Expression of Proteinase-Activated Receptor 2 on the Human Intervertebral Disc

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Introduction: Intervertebral disc (IVD) degeneration is characterized biochemically by the degradation of the extracellular matrix (mainly, proteoglycans and collagens). Recent studies suggest these changes in macromolecular assemblies are induced by matrix-degrading enzymes, such as the matrix metalloproteinases (MMPs), ADAMTS [1, 2] and serine proteinases [3] under the stimulation of proinflammatory cytokines [e.g., interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)]. Proteinase-activated receptor 2 (PAR2) is one of a unique subfamily of G protein-coupled receptors that are activated by several serine proteinases (e.g., elastase) [4]. The activation of PAR2, via protease cleavage and exposure of the tethered ligand, is known to exert an inflammatory reaction with upregulation of cytokines in several cell types [5]. The expression of PAR2 was also identified in articular cartilage and was thought to contribute to the pathogenesis of osteoarthritis [6]. Recent data indicated that PAR2 was expressed in rat IVD cells and, importantly, its expression was upregulated by IL-1β [7]. We hypothesized that PAR2 is expressed in IVD cells and is involved in the regulatory mechanism of IVD degeneration. The expression of PAR2 in the human IVD remains unknown.

The purpose of this study was (1) to determine if human IVD cells express PAR2, and (2) to quantify the PAR2-expressing cells in human IVD tissues of early stage and advanced stages of degeneration.

Materials and Methods: Human disc tissue: Human lumbar IVDs were obtained from (a) cadaveric human spines (10 donors, 12 discs) and (b) tissues obtained from spine surgery (6 patients, 6 discs) (Total: 18 discs; mean age: 64.2 years [range 43-76]; MRI: Thompson grade: average 3.1 [range 1-4]). Tissue preparation for histology: The specimens were fixed in 4% paraformaldehyde and then embedded in paraffin. 5-μm-thick sections were stained with Safranin-O and hematoxylin and eosin (H-E). Serial sections adjacent to the sections stained with Safranin-O/H-E were used for immunohistochemical analysis.

Immunohistochemistry: The specimens were incubated with a mouse monoclonal anti-human PAR2 antibody overnight at 4°C. Mouse IgG was used as an isotype control. Color was developed with an immuno-peroxidase polymer.

Immunoblotting for PAR2: Two IVD tissues (mean age 48.5 years; range 46-51) obtained from surgery were used for western blot analysis. The specimens were homogenized and lysed with RIPA buffer, including protease inhibitors, for seven days at 4°C. Proteins (30 μg per well) were separated by SDS-PAGE (7.5% acrylamide) under reducing conditions, followed by western blotting using the anti-PAR2 antibody. Quantification of PAR2 expression: Human IVDs were divided into two groups; early stage degeneration (Early group, MRI: Thompson grades 1 and 2) and advanced stage degeneration (Advanced group, grades 3 and 4). The number of samples used in each group was as follows: early group (NP: n = 6, AF: n = 11); advanced group (NP: n = 6, AF: n = 12). PAR2-immunoreactive cells were divided into three groups according to the intensity of staining (none: -: moderate: +; strong: ++). Under light-microscopy, five visual fields (x200) on each sample were randomly chosen, and the total number of cells and PAR2-positive cells were manually counted. The percentage of PAR2-immunoreactive cells was calculated as follow: the number of PAR2-positive cells/total number of cells x 100 (%).

Statistical Analysis: The evaluation of statistical differences between the groups was determined by the unpaired student t-test. P values less than 0.05 were considered significant.

Results: PAR2 expression in human IVD tissues: Immunoreactivity to the anti-PAR2 antibody was clearly identified in human IVD cells, showing a punctate cytoplasmatic staining pattern (Fig. 1). PAR2-immunoreactive cells were distributed in both AF (anterior and posterior) and NP tissues.

Immunoblotting for PAR2: A single protein band with a molecular mass of about 55 kDa was found (Fig. 2).

Discussion: We have demonstrated for the first time that PAR2 is expressed in human IVD tissues. Importantly, the number of PAR2-immunoreactive cells in the AF was increased in tissues with an advanced stage of degeneration when compared to tissues with an early stage of degeneration. This increase may suggest a role for PAR2 as one of the factors involved in, or as a phenotypical marker for, the degenerative disc, especially in the AF.

Because the serine proteinases that are capable of activating PAR2 are known to exist in the degenerated human IVD [3], PAR2 activation may possibly result in an upregulation of cytokine cascades, as previously reported in other cell types [5], and thus induce disc degeneration. However, it remains to be proven if PAR2 activation does indeed exert a biological effect on disc cells. Studies on the metabolic changes resulting from the inhibition of the PAR2 pathway or the activation of PAR2 agonists in disc cells may elucidate the mechanisms for the involvement of PAR2 in disc degeneration.


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