

Towards *in vitro* Generation of “Intervertebral-Disc-Like” Cells

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INTRODUCTION: Two regenerative approaches of the degenerative lumbar disc are currently debated; i) injection of growth factors, and ii) application of autologous bone-marrow derived stem cells (BMSCs). However, unconditioned BMSCs have a poor chance of survival in the “harsh” conditions of an intervertebral disc, which are low pH and low concentrations of oxygen and glucose [1-3]. We address this issue and precondition human BMSCs by co-culture with bovine nucleus pulposus cells (NPCs) and/or rhGDF-5. Recently, keratin-19 was proposed as potential NPC-specific marker from whole human genome microarray data [4-5].

METHODS: BMSCs were isolated from human bone-marrow aspirates by density gradient procedure and selection by plastic adherence. The procedure and samples were ethically approved by the Swiss Ethical Committee. Cells were passaged up to P4 before seeding in alginate beads. NPCs were isolated from caudal discs of 6-12 months old calves and seeded in alginate beads. Co-cultures were conducted using culture inserts (0.4 μ m pore size, Becton, Dickinson and Company [BD]). Cells were cultured serum-free under four different conditions in 12-well plates (BD): BMSCs, BMSCs + NPCs, BMSCs + rhGDF-5, and BMSCs + NPCs + rhGDF-5 (BMSC:NPC ratio 1:1). BMSCs were analyzed by RT-PCR at selected loci, i.e. aggrecan (ACAN), collagen II (col2A1), collagen 1 (col1), keratin-19 (KRT-19), and SOX-9, at 4 time points during 12 days.

RESULTS: Co-culture of hBMSCs with bNPCs promotes differentiation of BMSCs as was found in other studies. We also show that ACAN and col2A1 expression can be stimulated with rhGDF-5. Remarkably, KRT-19 was stimulated in the presence of rhGDF-5 and was found significantly down-regulated in the chondrogenic pathway (i.e. treatment with TGF β ₁ and Dexamethasone) (Fig. 1).

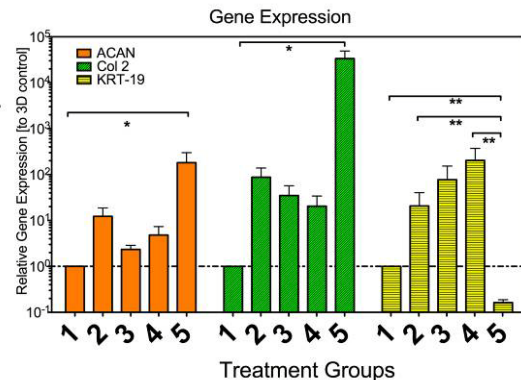


Fig. 1: Relative Gene Expression (relative to MSC3D control) at 3 gene loci on day 12.

1 = hBMSC; 2 = hBMSC+GDF-5; 3 = hBMSC+bNPC; 4 = hBMSC+bNPC+GDF-5; 5 = hBMSC+TGF β ₁+Dexamethasone.

DISCUSSION & CONCLUSIONS: We demonstrate a down-regulation of KRT-19 in presence of TGF- β ₁, indicating that this potential NPC-specific marker is negatively influenced in the TGF- β -pathway.

A trend to up-regulate KRT-19 was found in the presence of rhGDF-5, suggesting differentiation towards the disc cell-like phenotype.

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