Influence of sarcomeric protein mutations on myocardial function in a 3D-disease model of human iPSC-derived bioartificial cardiac tissue

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Background: Peripartum cardiomyopathy (PPCM) is a rare type of cardiomyopathy that occurs in women without prior history of heart disease at later stages of pregnancy or several months after childbirth (1). The disease is characterized by lowered left ventricular ejection fraction (LVEF < 45%) and can lead to heart failure if left untreated (1). Approximately 20% of PPCM patients harbor sarcomeric mutations, from which titin truncating variants (TTNtv-s) have the highest pathogenic potential (2). However, the role of such genetic defects of the contractile apparatus on PPCM pathogenesis is not well understood.

Purpose: The study aims to establish an in vitro 3D disease model of PPCM to elucidate the role of sarcomeric mutations, including titin mutations, in its etiology. We hypothesize, that the disease phenotype can be uncovered in vitro via the stimulation of PPCM patient-derived Bioartificial Cardiac Tissues (BCTs), utilizing pregnancy-related factors in our custom-made bioreactors (3).

Methods: We selected three patients carrying previously unstudied mutations in the TTN gene for the study. The iPSC reprogramming was performed using a commercial, Sendai virus-based kit. Pluripotency was confirmed via flow cytometry and immunofluorescence stainings. The presence of mutations was confirmed by exome sequencing. Cardiomyocyte differentiation (4) and BCT generation (3) were carried out via established protocols.

Following maturation, BCTs were stimulated with the pregnancy-related factors, and tissue forces were measured in the bioreactors. These forces include intrinsically generated (spontaneous) and electrically induced (paced) contraction forces (mN), as well as passive forces, which correlate with tissue stiffness.

Results: We successfully generated two clonal induced pluripotent stem cell (iPSC) lines from each patient. We confirmed that all of the cell lines were >90% positive for pluripotency markers. After differentiation, CMs contracted spontaneously from day 7 onwards and were >95% positive for cardiac markers (SA, MF20, cTnT). The BCTs remodeled and started contracting on days 1-2 after generation, similarly to wild-type tissues in the baseline conditions.

Prolactin treatment of Patient A, harboring a missense mutation in titin, did not show significant functional effects. We confirmed, that patients B and C harbor TTNtv-s, and tissues from these patients show a trend of altered stiffness after stimulation with repeated mechanical stretch cycles or β-adrenergic agonist isoprenaline. Further analysis of the underlying mechanisms investigating metabolic changes, signaling pathways, and transcriptomics are ongoing.

Conclusion: The established iPSC and tissue engineering technologies offer a valuable tool for the development of an iPSC-CM-based 3D in vitro disease model for PPCM, which can be used for risk stratification of individual and combined sarcomeric mutations and the development of tailored therapeutic strategies.