IMPROVING PRODUCTION AND MATURATION OF CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS: AN "-OMICS" DRIVEN APPROACH

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The production of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) holds great promise for cardiotoxicity drug testing, disease modeling and cardiac regeneration. However, the complex nets of signaling pathways involved in cardiomyogenesis compromises the effectiveness of the existing differentiation protocols to reproducibly produce high-quality hPSC-CMs. Produced hPSC-CMs are immature compared with adult CMs, express typical fetal cardiac genes, use glucose as major energy source and have immature structural and electrophysiological properties. In this study we aim to overcome these hurdles by devising an integrated strategy for production and maturation of functional hPSC-CMs. hPSC (hiPSC and hESC lines) were differentiated into CMs, using a novel directed differentiation protocol. CM differentiation was monitored by flow cytometry, qRT-PCR and proteomic analyses. Different medium compositions were tested aiming to improve hPSC-CM maturation after differentiation. Metabolome, fluxome and transcriptome analyses as well as structural, ultrastructural and functionality analyses were used to evaluate the impact of feeding strategies on hiPSC-CM maturation.

We showed that our differentiation protocol originates pure 2D monolayers and 3D aggregates of hiPSC-CMs that present typical CM transcriptome and proteome profiles.

Combining a set of "-omics" tools (metabolomics, fluxomics and transcriptomics) with structural and morphological analyses we demonstrated that hiPSC-CM cultured in glucose depleted medium supplemented with fatty acids display features that resemble more mature CMs, than hiPSC-CMs cultured in standard glucose rich medium, namely: energetically efficient oxidative metabolism, transcriptional signatures closer to ventricular CM; more elongated morphologies; organized sarcomeric structures and higher myofibril density and alignment. Comprehensive physiological characterization further demonstrated improved calcium handling, enhanced contractility, and more physiological action potential kinetics within 10 days in fatty acid-containing media.
This work describes significant advances towards production of mature functional hPSC-CMs, meeting some of the needs of the cardiac regenerative medicine market and industrial field.