**Gut Microbial Extracts Modulate iPSC-Cardiomyocyte Electrical Activity and Expression of Genes Involved in Dilated Cardiomyopathy (DCM)**

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Background:

Previous studies suggest associations between DCM and differential gut microbial composition. However, potential causative roles of the gut microbiome in DCM progression remain unclear. Our objective was to develop an in-Vitro model with iPSC-CMs for studies into the role of gut microbial metabolites in DCM progression. This work will allow for future studies exploring the causative role of the gut microbiome in heart failure and may lead to microbiota-targeted therapies.

Methods:

Filtered cecal extracts from 22-week old wild-type (WT), germ-free, and LmnaH222P mutated mice were used to compare the impacts of healthy microbiota products, host metabolites, and metabolites from microbiota in mice with heart failure. iPSC-CMs were seeded in a 24-well multielectrode array plate and grown for 7 days. Then, cells were treated every 48 hours with gut extracts or PBS vehicle control diluted to 2.5% v/v (n=6 for each condition). Field potential and contractility measurements were obtained daily using a microelectrode array, imaging, and MuscleMotion analysis software. Differential gene expression was analyzed using the NanoString nCounter System following experiment termination and RNA extraction.

Results:

Cells remained viable and electrically active following 19 days of treatment with gut extracts at 2.5%. During this period, field potential duration remained unchanged across treatment groups. However, treatment with gut extracts significantly decreased beat rate and increased contraction duration, time-to-peak, and 90-to-90 transient time. Gene expression analysis revealed the upregulation of the NFKBIA gene and downregulation of LDLR and VEGFA genes in microbial extract groups, when compared to both vehicle and germ-free controls. These changes have all been implicated in the progression of cardiovascular disease (CVD).

Conclusions:

This study establishes a model for treatment of cardiomyocytes with gut microbial extracts that allows for long-term study of electrical, contractile, and gene expression changes. At 2.5% v/v, treatments did not appear toxic to iPSC-CMs. Yet, significant changes in electrical activity and the differential expression of genes implicated in CVD suggests a potential causative role of gut microbiota in the progression and severity of dilated cardiomyopathy.