Novel Mechanisms of Non-coding Genomic Regulation Identified in Cardiac Disease-in-a-dish Models

Aditya Kumar, Stephanie Thomas, Kirsten Wong, Kevin Tenerelli, Univ of California, San Diego, San Diego, CA; Valentina Lo Sardo, William Ferguson, Eric Topol, Kristin Baldwin, The Scripps Res Inst, San Diego, CA; Adam Engler, Univ of California, San Diego, San Diego, CA

Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) at gene loci that affect cardiovascular function, and while mechanisms in protein-coding loci are obvious, those in non-coding loci are difficult to determine. 9p21 is a recently identified locus associated with increased risk of coronary artery disease (CAD) and myocardial infarction. Associations have implicated SNPs in altering smooth muscle and endothelial cell properties but have not identified adverse effects in cardiomyocytes (CMs) despite enhanced disease risk. Using induced pluripotent stem cell-derived CMs from patients that are homozygous risk/risk (R/R) and non-risk/non-risk (N/N) for 9p21 SNPs and either CAD positive or negative, we assessed CM function when cultured on hydrogels capable of mimicking the fibrotic stiffening associated with disease post-heart attack, i.e. “heart attack-in-a-dish” stiffening from 11 kiloPascals (kPa) to 50 kPa. While all CMs independent of genotype and disease beat synchronously on soft matrices, R/R CMs cultured on dynamically stiffened hydrogels exhibited asynchronous contractions and had significantly lower correlation coefficients versus N/N CMs in the same conditions. Dynamic stiffening reduced connexin 43 expression and gap junction assembly in R/R CMs but not N/N CMs. To eliminate patient-to-patient variability, we created an isogenic line by deleting the 9p21 gene locus from a R/R patient using TALEN-mediated gene editing, i.e. R/R KO. Deletion of the 9p21 locus restored synchronous contractility and organized connexin 43 junctions. As a non-coding locus, 9p21 appears to repress connexin transcription, leading to the phenotypes we observe, but only when the niche is stiffened as in disease. These data are the first to demonstrate that disease-specific niche remodeling, e.g. a “heart attack-in-a-dish” model, can differentially affect CM function depending on SNPs within a non-coding locus.

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