Non-Coding Genomic Regulation Identified in Human Cardiomyocytes
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Introduction: Cardiovascular disease is multivariate, but a significant portion of the risk is genetic. 10^6 single nucleotide polymorphisms (SNPs) in the human genome have been identified. The vast majority of SNPs cluster in non-coding regions, complicating our understanding of their function. As a model system, we examined how a portion of the long non-coding RNA ANRIL, which arose only in primates and humans in the 9p21 locus, regulates cardiac phenotypes associated with fibrotic remodeling.

Materials and Methods: Induced pluripotent stem cell-derived cardiomyocytes (CMs) from patients that are risk/risk (R/R) or non-risk/non-risk (N/N) for 9p21 SNPs were cultured on methacrylated hyaluronic acid hydrogels (MeHA) capable of mimicking fibrotic remodeling, i.e. stiffening from 10 kилоPascals (kPa) to 50 kPa (Fig. 1A). To eliminate patient-to-patient variability, we obtained isogenic lines where the 9p21 locus was deleted from R/R lines, i.e. R/R KO. Calcium signaling was assessed using Fluo-4 AM dye and connexin 43 expression was determined via qPCR and immunofluorescence. Functional gap junction expression was determined by dye transfer assay. ANRIL and p16 expression was measured by qPCR.

Results and Discussion: While all CMs contracted synchronously on soft matrices, R/R CMs on stiffened hydrogels exhibited asynchronous contractions compared to N/N CMs; removal of the risk locus, i.e. R/R KO, reverted CMs to synchronous contraction (Fig. 1B). Similarly, dynamic stiffening reduced connexin 43 expression in only R/R CMs (Fig. 1C) and decreased dye transfer, pointing to altered gap junction expression. A kinase screen identified JNK and CREB activation in response to stiffening. JNK activation in R/R CMs after stiffening was validated via western blot and JNK inhibition improved synchronicity, connexin 43 expression, and dye transfer. This data was coupled with increased ANRIL and decreased p16 expression in R/R CMs, which led to the pathway described in Fig. 1D based on data and literature.

Conclusions: 9p21 appears to repress connexin transcription via altered JNK signaling, but only when the niche is stiffened as in disease. These data are the first to demonstrate that disease-specific niche remodeling can differentially affect CM function depending on SNPs within a non-coding locus.