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Targeting the hippo signalling pathway to enhance the therapeutic potential of iPS-derived cardiomyocytes

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Introduction: Cell based therapy using stem cell derived cardiomyocytes, has emerged as a potential therapeutic approach for cardiac diseases such as myocardial infarction and heart failure. Adult skin fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSC) which could be an ideal source of iPS-derived cardiomyocytes (iPS-CM). Challenges facing cell therapy include the high number of viable cells needed to survive in pathological conditions. The Hippo signalling pathway has been described as a key pathway involved in regulating cardiomyocyte proliferation and survival in both embryonic and adult hearts. The purpose of this study is to test whether modification of the Hippo pathway will enhance the efficiency of iPS-CM generation and will increase iPS-CM survival and viability in pathological conditions.

Methods: Skin fibroblasts were reprogrammed to iPSC cells and then differentiated to cardiomyocytes. The Hippo signalling pathway was modified by genetic ablation of MST1, a major upstream regulator of the Hippo pathway, or by overexpressing YAP, the main downstream effector of the pathway. Cell proliferation was analysed using an EdU incorporation assay and staining for cytokinesis markers Ki67 and phospho-histone H3. Cell death and viability were analysed by measuring caspase 3/7 and MTT activity and by trypan blue staining in both normal and hypoxic conditions.

Results: Analysis of cell proliferation showed that genetic ablation of Mst1 leads to significantly increased proliferation (1.5 ± 1.5% P<0.001), survival and viability (20 ± 4.3% P<0.005) of iPSCs in both normal and hypoxic conditions compared to controls. In addition overexpression of YAP, which is normally inhibited by upstream Hippo pathway components, and overexpression of mutated constitutively active form of YAP (S127A) increases cell proliferation in iPSC-CM compared to control iPSC-CM as shown with EdU assay (+20.8 ± 1.6% P=0.001) and Ki67 staining (49 ± 9.3% P<0.001). Overexpression of YAP leads to up regulation of genes associated with inhibition of apoptosis and promotion of cell proliferation.

Conclusion: Targeting the Hippo pathway in iPSC cells and iPSC-CM significantly increases proliferation and survival in both normal and hypoxic conditions. Therefore, modulation of the Hippo pathway could become a new strategy to enhance the therapeutic potential of iPSC-CM.