

Chondrogenic Differentiation in Adipose Tissue Derived Mesenchymal Stem Cells: Effects of Growth Factors and PTHrP

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INTRODUCTION: Due to their remarkable abilities in proliferation and differentiation mesenchymal stem cells are an attractive cell source for application in tissue engineering and regenerative therapy. Special interest has been paid on mesenchymal stem cells originating from adipose tissue (ATSC) as they are easily available in great amounts. Though many similarities to bone marrow derived mesenchymal stem cells (BMSC) have been reported, the chondrogenic differentiation potential of ATSC is inferior to that of BMSC using common TGF- β driven protocols (Winter et al. 2003). Further, chondrogenic induction of BMSC with standard induction medium results in a hypertrophic phenotype. The aim of this study was to improve the chondrogenic differentiation of ATSC without inducing hypertrophy by applying different growth factors and growth factor combinations.

METHODS: ATSC were derived by liposuction surgery after informed consent. After expanding the cells for up to 8 passages in a standard expansion medium, differentiation was induced by incubation in high density pellet culture in serum-free medium containing ascorbate and 10ng/ml of TGF- β , BMP2, 4, 6, 7, FGFa, FGFb, IGF-1 or PTHrP, respectively. The same growth factors were also applied in combination with TGF- β (10ng/ml). Success of chondrogenic differentiation was analyzed by immunohistological staining and gene expression profiles were determined by cDNA-array analysis and real-time RT-PCR.

RESULTS: The combination of TGF- β and BMP6 was most effective for chondrogenic induction of ATSC. Though the combination of TGF- β with BMP2 or BMP4 also resulted in proteoglycan deposition and collagen type II immunostaining of ATSC spheroids, it did not lead to a complete differentiation. Both, TGF- β and BMP6 had to be administered simultaneously for successful chondrogenesis. The gene expression profile of ATSC induced with TGF- β and BMP6 was similar that of BMSC induced with TGF- β showing expression of cartilage relevant molecules like

Col2, aggrecan, CRTL-1, COMP, PRELP and fibromodulin. Like BMSC, ATSC showed an early upregulation of hypertrophic markers like Col10A1 and enhanced alkaline phosphatase (AP) activity from day 14 on. When PTHrP, a known inhibitor of chondrocyte hypertrophy, was added to the TGF- β and BMP6 supplemented chondrogenic induction medium, there was no immunostaining for collagen type X and no upregulation of AP enzyme activity over 6 weeks. There was, however, also no staining for collagen type II, indicating an inhibition of chondrogenic differentiation. Interestingly, mRNA of Col2 and Col10 was still detected after addition of PTHrP.

DISCUSSION & CONCLUSIONS:

Application of TGF- β and BMP6 completely eliminated the reduced chondrogenic potential of ATSC. Like BMSC they can be differentiated into chondrocyte like cells, which, however, express hypertrophic markers. Attempts to suppress the hypertrophic phenotype by PTHrP prevented the differentiation of ATSC and BMSC to chondrocyte like cells. Addition of PTHrP to standard chondrogenic induction media, thus, is no means to obtain phenotypically stable chondrocytes known from articular cartilage.

REFERENCES: Winter et al. (2003) *Arthritis & Rheumatism* 48:418-429

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