Cardiac Ischemia/Reperfusion Injury

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Objective: Reperfusion injury accounts for ~50% of myocardial infarct size, and meaningful clinical therapies targeting this do not exist. We have shown that HDAC inhibition-enhanced cardiomyocyte autophagy blunts ischemia/reperfusion (I/R) injury given at the time of reperfusion. However, as HDAC inhibition may have off-target effects, we set out to test whether augmentation of autophagy protects myocardium through maintenance of mitochondrial homeostasis and reduction of oxidative stress during reperfusion injury.

Methods: 10-week-old, wild-type, C57BL6 mice were randomized into 3 groups: vehicle control, or exposed to a Tat-Scrambled (TS) peptide, or a Tat-Becn1 (TB, autophagy-inducing molecule) peptide. Each group was subjected to I/R surgery (45min coronary ligation, 24h reperfusion). Infarct size, systolic function, and mitochondrial dynamics were assayed. Cultured neonatal rat ventricular myocytes (NRVMs) were exposed to TB during simulated ischemia/reperfusion injury.

ATG7 knockout (ATG7 KO) mice and ATG7 knockdown by siRNA in NRVMs was used to evaluate the role of autophagy.

Results: TB treatment at reperfusion reduced infarct size by 20.1% (n=23, p<0.05) and improved systolic function (n=11, p<0.05). Improvement correlated with increased autophagic flux in the border zone with less oxidative stress. ATG7 KO mice did not manifest TB-promoted cardioprotection during I/R. TB increased mtDNA content in the border zone (n=10, p<0.05). In NRVMs subjected to I/R, TB reduced cell death by 41% (n=12, p<0.001), reduced I/R-induced mtDNA damage, and increased mtDNA content by >60% (n=3, p<0.05). Moreover, TB promoted expression of the gene coding for PGC-1 α , which controls mitochondrial biogenesis, in the border zone (n=10, p<0.05) and in NRVMs subjected to I/R (n=3, p<0.05), along with expression of the mitochondrial dynamics genes *Drp1*, *Fis1* and *MFN1/2* (n=9, p<0.05). Conversely, ATG7 knockdown in NRVMs abolished these beneficent effects of TB on mitochondria.

Conclusions: Autophagy is a sufficient and essential process to mitigate reperfusion injury through maintenance of mitochondrial homeostasis. Augmentation of autophagic flux may emerge as a viable clinical therapy to reduce reperfusion injury in myocardial infarction

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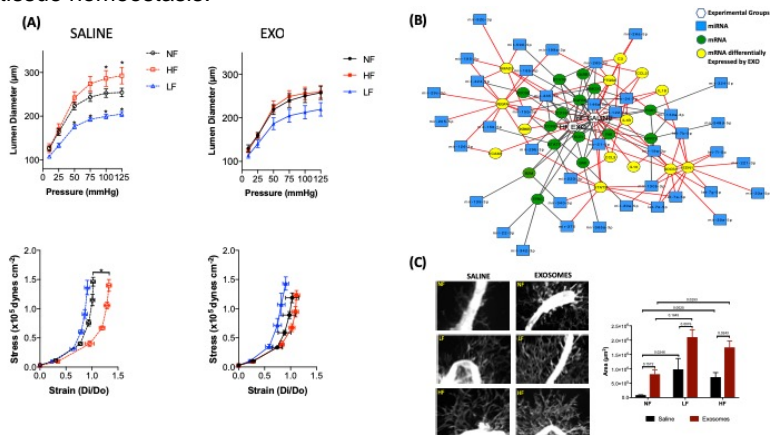
Poster Session 3 and Reception

Exosomes Derived From Endothelial Progenitor Cells Modulate Flow-Induced Remodeling and Increase Angiogenesis/Arteriogenesis in Mesenteric Arteries of Mice

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Exosomes are key regulators of cell-to-cell communication, becoming valuable tool as disease biomarker and in the development of new therapeutic strategies. We aimed to determine the role of endothelial progenitor cell (EPC)-derived exosomes (EXO) in vascular remodeling induced by flow. C57BL/6 mice underwent chronic changes in flow by mesenteric resistance arteries ligation. Arteries were thus submitted to high flow (HF), low flow (LF), or normal flow (NF). The day before surgery mice received I.P. injection with saline or exosomes (3x10⁹particle/kg), following the treatment every 3 days until sacrifice. After 14 days, arteries were studied in vitro in a pressure arteriograph. Increase in diameter and compliance by pressure found in HF arteries were not seen in EXO treated mice (Fig A). Decrease in diameter observed in saline treated LF arteries, was also abolished by EXO treatment. Network analysis of miRNA content in exosomes and arterial mRNA expression revealed an increased expression of components involved in angiogenesis/arteriogenesis (Fig B), which could contribute to flow maintenance in the tissue. Mesenteric arteries submitted to LF, HF and NF were isolated and placed in Matrigel matrix for angiogenesis analysis. We observed that change in flow is a trigger for vasculogenesis, which is enhanced by EXO treatment (Fig C). This study establishes the potential role of EPC-derived exosomes to regulate vascular remodeling during changes in flow. The mechanisms for these effects involve exosome-derived miRNA regulation of a network of mRNAs implicated in collateral angiogenesis/arteriogenesis, which in turn, may contribute to maintenance of

tissue homeostasis.



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Vascular MicroRNA-204 Promotes Diabetes-Associated Endothelial Dysfunction

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Background: The failure to achieve a decline in blood pressure at rest in type 2 diabetes (T2D) patients is believed to reflect endothelial dysfunction, and it increases the risk of coronary artery disease. Previously we discovered that the intestinal microbes govern vascular endothelial function through microRNA-204 (miR-204). The objective of this study was to investigate whether miR-204 contributes to diabetes-associated vascular endothelial dysfunction.

Methods: To test miR-204's role in diabetes we generated leptin receptor mutant genetically diabetic mice lacking miR-204 (db/db-miR-204^{-/-}). The vascular function was assessed by determining the vessel relaxation of phenylephrine-induced precontracted aortic rings. To ascertain the vascular miR-204 role in endothelial function *ex-vivo* experiments were performed, on aorta isolated from db/db mice, using miR-204 inhibitor. The miR-204 expression and localization were done using quantitative real-time PCR (qRT-PCR) and In-situ hybridization (ISH) assays.

Results: A significant increase in the vascular miR-204 expression was observed in the thoracic aorta of db/db mice compared to the age-matched control (208.80 ± 45.60 vs. 80.65 ± 13.11; p<0.05). The genetic deletion of miR-204 rescues endothelial function (improved vascular relaxation; 36.17 ± 7.42 vs. 76.56 ± 4.43; p<0.001). The db/db-miR-204^{-/-} mice develop obesity but are protected against diabetes (as evidenced by better glycemic control). *Ex-vivo* inhibition of miR-204 rescues diabetes-associated impairment of endothelium-dependent vasorelaxation (18.10 ± 2.16 vs. 50.64 ± 7.66; p<0.01). Next, we plan to identify the molecular mediator of miR-204 effects on the vascular endothelial function using Argonaute 2-crosslinked immunoprecipitation (Ago2-CLIP) assay.

Conclusions: Type 2 diabetes upregulates vascular miR-204, and genetic deletion of miR-204 rescues diabetes-associated impairment in endothelial function.

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Phosphorylation of RNA Binding Motif 20 is a Novel Target to Reduce Myocardial Stiffness in Diastolic Dysfunction

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Molecular Med, Berlin, Germany; Ying Ge, Univ of Wisconsin-Madison, Madison, WI; **Wei Guo**, Univ of Wyoming, Laramie, WY

Introduction: Published work has shown that RNA binding motif 20 (RBM20) can improve ventricular stiffening through altering titin sizes in HFpEF, a syndrome with no effective therapeutic options. Reducing RBM20 activity to treat diastolic dysfunction is promising, but it may cause side effects due to its multiple targets except for titin. RBM20 is one of the serine-arginine (SR) proteins of which the phosphorylation plays a critical role in gene splicing. Here we investigate the phosphorylation status of RBM20 and its potential role in diastolic dysfunction. **Methods:** Insect cell expression system was utilized for RBM20 expression. Purified RBM20 was subjected to the middle-down mass spectrometry (MS) analysis. Identified phosphorylation sites were mutated for *in-vitro* splicing and dual luciferase splice reporter assay through co-transfection with titin mini-gene. *In-vitro* kinase and co-IP assays were performed to identify the kinases that phosphorylate RBM20. Since phosphorylation is also important for translocation of RNA binding proteins, the confocal microscope was applied to estimate RBM20 trafficking. **Results:** Sixteen phosphorylation sites with four of them on SR domain of RBM20 were identified with MS analysis. *In-vitro* splicing and dual luciferase reporter assays revealed that two phosphorylation sites on SR domain play a major role in titin mini-gene splicing. *In-vitro* kinase assay indicated that kinases SRPK, CLK, and AKT can all phosphorylate RBM20, and co-IP confirmed the interaction between kinases and RBM20. Co-transfection of these kinases with RBM20 and titin mini-gene revealed that splicing pattern was different from co-transfection of mutated RBM20. Interestingly, two-phosphorylation sites mutation on SR domain facilitated RBM20 trafficking from the nucleus to the cytoplasm and thus, form RNA granules, suggesting the new role of RBM20 phosphorylation in diastolic dysfunction. *In-vivo* studies with mutation knock-in mouse model will be warranted in near future. **Conclusion:** These results suggest that RBM20 phosphorylation plays an important role in titin splicing, which is a potential target to treat diastolic dysfunction. RBM20 trafficking could be another new pathway to treat HFpEF.

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Poster Session 3 and Reception

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Ablation of MiR-144 Increases Vimentin Expression and Atherosclerotic Plaque Formation

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Objective—It has been suggested that miR-144 is pro-atherosclerotic via effects on reverse cholesterol transportation targeting the ATP binding cassette protein 1 (ABCA1). This study used proteomic analysis to identify additional cardiovascular targets of miR-144, and subsequently examined the role of a newly identified regulator of atherosclerotic burden in miR-144 KO mice receiving a high fat diet (HFD). *Approach and Results*—To identify affected secretory proteins, miR-144 treated endothelial cell culture medium was subjected to proteomic analysis including two-dimensional gel separation and nanospray liquid chromatography coupled to tandem mass spectrometry. We identified 5 gel spots representing 19 proteins that changed consistently across the biological replicates. One of these spots, was identified as vimentin. Atherosclerosis was induced in miR-144 KO mice by HFD and vascular lesions were quantified by Oil Red-O staining of the serial sectioned aortic root and from en-face views of the aortic tree. Unexpectedly, HFD induced extensive atherosclerosis in miR-144 KO mice in aortic root (WT 100% ± 22.71 vs miR-144 KO 256.88% ± 58.19, p < 0.05, n = 10) and arch areas (WT 100% ± 29.67 vs miR-144 KO 371.74% ± 73.48, p < 0.01, n = 10), and was accompanied by severe fatty liver disease compared with wild type littermates (WT). Vimentin levels were reduced by miR-144 and increased by antagomiR-144 in cultured cardiac endothelial cells without affecting vimentin mRNA levels. Compared with WT, ablation of the miR-144/451 cluster significantly increased plasma vimentin, while vimentin levels were decreased in control mice injected with synthetic miR-144 as double confirmed with Western blot and ELISA assays. Furthermore, increased vimentin expression was seen in the commissural regions of the aortic root (WT 100% ± 10.02 vs 164.19 ± 13.90, p < 0.01, n = 10) and collocated with other areas of aortic atherosclerosis. In contrast, neither liver ABCA1 nor circulating cholesterol levels were different between WT and miR-144/451 KO mice. *Conclusion*—We conclude that miR-144 is a potential regulator of the development of atherosclerosis via changes in vimentin signaling and both miR-144 and vimentin are potential therapeutic targets for atherosclerosis.

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MicroRNA-448 Regulates the Cardiac Sodium Channel During Ischemia

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Background: The cardiac voltage-gated sodium channel (*SCN5A*; encoding Nav1.5) plays a key role in cardiac conduction. The sodium channel is downregulated in cardiomyopathy, contributing to arrhythmic risk. In part, this downregulation is because of abnormal mRNA splicing and reduced mRNA stability. The reason for decreased mRNA stability is unclear, however.

Objective: Here, we examined whether microRNA-448 (miR-448) can contribute to *SCN5A* mRNA instability.

Methods: Datasets from the Gene Expression Omnibus database were utilized to screen miR-448 in human and mouse heart tissues. The relationship between *SCN5A* and miR-448 was predicted by three independent prediction tools. The expressions of *SCN5A* and miR-448 were determined by quantitative real time polymerase chain reaction. Protein levels of Nav1.5 were determined by Western blot. The effect of miR-448 mimic on sodium current was determined in induced pluripotent stem cells-derived cardiomyocytes. Mouse myocardial infarction was created by left anterior descending coronary artery ligation.

Results: The expression of miR-448 is increased in stressed heart after myocardial infarction. The binding sequence for miR-448 is well-conserved in 3'-UTR of *SCN5A* and miR-448 directly bound to a consensus cis element, suppressing *SCN5A* expression and sodium currents. miR-448 expression was increased by hypoxia. Activated by hypoxia, HIF1 α and NF- κ B activated were major transcriptional regulator for *MIR448*.

Conclusion: These results indicated that miR-448 contributed to post-transcriptional modification of *SCN5A*. miR-448 is likely one cause of sodium channel downregulation during ischemia and may represent a target for antagomir therapy to reduce arrhythmic risk associated with cardiomyopathy..

Keywords: *SCN5A*, miR-448, Cardiomyocyte, Hypoxia, NF- κ B, HIF1 α

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Inhibition of Long Noncoding RNA IncExACT1 Induces Physiological Cardiac Hypertrophy and Protects Against Pathological Hypertrophy

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Long noncoding-RNAs (lncRNAs) are critical regulators of cardiac development as well as pathological hypertrophy and heart failure (HF). However, their roles in exercise-induced physiological hypertrophy are unclear. Here, we used RNAseq to identify a novel class of cardiac lncRNAs that are dynamically regulated by exercise. We call

these long noncoding Exercise Associated Cardiac Transcripts (IncExACTs). Among them, IncExACT1, a highly conserved lncRNA, is down-regulated in exercised hearts but upregulated in transverse aortic constriction (TAC)-induced pathological hypertrophy and HF. In primary neonatal cardiomyocytes (CMs), transfection of LNA antisense oligonucleotide complementary to IncExACT1 (GapmeR) was sufficient to inhibit IncExACT1 expression and increase CM size with a gene expression pattern consistent with physiological hypertrophy (increased PCG1 α and α / β MHC ratio, all $p < 0.05$ vs. control). In contrast, lentiviral overexpression of IncExACT1 in primary CMs induced a pathological gene expression pattern (decreased PCG1 α and β / α MHC ratio, with increased ANP and BNP, all $p < 0.05$ vs. control). In vivo, GapmeR treatment for two weeks reduced cardiac IncExACT1 expression (0.5-fold at 2 weeks), increased ventricular wall thickness and fractional shortening (FS, $p = 0.034$ vs. control), increased heart weight to tibial length ratio (HW/TL) and α / β MHC. In contrast, injection of AAV9-IncExACT1 increased cardiac IncExACT1 (6-fold at 5 weeks) increased HW/TL as well as β MHC and ANP. Further, IncExACT1 acted as a sponge for microRNA-222 (a microRNA we previously reported is necessary for physiological cardiac growth) thereby increasing expression of microRNA-222 targets, including p27, a cell cycle inhibitor protein. Moreover, GapmeR injection reduced TAC-induced increase of interventricular septal end diastole (IVSd) and left Ventricular Posterior Wall Dimensions (LVPWd) without affecting FS. We conclude that inhibition of IncExACT1 is sufficient to induce physiological hypertrophy and protect against pathological hypertrophy, while induction of IncExACT1 promotes pathological hypertrophy. IncExACT1 appears to mediate these effects, at least in part, by acting as competing endogenous RNA for microRNA-222.

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The Role of Poly(rC)-Binding Protein-1 in Heart Development

Yao Wei Lu, Tiago Fernandes, Haipeng Guo, Xiaoyun Hu, Ramon A Espinoza-Lewis, Da-Zhi Wang, Dept of Cardiology, Boston Children's Hosp, Harvard Medical Sch, Boston, MA

Poly(rC)-Binding Protein-1 (Pcbp1) belongs to the KH homology superfamily of nucleic acid-binding proteins, which has been implicated in a vast array of biological processes such as iron transport, RNA processing, post-transcriptional and translational regulations. Germline deletion of Pcbp1 results in embryonic lethality before embryonic day (E) 8.5. To investigate the role of Pcbp1 in heart development, we generated cardiac-specific conditional deletion of Pcbp1 (Pcbp1-cKO) by crossing the Pcbp1-flox with cTNT-Cre mice. The observed frequencies for Pcbp1-cKO at E12.5 and E16.5 are normal, but no surviving Pcbp1-cKO mice were observed at weaning, suggesting the Pcbp1-cKO is perinatal lethal. E12.5 Pcbp1-cKO hearts are smaller with thin myocardium. At E16.5, Pcbp1 cKO hearts displayed ventricular non-compaction and abnormal ventricular apex formation. To understand molecular mechanisms, we perform RNA sequencing follow by differential gene expression analysis in Pcbp1-cKO hearts at E16.5. We found 241 genes were significantly dysregulated and identified unfolded protein response as a key pathway dysregulated. Furthermore, through alternative splicing analysis, we identified 168 uniquely spliced junctions in 139 genes. Of these, 10 differentially spliced genes are also differentially expressed. Future studies will be performed to better understand the biological function and mechanism of Pcbp1 in the heart. Together, this study suggests Pcbp1 is an important regulator of heart development.

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Cardiomyocyte-derived Mir-200c-3p In Exosomes Affects Endothelial Angiogenic Capacity And Impairs Cardiac Function

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While cardiomyocytes (CMs) have been the main subject of extensive research, the role of other cardiac cell types, such as fibroblasts and endothelial cells (ECs), received considerable less attention in the pathogenesis of heart failure (HF). MiRNAs have recently emerged as mediators of paracrine signaling by being selectively incorporated in exosomes and exchanged between different cell types. The aim of our study is to investigate a potential paracrine miRNA crosstalk between CMs and cardiac ECs and assess the consequences of such miRNA transfer for cardiac vascular remodeling under pathological conditions. We isolated and characterized exosomes from CMs at baseline or after pathological stimulation with phenylephrine and isoproterenol. Although baseline and stressed CMs secrete miRNA-enriched exosomes at similar rates, comparative analysis of extracellular vesicles from both conditions revealed differential miRNA levels, with miR-200c-3p being highly enriched under stress conditions. Direct transfection of ECs with miR-200c-3p precursor molecules or indirect overexpression through transwell co-culture with stimulated CMs leads to diminished angiogenesis reflected by reduced capacity of ECs to proliferate, migrate, and form tubes. This effect was abrogated when we treated CMs with GW4869, an inhibitor of exosomal biogenesis and release. Next, we tested *in vivo* two doses of specific miR-200c-3p antagomir, Cy3 labelled, to assess specific target of ECs. FACS analysis on cardiac cells derived from the injected mice, confirmed that a low antagomir dose targets ECs whereas, the high dose of antagomir targets all different cardiac cell types. Moreover, when we treated mice subjected transverse aortic constriction (TAC)-induced cardiac pressure overload with miR-200c-3p antagomir, the animals developed a milder hypertrophic phenotype, smaller fibrotic areas, higher amount of capillaries and preserved cardiac ejection fraction, when compared to untreated pressure overloaded mice. Altogether, our results showing exosomal transfer of miR-200c-3p from CMs to ECs indicate the importance of cardiac intercellular communication in the pathophysiology of HF and identify a potential new therapeutic target for intervention strategies.

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Poster Session 2 and Reception

Tuesday, July 30, 2019, 4:30 pm - 7:00 pm

900

Troponin I Tyrosine 26 Phosphorylation Accelerates *in vivo* Myocardial Relaxation

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Heart failure results in depressed cardiac systolic contraction and diastolic relaxation, both of which limit the heart's ability to pump blood. Currently there is no therapy to accelerate the detrimental slowed relaxation in heart failure. A fundamental mechanism of the heart to modulate cardiac relaxation is through serine/threonine kinase-mediated phosphorylation of the contractile regulatory protein troponin I (TnI). Non-receptor tyrosine-specific kinases are expressed in the heart and their activity is altered in disease; yet, the role of tyrosine kinase-mediated phosphorylation to directly modulate cardiac relaxation has not been described. TnI is phosphorylated at tyrosine 26 (Tyr26) in the heart and we previously demonstrated TnI Tyr26 phosphorylation accelerates myofilament deactivation. TnI Tyr26 phosphorylation is therefore the first tyrosine phosphorylation identified to directly modulate cardiac myofilament function, however the effects of tyrosine phosphorylation on *in vivo* function of the heart are unknown. To determine the effect of tyrosine phosphorylation on heart function, we generated a TnI Tyr26 phosphorylation (Tg Y26E) mouse. At the muscle level, myofibrils from Tg Y26E mice exhibit accelerated myofibril relaxation. Echocardiography and pressure-volume hemodynamic measurements demonstrate Tg Y26E mice have enhanced diastolic function evident in accelerated myocardial relaxation, without depressed systolic function or altered morphology. As a whole these data support the phosphorylation of TnI at Tyr26 as a novel tyrosine signaling mechanism to accelerate *in vivo* diastolic function without depressing systolic contraction.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

901

Muscle-specific Stress Fibers Give Rise to Sarcomeres in Cardiomyocytes

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The sarcomere is the contractile unit that drives muscle contraction. Despite its importance, little is understood about how a disordered acto-myosin distribution converts into an ordered contractile array during sarcomere assembly. Here, we take advantage of a sarcomere assembly assay we developed using human induced pluripotent stem cell derived cardiomyocytes to image the formation of sarcomeres, using live-cell high resolution microscopy. Our data show that a population of muscle specific stress fibers (MSFs) are essential sarcomere precursors. Interestingly, MSFs are formed at the leading edge of cells, undergo retrograde flow, and transition into sarcomere-containing myofibrils on the dorsal surface of cardiomyocytes. This is in direct contradiction to a recent report claiming sarcomeres are formed from adhesions on the ventral surface of cardiomyocytes. We have been able to recapitulate this other group's published experiments and definitively show that sarcomeres are not forming from the bottom of the cells or streaming out of adhesions. Instead, our 3D microscopy data shows that this group was imaging sarcomeres which were already formed on the dorsal surface and were traveling to the ventral surface of the cells. After this important clarification, we used our assay to show that the transition of MSFs to sarcomere-containing myofibrils requires formin-mediated actin polymerization and the non-muscle myosin IIA and myosin IIB. We conclude that sarcomeres form by a "templating" mechanism similar to that originally postulated by Howard Holtzer >30 years ago. Furthermore, our data show short β cardiac myosin II filaments are themselves templated by "non-muscle" myosin II filaments. Subsequently, the short β cardiac myosin II filaments grow to form ~1.5 μ m long filaments that then

“stitch” together to form the stack of filaments at the core of the sarcomere (i.e., the A-band). Taken together, our data show that the differentiation of cardiomyocytes from stem cells is a powerful tool for dissecting the mechanisms controlling sarcomere assembly.

D.T. Burnette: None. **A.M. Fenix:** None. **A.C. Neininger:** None. **N. Taneja:** None. **K. Hyde:** None. **M.R. Visetsouk:** None. **R. Garde:** None. **B. Liu:** None. **B.R. Nixon:** None. **A.E. Manalo:** None. **J.R. Becker:** None. **S.W. Crawley:** None. **D.M. Bader:** None. **M.J. Tyska:** None. **Q. Liu:** None. **J.H. Gutzman:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

902

Leveraging Natural Cardiomyocyte Variability to Investigate Downregulation of β -1 Adrenoceptors Following Dobutamine Treatment

J. Alexander Clark, Jonathan Weiss, **Stuart Campbell**, Yale Univ, New Haven, CT

In heart failure, the most consistent effect on the myocardial beta-adrenergic pathway is a downregulation of β -1 adrenoceptors (β 1AR). This loss of receptors impairs the ability of the heart to respond to increases in cardiac demand, and can lead to decreased myocyte function even at rest. As a model of the process that leads to downregulation of β 1AR, we measured intracellular Ca^{2+} transients and unloaded sarcomere shortening in electrically stimulated isolated adult rat cardiomyocytes undergoing 30 minutes of exposure to 40 nM dobutamine. These experiments were facilitated by a custom device containing micro-patterned wells that orient and immobilize the cardiomyocytes, allowing for rapid and consistent measurements over an extended time period. After functional measurements, cells are picked up individually with a computerized micro-aspirator and subjected to single-cell RT-qPCR to quantify abundance of transcripts of interest. In this way, the physiological responses of individual cells to β 1AR stimulus can be correlated to gene expression levels. We found a surprising diversity in the single-cell responses to dobutamine exposure. Although average cardiomyocyte contractility increased significantly, several apparently healthy cells registered no change or even a decrease in sarcomere shortening in the presence of dobutamine. Gene expression was quantified in cardiomyocytes representing a spectrum of sensitivities to dobutamine ($n = 7$). The transcript for β 1AR was significantly downregulated in all cells after 30 minutes, indicating consistent adaptation to chronic stimulus. However, abundance of the gene encoding PKA was negatively correlated with dobutamine-triggered augmentation of Ca^{2+} release (p -value: 0.0201). In other words, PKA expression was reduced in cells that experienced potent increases in Ca^{2+} release after dobutamine treatment. These data suggest the presence of gene regulatory circuits that modulate individual components of the β 1AR pathway independent of overall β 1AR abundance. These circuits may be critical to restoring β 1AR sensitivity in the failing heart.

J. Clark: None. **J. Weiss:** None. **S. Campbell:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

903

Rapamycin Treatment Reduces Myocardial Stiffness and Promotes Cardiomyocyte Relaxation to Restore Diastolic Function in Old Murine Hearts

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Aging is associated with a decline in diastolic function and is a strong risk factor for heart failure with preserved ejection fraction (HFpEF), which has no effective treatment. We previously showed that late-life rapamycin treatment can reverse age-related cardiac dysfunction in mice. However, the mechanisms of the reversal of diastolic dysfunction have not been established. The objective of this study is to determine the mechanisms by which rapamycin reverses age-related diastolic dysfunction.

To study the effects of rapamycin on myocardial stiffness, we assessed the passive length-tension relationship of demembrated trabecular muscle from young, old control and old rapamycin-treated mice. We observed a substantial increase in the slope of the length-tension curve with aging, indicating an age-related increase in myocardial stiffness. The age-related increase in myocardial stiffness was significantly reduced by rapamycin treatment, by a mechanism independent of titin isoform shift. We measured the force-calcium relationship of the demembrated trabeculae and revealed an age-

related increase in Ca^{2+} sensitivity, as indicated by a left-shift of the force-pCa curve, which was partially restored after rapamycin treatment (pCa50: 5.59 ± 0.04 for young controls; 5.76 ± 0.04 for old controls; and 5.65 ± 0.04 for old rapamycin-treated group). To investigate the changes at myofibril level, we assessed kinetic properties of control and rapamycin-treated myofibrils following maximal (pCa 4.0) and submaximal (pCa 5.6) Ca^{2+} activation. Myofibrils from rapamycin-treated mice displayed increased rate of the fast phase of relaxation (kREL, fast) compared to old control at both maximal and submaximal Ca^{2+} levels, potentially due to reduced myofibril Ca^{2+} sensitivity. Studies have detected age-related reductions in expression and activity of SERCA2, which is responsible for SR calcium reuptake during diastole. In this study, we showed that rapamycin increased SERCA2 expression, which may improve cardiomyocyte relaxation. In summary, our results suggest that rapamycin normalizes age-related increases in myocardial stiffness and Ca^{2+} sensitivity and improves myofibril relaxation kinetics, therefore, improving diastolic function.

Y. Chiao: None. **K. Kooiker:** None. **Y. Cheng:** None. **J. Powers:** None. **M. Razumova:** None. **H. Granzier:** None. **M. Regnier:** None. **P. Rabinovitch:** None. **F. Moussavi-Harami:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

904

Disrupted Mechanobiology Links the Molecular and Cellular Phenotypes in Familial Dilated Cardiomyopathy

Michael J Greenberg, Sarah R Clippinger, Paige E Cloonan, Melanie Ernst, Tom Stump, Lina Greenberg, Washington Univ, Saint Louis, MO

Familial dilated cardiomyopathy (DCM) is a leading cause of sudden cardiac death and a major indicator for heart transplant. The disease is frequently caused by mutations of sarcomeric proteins; however, one of the outstanding challenges in the field has been connecting mutation-induced changes in molecular function with the phenotype seen in cardiomyocytes. Many of the DCM mutation-induced changes in contractility at the molecular scale are subtle, begging the question of what other factors could link molecular-scale changes in contractile proteins with the cellular phenotype. We hypothesized that disease-causing mutations of sarcomeric proteins would likely affect not only contraction, but also how cardiomyocytes sense and respond to changes in their mechanical environment associated with aging and disease. To test this hypothesis, we studied the molecular and cellular consequences of a DCM mutation in troponin-T, deltaK210. We determined the molecular mechanism of deltaK210 using biochemical and biophysical tools, and then we used computational modeling to predict that the mutation should reduce the force per sarcomere in cells. In mutant human stem cell derived cardiomyocytes, we found that deltaK210 not only reduces contractility, but also causes cellular hypertrophy and impairs cardiomyocytes' ability to adapt to changes in substrate stiffness (e.g., heart tissue fibrosis that occurs with aging and disease). These results link the molecular and cellular phenotypes and implicate impaired mechanosensing as an under-appreciated mechanism in the pathogenesis and progression of dilated cardiomyopathies. These results also have important implications for our understanding of multiple heart diseases and the design of precision therapeutics.

M.J. Greenberg: None. **S.R. Clippinger:** None. **P.E. Cloonan:** None. **M. Ernst:** None. **T. Stump:** None. **L. Greenberg:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

905

Length Dependent Activation in Porcine Cardiac Myofilaments is Modulated by Mavacamten

Marcus Henze, Myokardia Inc, South San Francisco, CA; Weikang Ma, Illinois Inst of Technology, Chicago, IL; Fiona Wong, Myokardia Inc, South San Francisco, CA; Henry Gong, Illinois Inst of Technology, Chicago, IL; Robert Anderson, Carlos del Rio, Myokardia Inc, South San Francisco, CA; **Thomas Charles Irving**, Illinois Inst of Technology, Chicago, IL

Length dependent activation (LDA) is a property of muscle where increased sarcomere length (SL) leads to increased force of contraction. Despite its key role in both normal and pathological states, the molecular mechanisms underlying LDA are not understood. Previous studies suggest that increased titin-based passive tension at longer SL triggers structural changes in the troponin complex leading to increased Ca^{2+} sensitivity. Stretch also appears to release myosin heads from the OFF or super-relaxed state (SRX) increasing the number of available heads for cross-bridge formation, and, hence, maximum force. The small molecule inhibitor of myosin, mavacamten (mava) has been shown to stabilize the SRX state. Here, we used both stretch and mava on permeabilized porcine myocardium for force/pCa measurements coupled with x-ray diffraction to

interrogate the mechanisms underlying LDA. Increasing SL from 2.0 to 2.3 μm increases Ca^{2+} sensitivity and elevates both diastolic and maximal tension (i.e. LDA). Mava abolished LDA while blunting the passive stiffness/tension relationship, suggesting increased compliance. The equatorial x-ray reflection intensity ratio, $I_{1,1}/I_{1,0}$, increases with stretch, indicating increased myosin head displacement towards actin. Stretch also decreases the intensities of both the m3 meridional and the MyBPC reflections, suggesting stretch-induced disordering of the thick-filament. Surprisingly, these findings in pig skinned muscle are opposite to those previously reported in intact rat muscle despite robust LDA in both preparations. Consistent with previous studies, stretch increases thick filament strain (indicated by changes in the m6 meridional spacing) and changes in the structure of the troponin complex (indicated by increased intensity of the third order troponin reflection), suggesting that the mechanism for increased Ca^{2+} sensitivity are similar in both muscle systems. Interestingly, all diffraction pattern changes that occurred with stretch moved in the opposite direction in the presence of mava. Together, these data indicate that mava is not only capable of stabilizing the SRX state of myosin, but can modulate inter-filament interactions responsible for increasing Ca^{2+} sensitivity and force.

M. Henze: 1. Employment; Significant; Myokardia Incorporated. **W. Ma:** None. **F. Wong:** 1. Employment; Significant; Myokardia Incorporated. **H. Gong:** None. **R. Anderson:** 1. Employment; Significant; Myokardia Inc. **C. del Rio:** 1. Employment; Significant; Myokardia Incorporated. **T.C. Irving:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

906

Age-Dependent Modifications in Cardiac Myofilament Proteins are Graded by Overall Health, Measured as Frailty, in Aging Male and Female C57BL/6 Mice

Alice Kane, Harvard Medical Sch, Boston, MA; Elise S Bisset, Kaitlyn Keller, Dalhousie Univ, Halifax, NS, Canada; W Glen Pyle, Univ of Guelph, Guelph, ON, Canada; Susan E Howlett, Dalhousie Univ, Halifax, NS, Canada

Previously, we showed that the age-dependent decline in ventricular function in male mice is proportional to overall health, as quantified with a frailty index (FI) tool. This tool is based on the accumulation of deficits in health across many systems, but not the cardiovascular system *per se*. Here we investigated whether frailty grades age-dependent modifications in myofilaments in young and aged mice of both sexes. Myofilaments were isolated from ventricles of adult (12-13 mos; n=5 males, 5 females) and aged (22-23 mos; n=6 males, 5 females) mice. FI scores were obtained from all mice. Actomyosin Mg-ATPase activity as a function of increasing calcium concentration and phosphorylation of major myofilament proteins were quantified and compared between groups. Results showed that myofilament calcium sensitivity (EC_{50} values) was similar regardless of age or sex and was not affected by FI score. Maximal actomyosin Mg-ATPase activity increased with age in females, but this was not linked to frailty. Hill coefficients declined with age in males only and were lower than females in the older group ($p < 0.05$). However when all mice were examined, Hill coefficients declined as frailty increased ($r = 0.68$, $p = 0.001$). Total phosphorylation levels of myosin-binding protein C (MyBP-C), desmin, tropomyosin and essential myosin light chain (ELC) increased with age, but only in males. Interestingly, phosphorylation of MyBP-C ($r = 0.49$; $p = 0.03$), desmin ($r = 0.65$; $p = 0.003$), tropomyosin ($r = 0.62$; $p = 0.004$) and ELC ($r = 0.71$; $p = 0.001$) were highly correlated with overall health (FI scores). By contrast there was no link between frailty and phosphorylation of actin, troponin T and troponin I. These findings suggest that poor overall health, quantified in an FI, predicts changes in the myofilaments across the life course in both sexes. This may contribute to changes in cardiac contractile function in frail older adults.

A. Kane: None. **E.S. Bisset:** None. **K. Keller:** None. **W.G. Pyle:** None. **S.E. Howlett:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

907

Understanding Cardiomyocyte Mechanosensing Utilizing CRISPR-Cas9 Based Screening Methods

Feria Ladha, Anthony Pettinato, Univ of Connecticut Sch of Med, Farmington, CT; Ketan Thakar, The Jackson Lab for Genomic Med, Farmington, CT; J. T. Hinson, Univ of Connecticut Sch of Med, Farmington, CT

Heart failure (HF) is a leading cause of premature death that affects approximately 6.5 million patients in the United States and costs over \$30 billion annually. HF is a complex disease that involves genetic and environmental factors, and is characterized by gradual deterioration of cardiac pump function. A key process implicated in the development of HF is mechanotransduction—the ability of cardiomyocytes to sense and respond to mechanical forces. While

mechanotransduction signaling may play an adaptive role in response to changes in mechanical load, this process can become maladaptive, and ultimately lead to pathological hypertrophy and eventual HF. Gaining insight into cardiac mechanotransduction would solidify our understanding of how cardiomyocytes sense mechanical perturbations and will provide novel targets for the prevention and management of cardiomyopathy and HF.

To study perturbations in cardiomyocyte mechanosensing, we have developed a B-type natriuretic peptide (BNP) reporter assay in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), which uses bright red fluorescent protein (tdTomato) to parallel endogenous BNP expression. Our reporter has been validated as a robust, scalable, and quantitative molecular marker of sarcomere function (inside-out) and substrate stiffness (outside-in). We have also generated a custom CRISPR guide RNA (gRNA) library targeting 75 extrinsic and intrinsic components of the cardiomyocyte mechanotransduction apparatus, enabling efficient analysis of single gene knockouts in our BNP reporter and providing a novel high-throughput model for interrogating the mechanical regulation of BNP expression. These gene candidates will be further tested in an *in vitro* HF model to assess functional response and the potential for phenotypic correction.

F. Ladha: None. **A. Pettinato:** None. **K. Thakar:** None. **J.T. Hinson:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

908

Mechanism of Calcium Sensitivity Modulation of Cardiac Troponin C by Small Molecules Illuminated by Umbrella Sampling Simulations

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Cardiac troponin C (cTnC) binds intracellular calcium and subsequently cardiac troponin I (cTnI), initiating cardiac muscle contraction. Due to its role in contraction, cTnC has been a therapeutic target in the search for small molecules to treat cardiomyopathies that interfere with normal muscle contraction. Structural studies have shown the binding location of small molecules such as bepridil, dfbp-o, 3-methyldiphenylamine (DPA) and W7 to be a hydrophobic pocket in the regulatory domain of cTnC (cNTnC) but have not shown the influence of these small molecules on the dynamics of opening this domain. Here we describe an application of an umbrella sampling method used to elucidate the impact these calcium sensitivity modulators have on the free energy of cTnC hydrophobic patch opening. We found that all these molecules lowered the free energy of opening in the absence of the cTnI, with bepridil facilitating the least endergonic transformation. In the presence of cTnI, however, we saw a stabilization of the open configuration due to DPA and dfbp-o binding, and a destabilization of the open configuration imparted by bepridil and W7. Predicted poor binding molecule NSC34337 left the hydrophobic pocket in conventional MD simulations suggesting that only hydrophobic patch binders stabilized the open conformation. Additionally, differences in the free energy of hydrophobic patch opening of hypertrophic (HCM) and dilated cardiomyopathy (DCM) cTnC systems were investigated. Molecular dynamics and umbrella sampling simulations revealed a lower free energy of opening for the HCM mutations A8V and A31S, as well as the calcium-sensitizing mutation L48Q. The DCM mutations, Y5H, Q50R, and E59D/D75Y, all exhibited a higher free energy of opening. In conclusion, our developed simulation protocol presents a novel approach to study calcium sensitivity modulation by small molecules and mutations and facilitates a molecular understanding of cardiac muscle contraction.

S. Lindert: None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

909

Determining the Role of the Co-chaperone BAG3 at the Cardiac Myofilament

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Bcl-2-associated athanogene 3 (BAG3) is an adaptor protein often associated with protein quality control. In the heart, decreased BAG3 expression is linked to dilated cardiomyopathy. Mutations to BAG3 can result in myofibrillar disarray, indicating a possible role at the sarcomere. One previous study found BAG3 has a role in maintaining sarcomere structure, however this was in neonatal myocytes. Whether BAG3 has any role in the myofilament of the adult myocyte is unknown. To determine if BAG3 is present in the cardiac sarcomere and identify the myofilament-BAG3 interactome, we used co-immunoprecipitation of the myofilament fraction from mouse LV and mass spectrometry. This revealed a robust interaction of

BAG3 with the myofilament. Furthermore, BAG3 interacted with a heat shock protein (HSPB8) known to assist in the removal of protein aggregates, which are abundant after MI. To determine the mechanistic role of BAG3, we overexpressed the protein in mice hearts using adeno-associated virus 9. At baseline, overexpression caused a slight decrease in skinned myocyte calcium sensitivity compared to WT. This effect may be due to BAG3's interaction with β -adrenergic receptors, which can cause PKA phosphorylation of thin filament proteins known to reduce calcium sensitivity. Preliminary data support this. We then exposed these mice to left anterior descending coronary artery ligation to induce MI. Compared to shams, MI decreased maximum calcium activated force (F_{max}) in the WT mice, but BAG3 overexpression restored F_{max} to near sham levels. We also studied the functional impact of a known disease-causing mutation discovered in humans, P209L. Transgenic overexpression of P209L BAG3 had no effect on F_{max} , calcium sensitivity, or cooperativity, suggesting the mutation may not interfere with domains in BAG3 required for its myofilament role. This work is the first to show a functional role for BAG3 in the adult myofilament. However, our findings reveal a complex interaction in which the effect of altered BAG3 expression varies with context. The P209L mutation findings suggest that not all BAG3 mutations have shared phenotypes, a conclusion supported by recent clinical work. Further research is necessary to understand BAG3's myofilament role in health and disease.

T.G. Martin: None. **V. Myers:** None. **M. Willis:** None. **A. Feldman:** None. **J.A. Kirk:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

910

Identification of Novel Cardiac Sarcomere Interactions Using BioID Proximity-labeling

Anthony M PETTINATO, Ferial Ladha, Univ of Connecticut Sch of Med, Farmington, CT; Ketan Thakar, The Jackson Lab for Genomic Med, Farmington, CT; Travis Hinson, Univ of Connecticut Sch of Med, Farmington, CT

Mutations in components of the sarcomere, the contractile unit of cardiomyocytes, are a leading cause of genetic cardiomyopathies, such as dilated cardiomyopathy (DCM), which is an important contributor to heart failure burden. Using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), our work has previously shown that DCM-causing mutations in titin, a major structural and functional component of the sarcomere, lead to diminished cardiac force production and impaired sarcomerogenesis. A classical model of sarcomerogenesis suggests that sarcomere assembly begins with pre-myofibrils containing beaded Z-disks composed of alpha-actinin, actin, and non-muscle myosin, with further assembly marked by addition of muscle myosin and titin. Once assembled, sarcomeres exhibit linear Z-disks and distinct protein markers. We are interested in understanding this stepwise process by probing sarcomere protein-protein interactions, with the objective of identifying novel developmental mediators and structural components of the sarcomere. More specifically, we would like to identify proteins that interact or localize near alpha-actinin at the Z-disk of the sarcomere. To do this, we have combined CRISPR/Cas9 genome-editing with BioID proximity-labeling to produce isogenic iPSC-CMs that express alpha-actinin fused with BirA, a promiscuous biotin ligase that biotinylates vicinal proteins. We have also generated a sarcomere-deficient iPSC-CM model that can readily reform sarcomeres on-demand, which we will use to further understand stage-specific interactions of sarcomere structure and development. Our results will not only provide novel insights into human sarcomere biology, but may also uncover novel targets for heart failure drug development.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

911

New Promoters to Improve the Efficiency of Cardiac Gene Therapies Using 2 Deoxy-ATP

Kalen Zeeh Robeson, Farid Moussavi-Harami, Jennifer Davis, Michal Regnier, Univ of Washington, Seattle, WA

Traditional inotropes work by altering intracellular calcium levels but have many off-target effects. Myosin activators have shown promise for treating the increasing number of heart failure patients today, with one drug in phase three clinical trials. These drugs work by increasing the affinity of myosin for actin. Our lab has demonstrated that the small molecule 2 deoxy-ATP (dATP) can improve both myosin binding to and myosin release from actin. We have also demonstrated the efficacy of a cardiac targeted gene therapy to improve heart function via increasing intracellular dATP levels. This was done by overexpressing ribonucleotide reductase (RNR)—the rate-limiting enzyme in de novo dNTP synthesis. This cardiac therapy, using the cardiac troponin T (cTnT) promoter, was effective in a mouse model but provided variable results in a large animal

model due to delivery challenges. Therefore, we are now investigating alternative promoters to deliver dATP to cardiomyocytes and/or other striated muscles. Here we present our investigation of the CK8 promoter for striated muscle specific delivery of RNR. We report that the CK8 promoter was able to produce a greater than fourfold increase in mouse ventricular tissue dATP levels above that seen with the cTnT promoter (2.0 to 8.4 pmol/mg; $P < 0.01$). RNR was also delivered to skeletal muscle with a twofold increase in gastrocnemius dATP levels (0.62 to 1.31 pmol/mg; $P < 0.01$). We have also been investigating cardiac fibroblasts as a route to deliver dATP to cardiomyocytes. We have shown that *in vitro* fibroblasts can transmit dATP to coupled cardiomyocytes through gap junctions, indicating that overexpressing RNR in cardiac fibroblasts could increase dATP levels in both cell types. Previous research has shown that proliferating fibroblasts have increased levels of dATP, and preliminary results in our lab show that differentiated myofibroblasts have decreased levels of dATP. We therefore hypothesize that increased dATP in cardiac fibroblasts will not only improve contraction in coupled cardiomyocytes but may also inhibit fibroblast transdifferentiation into collagen producing myofibroblasts.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

915

Dysregulation of the Myosin and Myosin Binding Protein-C interaction in Hypertrophic Cardiomyopathy

Rohit R Singh, James W McNamara, Sakthivel Sadayappan, Univ of Cincinnati, Cincinnati, OH

Rationale: Affecting 1 in 300 individuals, hypertrophic cardiomyopathy (HCM) is a genetic heart disease characterized by left ventricular hypertrophy, myocardial disarray, and sudden cardiac death. Often, HCM is associated with myocardial hypercontractility. The subfragment-2 (S2) of beta-myosin heavy chain contains a cluster of missense and deletion mutations associated with severe HCM. Interestingly, myosin S2 interacts with the C0-C2 region of cardiac myosin binding protein C (cMyBP-C) in a phosphorylation-dependent manner to regulate sarcomere contractility. However, the nature of myosin S2 and cMyBP-C interactions and the mechanism(s) by which mutations in myosin S2 cause HCM remain to be elucidated. **Objective:** To determine whether mutations in myosin S2 weaken its interaction with cMyBP-C, resulting in enhanced myofilament contractility. **Methods and Results:** Myosin S2 proteins (126 amino acids) containing three clinically relevant mutations (R870H, E924K, E930del, or wild type), and recombinant C0-C2 region of cMyBP-C were produced and purified by metal affinity chromatography. Solid-phase binding assays and isothermal calorimetry experiments revealed a significantly dampened binding to C0-C2 for these three mutants in myosin S2 (30% lower than wild type, $p < 0.002$), suggesting that mutations in S2 regions reduce their bindings to cMyBP-C. Conversely, upon protein kinase A phosphorylation of C0C2, these S2 mutants displayed an increased affinity to cMyBP-C, an effect opposite that of wild-type S2. Structural analyses of these mutations within myosin S2 are predicted to reduce the alpha-helical content, thereby reducing stability of the critical coiled-coil structure. **Conclusions:** Mutations in myosin S2 result in reduced binding to cMyBP-C. Functionally, this would result in a greater attachment of cross-bridges, and thus enhance myofilament contractility. Strikingly, these mutations increase their affinity to cMyBP-C upon phosphorylation, demonstrating fundamental changes to the regulation of contractile function.

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916

Evidence of Cardiac Myosin Dephosphorylation by Two Distinct Pools of Myosin Light Chain Phosphatases

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Muscle myosins are hexameric proteins comprised of two each of the heavy chain, essential light chain, and regulatory light chain (RLC). In normal beating hearts, cardiac myosin RLC is phosphorylated, and the extents maintained at ~0.45 mol phosphate/mol RLC by the balanced activities of cardiac-specific myosin light chain kinases (cMLCK) and phosphatases (cMLCP). Hearts in failure have decreased cMLCK expression and RLC phosphorylation, indicating maintenance of near half-maximal RLC phosphorylation levels is necessary for normal function. However, how cMLCK and cMLCP activities are regulated is unclear. MLCP has been biochemically defined as a trimeric protein comprised of a regulatory myosin target subunit (MYPT1 in smooth muscles, and MYPT2 in striated muscles), a catalytic subunit PP1c β , and a small subunit M20.

Interrogations into the specificity and regulatory activity of smooth muscle MLCP using conditional MYPT1 knockout animals have provided evidence of MYPT1-independent RLC phosphatase activity, which contradicts long-standing dogma. Extending upon recent studies of PP1c β knockout in smooth muscles, we knocked out PP1c β from the cardiac muscle to test the hypothesis that a distinct MYPT-independent pool of PP1c β activity directly contributes to cRLC dephosphorylation. Two weeks post gene-ablation, PP1c β protein was reduced by >60%, and cRLC phosphorylation in beating hearts was increased to 0.6 mol phosphate/mol cRLC, without significant changes in MYPT1 or MYPT2 expression. Additionally, we measured MYPT-dependent and MYPT-independent PP1c β phosphatase activities toward cRLC dephosphorylation in mouse cardiac myofibrils. These studies provide further evidence that cardiac RLC is dephosphorylated by two distinct pools of MLCP activities.

A.N. Chang: None.

Poster Session 3 and Reception

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917

Actively Blocking Nuclear Localization of G Protein-Couple Receptor 5 Blunts Hypertrophy and Heart Failure After TAC

Ryan Coleman, Eric W Barr, Kurt Chuprun, Walter J Koch, Lewis Katz Sch of Med, Philadelphia, PA

Introduction: The induction of heart failure (HF) after aortic banding (TAC) in mice encompasses aberrations in gene regulation, leading to maladaptive cardiac hypertrophy and contractile dysfunction. Previously, our lab has shown that the nuclear targeting of GRK5, which is required for maladaptive hypertrophy, is controlled by its N-terminal (NT) calmodulin-binding domain.

Hypothesis: We hypothesize that expression of a peptide encoding the NT of GRK5 will act as a GRK5-calmodulin inhibitor preventing the nuclear accumulation of GRK5 and inhibit its pathological activities.

Methods: Neonatal rat ventricular cardiomyocytes (NRCM) overexpressing GRK5-NT or control virus were subjected to α -adrenergic stress with 50 μ M phenylephrine (PE) or vehicle for 30 minutes. Subcellular fractionation and western blotting were used to detect nuclear GRK5. Novel cardiac-specific transgenic mice were generated expressing HA-tagged GRK5-NT and these mice along with their control mice were characterized 8 weeks after TAC or sham surgery. Echocardiography, fibrosis, and lung and heart weight to tibia length metrics were used to evaluate hypertrophy and heart failure response post-TAC. Other cohorts were subjected to TAC for 1 week followed by Western blotting to assess acute signaling and nuclear accumulation of GRK5.

Results: In vitro, expression of the GRK5-NT peptide reduced PE-induced nuclear accumulation of GRK5. In both NRCM and tissue, the GRK5-NT peptide was found to bind to calmodulin, which disrupts the endogenous calmodulin-GRK5 interaction. GRK5-NT expression abrogates nuclear GRK5 accumulation following hypertrophic stress in vitro and in vivo. Following 8 weeks of TAC, cardiac function, hypertrophy, and pulmonary congestion were significantly attenuated in GRK5-NT mice compared to NLC mice.

Conclusion: We show that the GRK5-NT peptide binds to calmodulin and prevents nuclear accumulation of GRK5 following hypertrophic stress. Our in vivo data show that cardiac-restricted expression of GRK5-NT prevents HF by partially restoring cardiac dysfunction, hypertrophy, and pulmonary congestion following 8 weeks of TAC. Further in vitro work will reveal the calmodulin-dependence of GRK5-NT and the peptide's effects on G-protein signaling.

R. Coleman: None. **E.W. Barr:** None. **K. Chuprun:** None. **W.J. Koch:** None.

Poster Session 3 and Reception

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918

Pre-B-cell Leukemia Homeobox Interacting Protein 1 is a Novel Regulator of Growth Signaling in the Heart

Kelly M Grimes, Anna Pyo, Jeffery D Molkenin, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH

The cellular mechanisms that allow for cardiomyocyte growth in different directions may yield important information about the development of hypertrophic and dilated cardiomyopathies, diseases which include the widening and lengthening growth of cardiomyocytes, respectively. We have previously found that genetic manipulations of the mitogen-activated protein kinase kinase 1 (MEK1) and extracellular signal-related kinase 1/2 (ERK1/2) signaling pathway cause cardiac hypertrophy and cardiomyocyte widening when the pathway is activated or dilated cardiomyopathy and cardiomyocyte lengthening when it is inhibited. The downstream effectors of the directional growth response controlled by these kinases have yet to be studied.

Therefore, an unbiased phosphoproteomic screen was conducted on cardiomyocytes subjected to activation or inhibition of MEK1-ERK1/2 signaling. After validation of the screening hits with western and Phos-tag blots, Pre-B-cell leukemia homeobox interacting protein 1 (PBXIP1) emerged as an interesting target. Although it is known as a microtubule binding protein and regulator of proliferation in cancer cells, its function has never before been studied in the heart. Inhibition of MEK1-ERK1/2 increases phosphorylation of PBXIP1. However, activation of the pathway increases PBXIP1 protein expression, and as such we sought to determine the effects of PBXIP1 overexpression in the heart via adeno-associated virus 9 (AAV9) treatment. At four months of age, mice overexpressing PBXIP1 have significantly larger hearts than controls without a change in cardiac contractility. Intriguingly, histological analysis showed that cardiomyocytes highly infected with PBXIP1 AAV9 are significantly smaller in size compared to uninfected cells. Current studies are manipulating the PBXIP1 phosphorylation sites identified in the phosphoproteomic screen to determine the exact roles they may play in the cardiac growth response.

K.M. Grimes: None. **A. Pyo:** None. **J.D. Molkentin:** None.

Poster Session 3 and Reception

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919

Intercalated Disk Protein Xin-beta is Required for the Hippo/YAP Signaling in the Heart

Haipeng Guo, Yao Wei Lu, Zhiqiang Lin, Zhanpeng Huang, Jianming Liu, Xiaoyun Hu, Yi Wang, Tiago Fernandes, Yuxuan Guo, Dept of Cardiology, Boston Children's Hosp, Harvard Medical Sch, Boston, MA; Jenny L.-C Lin, Jim J.-C Lin, Dept of Biology, Univ of Iowa, Iowa City, IA; Francisco Naya, Dept of Biology, Boston Univ, Boston, MA; William T Pu, Da-Zhi Wang, Dept of Cardiology, Boston Children's Hosp, Harvard Medical Sch, Boston, MA

Cardiovascular diseases continue to be a leading cause of death and disability. Despite this alarming fact, there is lack of effectual treatment and the molecular mechanisms underlying these devastating diseases remain elusive. Intercalated disk (ICD) is not only essential for the integrity of cardiomyocytes to withstand the strong mechanical forces imposed by constant heart beating; it is also critical for the dissemination of the electrical signal that initiates cardiac contraction. However, relatively less is known about how ICD transmits pathophysiological signals in cardiomyocytes to modulate gene expression and cardiac function. The Xin- α and Xin- β , belongs to a family of Xin-repeat containing proteins, are primarily located at the ICD of adult cardiomyocytes. They play an important role during heart development. Interestingly, the human homologue of the mouse Xin- β gene was mapped to a locus associated with cardiomyopathy; most importantly, mutations in Xin- α and Xin- β have been found in patients with cardiomyopathy, underscoring the importance of Xin genes to cardiac disease. Our previous studies have shown that Xin- β KO mice die postnatally with severe cardiomyopathy. Here, we report that loss of Xin- β results in defect in cardiomyocyte proliferation. Unbiased transcriptome analyses reveal that gene program related to the Hippo/Yap pathway is altered, leading to the hypothesis that Xin- β regulates cardiomyocyte proliferation and cardiac function by modulating the Hippo/Yap signaling. We identify physical and genetic interaction between Xin- β and components of the Hippo/Yap pathway. We further show that the expression of Xin- β is transcriptionally regulated by Mef2a, Yap and Tead1, suggesting the presence of a Xin- β /Yap feedback regulatory network in the heart. Strikingly, cardiac-specific overexpression of Yap markedly rescues cardiac defects in Xin- β KO mice; indicating a functional and genetic interaction between Xin- β and Yap. Together, our study reveals a novel molecular mechanism by which the ICD protein Xin- β modulates important pathophysiological Hippo/Yap signals to control heart development and cardiac function. Molecules uncovered here will become candidate targets for therapeutic treatment of cardiac disease.

H. Guo: None. **Y. Lu:** None. **Z. Lin:** None. **Z. Huang:** None. **J. Liu:** None. **X. Hu:** None. **Y. Wang:** None. **T. Fernandes:** None. **Y. Guo:** None. **J. Lin:** None. **J. Lin:** None. **F. Naya:** None. **W. Pu:** None. **D. Wang:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

920

Mutual Potentiation Between Myofibril Assembly and Serum Response Factor in Cardiomyocyte Maturation

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Cardiomyocyte (CM) maturation is characterized by transcriptional, morphological and functional specializations that are essential for robust and sustained CM contractions throughout lifetime. The signal networks that govern CM maturation

remain poorly defined, which obscures the role of CM maturation in inherited cardiomyopathies and myocardial regeneration and impairs efforts to engineer mature cardiac tissues *in vitro*. Our prior studies established the transcription factor serum response factor (SRF) as a key regulator of CM maturation: SRF regulates major CM maturation events including myofibril expansion, mitochondria biogenesis, transverse-tubule formation, and cellular hypertrophy. Myofibrillar genes were identified as direct SRF downstream targets that are required for other aspects of CM maturation. To further understand the role of myofibrils in CM maturation, here we report the generation and investigation of a floxed allele of *Actn2* in mice, which encodes a central component of sarcomere Z-lines. We applied to these mice a low dose of adeno-associated virus that expressed Cre recombinase specifically in neonatal CMs to generate hearts with mosaic *Actn2* mutation. This approach circumvented the confounding secondary effects of cardiac dysfunction in *Actn2* mutants and revealed cell-autonomous gene functions. Strikingly, *Actn2* ablation triggered dramatic transcriptional dysregulation in addition to the expected myofibrillar disassembly phenotypes in CMs, which strongly correlated with observations in SRF-depleted CMs. *Actn2* mutation increased the amount of monomeric actin in CMs, which perturbed the nuclear localization of SRF cofactors MRTF-A/B. Overexpression of a dominant-negative MRTF-A isoform was sufficient to recapitulate the transcriptional and morphological defects in *Actn2* or *Srf* mutant CMs. Together, these data demonstrate mutual potentiation between myofibril assembly and MRTF-A/B-SRF signaling in CM maturation. This positive feedback loop underlies a novel mechanism by which mechanical forces regulate CM maturation, disruption of which likely contributes to cardiomyopathies caused by sarcomere gene mutations.

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Poster Session 3 and Reception

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Carvacrol Protects Against Diabetes-induced Hypercontractility in the Aorta Through Activating PI3k/akt Pathway

Yun Liu, Kai-Ting Ma, Jie Wei, Yun-Pei Mai, Xiao-Xia Qiu, Han Wei, Ning Hou, Jian-Dong Luo, Guangzhou Medical Univ, Guangzhou, China

Vascular complications induced by diabetes constitute the principal cause of morbidity and mortality in diabetic patients. It's reported that carvacrol (CAR) possess a wide range of biological activities. The effect of CAR on diabetes-induced vasculopathy remains unknown. In this study, diabetic mice were created by intraperitoneal injection of streptozotocin to investigate whether CAR provided a protective effect against diabetes-induced vasculopathy and the underlying mechanisms. We found that CAR decreased blood glucose level of diabetic mice. Moreover, CAR ameliorated diabetes-induced aorta morphological alteration, evidenced by increased thickness of intima-media width and increased number of vascular smooth muscle cell (VSMC) layer. Further studies revealed that CAR inhibited the hypercontractility in the aorta of diabetic mice and VSMC in response to hyperglycemia, evidenced by decreased expression of smooth muscle (SM)- α -actin, and increased expression of Ki67 and PCNA. Furthermore, PI3K/Akt signal pathway was inhibited in the aorta of diabetic mice and VSMC in response to hyperglycemia, while CAR treatment activated PI3K/Akt signaling pathway. In conclusion, our results strongly suggested that CAR played a protective role in diabetes-induced aorta hypercontractility, possibly by activating PI3K/Akt signaling pathway. CAR is a potential drug for the treatment of diabetic vasculopathy.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

922

Cardiac-specific Deletion of Orai3 Channel Causes Dilated Cardiomyopathy

Salvatore Mancarella, Samuel Kamatham, Univ of Tennessee, Memphis, TN

Background. Calcium (Ca²⁺) signalling is a vital regulator of cardiac myocyte function. Defective cellular calcium handling is widely recognized as a significant patho-physiological event in the contractile dysfunction of the failing heart. Orai3 is a newly discovered calcium channels that participate in the voltage-independent calcium currents in the myocardium. However, its role in the heart remain uncharacterized. Methods and Results. Here, we show that disruption of Orai3 function in murine cardiomyocytes leads to decompensated dilated cardiomyopathy. Echocardiographic analysis supported by histological examinations revealed that 4-month-old Orai3 knockout (KO) mice have signs of dilated cardiomyopathy denoted by a loss of EF and a thinner left ventricular posterior wall and septum, with significant fibrosis, whereas in the younger animals only a

thinner septum could be observed. In both genders, most mice died within six months after birth. Myocardial samples that underwent electron microscopy and immunohistochemical analysis showed myofilament changes in Orai3 KO mice with misexpression of cardiac contractile proteins and profound sarcomere disarray. Transverse aortic constriction (TAC) was used as a chronic stressor on the younger mice to determine whether the ability to compensate against a pathologic insult is compromised in the Orai3 KO heart. Orai3 KO mice presented with significantly reduced systolic function and ventricular dilation that deteriorated into congestive HF within 4 weeks post-surgery, while constricted WT hearts remained well-adapted throughout. Conclusions. Thus, our results identify Orai3 critical role in adult cardiac function and demonstrate a novel cardioprotective role of Orai3 against cardiac pressure overload-induced heart failure.

S. Mancarella: None. **S. Kamatham:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

923

Pharmacological Activation of Ampk With a Direct Pan-ampk Activator Results in a Worsening of a Hf Phenotype in Mice

Russell A Miller 02026, Kimberly Cameron, Declan Flynn, Pfizer, Inc, Cambridge, MA

The absence of effective therapeutic treatments that can restore function in patients with heart failure necessitates the identification of new mechanisms and strategies to improve outcomes. Strategies that directly target the cardiomyocyte and have potential to increase metabolic capacity hold promise as treatment modalities. We sought to evaluate direct activators of the AMP-Activated Protein Kinase (AMPK) in a model of HF to test for improvements. The pan-AMPK activator PF-739 resulted in activation of AMPK, phosphorylation of the downstream target ACC, and increases in glucose uptake after a single dose in vivo. PF-739 resulted in an increase in the gene expression of relevant transcriptional regulators PGC1 α and NR4a1, suggesting potential for beneficial effects on substrate metabolism in a HF model. PF-739 and the ACE inhibitor Enalapril were used in a combined TAC/MI model of mouse HF. 4 weeks after injury the animals treated with PF-739 had an increase in end systolic and diastolic volume and ejection fraction compared to vehicle animals. Enalapril treatment resulted in modest improvements in echo heart assessments and a reduction in heart weight compared to vehicle animals. To understand the mechanism for impaired heart function in HF we assessed the impact of PF-739 on systemic blood pressure, reasoning this could impact HF progression in the model. PF-739 caused an increase in blood pressure after acute dosing in mice and rats. A small molecule analogue of PF-739 differing in one methyl group with dramatically reduced AMPK activity was shown to have no detectable blood pressure effect, suggesting the blood pressure effects are related to AMPK activity. Contrary to expectation, treatment with PF-739 caused impairment in heart function in a mouse model of HF, an effect that may be the result of a systemic blood pressure effect of the compound.

R.A. Miller: 1. Employment; Significant; Pfizer Inc. **K. Cameron:** 1. Employment; Significant; Pfizer. **D. Flynn:** 1. Employment; Significant; Pfizer.

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924

The Role of Protein Kinase C Isoforms in Cardiomyocyte Hypertrophy

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Background: Protein kinase C (PKC) has been linked to cardiomyocyte hypertrophy. However, the exact role of different PKC isoforms in mediating the hypertrophic response remains controversial and both classical and novel isoforms have been suggested to play a critical role. The aim of this study was to characterize the role of PKC isoforms in cardiomyocyte hypertrophy using pharmacological tools and high content screening (HCS).

Methods: Neonatal rat ventricular cardiomyocytes (NRVCs) isolated from 1-3 days old Wistar rats and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) differentiated from iPS(IMR90)-4 line were exposed to endothelin-1 (ET-1) and PKC modulators for 48 h followed by fixing and immunofluorescence staining for nuclei, α -actinin and F-actin. Imaging and morphological analysis of thousands of cells were carried out using automated HCS platform.

Results: NRVCs exposed to ET-1 (100 nM) or PKC agonists (HMI-1b11 at 10 μ M or bryostatin-1 at 10 nM) or inhibitor of

classical PKC isoforms, Gö6976 (1 μ M), showed a hypertrophic phenotype measured by increased area and number of alpha-actinin and F-actin fibers in HCS. In contrast, inhibition of all PKC isoforms with Gö6983 (1 μ M) attenuated ET-1 and PKC agonist-induced hypertrophy similarly to mitogen-activated protein kinase kinase 1/2 (MEK1/2) inhibitor U0126 (10 μ M). In turn, these HCS parameters did not show ET-1 or PKC agonist -induced hypertrophy in hiPSC-CMs. However, qRT-PCR analysis of the expression of *NPPA* and *NPPB* genes showed hypertrophic responses to ET-1 and PKC agonists also in hiPSC-CMs.

Conclusions: These results confirm the central role of PKC in cardiomyocyte hypertrophy and indicate that either classical PKC isoforms have a direct anti-hypertrophic role in cardiomyocytes or, when classical PKCs are inhibited, the balance is moved towards activation of novel isoforms, which mediate the hypertrophic response. Due to the morphological differences between NRVCs and hiPSC-CMs, similar phenotypic analysis readouts cannot be reliably applied to compare hypertrophic responses in these cell types. These findings may help in developing new drugs that target specific PKC isoforms to treat cardiac hypertrophy.

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Poster Session 3 and Reception

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Insulin Inhibits β -adrenergic Receptor Resensitization Through PI3K
Insulin Inhibits β -adrenergic Receptor Resensitization Through PI3K

Anita Sahu, Maradumane Mohan, Sathyamangla V. Naga Prasad, Lerner Res Inst, Cleveland Clinic, Cleveland, OH

Insulin regulates cardiac metabolism, myocyte survival, and cardiac growth. In addition, insulin is also known to modulate beta adrenergic receptors (β ARs) function in the heart regulating cardiac function. β AR function in response to its agonist epinephrine/norepinephrine is regulated by G-protein coupled receptor kinase (GRKs) driven desensitization and protein phosphatase 2A (PP2A) mediated resensitization. Although insulin is known to modulate β AR function through GRKs, less is known about role in insulin in regulating resensitization mechanisms. Phosphoinositide 3-kinase γ (PI3K γ) is a key regulator of resensitization as it inhibits PP2A activity and therefore, we tested whether insulin could mediate β AR dysfunction through inhibition of resensitization. Insulin stimulation of HEK 293 cells stably expressing β_2 ARs resulted in significant β_2 AR phosphorylation. This β_2 AR phosphorylation was completely abolished with use of pharmacologic inhibitors of PI3K wortmannin or LY 294002 and knock-down of PI3K γ showing an essential role for PI3K γ in regulation of β AR function by insulin. Activation of insulin receptor induces downstream signaling via recruitment of the adaptor insulin receptor substrates (IRS). Interestingly, our data suggests that cross talk between the IRs and β_2 ARs are dependent on interaction between PI3K γ , GRK2 and IRS as identified by co-immunoprecipitation studies and use of surface plasmon resonance using purified proteins. Since cardiomyocytes and fibroblasts represent major cell-types in the heart, primary adult cardiomyocytes and cardiac fibroblasts (CFs) were isolated from WT and PI3K γ KO hearts for insulin stimulation. While β_2 AR phosphorylation was completely abolished in primary CFs from PI3K γ KO compared to WT CFs, cardiomyocytes did not show any differences in β_2 AR phosphorylation in response to insulin. These data suggest that PI3K γ may differentially regulate pathways in cardiomyocytes and CFs in the heart indicating the existence of cell-specific signaling mechanisms that may underlie response to insulin and thereby the cardio-metabolic process.

A. Sahu: None. **M. Mohan:** None. **S. Prasad:** None.

Poster Session 3 and Reception

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926

Regulation of Fibrosis by Phospholipase C α 949;in Cardiac Fibroblasts

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Cardiac fibrosis is commonly associated with heart diseases, such as cardiac hypertrophy and myocardial infarction. Upon injury to the heart, cardiac fibroblasts transition to an activated myofibroblast state, driven by TGF β or angiotensin II (AngII) signaling, characterized by expression of α -smooth muscle actin (α SMA). Fibrosis is driven by the activity of both cardiac myofibroblasts and inflammatory cells. Activation of cardiac fibroblasts leads to the induction of pro-inflammatory cytokines that are important for recruitment of immune cells and regulation of cardiac remodeling. Monocyte Chemoattractant Protein (MCP)-1/CCL2 is a chemokine expressed by cardiac myofibroblasts through activation of Nuclear Factor- κ B (NF- κ B) that

leads to recruitment of mononuclear cells. Phospholipase C ϵ (PLC ϵ) is highly expressed in cardiac fibroblasts and is activated by multiple upstream stimuli including GPCRs and RTKs, leading to protein kinase C (PKC) and D (PKD) activation and Calcium release. In this study to elucidate the role of PLC ϵ in neonatal rat cardiac fibroblasts adenovirus encoding for a PLC ϵ -shRNA was used to knockdown PLC ϵ in fibroblasts. Treatment with PLC ϵ -shRNA strongly inhibited the transition to myofibroblasts induced by TGF β as assessed by staining of α SMA. PLC ϵ knockdown did not inhibit the ability of TGF β to induce SMAD nuclear translocation. PLC ϵ depletion also strongly inhibited, basal, Thrombin, and AngII mediated PLC activity in cardiac fibroblasts, indicating PLC ϵ is a dominant regulator of phosphoinositide hydrolysis in these cells. PLC ϵ shRNA also decreased expression of MCP-1 mRNA and reduced the ability of pro-fibrotic stimuli to induce MCP-1 mRNA expression. Knockdown of PLC ϵ or inhibition of PKD with the small molecule inhibitor NB 142-70 reduced the ability of thrombin to activate NF- κ B. These data indicate that PLC signaling, and PLC ϵ in particular, plays an important role in pro-fibrotic processes in cardiac fibroblasts, through both cell autonomous and paracrine mechanisms. Future studies will focus on further elucidating the mechanistic role of PLC ϵ in the transition to myofibroblasts, and will study in *in vivo* models of cardiac fibrosis.

L.M. Brown: None. **A.V. Smrcka:** None.

Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

930

Aerobic Dose Intensity and the Human Exercise Plasma Proteome

James Sawalla Guseh, Timothy W Churchill, Ashish Yeri, Claire Lo, Marcel Brown, Anthony Rosenzweig, Aaron Baggish, Massachusetts General Hosp, Boston, MA

Aerobic exercise confers myriad benefits to human health but the mechanism by which it does so remain incompletely understood. We hypothesize that exercise works in part through circulating protein signaling. Healthy adult men (n, 12) participated in treadmill running sessions at both low (6 mph) and high intensity (maximal effort). We used pre- and post-exercise plasma and a high-throughput aptamer-based assay (SomaScan) to examine the acute impact of exercise on the plasma proteome.

Acute aerobic exercise consistently alters the resting plasma proteome in an intensity-dependent fashion. Of 1,305 proteins assayed 184 (14%) and 598 (46%) change at low and high intensity respectively (FDR $p < 0.05$). 159 protein species (12%) are common to both intensities. Gene ontology analysis revealed enrichment of pathways associated with leukocyte chemotaxis and chylomicron metabolism at low intensity and Wnt signaling, neuronal axonogenesis, and nitric oxide metabolism pathways at high intensity. We used human sequencing data from the GTEX Consortium (Broad, Cambridge MA) to computationally infer the sources of increasing proteins and found major contributions from the cardiovascular, gastrointestinal, and nervous systems. We identified 43 cis-SNPs that approximate the upregulated proteomic response to acute exercise and used Mendelian randomization to infer a causal relationship between the exercise proteome and decreased muscle wasting in a UK Biobank cohort.

Although guidelines present low and high intensity exercise as equivalent alternatives for health, these data suggest that distinct exercise intensities might offer common and distinct exercise benefits.

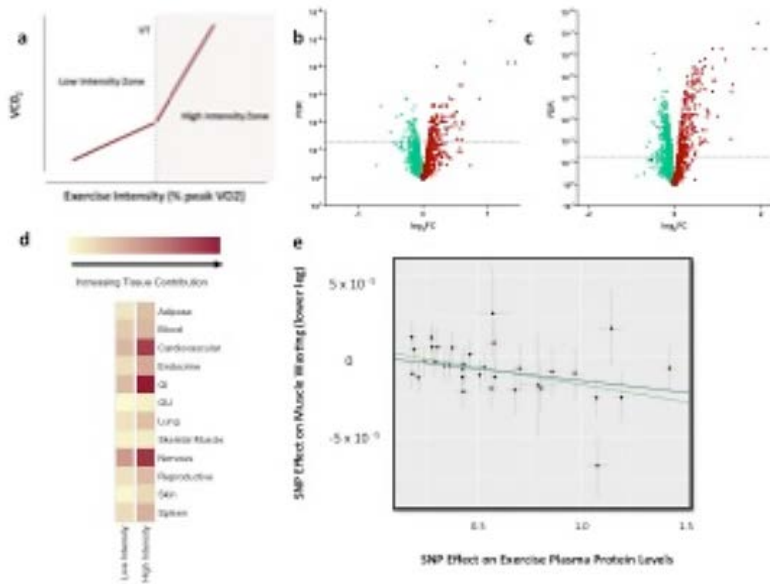


Figure 1. Aerobic Dose Intensity and the Human Exercise Plasma Proteome a, Participants underwent single session 5 mile runs at discrete exercise intensities below their ventilatory thresholds (white zone; low intensity) and above their ventilatory thresholds (red zone, high intensity). Volcano plots reveal the effect on the plasma proteome at low intensity (b) includes 184 increased and 62 decreased proteins. In contrast, at high intensity (c) there were 253 increased and 345 decreased protein species that reflect the acute plasma proteome. d, heat map using computational interference based upon human sequencing data reveal that the dominant contributors of protein species diversity come from the cardiovascular, GI, and nervous systems. e, Mendelian randomization supports the hypothesis that the proteomic effect of exercise attenuates muscle wasting in a UK biobank cohort (IVW p, 7.12×10^{-5})

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Poster Session 3 and Reception

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931

Compensatory Response of Perivascular Adipose Tissue to Vascular Dysfunction in Metabolic Syndrome Rats Involves Apelin

Satomi Kagota, Miho Shimari, Kana Maruyama-Fumoto, Saki Iwata, Kazumasa Shinozuka, Mukogawa Women's Univ, Nishinomiya, Japan

Perivascular adipose tissue (PVAT) regulates vascular homeostasis including vascular tone via release of adipokines. Using SHRSP.Z-*Lep^{fl/fl}/IzmDmcr* (SHRSP.ZF) rats, an animal model of metabolic syndrome (MetS), we have demonstrated that mesenteric PVAT enhances vasodilation under impaired vasodilation, in response to nitric oxide. We have also shown that the compensatory effect of PVAT disappears later in the course of MetS. Apelin is an adipokine that is produced at high levels in obesity and acts as a vasorelaxing factor. In this study, we evaluated the role of apelin in the enhancing effect of mesenteric PVAT on relaxations in SHRSP.ZF rats, by examining whether apelin can enhance vasorelaxation in the mesenteric arteries of SHRSP.ZF rats without PVAT, as it does in those with PVAT. Furthermore, we investigated whether apelin expression changes with increasing age. SHRSP.ZF rats at 17, 20, 23, and 30 weeks old were used. The mesenteric artery and surrounding adipose tissue were isolated from each rat; PVAT samples were isolated from surrounding adipose tissue; and the mRNA transcript level of apelin was examined by quantitative real-time PCR assays. Mesenteric arterial ring preparations with and without PVAT were made, and after a stable contraction was obtained by adding phenylephrine, relaxation was elicited using acetylcholine in the presence or absence of apelin (100 ng/mL, 20 min). There was no significant difference in apelin mRNA levels between 17 and 20 weeks, but there was a decrease at 23 and 30 weeks of age, which was when the enhancing effect of PVAT on vasodilation in SHRSP.ZF rats was impaired. In the presence of apelin, acetylcholine-induced relaxation in arteries without PVAT was significantly increased to the level of the response in arteries with PVAT in SHRSP.ZF rats at 20 weeks. These findings suggest that apelin is involved in the enhancing effect of PVAT in MetS rat mesenteric artery, and decreases in the level is associated with deterioration of PVAT function. Further study to investigate the mechanisms underlying the decrease in apelin production with increasing age in metabolic syndrome is

needed, but the apelin level could be one of the predictors for development of cardiovascular complications in patients with MetS.

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Translated Alternative Isoforms Coincide With Disordered Sequences in the Cardiac Proteome

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Introduction

Alternative splicing is widely implicated in cardiac development and diseases, but few isoform transcripts have ever been empirically observed as translated proteins. We aim to understand how alternative splicing diversifies proteome function.

Method

We show an RNA-seq guided proteomics approach, comprising a custom pipeline of sequence read alignment, splice junction determination, and in silico filtering and translation to identify alternative isoforms across multiple cell types and anatomical regions of the human heart. We further applied the method to a time course study of human iPSC differentiation into cardiomyocytes, collecting isotope-tagged quantification data on a Q-Exactive HF mass spectrometer to examine isoform expression patterns during myocyte differentiation. We cataloged the translated non-canonical isoforms in the heart, and characterized sequence features upon which the empirically observed alternative sequences impinge.

Result

We identified 1,216 distinct non-canonical isoform sequences at 1% FDR, including 143 sequences from 107 genes not found in the comprehensive databases TrEMBL and RefSeq. We observed tissue-specific and differentiation stage-specific differences in isoform expression patterns. The data show a proteome-wide enrichment of translated alternative isoform sequences at intrinsically disordered regions of proteins with implication on isoform function. We observed examples where protein PTMs may be modulated by the exclusion of modification sites via splicing including a novel isoform we found in MYBPC3 which coincides with PKA-mediated phosphorylation implicated in diastolic function regulation in animal models.

Conclusion

This is the first study to combine experimental RNA-seq and proteomics to investigate how splicing impacts the human cardiac proteome. The approach may avail understanding of the functional outcome of splicing and its role in disease mechanisms.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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Integrated Omics Analysis of Diabetic Heart Failure in Human Myocardium

Stephani Page, Lydia Kwee, William Kraus, Phillip White, Adam Devore, Robert Mentz, Duke Univ, Durham, NC; Michael Bristow, Univ of Colorado, Aurora, CO; Carmelo Milano, Dawn Bowles, Olga Ilkayeva, G. Michael Felker, Bridgette Christopher, Christopher Holley, Robert McGarrah, Svati Shah, Duke Univ, Durham, NC

Background. Patients with diabetes mellitus (DM) have a higher risk of heart failure (HF). While metabolic dysregulation has been well-established in HF and DM separately, molecular mechanisms associated with diabetic HF are incompletely understood. Thus, we sought to test the overarching hypothesis that cardiac metabolic programming is distinct in diabetic HF compared to DM and HF individually. **Methods.** RNA-sequencing and targeted metabolomics profiles were completed on human myocardial tissue from 54 unique individuals received from the Duke Human Heart Biorepository and the University of Colorado. Multi-omics profiles of cardiac tissue from 4 groups were compared: a) controls with no clinical evidence of DM or HF (n = 12), b) DM-only (n = 15), c) HF-only (n = 10), and d) HF+DM (n = 17). **Results.** After using statistical criteria to prioritize genes, we looked for genes that function together or modulate the same activity. Genes encoding producers of reactive oxygen species (ROS), DUOX2, DUOX2, and DUOX1, were differentially upregulated in diabetic-HF, compared

to DM or HF alone (FDR HF+DM vs DM-only/HF-only = $6.6 \times 10^{-7}/2.2 \times 10^{-4}$, $7.7 \times 10^{-3}/6.7 \times 10^{-3}$, and $3.6 \times 10^{-6}/0.013$, respectively). Modulators of mTOR signaling, ERBB3, ERBB4, and PI3K3R3, were also differentially regulated in diabetic-HF (FDR HF+DM vs DM-only/HF-only = $1.4 \times 10^{-3}/0.010$, $0.012/7.4 \times 10^{-3}$, and $0.011/9.3 \times 10^{-3}$, respectively). Gene set enrichment analysis supported our focus on these genes; top enriched gene sets included mTOR signaling and processes related to oxidative stress response. In our metabolomics data, long chain acylcarnitines (AC) and ceramides (Cer) are decreased in diabetic-HF. For example, C20:1 AC can be converted to C20:1 Cer and accumulation of both is diminished in diabetic-HF (FDR = 0.0032 and 0.035, respectively). Conclusions. We found that diabetic-HF was characterized by a distinct omics profile consistent with increased ROS production, diminished AC and Cer accumulation, and modulation of mTOR signaling. The fact that ceramide accumulation has been previously associated with oxidative stress and also linked to ERBB and PI3K pathways suggests that these data may reveal a unique regulation of these pathways in diabetic-HF.

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Poster Session 3 and Reception

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Physical and Genetic Interaction Analyses in Human Pluripotent Stem Cell-Derived Cardiomyocytes to Study Protein Quality Control Disease in the Heart

Juan A Perez-Bermejo, Luke M Judge, Kenneth Wu, Christina L Jensen, Robyn M Kaake, Danielle Swaney, Po-Lin So, Nevan J Krogan, Bruce R Conklin, Gladstone Insts, San Francisco, CA

Cardiomyocytes must maintain constant contractile function of the heart throughout a human lifetime. To achieve this, a complex network of chaperones and other proteins maintains the homeostasis of the proteins in the cell. Protein quality control pathways are increasingly recognized for their importance in both inherited and sporadic cardiac disease, and as potential therapeutic targets. The co-chaperone BAG3 is particularly interesting, because genetic evidence suggests that variants of BAG3 can be both pathologic and protective. However, understanding the molecular mechanism underlying most of these disease-genetic variant associations remains a major challenge. Unbiased analysis of physical and genetic interaction networks holds the potential to provide critical information to fill in this knowledge gap. Using genome engineering tools, we have generated a series of isogenic human induced pluripotent (iPS) cell lines bearing different variants on the BAG3 gene, along with an epitope tag fusion. Using mass spectrometry methods, we have been able to identify interacting partners for the BAG3 protein expressed at endogenous levels in iPS-derived cardiomyocytes. Our results show a surprisingly high number of cardiac-specific interactions. In addition, some BAG3 protein variants present distinct gain or loss of specific interactions, allowing us to narrow down the list of candidate hits. Using microscopy image analysis, we performed a targeted knockdown screening on BAG3 interaction partners to dissect pathways of BAG3 interactors that are involved in the development disease phenotype. We hope the information obtained from this study will improve our understanding of the heart proteostasis network, enable the identification of potential therapeutic targets, and provide clues towards a broader understanding of the role of genetic variation in complex disease.

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Poster Session 3 and Reception

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Entropy of Cardiac Rhythm Uniquely Predicts Mortality in a Multi-site Polysomnography (psg) Study of Ambulatory Asymptomatic Community Adults With Heart Failure

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INTRODUCTION: Heart failure (HF) is associated with high rates of mortality and hospital readmission. Current strategies for risk stratification are limited. Recently, we introduced EntropyX, a novel measure of non-linear patterns underlying physiological variability using newer concepts of entropy estimation and machine learning. EntropyX of cardiac repolarization (EntropyX_{QT}) enhanced the predictive value of all established risk factors in a multicenter study of HF patients (PMID: 27044982). Herein, we test the hypothesis that EntropyX of ventricular activation (EntropyX_{RR}) during sleep enhances the performance of established mortality risk factors in asymptomatic community adults with HF. We interpret our results in context of fundamental mechanistic studies in animal models and modern theories of systems biology.

METHODS & RESULTS: We followed 96 NYHA class I HF adults in sinus rhythm for 6.5±3.0 years (1994-2011; SHHS NCT#00005275). Baseline exposures included demographics, history, medications, labs, PSG metrics [e.g., sleep disordered breathing (SDB)], ECG analyses [e.g., heart rate (HRV), QT variability (QTV)], and EntropyX_{RR}. The cohort had mean age of 70±10 years, 49% women, 11% African Americans, and 46 deaths (48%; N=35 from cardiovascular events) over 4.6±2.6 years. After adjusting for exposures, the adjusted hazard ratios (4th to 1st quartile) for mortality for EntropyX_{RR} was 2.3 (95% CI, 1.1-4.6) and age was 4.5 (1.9-10.3), consistent with machine learning-based classification and regression tree analysis. Addition of EntropyX_{RR} to a multivariate model (comprised of age, diabetes, myocardial infarction, SDB) improved continuous net reclassification by 43% (37-48).

CONCLUSIONS: EntropyX_{RR} during sleep predicts mortality over follow-up of asymptomatic community adults with HF, independent of conventional risk factors and linear/deterministic measures (e.g., HRV, QTV, SDB). Unlike simpler concepts of RR variability, EntropyX_{RR} is a fundamentally different measure, reflecting the complexity of multi-directional physiological network properties that regulate homeostatic function under changing conditions. This new paradigm complements conventional measures of risk and has potential for broad application.

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Etv2 Regulates Endothelial Gene Expression With Its Novel Binding Partner Vezf1

Satyabrata Das, Bhairab N Singh, Wuming Gong, Erik Skie, Daniel J Garry, Mary G Garry, Vinayak Gupta, Joshua Theisen, Univ of Minnesota, Minneapolis, MN

Ets transcription factors function as important developmental regulators and are known to be modulators of cell fate. Previously, in conjunction with co-factors, we have described Ets variant 2 (Etv2) as an essential regulator of the hematopoietic and endothelial lineages. But the mechanism and the Etv2 interacting partners involved in achieving this critical function remains poorly understood. Through Yeast two-hybrid analysis we identified Vascular Endothelial Zinc Finger 1 (Vezf1) as an interacting factor with Etv2. Vezf1 is a conserved C2H2 zinc finger transcription factor known to regulate the formation, proliferation, and migration of endothelial cells through several gene targets. We verified Vezf1 as a binding partner of Etv2 through co-immunoprecipitation and GST-pull down studies. Bioinformatic analysis of ChIP-seq and Etv2-expressing single cell RNA sequencing was conducted to identify candidate genes containing both Etv2 and Vezf1 binding motifs in their regulatory regions. Histone deacetylase 7 (Hdac7) and angiomin like protein 2 (Amotl2) were identified as genes of interest involved in endothelial development. Hdac7 is a conserved class II deacetylase that functions in endothelial cell adhesion and vascular integrity. Amotl2 is a Motin family protein that functions in angiogenesis, endothelial cell polarity and migration. RT-qPCR analysis showed upregulation of Hdac7 and Amotl2 in response to doxycycline inducible Etv2 and Vezf1; whereas significant reduction of expression of these two genes was observed in the Etv2 and Vezf1 knockout cells. Chromatin immunoprecipitation (ChIP) and electrophoresis mobility shift assays (EMSA) confirmed Etv2-Vezf1 adjacent binding sites in the promoters of Hdac7 and Amotl2. Histone Acetyl transferase (HAT) assays was performed to investigate Etv2-Vezf1 on global histone acetylation conditions in doxycycline inducible embryoid bodies. Vezf1 overexpression results in a significant reduction of acetylated histone. In summary, this study identifies Vezf1 as a novel binding partner of Etv2 and their combined role in regulating downstream target genes Amotl2 and Hdac7 in hematoendothelial development.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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BET Bromodomain Protein 4 (BRD4) Governance of Cardiovascular Disease Stress-related Cardiomyocyte Remodeling

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During the past decade, epigenetic control of cardiac remodeling has attracted attention as a major mechanism contributing to heart failure. The BET family of bromodomain proteins (BRD) functions as “readers” of acetylated chromatin. Pharmacological inhibition of BRD proteins prevents cardiac hypertrophy and heart failure in preclinical models, yet mechanistic studies elucidating the biology of BRD proteins in the heart are lacking. Here, we investigated the role(s) of BRD4 in cardiac physiology and pathophysiology. By engineering a cardiomyocyte-specific BRD4 knockout mouse, we discovered that loss of BRD4 triggered progressive decline in ventricular contractile function, culminating in dilated cardiomyopathy in both post-natal and adult hearts. To identify early transcriptomic changes in BRD4-ablated heart, we conducted a global transcriptome analysis in KO hearts prior to the onset of ventricular dysfunction. RNA sequencing analysis of BRD4 KO hearts revealed disruption of genes essential to mitochondrial energy production and homeostasis, as well as key cardiac sarcomeric components. Computational analysis identified key transcription factors involved in BRD4-mediated gene regulation, including MEF2 complex, ERR1, SRF, and Nkx2.5. By studying isolated cardiomyocytes maintained in primary culture, we confirmed that disruption of the candidate TF complexes by BRD4 ablation altered mitochondrial morphology and provoked progressive contractile dysfunction. In aggregate, we describe for the first time a critical role of BRD4 in regulating key cardiomyocyte gene networks, in particular genes involved in mitochondrial homeostasis and contractility, to maintain cardiac function. Moreover, our data suggest that BRD4 is a novel regulator of epigenetic events in dilated cardiomyopathy. Elucidating these novel roles of BRD4 in cardiomyocyte remodeling will provide critical insights into the epigenetic control of stress-related cardiac events with potential clinical relevance.

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Mdm2 Induces Vascular Calcification Through Its E3 Ligase Activity

Hyun Kook, Duk-Hwa Kwon, Nakwon Choe, Sera Shin, Hosouk Joung, Juhee Ryu, Young-Kuk Kim, Chonnam Natl Univ Med Sch, Hwasun, Korea, Republic of

Rationale: Calcium deposition to the vascular smooth muscle matrix, or vascular calcification (VC), makes vessels rigid, increasing morbidity and mortality in patients with cardiovascular and renal diseases. Previously, we suggested that histone deacetylase (HDAC) 1 prevents VC, whereas its E3 ligase, mouse double minute 2 homolog (MDM2), exaggerates VC by inducing the polyubiquitination of HDAC1. **Objective:** In the present study, we extend our results to investigate whether MDM2-induced VC is dependent on its ubiquitination activity. **Methods and Results:** Using cellular and animal models with genetically engineered mice, we observed that vascular smooth muscle cell-specific conditional knockout of Mdm2 blunted vitamin D3-induced VC. We generated both MDM2 Y489A, which lacks ubiquitination activity, and MDM2 Δ R, a RING domain-deleted truncated mutant. Compared with the activity of wild-type (WT) MDM2, the HDAC1-ubiquitination activities of both Y489A and Δ R were significantly reduced. WT MDM2 potentiated inorganic phosphate-induced VC by inducing runt-related transcription factor 2 (Runx2), whereas Y489A and Δ R failed to do so. We generated three different transgenic lines to overexpress WT, Y489A, and Δ R MDM2. TgMDM2 WT elicited calcium deposition, whereas TgMDM2 Y489A and TgMDM2 Δ R did not. **Conclusions:** Taken together, our results suggest that MDM2-induced VC is dependent on the ubiquitination activity of MDM2 to degrade HDAC1. Accordingly, blockade of MDM2 ubiquitination activity might have beneficial effects in the treatment of VC.

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Poster Session 3 and Reception

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Global Analysis of Histone Modifications and Long-range Chromatin Interactions Revealed the Differential Cistrome Changes and Novel Transcriptional Players in Dilated Cardiomyopathy

Chia-Feng Liu, Armen Abnoui, Peter Bazeley, Ying Ni, Cleveland Clinic, Cleveland, OH; Michael Morley, Univ of Pennsylvania, Philadelphia, PA; Christine S Moravec, Ming Hu, WH Wilson Tang, Cleveland Clinic, Cleveland, OH

Background: Acetylation and methylation of histone alter the chromatin structure and accessibility that allows or prevents transcriptional regulators to bind to enhancers or promoters. The binding of transcriptional regulators enables the interaction between enhancers and promoters, thus affecting gene expression. However, our knowledge on the regulation of promoter and enhancer interactions and histone modifications in patients with heart failure remains limited.

Methods and Results: We performed chromatin immunoprecipitation followed by sequencing (ChIP-seq) for major histone modifications and proximity ligation-assisted ChIP-seq (PLAC-seq) for long-range chromatin interaction for enhancer and promoter (E-P) using left ventricular tissues from dilated cardiomyopathy (DCM) patients and non-heart failure (NF) donors (n=2-3). We detected 37,531, and 8,587 differential H3K27ac and H3K4me3 regions between NF and DCM respectively (FDR <0.05). While the average read density (ARD) for H3K27ac, an active enhancer histone mark, is similar between NF and DCM, we observed the ARD of H3K4me3, which marks the active promoter region of the genes, is significantly lower in DCM samples than in NF ones (p<0.01). Super-enhancer (SE) analysis revealed that 481 and 119 genes are associated with NF- and DCM-specific SE respectively. Moreover, the differential E-P interactions were observed in the known heart failure gene loci, e.g., *CTGF* and *NPPA-NPPB* and these alternation interactions are correlated with the gene expression levels. Motif analysis identified known cardiac factors, such as MEF 2D and A and possible novel players, such as SOX transcription factors, namely SOX4 and SOX8, are enriched within enhancers and promoter regions identified in DCM samples. Their expression levels were confirmed with the whole-transcriptome analysis and protein assays.

Conclusions: We have established cistrome for major histone modifications and long-range chromatin interaction for enhancer and promoter in NF and DCM tissues. The differential histone modifications and E-P interactions were found in DCM and these differences were associated with the gene expression level of a subset of disease-associated genes in human heart failure.

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Poster Session 3 and Reception

Wednesday, July 31, 2019, 4:30 pm - 7:00 pm

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LncRNA HBL1 Regulates Human Cardiogenesis Through Interacting MIR1 and PRC2 Complex

Juli Liu, Indiana Univ, Indianapolis, IN

Cardiogenesis processes in human and animals have differential dynamics, suggesting the existence of species-specific regulators during heart development. However, it remains a challenge to discover the human-specific cardiac regulatory genes, given that most coding-genes are conserved. Here, we report the identification of a human-specific long noncoding RNA, Heart Brake LncRNA 1 (*HBL1*), which regulates cardiomyocyte development from human induced pluripotent stem cells (hiPSCs). Overexpression of *HBL1* repressed, whereas knockdown and knockout of *HBL1* increased cardiomyocyte differentiation from hiPSCs. Cytoplasmic *HBL1* physically interacted with miR-1 and functioned as an endogenous miR-1 sponge. Disruption of miR-1 binding sites in *HBL1* showed similar phenotype as *HBL1*^{null} hiPSCs. Additionally, we found nuclear *HBL1* directly interacted with EED to form an *HBL1*-EED/PRC2 complex, which occupied key cardiogenic promoters to repress transcription. *SOX2* bound to *HBL1* promoter and activated its transcription. Knockdown of *SOX2* in hiPSCs lead to decreased *HBL1* expression, and increased cardiomyocyte differentiation efficiency. Altogether, *HBL1* adds a human-specific regulatory mechanism on top of conserved mechanisms to orchestrate early human cardiac development.

J. Liu: None.

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Circulating Mirnome in Obese and Lean Heart Failure Patients: A Case-control Study

Santiago Alonso L Tobar, Douglas dos Santos Soares, Hosp De Clinicas De Porto Alegre, Porto Alegre, Brazil; Rodrigo Hass Bueno, Univ Federal Do Rio Grande Do Sul, PORTO ALEGRE, Brazil; Raquel Calloni, Mariana Recamonde Mendoza, Univ Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; Michael Andrades, Luis Eduardo P Rohde, Andreia Biolo, Hosp De Clinicas De Porto Alegre, Porto Alegre, Brazil

Background: Obesity is a risk factor for cardiovascular diseases; however, in obese heart failure (HF) patients live longer than lean HF patients, this observation is known as obesity paradox. MicroRNAs (miRs) regulate processes involved in both cardiac remodeling and obesity.

Objective: We investigated whether the levels of circulating miRs in HF patients are influenced by obesity.

Methods: In this case-control study, twenty HF patients (10 obese and 10 lean) and 10 healthy control individuals were analyzed using Affymetrix GeneChip miRNA 4.0 Array following manufacturer's instruction. Raw data was normalized using the Robust Multiarray Average method, batch effect was adjusted with Surrogate Variable Analysis, pairwise differential expression analysis was carry-out with Limma R package, and miRPath v3.0 was used to interrogate pathways enriched for the dysregulated miRNAs based on validated targets from Tarbase v7.0 and KEGG pathways annotation database. Bioinformatics and statistical analysis were performed using R and a p-value <0.01 was considered significant.

Results and conclusions: We discovered a set of 36 and 48 miRNAs that were differentially expressed in obese HF and lean HF, respectively, in relation to controls. Thirteen miRNAs were commonly dysregulated in both HF groups. In addition, we found hsa-miR-451a to be up-regulated in obese HF in relation to controls, as well as to lean HF patients, suggesting that obesity may accentuate its dysregulation. On the other hand, hsa-miR-4738-5p, hsa-miR-1260a, and hsa-miR-98-5p were differentially expressed in lean HF in relation to both remaining groups. Pathways enrichment analysis suggested that hsa-miR-451a regulates genes from the mTOR signaling pathway (p=0.002), whereas hsa-miR-1260a modulates Hippo (p=0.02) and AMPK (p=0.03) signaling pathways, all of which have been previously related to cardiac hypertrophy. Further investigation and validation of their targets may contribute to a better understanding of the obesity paradox.

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Differential Dna Methylation Co-segregates With the Severity of Heart Failure

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Heart failure (HF) is a leading cause of morbidity and mortality worldwide. As a potential epigenetic biomarker, DNA methylation differs between healthy and diseased hearts. However, its association according to disease severity has not yet been studied. Here, we sought to investigate how DNA methylation (DNAm) differs across HF disease severity, and study the use of a DNMT methyltransferase inhibitor RG108 on DNAm and its effect on heart function. A fixed dose of Isoprenaline (ISO), or saline control (SAL), administered to a hybrid mouse diversity panel (HMDP) consisting of 85 classical and recombinant inbred mouse strains produced a range of cardiac hypertrophy and/or LV dilatation. Left ventricles were harvested and subjected to genome-wide cardiac DNAm profiling by Reduced Representation Bisulfite Sequencing (RRBS). Unsupervised clustering of the top 1% most variable CpG methylation segregated strains to their genetic origin. Disregarding strain-specific methylation differences, differential methylation between ISO and SAL unexpectedly categorised mice into mild and severe disease responders. In the severe-responder strain BTBRT, the pharmacological DNMT methyltransferase inhibitor, RG108, rescued disease from ISO-response, with accompanying evidence of gene expression recovery. This work establishes the range of cardiac differential DNAm correlating according to disease severity. It displays the involvement of DNA methylation-dependent gene expression changes that turns out to be unique, despite different mouse strain backgrounds. This gives further proof of principle that cardiac DNAm changes represent novel biomarkers for disease stratification and consequent targeted therapy.

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Poster Session 3 and Reception

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A Novel Role of Cardiac DNA Methylation as a Regulator of Fibrosis in Human Diabetic Heart Failure

Luke A. Potter, Mark E. Pepin, **Adam R. Wende**, Univ of Alabama at Birmingham, Birmingham, AL

Human heart failure (HF) is accompanied by changes in cardiac gene expression; however, the impact of diabetes mellitus on this transcriptional regulation remains unclear. Our laboratory studies the role of epigenetics in HF and focuses on DNA methylation, commonly accepted as a negative regulator of gene expression when present in a gene's promoter. In the current study, we hypothesized that diabetes alters cardiac DNA methylation and the transcriptome. We performed an integrated analysis of RNA-sequencing and DNA methylation using human left ventricle biopsies from HF patients to determine the effect of diabetes on this reprogramming. Our analysis identified global changes in cardiac DNA methylation as well as mRNA expression sufficient to distinguish diabetic from non-diabetic patients. Specifically, we identified significant regulation of 1,133 genes (|1.5| fold-change, $P < 0.05$) and 9,302 methylation sites (|5%| methylation, $P < 0.05$). Within the 391 co-regulated genes, only 174 genes had altered promoter-associated methylation. Consistent with the accepted dogma that promoter methylation inversely regulates gene expression, we found that 119 of those 174 genes displayed this inverse relationship; of these, an overwhelming majority (102) were hypomethylated in diabetic relative to non-diabetic hearts. These findings contrast the cardiac hypermethylation we have reported for ischemic HF. Gene set enrichment of the 119 inversely-regulated genes identified numerous pathways involved in fibrosis, including extracellular matrix organization (FDR < 0.05, 3.9% enriched) and collagen biosynthesis (FDR < 0.05, 6.3% enriched), which suggest that DNA methylation contributes to the adverse cardiac remodeling associated with diabetic heart failure. To identify putative regulators of these transcriptional and epigenetic differences, a candidate gene approach was used to reveal induction (2.5-fold, $Q < 0.05$) and demethylation (20.5%, $P < 0.05$) of *EGR2*, a known regulator of fibrosis. Furthermore, we found induction of *GADD45beta* (2.6-fold, $Q < 0.05$) a known regulator of DNA demethylation. Taken together, these observations suggest that epigenetic mechanisms underlie an etiology-specific reprogramming of cardiac fibrosis in diabetic HF.

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Poster Session 3 and Reception

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Aberrant Activation of Nitric Oxide Synthase 1 in the Heart Accelerates Diastolic Dysfunction Through Protein S-nitrosylation

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Heart failure refers to an inappropriate blood supply to the periphery. Among subtype of heart failure, pathophysiology and effective therapeutic strategy of systolic heart failure is well established. However, molecular mechanism and specific regimens for diastolic heart failure (DHF) still remains unclear. Here we propose nitric oxide synthase (NOS) 1 mediated exacerbation of DHF and therapeutic potential for novel therapeutics. We screened alteration of posttranslational modifications in DHF which were induced either by SAUNA (SAlty drinking water/Unilateral Nephrectomy/Aldosterone) model or transverse aortic constriction (TAC) model and observed that protein S-nitrosylation was dramatically increased. Total RNA sequencing revealed that transcription amount of NOS1 was aberrantly increased in DHF, whereas NOS2 or NOS3 was not changed. Either non-selective NOS inhibitor or specific NOS1 inhibitor effectively blocked diastolic dysfunction. To elucidate the molecular target of S-nitrosylation, we performed biotin switch assay and requested protein sequencing which were newly appeared in the presence of GSNO. Among novel signal, we identified and confirmed that Histone deacetylase (HDAC) 2 was target molecule of NOS1. HDAC2 cysteine 262 and cysteine 274 were responsible residue of NOS1-mediated S-nitrosylation. We generated knock-in mice carrying S-nitrosylation defect HDAC2 mutant, HDAC2 C262A/C274A (2CA). HDAC2 2CA knock-in mice underwent SAUNA surgery or TAC and diastolic function of those mice was measured by early diastole (E) and mitral valve annulus movement (E'). When compared to wild type littermates, the diastolic function assessed by E/E' ratio in HDAC2 2CA knock-in mice were relatively well conserved both in SAUNA- and TAC-induced DHF model. Survival rate after TAC operation was also dramatically ameliorated in HDAC2 2CA knock-in mice. Taken together, we proposed novel molecular axis, NOS1/S-NO HDAC2, accelerating diastolic dysfunction and which implicated notable molecular target for treatment of DHF patients.

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