Recapitulation of Myocardial Infarction Coupled with Patient-Specific iPSC-Derived Cardiomyocytes for Characterization of Chromosomal Mutation 9p21

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Statement of Purpose: Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) at the chromosomal locus 9p21 as increasing the risk of coronary artery disease (CAD) and myocardial infarction (MI) susceptibility[1]. Associations have implicated SNPs in enhancing smooth muscle cell proliferation and endothelial permeability but have not identified adverse effects in cardiomyocytes (CM)[2]. Using induced pluripotent stem cell (iPSC)-derived CM from patients that are homozygous risk/risk (R/R), heterozygous risk/non-risk (R/N), and non-carrier non-risk/non-risk (N/N) for 9p21 SNPs and are either CAD positive (CAD+) or negative (CAD-), we assessed altered CM behavior when cultured in a bioreactor that mimics MI progression, e.g. hypoxia and fibrotic stiffening. These data establish a possible new cardiac phenotype for patients with 9p21 SNPs.

Methods: Patients from the Geneheart and Wellderly studies in the Scripps Health System were genotyped and peripheral blood mononuclear cells reprogrammed for R/R, R/N, and N/N patients that were CAD+ and CAD-. CM differentiation was induced using a modified monolayer culture protocol that has previously reported CM purity in excess of 90% [3]. CM were purified via lactate treatment from days 11-18 post CHIR99021 treatment before use in experiments. CM were seeded on methacrylated hyaluronic acid (MeHA) hydrogels that had been stiffened with 0.2% Irgacure and UV light as indicated to make hydrogels that had elastic moduli of 11 or 50 kiloPascals (kPa) or hydrogels that transitioned from 11 to 50 kPa on day 5 from a second UV and irgacure exposure. CM were seed at a density of 40,000 cells/cm^2. EDC and NHS chemistry was used to attach type I rat tail collagen to substrates. Cell maturation via immunofluorescence for α-actinin or calcium handling with Fluo-4 AM was assessed at day 10. Videos were analyzed in MATLAB to determine the power spectral density and beat synchronicity.

Results: To determine if 9p21 SNPs perturbed sarcomere assembly resulting in functional deficits, sarcomere spacing first was assessed for CMs derived from R/R CAD+ iPSCs. When plated on 11 kPa MeHa hydrogels, spacing matched typical sarcomere organization of R/N CAD- patients. Synchronous contraction could also be observed across the culture for CMs independent of genotype, though the power spectral density of Fluo-4 AM signaling, indicating more regular contractions, was higher for R/N CAD- patients.

Quite strikingly, on dynamically stiffened matrices (transitioning from 11 to 50 kPa on day 5 to mirror MI stiffening), R/R CAD+ CMs exhibited asynchronous contractions; correlation coefficients for beating were significantly higher for all cells cultured on 11 kPa gels, but R/N CAD- CMs cultured on dynamically stiffened gels maintained rhythmic contraction and had correlation coefficients that were significantly higher than R/R CAD+ CMs cultured under the same conditions (Figure 1).

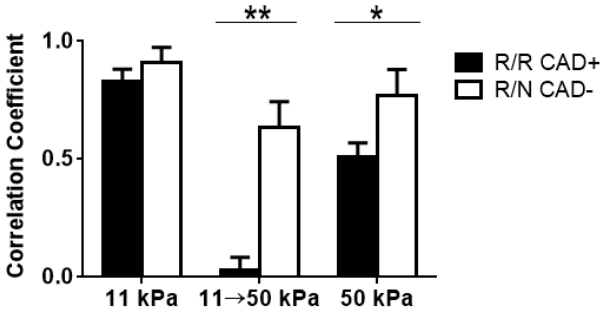

Figure 1. Correlation coefficients for R/R CAD+ and R/N CAD- CM cultured on different substrate stiffness. Statistical comparisons for correlation coefficient were obtained using Student's t-test. * denotes $p<0.05$ and ** denotes $p<0.01$ (n=3, 15 cells per video).

Conclusions: GWAS have demonstrated a link between recurrent MI and the 9p21 mutation[4], but this research is the first to demonstrate that specific MI niche changes differentially affect CM function depending on 9p21 SNP state. These data also suggests that altered cell-cell communication, perhaps due to pathological gap junction remodeling or defective calcium handling, may contribute to the dyssynchronous beating we observed and increased risk of recurrent MI.

References: [1]Helgadottir A., et al. Science. 2007;316 (5830):1491-1493. [2]Horne B., et al. Circ Cardiovasc Genet. 2008;1:85-92. [3]Lian X., et al. Nat Protoc. 2013;8(1):162-175. [4]Buysschaert I., et al. Eur Heart J. 2010;31:1132-1141.

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