Transfection of primary human mesenchymal stem Cells with growth and differentiation factor 5 (GDF-5) – A non-viral gene transfer therapy for the disc?

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INTRODUCTION: Mesenchymal stem cells (MSC) harvested from human bone marrow show great promise for therapeutic interventions. The relatively easy isolation protocol, the high proliferation capacity and their ability to differentiate into different cell types of the mesenchyme puts them into primary focus of regenerative medicine.[1] Insertion of targeted DNA into MSCs gives raise to new therapeutic strategies. The phenotype of intervertebral disc (IVD) cells is still obscured. Recent evidence demonstrated that growth and differentiation factor 5 (GDF-5) is a promising cytokine pushing stromal progenitor cells possibly towards a disc-like phenotype.[2,3] The aim of this study was to transfect MSCs with a plasmid containing the full open reading frame (ORF) of GDF-5. The procedure included a combination of electroporation (nucleofection) and sub-sequent lipofection with the aim to differentiate MSCs to IVD cells with the over-expression of GDF-5. Nucleofection is a straightforward method to insert genes of interest into target cells without the need of silenced viruses, which are not well accepted as therapeutic vectors.[4].

METHODS: MSCs were harvested from bone marrow aspirations of 4 patients undergoing spine surgery and isolated through histopaque density centrifugation and plastic adhesion (Ethical permit #187/10 of ethical authorities of the canton of Bern). These isolated cells were cultured and expanded for 2 weeks and then transfected with a GFP-tagged ORF clone of the GDF-5 gene by nucleofection using the nucleofector from Amaxa (Lonza, Basel, Switzerland). In addition, lipofection (Invitrogen, Basel, Switzerland) was applied and the cells were analyzed for RT-PCR, immunohistochemistry over time to see the effect of the GDF-5 transfection.

RESULTS: Increased levels of GDF-5 gene expression by nucleofected-MSC were confirmed using RT-PCR (Figure 1). We found ACAN upregulated but not collagen type 2. GDF-5 specific antibody confirmed presence of intracellular GDF-5 compared to untransfected control cells by immunohistochemistry.

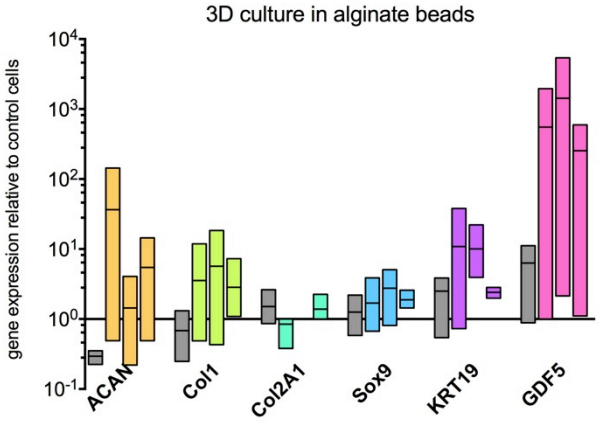

Fig. 1 *Gene expression of MSC nucleofected with plasmid GDF-5 and encapsulated in alginate. Expression levels were adjusted to control cells. Per gene 3 time-points (colorized bars) were monitored: from left to right day 7, day 14, day 21. Gray bars correspond to day 0 (day 7 in monolayer culture). Bars represent min to max with a line at the mean. N=4).*

DISCUSSION & CONCLUSIONS: These results suggest that gene delivery by nucleofection of GDF-5 is an attractive approach for the release of GDF-5, which was produced by the cells themselves. Previous studies point towards an IVD-relevant role of GDF-5 to rescue IVD degeneration. Future studies will have to demonstrate longevity of the over-expression of GDF-5 *in vitro* and *in vivo*. Injection of transfected MSCs into 3D organ culture will show performance of cells in the IVD environment. Non-viral gene therapy seems a more promising approach from a translational medicine perspective than viral methods.[4]

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