Myocardial Reductive Stress

Ivor J. Benjamin, MD, FACC, FAHA
Chief-Division of Cardiovascular Medicine
Vice Chair-Translational Research
Director-Cardiovascular Center

Medical College of Wisconsin
Milwaukee, WI
July 15, 2014
OXIDATIVE STRESS THEORY IN DISEASE: Implications from Clinical Trials

Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention

Bjelakovic, G. et al. JAMA 2007;297:842-857
REDUCTIVE STRESS IN DISEASE PATHOGENESIS: Implications for Clinical Trials

Human αB-Crystallin Mutation Causes Oxido-Reductive Stress and Protein Aggregation Cardiomyopathy in Mice

Namakkal S. Rajasekaran,1 Patrice Connell,2 Elisabeth S. Christians,2,3 Liang-Jun Yan,2 Ryan P. Taylor,1 András Orosz,1 Xiu Q. Zhang,1 Tamara J. Stevenson,1 Ronald M. Peshock,2,4 Jane A. Leopold,5 William H. Barry,1 Joseph Loscalzo,5 Shannon J. Odelberg,1 and Ivor J. Benjamin1,2,*

Involvement of Reductive Stress in the Cardiomyopathy in Transgenic Mice With Cardiac-Specific Overexpression of Heat Shock Protein 27

Xia Zhang, Xiaoyan Min, Chuanfu Li, Ivor J. Benjamin, Bo Qian, Xiaojin Zhang, Zhengnian Ding, Xiang Gao, Yuzhen Yao, Yujie Ma, Yunling Cheng, Li Liu

Elimination of NADPH Oxidase Activity Promotes Reductive Stress and Sensitizes the Heart to Ischemic Injury

Qiujun Yu, MD, PhD; Chi Fung Lee, PhD; Wang Wang, MD, PhD; Georgios Karamanlidis, PhD; Junya Kuroda, MD, PhD; Shouji Matsushima, MD, PhD; Junichi Sadoshima, MD, PhD; Rong Tian, MD, PhD

Zhang et al Hypertension. 2010
Rajasekaran: Cell 130: 437, 2007
Yu et al J Amer Heart Assoc. 2014
Outline for Today’s Presentation

• Myofibrillar diseases as a novel mechanism involving redox metabolic remodeling;

• Mitochondrial-nuclear crosstalk and oxido-reductive stress in mice, flies and *in vitro* systems;

• Perspectives on the therapeutic targeting of oxido-reductive stress in health and disease.
αB-Crystallin (CryAB) Mutations Cause Multisystem Disorders Including Myofibrillar Myopathy

- Small molecular weight heat shock protein that functions as a chaperone.
- 175 amino acids, 22 kDa
- Tissue specific expression predominantly in the lens, heart, and skeletal muscle.
- Translocates to the Z-disc during muscle contraction and is involved in myofibrillar remodeling following damage to the sarcomere.
- Acts as a chaperone for structural myofibrillar proteins including desmin and titin.
- Redox modulator- increases the levels/activity of ROS scavenging enzymes.

- Dominant and recessive
- Loss of function or gain of toxic function
- Variable penetrance and expressivity
- Tissue specificity


(Golenhofen et al. J Mol Cell Cardiol 2002. 34: 309-319)
Hilton et al. 2013
Human $\alpha$B-Crystallin Mutation Causes Oxido-Reductive Stress and Protein Aggregation Cardiomyopathy in Mice

CryAB $^R_{120G}$

Control Cry AB $^R_{120G}$

Rajasekaran: Cell 130: 437, 2007
Hemizygous G6PD deficiency (20% normal activity) rescues CryAB\textsuperscript{mut} pathology & reductive stress in mice.

![Graph](image_url)

- **Control**
- **CryAB\textsuperscript{mut}**
- **CryAB\textsuperscript{mut}/G6PD\textsuperscript{+/−}**
ROS Pathways: Implications for Reductive Stress

Aon and O’rourke. BBA 2010: 865-877
Modeling CryAB\textsuperscript{mut} Pathology in Flies

The NADPH Metabolic Network Regulates Human αB-crystallin Cardiomyopathy and Reductive Stress in Drosophila melanogaster

Heng Xie
Ken Golic
Anthony Cammarato

Small Molecule Inhibitor

"Antireductant"

NADPH-dependent Pathways as a Hub for Modifying Reductive Stress Networks in Mice and Flies

O$_2^-$

SOD

H$_2$O$_2$

CAT

H$_2$O ($+$ O$_2$)

GPX

GSH

GSSG

GR

NADP$^+$

NADPH

G6PD

IDH

PGD

MEz

CryAB$^{\text{mut}}$

Oxidative Damage

Reductive Toxicity

Small Molecule Inhibitor

“Antireductant”

Xie at al: PLoS Genetics, 2013
R120G CryAB Expression on Nuclear erythroid-2 related factor 2/Kelch-like AP1 Pathways

Cell.130: 427-49, 2007:
PNAS. 105: 9745-50, 2008
Phys Genom. 35: 165-72, 2008
JMCC 49:918-30, 2010
ARS. 20: 2891-906, 2014

Amino acid substitutions perturb lamin Ig-fold tertiary structure (Lori Wallroth)

N C
head rod NLS Ig-fold domain

G449V  L489P  W514R

PDB1IVT
The oxidative state of glutathione reflects the redox status.

oxidative stress

reduced glutathione (GSH)

oxidized glutathione (GSSG)
Mutant lamins increase levels of NADPH.
Elevated levels of reduced glutathione suggest “reductive stress”.

Caused by cytoplasmic protein aggregation

Results in dilated cardiomyopathy in mouse models

Rajasekaran et al., Cell 2007
http://www.yalescientific.org
Mutant lamins increase nuclear levels of Nrf2 (CncC).

Antibodies a kind gift from H. Deng and T. Kerppola
There is an alternative mechanism for Nrf2 target gene activation.

Komatsu et al., Nat Cell Biol 2010
Mutant lamins increase levels of p62.
Mutant lamins increase levels of nuclear Nrf2 and p62 in human muscle.
Mutant lamins cause reductive stress and activate the Nrf2 pathway.
Aggregation-Prone iPS-derived Cardiomyocytes with R120 CryAB exhibit Cellular Hypertrophy

Effects of Dose-Dependent R120G on Cellular Hypertrophy in iPS-derived CMs

The redox environment is the summation of redox couples of the electron transport chain and intracellular subcompartments (i.e., NAD(P)H, GSH/GSSG, Cys/CySS).

Hansen et al, Annu Rev Pharm Tox, 2006
Human myofibrillar diseases such as mutations in CryAB trigger a ‘toxic’ gain-of-function mechanism.

Metabolic redox pathways with high NADPH generation are genetic modifiers of reductive stress-induced pathology.

Pathways linked to NRF2-dependent regulation and proteostasis are likely necessary and sufficient to drive disease pathogenesis.
Investigators:
Liang-jun Yan
Michael Riedel
Heng Xie
Raj Soorappan
Huali Zhang
Elisabeth Christians
Soumyajit Mustafi
Pat Limphong
Anthony Cammarato
Takahiro Ishiwata
Xiaohui Wang

Collaborators:
Kent Golic & Lori Wallrath (U Iowa)
Deepak Srivastava (Gladstone)
Kenneth Chien (MGH/HSCI)
James Cox
Raj Soorappan
Sandy Bernstein (SDSU)

NIH Pioneer, NHLBI, Leducq, Catalyst, SYNERGY & AHA.
Modeling Protein Misfolding Diseases in Flies: Cardiovascular Phenotyping