Transplantation of adipose mesenchymal cells overexpressing telomerase and myocardin preserved cardiac function and promoted tissue repair in murine myocardial infarction

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Rationale: The success of stem cell therapies for acute myocardial infarction (AMI) has been hampered by poor cellular engraftment and survival, which may be partly due to the harmful microenvironment within ischemic myocardium. However, it may be possible to promote survival and cardiomyogenesis of transplanted adult mesenchymal cells by inducing the overexpression of myocardin (MYOCDD), a prymogenic transcription factor with anti-apoptotic activity, and talonemurase reverse transcriptase (TERT), an antisenescent protein.

Objectives: We used a murine model of AMI to assess the efficacy of transplanted adipose tissue-derived mesenchymal stromal cells (AT-MSCs) engineered to overexpress TERT and TERT.

Methods: Twelve-month-old C57BL/6 mice underwent coronary artery ligation to induce AMI and were randomized into 3 treatment groups: phosphate-buffered saline (PBS) (20μL n=7), mock-transduced AT-MSCs (2.5x105 cells in 20μL n=5), or AT-MSCs overexpressing TERT and MYOCDD (2.5x105 cells in 20μL n=7). Sham-operated mice (n=7) were used as controls. The AT-MSCs were obtained from 12-month-old male green fluorescent protein-transgenic C57BL/6 mice and transduced with lentiviral vectors encoding TERT and MYOCDD.

Results: When transplanted into the infarcted hearts of C57BL/6 mice, AT-MSCs overexpressing TERT and MYOCDD preserved myocardial fractional shortening (Figure A), increased arteriogenesis and cell engraftment and decreased fibrosis formation (Figure B), compared with PBS alone or mock-transduced AT-MSCs (2.5x105 cells in 20μL n=7). Enhanced proliferation (12.8 ± 1.5% P < 0.001), survival and viability (20.4 ± 3.3% P < 0.001) of iPS-CM were observed in both normal and hypoxic conditions. Therefore, modulation 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 assessed using an EdU incorporation assay and staining for cytokinesis markers Ki67 and phospho-histone H3. Cell death and viability were assessed 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 (12.8 ± 1.5% P < 0.001), survival and viability (20.4 ± 3.3% P < 0.001) of iPS-CM 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 iPS-CM compared to control PS-CM as shown with EdU assay (+20.8 ± 1.6% P < 0.01) and Ki67 staining (4.9 ± 0.9% 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 iPS cells and iPS-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 iPS-CM.