

## Effect of TGF- $\beta$ and BMP-2 on rat mesenchymal stem cell differentiation into disc-like cells *in vitro*

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**INTRODUCTION:** Current treatment for degenerated discs is limited and alternative methods have to be found. Mesenchymal stem cells (MSCs), differentiated into cartilage-like disc cells, are a promising candidate for inducing intervertebral disc regeneration and need further development in animal models [1, 2]. *In vitro* protocols to induce differentiation into proteoglycan (PG) producing cells have been established for human MSCs using TGF- $\beta$  [3], but TGF- $\beta$  alone does not induce chondrogenesis in rat periosteal cells [4]. Therefore, we hypothesized that rat MSCs would differentiate into cartilage-like cells with a combination of TGF- $\beta$  and BMP-2.

**METHODS:** MSCs were harvested from the bone marrow of 13 month old Wistar rats. Cells were expanded to the third passage and seeded at a density of 10 Mio/ml into a Fibrin carrier (45 mg Fibrinogen and 1 IU Thrombin/ml; provided by Baxter). Carriers were disc shaped with a diameter of 5 mm and a height of 2 mm. They were cultured in the presence of either  $\alpha$ -MEM and 1% ITS+ (control) or with additional dexamethasone, proline, ascorbic acid and TGF- $\beta$  or BMP-2, or both (experimental groups). Samples were harvested at day 1, day 14 and day 21 and analysed for PG content spectrophotometrically (normalized to DNA content) and histologically (cryostat sections, Toluidine blue staining). RT PCR for collagen I, II and X, aggrecan, SOX-9 and osteocalcin was performed. All data was normalized to day 1. In case of a sufficiently high 18s value (housekeeping gene), but no detectable gene specific mRNA, the maximum number of cycles was taken for the analysis.

**RESULTS:** Spectrophotometrically undetectable to very low amounts of PG were found in the control groups. In all experimental groups similar amounts were found (0.039 to 0.070  $\mu$ g PG per  $\mu$ g DNA). Histological sections showed faint metachromasia around cells (not shown). Collagen I was slightly upregulated on days 14 and 21 relative to day 1 in all groups (up to 21 fold). Collagen II and aggrecan were only slightly upregulated in the control and BMP-2 group (less than 15 fold),

but both genes were clearly upregulated in experimental groups with TGF- $\beta$  alone or in combination with BMP-2. In both groups values were highest at day 21. There was a synergistic effect of BMP-2 versus TGF- $\beta$  alone (figure 1). Results from collagen X, SOX-9 and osteocalcin are pending.

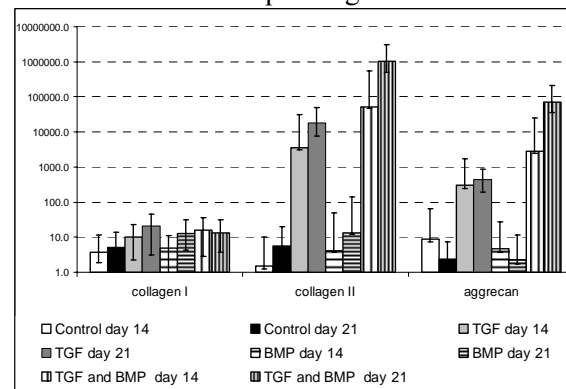


Fig. 1: Gene expression relative to control day 1. (Avg  $\pm$  SD; n=4).

**DISCUSSION:** In contrast to the results of Hanada et al [4] TGF- $\beta$  alone did induce the expression of collagen II in rat MSCs, but BMP-2 did not. Hence, rat MSCs behave more like human MSC than periosteal cells. Expression of collagen X and osteocalcin will determine, if the combination of both growth factor influences the amount and the kind of gene expression. Although we have a clear upregulation of the relevant genes our detection of PG was limited. In aggregates of human MSCs, 21 days have been shown to be sufficient to induce detectable amounts of PG [3]. However, this period may be too short for cells embedded within a fibrin carrier at the density we have used. We showed that TGF- $\beta$  and BMP-2 have different effects upon gene expression of rat MSCs and that the combination of both is synergistic. Future studies will evaluate the interaction between these biological factors with mechanical ones.

**REFERENCES:** <sup>1</sup> YG. Zhang, et al (2005) *Clin Orthop Relat Res* 430:219-26. <sup>2</sup> G. Crevensten, et al (2004) *Ann Biomed Eng* 3:430-4. <sup>3</sup> AM. Mackay, et al (1998) *Tissue Eng* 4:415-28. <sup>4</sup> K. Hanada, B. et al (2001) *J Cell Biochem* 81(2):284-94.