490 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 (MYOCD), a prionogenic transcription factor with anti-apoptotic activity, and telomerase reverse transcriptase (TERT), an antisenescence 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 MYOCD 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) (20 μL, n=7), mock-transduced AT-MSCs (2.5x10^5 cells in 20 μL, n=5), or AT-MSCs overexpressing TERT and MYOCD (2.5x10^5 cells in 20 μL, 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 MYOCD.

Results: When transplanted into the infarcted hearts of C57BL/6 mice, AT-MSCs overexpressing TERT and MYOCD 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. These effects were accompanied by increased numbers of Ki-67+ cells and c-kit+ cells (Figure B) and enhanced expression of cardiac actin, GATA4, Nkx2.5, and myocardin (Figure C).

Conclusions: Delivering TERT and MYOCD genes into AT-MSCs before transplantation promotes activation of the cardiomyogenic pathway, vasculogenesis, and stem cell survival in a murine model of AMI.

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 iPSC-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 iPSC–CM generation and will increase iPSC–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 cytokerin and phospho-histone H3. Cell death and viability were determined using annexin V and 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 ± 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 as compared to control iPSC–CM as shown with EdU assay (+20.8 ± 1.6% P<0.01) and Ki67 staining (+49 ± 9.9% P<0.01). 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.

492 Porous silicon nanoneedles for localised in situ gene transfer for cardiac therapy

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Introduction: Post-infarction functional recovery is severely limited by the lack of myocardial regeneration. Novel reparative routes aim at promoting regeneration by viral overexpression of therapeutic genes in the cardiac tissue. However, viral therapies pose a risk of immunological response and ectopic spreading of the particles.

Purpose: Here we develop porous silicon nanoneedles (nN) as a nanomaterial approach for the localised intracellular delivery of integrative plasmid DNA carrying genes for in situ gene therapy of the heart. The geometry of nN arrays allows for the ideal interfacing of the epicardium, which is known to be a source of reparative cells after myocardial infarction [1]. The development of a technological strategy for the targeting of this key tissue with localised gene therapy will open novel therapeutic routes.

Methods: Porous silicon nanoneedle arrays were fabricated as previously described (2); loading of plasmid DNA was assessed in high salt concentration and close to DNA isoelectric point. Primary epicardial cells and cardiac slices were nanoinjected by means of a force-feedback linear motor (Bose Electroforce 3200) applying forces ranging from 0.1N to 1N. Gene-reporter expression was tested by immunofluorescence and qPCR.

Results: Maximum loading efficiency and release in physiological buffer were achieved by loading with a glycine buffer at pH5. In vitro, the application of nN at 4N force on epicardial cells determined overexpression of the plasmid encoded genes to a level comparable to viral infection (90 to 100-fold upregulation), demonstrating the feasibility of nN-mediated integrative gene therapy. Following nanoinjection we detected upregulation of downstream genes, indicating the functionality of the delivered transgenes. On ex vivo heart explants, nanoneedles efficiently delivered plasmid DNA intracellularly within the tissue in a localized and spatially defined manner.

Conclusions: Nanoneedle arrays are an efficient method of stable and efficient gene transfer for large transcripts. Nanoinjection produces highly localized and safe gene delivery to the cardiac tissue.

491 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 iPSC-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 iPSC–CM generation and will increase iPSC–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 cytokerin and phospho-histone H3. Cell death and viability were determined using annexin V and caspase 3/7 and MTT activity and by trypan blue staining in both normal and hypoxic conditions.

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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.

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