IMPROVING FUNCTIONAL MATURATION OF HUMAN PLURIPOTENT STEM CELLS DERIVED CARDIOMYOCYTES THROUGH METABOLIC UNDERSTANDING

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In vitro differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CMs) is a crucial process to enable their application in cell therapy and drug discovery. Nevertheless, despite the remarkable efforts over the last decade towards the optimization of cardiac differentiation protocols, generated hPSC-CMs are still immature, closely reminiscent of fetal cardiomyocytes in what regards structure, metabolism and function. In this study, we aim to overcome this hurdle by devising a novel metabolic-based strategy to improve hPSC-CMs generation and functionality. Noteworthy, we integrated structural and functional analyses of hPSC-CM with powerful "omics" technologies (proteomics, transcriptomics, metabolomics and fluxomics) as complementary analytical tools to support process optimization and product characterization.

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 ^{13}C-metabolic flux analysis demonstrated that a three-dimensional (3D) and agitated-based culture environment enhances metabolic maturation of hPSC-CMs. When compared to static monolayer, 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 improved 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. "-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 demonstrated that metabolic shifts during differentiation/maturation of hPSC-CM are a cause, rather than a consequence, of the phenotypic and functional alterations observed. The metabolic-based strategy established herein holds technical and economic advantages over the existing protocols due to its scalability, simplicity and ease of application. Improved maturation and functionality of *in vitro* generated hPSC-CM will excel their application in cell therapy, drug discovery and cardiac disease modeling.

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