

STEM CELL APPLICATIONS IN CELL THERAPY FOR NUCLEUS REPAIR

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INTRODUCTION: There has been an increasing rise of interest in stem cell therapy, since it has provided new option in broad range of diseases. However, stem cell application to treat intervertebral discs has just begun. We have reported the regenerative effect of autologous mesenchymal stem cell (MSC) transplantation in treatment of disc degeneration. Despite the effectiveness of the procedure, its pathogenesis was unclear. In order to obtain evidence to clarify the mechanism in regenerative effect of MSC transplantation, we conducted an *in vitro* and *in vivo* study investigating differentiation and fate of the transplanted MSCs.

METHODS: *In vitro* induction study. Since differentiation ability of stem cells associates with microenvironment in which they are placed. Human NP cells or AF cell were cocultured with MSCs possessing GFP gene for 3 weeks in alginate beads. Using FACS Vantage cell sorter, only MSCs were retrieved and examined by flow-cytometry, immunocytochemistry, cDNA microarray and RT-PCR. In order to clarify cytokines related to induction, culture medium was analyzed by cytokine array. *In vivo* study. NZL white rabbits were divided into three groups (normal control; degenerative disc; and MSC transplantation models evaluated at 2, 4, 8, 16, 24 and 48 week). Autologous MSCs were isolated from marrow and were infected with retroviral vector expressing GFP. MSCs were embedded in atelocollagen gel and transplanted. MRI and radiographic evaluations, immunohistochemistry for C-S, K-S, type I, II, IV collagen, HIF-1 alpha and beta, HIF-2 alpha and beta, GLUT-1 and GLUT-3, and MMP-2 and RT-PCR for aggrecan, versican, type I, II collagen, IL-1 beta, IL-6, TNF-alpha, MMP-9 and MMP-13 were studied.

RESULTS: *In vitro* study. From flow-cytometric analysis, MSCs cocultured with NP cells in alginate expressed similar cell size and internal intensity with fairly large cells dominantly stained with keratin sulfate as

observed in NP cells, whereas MSCs cocultured with AF cells showed that MSCs remained small in size and relatively low in intensity with no dominance in expression of proteoglycan epitopes. These findings were compatible with immunocytochemistry and RT-PCR results with turn over of type I and type II collagen expression. Several genes were found to be candidate for specific markers by microarray. Cytokine array results confirmed importance of TGF-beta, PDGF, IGF-1 and EGF during induction. *In vivo* study. MRI and radiograph results confirmed regenerative effects of the procedure. GFP-positive cell were detected in the nucleus throughout all periods with its percentage rising from 21±6% in 2 weeks to 55 ± 8% in 48 weeks, which proved survival and proliferation of MSCs. Immunohistochemistry showed positive staining of all proteoglycan epitopes and type II collagen in some of the GFP-positive cells. MSCs expressed HIF-1alpha, MMP2 and GLUT-3 expressing compatible phenotypic activity with nucleus pulposus cells. RT-PCR results demonstrated significant restoration of aggrecan, versican and type II collagen genes and significant suppression of TNF-alpha and IL-1beta genes in transplantation group.

DISCUSSION & CONCLUSIONS: Our data show that MSCs are capable of differentiating into cells expressing some of the major phenotypical characteristics of disc cells *in vitro*. Furthermore, MSCs transplanted to degenerative discs not only survive but also proliferate and differentiate into cells expressing phenotypes of nucleus pulposus cells with suppression of inflammatory genes *in vivo*. Results of the current study demonstrate some possible explanation for regenerative process of MSC transplantation to the degenerate disc.

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