ENGINEERING CULTURE ENVIRONMENT OF HUMAN PLURIPOTENT STEM CELLS TO DIRECT THEIR COMMITMENT AND MATURATION TOWARDS FUNCTIONAL CARDIOMYOCYTES: AN "-OMICS" DRIVEN APPROACH

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The immature phenotype of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) constrains their potential in cell therapy and drug discovery applications. In this study, we aim to overcome this hurdle by devising a novel strategy for generation and maturation of functional hPSC-CM.

We relied on the aggregation of hPSC-derived cardiac progenitors to establish a scalable differentiation protocol capable of generating highly pure CM aggregate cultures. Whole-transcriptome analysis and 13C-metabolic flux analysis demonstrate at both molecular and fluxome levels that a 3D culture environment enhances metabolic maturation of hPSC-CMs. When compared to 2D, 3D cultures of hPSC-CMs displayed down-regulation of genes involved in glycolysis and lipid biosynthesis and increased expression of genes involved in OXPHOS. Accordingly, 3D hPSC-CMs had lower fluxes through glycolysis and fatty acid synthesis and increased TCA-cycle activity.

We then assessed if alteration of culture medium composition to mimic in vivo substrate usage during cardiac development improves further hPSC-CM maturation in vitro. Our results showed that shifting hPSC-CMs from glucose-containing to galactose- and fatty acid-containing medium promotes their fast maturation into adult-like CMs with higher oxidative metabolism, transcriptional signatures closer to those of adult ventricular tissue, higher myofibril density and alignment, improved calcium handling, enhanced contractility, and more physiological action potential kinetics. Integrated "-Omics" analyses showed that addition of galactose to culture medium improves total oxidative capacity of the cells and ameliorates fatty acid oxidation avoiding the lipotoxicity that results from cell exposure to high fatty acid levels. This study provides an important link between substrate utilization and functional maturation of hPSC-CMs facilitating the application of these cells in preclinical research and regenerative medicine.

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