

## EXPANSION IN FGF2 PROMOTES MATRIX SYNTHESIS DURING CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL CELLS WITHOUT UPREGULATING SOX9

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**INTRODUCTION:** Bone marrow mesenchymal cells can be manipulated *in vitro* to differentiate towards a number of different cellular fates and offer a potential source of cells in a variety of tissue engineering applications. Differentiation of chondrocytes *in vivo* is mediated through interactions with growth factors, neighbouring cells and the extracellular matrix, though the detailed mechanisms are unclear. Here we have induced chondrogenesis in human bone marrow mesenchymal cells in a pelleted cell system [1], and show that upregulation of matrix synthesis does not correlate with expression of the cartilage regulating transcription factor SOX9.

**METHODS:** Human bone marrow mononuclear cells that were adherent after 24 hours on tissue culture plastic were expanded in monolayer culture with or without FGF2 at 1 or 5 ng/ml [2]. Confluent flasks were split 1:3 and cells were used at either passage 2 or 3. For pellet cultures, aliquots of 500,000 cells were pelleted in serum-free defined chondrogenic medium [3] without FGF2 in 15 ml Falcon tubes. The medium was changed on the resulting contracted pellets every 2-3 days for up to 14 days. Pellet chondrogenesis was assessed by changes in wet weight, histology and total GAG and DNA content of pellet extracts. Gene expression in RNA extracted from monolayers and pellets was examined by quantitative real time PCR.

**RESULTS:** Isolation of human bone marrow mesenchymal cells in medium containing FGF2 gave a cell population with a robust chondrogenic response when tested in the pellet culture system. This method gave a similar result in all different donor samples tested to date (n=9), whereas cells isolated from the same samples without added FGF2 were not overtly chondrogenic. At 14 days there were much higher pellet wet weights in the treated samples, probably due to greater extracellular matrix (GAG) production. Preliminary results suggested that there was also a net gain in cell number in the pellets derived from FGF2 treated monolayers that would also contribute to the weight difference. Sections from pellets derived

from FGF-treated cells stained strongly with Safranin O, whereas those from non FGF-treated cells did not. There was a massive upregulation of collagen II mRNA expression (one hundred thousand fold) in pellets from FGF pre-treated cells, but this was not coupled to an increase in SOX9 expression in the same pellets.

**DISCUSSION & CONCLUSIONS:** Expansion of human bone marrow mesenchymal cells in the presence of FGF2 gave a cell population with enhanced chondrogenic potential, as recently described [2]. This was most clearly shown in the upregulation of GAG synthesis and deposition, and in a massive upregulation of collagen II expression. However this was not matched by an increase in SOX9 expression, which has been proposed to enhance transcription of the COL2A1 gene. This suggested that there were alternative pathways of transcriptional control, or that the SOX9 level already present in the monolayer mesenchymal cells was permissive, but not alone sufficient for collagen II expression. Modest increases in L-SOX5 and SOX6 expression in the pellets may have provided the necessary levels of promoter complex for the massive induction of collagen II expression observed. It has not been established if the isolation procedure with FGF2 alters the overall gene expression of a non-homogenous mesenchymal cell population, priming them for differentiation, or selectively enriches the cultures with cells retaining differentiation potential, although there is some evidence for the latter [4]. This system shows that changes in the level of SOX9 mRNA expression are not controlling matrix production in these differentiating mesenchymal cells.

**REFERENCES:** <sup>1</sup> Johnstone B *et al* (1998) *Exp. Cell Res.* 238, 265-272. <sup>2</sup> Mastrogiacomo M *et al* (2001) *Osteoarthritis Cartilage* 9, S36-S40. <sup>3</sup> Mackay AM *et al* (1998) *Tissue Eng.* 4, 415-428. <sup>4</sup> Bianchi G *et al* (2003) *Exp. Cell Res.* 287, 98-105.

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