

## Notch 1 expression as a sign for proliferation in Anulus Fibrosus after trauma of the cervical spine? An histological, immunohistological and ultrastructural study.

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**INTRODUCTION:** We examined 90 samples from patients with lower cervical spine injury using histological, immunohistological and ultrastructural techniques to determine the effect of trauma on cell-signalling molecules.

**METHODS:** Anulus fibrosus (AF) from 30 patients (17y-78y) were removed during treatment for spinal trauma and fixed immediately. Fracture type according to Magerl's classification was recorded. Samples were fixed for routine histology and immunocytochemistry in paraformaldehyde and embedded in Paraplast. Sections were stained with haematoxylin-eosin and immunolabelled with goat anti-human Notch 1(C-20), rabbit anti-human Notch 2 (25-255), rabbit anti-mouse Notch 3 (M-134), rabbit anti-human Notch 4 (H-225) (Santa Cruz Biotechnology),  $\alpha$ -smooth muscle actin (Sigma) and PCNA (Sigma) in PBS for 1 hour and localised with appropriate fluorescent secondary antibodies. For TEM, tissue samples were fixed and processed using standard TEM procedures.

**RESULTS:** Histologically all samples contained cell clusters in the outer AF. Vessel invasion was apparent 26 days post trauma. Using TEM, damaged (necrotic) cells were prominent in the first week post trauma but after this time normal ultrastructure of disc cells were visible. No differences could be noted according to age or sex of the patients. Immunohistological labelling showed twenty patients to be Notch 1 positive whilst five samples were PCNA-positive. Most of the Notch 1 positive cells were found in cell clusters in the outer AF. Only five patients stained positive for Notch 2.  $\alpha$ -smooth muscle actin positive cells were only present in blood vessels in the outer AF.

**DISCUSSION & CONCLUSIONS:** Our results show that after trauma, cell clusters were apparent in the outer AF, however these cells

did not label for PCNA or alpha smooth muscle actin as has previously been observed (Johnson et al 2001; Hastreiter et al 2001). Our results suggest that the cell proliferation events leading to cluster formation occur very quickly after trauma (< 21 days). The expression of members of the Notch family of cell signalling molecules in these clusters would suggest that after proliferation the cells begin to differentiate along different pathways.

We do not know at the moment, if neovascularisation of the disc tissue is based on migrating endothelial cells and/or proliferation of endothelial cells. In TEM sections, cells with processes, similar to migratory cells, were present near capillaries. This observation suggests that some cells might migrate in the damaged disc tissue. Proliferation of disc cells leading to cluster formations and vessel ingrowths leading to granulation tissue seem to be part of a repair strategy of the traumatised disc.

**REFERENCES:** WEB Johnson, SM Eisenstein, S Roberts (2001). Cell cluster formations in degenerate lumbar intervertebral discs is associated with increased disc cell proliferation. *Connective tissue Research* 42(3): 197-207.

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