Strategies for Promoting Survival and Engratftment of Transplanted Mesenchymal Stem Cells

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Introduction

Ischaemia is a feature of many conditions including cardiovascular disease, neurodegenerative disorders, cerebrovascular trauma and stroke. It is characterised by a restriction of regional blood flow which causes hypercarbia, hypoxia and glucose deprivation with resultant metabolic acidosis. Short periods of ischaemia may be tolerated but the combined effects of tissue hypoxia and acidosis lead to cell death by necrosis and apoptosis resulting in permanent loss of tissue and organ function. Mesenchymal stem cell (MSC) transplantation is one therapeutic approach which aims to repair damaged tissue and replace lost cells. However, upon transplantation in the myocardial infarct model, we have observed that MSCs are modest in their therapeutic effects. This may be due to the harsh pro-apoptotic, inflammatory microenvironment of the infarcted heart which is not conducive to MSC survival and engraftment. Therefore, protection of MSCs against apoptosis is critical for successful stem cell therapy. During ischaemia, cell survival can be influenced by expression of genes that promote glycolysis, limit mitochondrial metabolism (both of which reduce oxygen demand), suppress reactive oxygen species and inhibit pro-apoptotic protein expression.

The objectives of this study are: (i) to determine if antioxidant and anti-apoptotic gene expression can protect MSCs from acute ischaemic stress in vitro and (ii) to assess the effect of this gene expression on transplanted MSC survival and engraftment in an in vivo model of myocardial infarction.

Materials and Methods

HIV-1 based, VSV-G pseudotyped, lentiviral vectors expressing human catalase, Hsp27, Hsp70, SOD1 and SOD3 genes were produced by standard transient transfection of 293T cells. Transgene expression was measured by immunofluorescent staining and western blot. In vitro experiments consisted of MSC exposure to conditions of ischaemia and its components which included oxidative stress, hypoxia and complete glucose knockdown. Viability and apoptosis were assessed by cell and nuclear staining, MTT assay and caspase activity. In vivo experiments were performed on female Sprague Dawley rats. Myocardial infarction was induced by complete ligation of the left anterior descending coronary artery. Male MSCs were injected 24h post infarct and hearts were harvested at 7 and 28 days post cell injection. Genomic DNA was extracted for analysis of engrafted MSCs by quantitative PCR for the SRY gene. Statistical significance was determined by one-way ANOVA using SigmaStat3.5.

Results

The in vitro data suggest these putative therapeutic proteins have a protective effect on MSCs exposed to conditions of oxidative stress, hypoxia and ischaemia when compared to non-transduced control MSCs. In particular, Hsp27-expressing MSCs demonstrated a statistically significant increase in viability and decrease in caspase activity in both hypoxia and ischaemia. In vivo, Hsp27-expressing MSCs demonstrated a higher percentage engraftment at both 7 and 28 days post cell injection when compared to control groups. This was statistically significant at the 7 day time point. Currently we are investigating any possible efficacious effect of implanted Hsp27-MSCs on heart function by analysis of infarct size and ejection fraction.

Discussion and Conclusions

Stable long term expression of transgene in MSCs was achieved using these vectors, and expression of the therapeutic genes increased cell survival in vitro. For these reasons, these vectors may be applicable for use in such areas as neurodegenerative and cardiovascular disease.

In vivo results indicate that over-expression of Hsp27 in MSCs may help transplanted cells persist for a longer period of time in the infarcted heart. This increased longevity may allow the MSCs to exert further positive effects in the infarct area either directly or by paracrine action.

In summary, these findings demonstrate enhancement of therapeutic potential by MSC modification, through over-expression of antioxidant and anti-apoptotic genes, which may prove effective by promoting cell survival.

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