

**NOTCH MODULATION IN ARTICULAR CARTILAGE.****GP Dowthwaite<sup>1,3</sup>, AS Williams<sup>1,2</sup> and CW Archer<sup>1,3</sup>**

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**INTRODUCTION:** We have previously described the isolation of a population of progenitor cells from the surface of articular cartilage. These cells express members of the Notch family of cell surface signalling molecules and several reports highlight the importance of Notch signalling in both articular cartilage and growth plate development. Mature Notch is presented at the cell membrane after S1 cleavage by a furin-like convertase. A second cleavage at the cell surface by members of the ADAM family (ADAM 10 and ADAM 17) allows Notch to interact with its ligands (Delta and Jagged). After activation, S3 cleavage by the gamma secretase complex releases the Notch Intracellular domain (NICD) which mediates transcription of various genes.

Here we report on the effect of interfering with Notch signalling at various steps in the Notch cleavage pathway using inhibitors of gamma secretase mediated S3 cleavage and ADAM-mediated S2 cleavage *in vitro* and discuss possible implications for clinical use

**METHODS:** Full depth articular cartilage explants were excised from 7 day-old bovine metacarpal-phalangeal joints and incubated in DMEM/F12 containing the gamma secretase inhibitor DAPT (50nM in DMSO) or rh TIMP 1 and 3 (0.1 and 0.01 uM) for up to 7 days. In some experiments explants were incubated with both rhIL-1 (1ng ml<sup>-1</sup>) and 50 nM DAPT for up to 7 days. Media samples were assayed for GAG content and explants were either fixed and processed for histology or digested with papain for GAG analysis using the DMMB assay.

Chondroprogenitor cells, were isolated using differential adhesion to fibronectin for 20 minutes and incubated in DMEM/F12 containing gamma secretase inhibitors as described for explants. Both initial adhesion and the colony forming efficiency of these cells was calculated.

Human osteoarthritic cartilage samples were obtained from patients undergoing knee arthroplasty. Full depth samples were fixed, wax embedded and labelled with antibodies to Notch family members.

Whole knee joints were obtained from rats/mice up to 14 days after the onset of antigen induced arthritis and wax embedded and labelled with antibodies to Notch family members.

**RESULTS:** In samples treated with DAPT, a hypocellular area was present in the transitional zone of the articular cartilage and this zone was very weakly stained with toluidine blue suggesting GAG loss from this region. DMMB assays revealed that GAG was not lost to the media with time and that the area of hypocellularity was due to inhibition of proliferation in the surface zone of the explants.

We also showed that the gamma secretase inhibitor DAPT did not prevent chondroprogenitor adhesion but did abolish colony forming efficiency. The use of S2 cleavage blockers (TIMP 1 and TIMP 3) similarly abolished the clonality of chondroprogenitors.

The addition of DAPT to IL-1 treated cartilage explants abolished the GAG loss associated with IL-1 treatment, suggesting that DAPT may be of possible use in the treatment of osteoarthritis.

Finally, immunohistochemical studies reveal the recapitulation of Notch signalling molecules in both human OA joint tissues and in a collagen induced model of arthritis in the mouse, particularly the expression of Notch 1.

**DISCUSSION:** These results highlight the importance of Notch signalling mechanisms during articular cartilage growth and development and the re-expression of Notch signalling molecules during both OA and RA highlight these molecules as possible targets for both pharmaceutical intervention and manipulation of the chondrocyte phenotype during *ex vivo* growth for tissue engineering.

**ACKNOWLEDGEMENTS:** This work was supported by the BBSRC and Smith and Nephew Research Centre.