# Enhancing the survival and angiogenic potential of mouse atrial mesenchymal cells

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Endogenous cardiac progenitor cells (CPC) have the potential to regenerate damaged tissue following myocardial infarction. Various CPC populations have been proposed but doubt remains as to which is the optimal population for regenerative therapy. In order to induce regeneration, progenitor cells expanded in vitro need to be robust enough to survive after transplantation into the peri-infarct region of the heart. We have isolated a mouse atrial cell population by collagenase and trypsin digestion. Here we have compared these cells (known as CT cells) to cardiosphere-derived cells (CDCs) and established the ability of both cell types to survive under serum-starvation. MicroRNA210 has been shown to have anti-apoptotic and pro-angiogenic effects in HL1 cardiomyocytes, so here we have investigated whether transfection with mIR210 increased the therapeutic potential of CT cells.

Cells from murine cardiac atria were expanded as CDCs by explanting atrial tissue on fibronectin and culturing explant-derived cells as cardiospheres in hanging drops, followed by monolayer culture on fibronectin; or as CT cells after digestion using 0.1% collagenase and trypsin for 1 hour, followed by slow adhesion and monolayer culture on fibronectin. Gene expression was assessed using qRT-PCR. Cell survival was determined after culture in serum-free medium for 72 hours and 10 days. VEGF release over 24 hours was measured by ELISA. CT cells were transfected with miR210 using DharmaFECT.

Atrial CDCs and CT were isolated from mouse atria (n = 6) and cultured to passage 4 over 2 months or 28 days, respectively. Both cell types had a mesenchymal phenotype with comparable expression of the progenitor and early cardiac genes Oct3/4, c-kit, TERT, Nkx2.5, GATA4, MEF2c. However expression of Sca1 was higher in CT cells than CDCs whilst CDCs had 10-fold higher expression of the epicardial marker WT1 than CT cells. Culture in serum-free medium induced cell death over 72 hours in both cell types (n = 3) but was more pronounced in CDCs than CTs after 72 hours. CDC cell number further decreased from 3 to 10 days, whereas CT cells continued to proliferate, albeit at a much slower rate than in normal medium containing 20% serum. VEGF release per cell was comparable from CDCs and CT cells after 72 hours in serum-free medium. Transfection of CT cells (n= 3) with miR210 resulted in a 1.5 fold increase the number of surviving cells after 10 days in serum-free medium and a 25% increase in VEGF release per cell.

The CT protocol generated a mesenchymal cell population that could be expanded rapidly from atrial tissue and which survived long-term culture in serum-free medium. Transfection with miR210 enhanced cell survival and release of VEGF to further increase the therapeutic potential of the cells.