

Spatial Pattern of the Delta-Notch Signalling Pathway in Osteoarthritic Cartilage

[LM Haughton](#)¹, [GP Dowthwaite](#)¹, [SN Redman](#)¹, [AS Williams](#)², [CW Archer](#)¹

¹[Connective Tissue Biology Labs](#), School of Biosciences, Cardiff University, Wales, GB

²Department of Rheumatology.

INTRODUCTION: Notch family members (Notch 1, 2, 3 and 4) are single-pass transmembrane proteins, consisting of a cytoplasmic and an extracellular domain. The extracellular domain binds its ligands, Delta and Jagged (Serrate in *Drosophila*).

The Notch signalling pathway involves three proteolytic cleavages and functions in cell fate decisions including apoptosis, proliferation and terminal differentiation during development¹. Notch is also involved in homeostasis of adult self renewing stem cells and is implicated in disease e.g. osteoarthritis, rheumatoid arthritis and Alzheimer's^{1,2,3}

Previous work has shown the presence of Notch family members within articular cartilage during mouse development³. Localisation of Notch 1 in 7-day-old bovine articular cartilage corresponds with a chondrogenitor population present at the articular surface⁵.

The aim of this study was to map expression of Notch family members and its ligands in articular cartilage from human osteoarthritic tibial plateaus in order to determine the role of Notch signalling in disease progression.

METHODS: Osteochondral plugs were removed from tibial plateaus obtained from consented patients undergoing TKR. Plugs were fixed in NBFS, decalcified (10% EDTA), wax embedded and sectioned. Sections were stained with toluidine blue and safranin O and immunolabelled for Notch 1-4 and Notch ligands Jagged and Delta and visualized using Vector Nova Red peroxidase substrate.

RESULTS: Localisation of various Notch receptors differed throughout the depth of the osteochondral plug from the surface zone to the underlying bone. Notch 1 was localized to the surface zone and the deep zone. Intense label for Notch 1 was also present within chondrocyte clusters. Notch 2 was absent throughout the depth of articular cartilage but both fibrocartilage and areas of vascular invasion within the underlying subchondral bone labelled positively for Notch 2.

Notch 3 and 4 showed similar patterns of Notch expression in that chondrocyte clusters and hypertrophic chondrocytes labelled positively. Expression of Notch 3 and 4 also occurred in

areas of vascular invasion and fibrous repair. Delta and Jagged labelled positively within chondrocyte clusters and also areas associated with vascular invasion within the underlying subchondral bone.

DISCUSSION & CONCLUSIONS: Notch 1 labelling in the surface zone of osteoarthritic cartilage mimics the expression pattern seen during murine development and in 7-day-old bovine tissue^{4,5}. Positive labelling of Notch 1 within chondrocyte clusters suggests Notch may play a role in cluster formation and, therefore, the pathogenesis of osteoarthritis

The absence of Notch 2 in osteoarthritic cartilage suggests that although Notch 2 plays a role during development, it may not play a role in reparative responses thus not recapitulating developmental processes.

Expression of Notch 3 within chondrocyte clusters appears to be a novel pattern. Previous work suggested a role for Notch 3 retarding the cell cycle which is contrary to its presence within rapidly proliferating osteoarthritic chondrocyte clusters⁴.

The presence of Notch 4 within chondrocyte clusters contradicts previous work which suggested its role in preventing vascular invasion as invasion from the underlying subchondral bone is a feature of OA and, therefore, may play an alternative role in the pathogenesis of osteoarthritis⁴.

The localisation of the ligands Delta and Jagged, further supports the evidence for a role of the Notch signalling pathway in osteoarthritic cartilage.

Therefore, further research of the Notch signalling pathway is necessary in order to investigate cluster formation through uncontrolled proliferation.

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