ENGINNERING CARDIAC TISSUE USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVATIVES: PROTEOMIC CHARACTERIZATION OF CO-CULTURES OF CARDIOMYOCYTES AND ENDOTHELIAL CELLS

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Prediction of cardiac toxicity effect is extremely relevant in the development of new drugs for different medical applications. In this way, it is important to develop more predictable human cell-based models which physiologically better mimic the human heart and allow the prediction of this toxic effect as well as establish the tools that enable the characterization of these complex cell models. To recreate engineered cardiac tissue, it is essential to reproduce the complexity of the heart by resorting to different cell types. Cardiomyocytes (CMs) are functional contractile units of the heart, and it is known that their communication with endothelial cells (ECs) is crucial for cardiac homeostasis. The aim of this study is to recreate a human pluripotent stem cells (hiPSC)-based cardiac tissue model and evaluate the impact of communication between both cell types on the phenotype of CMs.

Co-cultures of hiPSC-CM and hiPSC-EC were established and maintained for 12 days as confirmed by immunofluorescence microscopy. Quantitative whole-proteome analysis was performed using SWATH Mass Spectrometry tools to compare the conditions of hiPSC-CM mono-culture and the co-culture of hiPSC-CM and hiPSC-EC. Our data showed relative increase of expression ratios of morphological maturation-related cardiac proteins in hiPSC-CM co-cultures. In particular, the expression ratios of MYH7/MYH6, MYL2/MYL7, TNNI3/TNNI1 increased 2.4-, 5.1-, and 5-fold, respectively, when compared to the mono-culture condition, indicating that in the presence of hiPSC-EC, hPSC-CM display a more adult- and ventricular- like phenotype. Changes in the extracellular matrix composition were also observed, especially related with the increased expression of ECM proteins in co-culture condition namely, collagens I and III (8.6-fold and 6-fold, respectively), fibronectin (3.5-fold) and thrombospondin-4 (2.5-fold). Other growth factors attributed to the extracellular space (e.g. CTGF, PAI1, CRTAP, IGFBP7, and NPPB) that may be responsible for the communication between both cell types have also shown to be up-regulated in the co-culture condition. The presence of a SMA+ (myofibroblast-like) population in the co-culture condition was observed by immunofluorescence microscopy images, which is in agreement with the more complex and fibrotic extracellular matrix found by whole proteome analysis. Ultrastructure characterization of CMs was carried out by transmission electron microscopy. In both conditions, hiPSC-CM displayed aligned myofibrils composed by sarcomeres with organized Z-disks, A- and I-bands, intercalated discs between adjacent cells as well as abundant mitochondria. Noteworthy, sarcomere length was higher in hiPSC-CM cultured with hiPSC-EC, suggesting structural changes associated with cardiomyocyte maturation. Calcium imaging is being performed to evaluate calcium handling of hiPSC-CMs and their response to drugs.

All together our data revealed that promoting the communication of hiPSC-CM and hiPSC-EC induced structural changes in hiPSC-CM associated with maturation. This study provides important insights towards the development of more complex cardiac tissues and establishes potent analytical tools for the characterization of these models.

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