

## Notch family signalling and notch signalling modulation during chondrogenesis

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**INTRODUCTION:** It has been shown that expression of the Notch signalling pathway regulates many aspects of limb development and plays a vital role in chondrocyte maturation<sup>1</sup>. The Notch family of transmembrane signalling receptors comprises the Notchs 1-4 and their corresponding ligands Delta (D) and Jagged (J). In this study, we first demonstrate the differential distribution of the Notch family receptors during development and secondly, we investigate the effects of inhibiting the Notch pathway using the  $\gamma$ -secretase inhibitor DAPT<sup>2</sup> demonstrating the importance of Notch's role in chondrogenesis.

**METHODS:** Chick hind limbs (HH st26-st44) and micromass cultures were immunolabelled with antibodies to Notch 1-4, D1 and J1 (10 $\mu$ gml<sup>-1</sup>, Santa Cruz Biotech Inc, USA). Micromasses were established from wing mesenchyme of st23 chick embryos at a density of 2 x10<sup>5</sup> cells per 10 $\mu$ l as described<sup>3</sup>. Micromasses were treated with the  $\gamma$ -secretase inhibitor DAPT (Calbiochem,UK) to inhibit S3 cleavage of Notch preventing Notch signal activation. Controls consisted of the DAPT vehicle, DMSO. Media samples and micromasses were analysed for GAG content using the DMMB assay<sup>4</sup>. Results were expressed as total GAG content normalised to DNA. Micromasses were fixed in NBFS and stained with alcian blue pH1.0 overnight. Labelled nodules were counted. One way ANOVA was used for all statistical analysis.

**RESULTS:** The 4 Notch receptors and their ligands D1 and J1 showed distinct distribution throughout development of the chick limb. Notch 1 (N1) was seen extensively in the interzone region of the developing limb where D1 and J1 expression was also evident (Fig1). Post cavitation D1 and J1 were present in the cartilage surface but to a lesser extent than N1.

*In vitro* studies showed that on addition of the Notch inhibitor DAPT significant (P<0.05) increases in the total amount of GAG occurred. No change in DNA content was observed. A 3 fold increase in chondrogenic nodule number

was revealed by alcian blue staining in DAPT treated micromasses (Fig2).

Immunolabelling of DAPT treated micromasses showed a higher proportion of N1 label within the chondrogenic regions. D1 was seen to be expressed in the DAPT treated micromasses whereas J1 expression was lost.

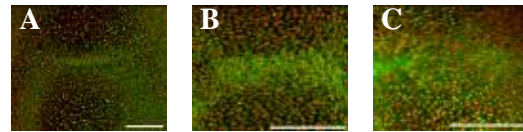


Fig.1 *St32 chick limb showed expression of A) N1; B) D1; C) J1. Scalebar=100 $\mu$ m.*

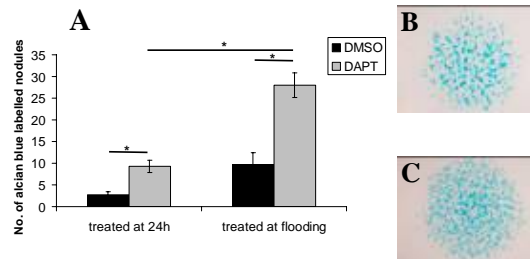


Fig.2 A) Numbers of alcian blue labelled chondrogenic nodules in DAPT/DMSO treated micromasses; Alcian blue stained micromasses treated with B) DMSO C) DAPT.

**DISCUSSION & CONCLUSIONS:** Mapping Notch family member expression in development of the avian limb shows a differential distribution pattern of expression suggesting Notch signalling is a necessary factor in the development and homeostasis of the cartilage anlagen. Inhibition of the Notch pathway using the  $\gamma$ -secretase inhibitor DAPT in mesenchyme micromass cultures demonstrates enhanced chondrogenesis that may reflect precocious cartilage differentiation of normally non-chondrogenic cells. This could be the result of a loss of lateral inhibition, suggesting that the Notch signalling pathway plays a vital role in chondrogenesis.

**REFERENCES:** <sup>1</sup>Crowe, R. *et al.* (1999). *Development* **126**, 987-98; <sup>2</sup>Lai, E.C. (2004). *Development* **131**, 965-73; <sup>3</sup>Dovey, H.F. *et al.* (2001). *Neurochem* **76**, 173-81; <sup>4</sup>Cottrill, C.P. *et al.* (1987) *Development* **131**, 503-515; <sup>5</sup>Farndale, R. *et al.* (1982). *Connect Tissue Res* **9**, 247-8.

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