

Regulation of Hypertrophic Gene Expression in Chondrogenic Cultures of Human Bone Marrow Mesenchymal Cells

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INTRODUCTION: Bone marrow mesenchymal cells (MSC) are a potentially useful source of progenitor cells for cartilage tissue engineering. Human MSCs can be directed in culture to assume a cell fate with some characteristics of articular chondrocytes; however the expression profile of these chondrogenic cultures has also been shown to include elements of a hypertrophic chondrocyte phenotype such as collagen type X [1]. In previous studies we have also demonstrated the expression of other molecules characteristic of chondrocyte terminal differentiation such as Indian hedgehog (IHH) and components of the parathyroid hormone-related peptide (PTHrP) signalling pathway in MSC chondrogenic pellet cultures. This study was performed to investigate the effects on MSC chondrogenic differentiation of molecules thought to be important mediators in the interacting pathways underlying the development and control of chondrocyte proliferation and hypertrophy (BMP2, FGF2 and PTHrP) [2].

METHODS: Adherent cultures of human bone marrow mesenchymal cells were derived from bone marrow mononuclear cells (Cambrex) and expanded in medium containing FGF2. Chondrogenic pellet cultures (5×10^5 cells) were performed as described [3] and were additionally supplemented with BMP2, FGF2 or PTHrP. Time course experiments were sampled at 1,3,7 and 14 days and pellets were analysed for DNA/Glycosaminoglycan (GAG) content, gene expression by quantitative RT-PCR, and immunohistochemistry.

RESULTS: Time course analysis of gene expression in the chondrogenic pellet cultures revealed an unexpected apparently co-ordinate expression of collagen II and X. Other markers of chondrocyte hypertrophy such as PTHrP receptor and Indian hedgehog (Ihh) were also upregulated over 14 days, though not in the temporal or spatial manner characteristic of the growth plate. Addition of BMP2 to the pellet cultures during the 14 day time course resulted in several key changes compared with controls. BMP2 treatment increased cellular proliferation and accelerated the onset of collagen II expression to a higher steady state level with a more even distribution of protein, whilst

having no effect on collagen I mRNA levels. However, collagen X expression was stimulated in a similar fashion to that of collagen II, and other markers of the hypertrophic phenotype (Ihh, PTHrP-R) were upregulated earlier in the time course. In contrast, when FGF2 was present during the time course there was a small degree of inhibition of proliferation and a reduction of about one half in the amount of GAG deposited into the matrix on a per cell basis at day 14. FGF treatment reduced expression of the hypertrophic marker genes, but also lowered the expression of collagen II and aggrecan. PTHrP treated pellet cultures accumulated size and weight at the same rate as controls. Despite this, GAG appeared to be deposited more slowly in the PTHrP treated cultures. The PTHrP treatment completely down regulated expression of Ihh and lowered the expression level of collagen X mRNA by 100 fold at day 14. Up regulation of collagen II expression was also inhibited by PTHrP addition, though to a lesser degree - a 20 fold lower steady state level was seen at day 14.

DISCUSSION & CONCLUSIONS: The 14 day chondrogenic cultures of hMSC appeared to be a composite of articular and hypertrophic chondrocyte phenotypes. This apparent hypertrophy might represent a problem in an implanted articular tissue engineered device, if local influences in the joint environment were unable to reverse or control the differentiation state of the cells. Our results showed that cells in the pellet cultures were able to respond to at least some of the pathways thought to control chondrocyte terminal differentiation and suggest the promise that the differentiation of hMSCs into chondrocytes could be controlled by appropriate factors if they were applied in the correct temporal and spatial manner.

REFERENCES: ¹ J.U. Yoo, T.S. Barthel, K. Nishimura et al. (1998) *J. Bone Joint Surg. Am.* **80**:1745-1757. ² E. Minina, C. Kreschel, M.C. Naski et al (2002) *Dev. Cell* **3**:439-449. ³ A.M. Mackay, S.C. Beck et al. (1998) *Tissue Eng.* **4**:415-428.

ACKNOWLEDGEMENTS: This work was funded by BBSRC, MRC and EPSRC.