

Evidence of a Chondroprogenitor Population in Human Osteoarthritic Cartilage

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INTRODUCTION: We have previously reported the presence of a chondroprogenitor population that resides in the superficial zone of immature bovine articular cartilage (1). There is much interest surrounding the therapeutic potential of this cell in terms of generating hyaline-like tissue in articular cartilage repair procedures such as autologous chondrocyte implantation. The question remains, however, as to whether this cell is present in mature human articular cartilage or during a disease state such as osteoarthritis. Alsalameh *et al* (2) have reported the presence of a mesenchymal progenitor cell population in normal and osteoarthritic articular cartilage, based upon the co-expression of cell surface markers CD105 and CD166. Here, we describe the isolation and expansion of clonally derived chondroprogenitor cells from human osteoarthritic articular cartilage.

METHODS: Articular cartilage was excised under sterile conditions from tibial plateaus obtained from patients undergoing total knee replacement for osteoarthritis. The articular cartilage was either subjected to a sequential digestion with pronase and collagenase or cryopreserved for cryosections. The isolated cells were then subjected to a differential adhesion assay to fibronectin or PBS (control) coated 6-well plates for 20 minutes after which non-adherent cells were removed (1). The adherent cells were cultured for up to 10 days in DMEM/F12 containing 10% FCS. Distinct colonies >32 cells were then cloned and cultured in DMEM/F12 containing 10% FCS in the presence or absence of 5ng/ml bFGF. Colonies of more than 32 cells were also fixed in 95% ethanol or pre-treated with monensin and then fixed in 95% ethanol for immunohistochemistry. Cartilage cryosections and fixed colonies were immunolabelled for Notch-1, CD166 and CD105. Colonies pre-treated with monensin were immunolabelled for type II, type IIA and type I collagen.

RESULTS: Following differential adhesion to fibronectin, colonies of greater than 32 cells formed. Clonally derived chondrocytes cultured in the presence bFGF were expanded through 10 population doublings. Immunolabelling of the tissue cryosections showed strong Notch-1 (fig1b) and CD166 labelling in the chondrocyte clusters. Immunolabelling of the colonies demonstrated that all cells in the colonies were Notch-1 positive (fig 1a). The colonies were also positive for CD166. Monensin blocked colonies demonstrated the presence of type I and type IIA collagen, however, they were negative for type IIB collagen

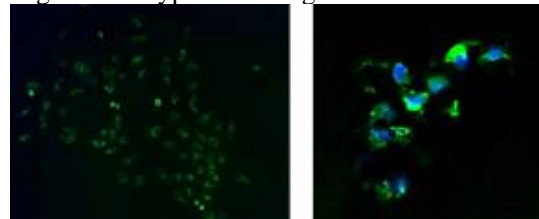


Figure 1: Notch 1 labelling of colonies (a) and clusters in tissue (b).

DISCUSSION & CONCLUSIONS: We have used a previously published method (1) to isolate a chondroprogenitor population from osteoarthritic articular cartilage that can be expanded successfully under the influence of specific growth factors. Immunohistochemical analysis of colonies formed from these cells demonstrates they may represent an immature chondrogenic phenotype. Notch-1 is involved cell fate selection and in combination with CD166 and CD105 immunolabelling may represent a marker of the chondroprogenitor cells *in situ*. The implication that a chondroprogenitor population is present within adult diseased articular cartilage presents many possibilities for the use of autologous tissue in the repair of articular cartilage

REFERENCES: ¹G. Dowthwaite *et al* (2004) *J Cell Sci* **12**: 106-16. ²S. Alsalemeh *et al* (2004) *Arthritis Rheum* **50**: 1522-32