ANALYSIS OF THE STRUCTURAL HETEROGENEITY OF VERSICAN IN HUMAN INTERVERTEBRAL DISC

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Relevance to Musculoskeletal Conditions - The presence of versican may be important for intervertebral disc function, and changes in its structure may be associated with abnormal function and disc degeneration.

Introduction - The intervertebral disc contains many proteoglycans within its extracellular matrix, the most abundant of which is aggrecan, a member of the hyaluronate-binding family of proteoglycans, whose structure and abundance change considerably with disc site and age. The disc also contains a second member of this proteoglycan family, versican, though nothing has yet been reported on variation in its structure or abundance within the tissue. Versican possesses two structural domains of particular interest - an amino terminal globular domain (G1) responsible for the interaction with hyaluronate and a central chondroitin sulfate attachment region. This latter domain is encoded by two exons (7 and 8 in the human) which may be subject to alternative splicing. Splice variants have been described possessing both exons (Vo), only exon 8 (V1), only exon 7 (V2), or neither exon (V3), depending on the tissue being studied. In addition, it is likely that versican exhibits structural heterogeneity due to proteolytic processing, in a manner similar to aggrecan, where the G1 domain is often separated from the remainder of the proteoglycan. The purpose of the present study was to determine which splice variants of the versican gene are expressed in the human intervertebral disc, to what extent the gene products might be proteolytically processed during growth and aging, and whether similar structural heterogeneity is present in both the annulus fibrosus and nucleus pulposus regions of the disc.

Methods - Human intervertebral discs were obtained from the lumbar spine of individuals ranging in age from the neonate to the mature adult. The discs were divided into three regions - the anterior annulus, the posterior annulus and the central nucleus - and the tissue was used for either direct extraction of total RNA with guanidinium isothiocyanate or extraction of matrix proteoglycans with guanidinium chloride. The RNA was purified by phenol/chloroform extraction and used to generate cDNA, from which the presence of the four splice variants could be studied by PCR analysis. The matrix proteoglycans were treated with chondroitinase ABC and keratanase to determine the presence of versican G1 domains, in the nucleus. Sizes ranged from that of an isolated G1 domain to the intact core protein, and there was no major change in the pattern of structural heterogeneity with age. A similar range of structural heterogeneity was also observed in both the anterior and posterior regions of the annulus. In the annulus there was a trend towards lower detection of versican in the older adult specimens. When extracts of more mature adult intervertebral disc were analyzed under equivalent conditions, it was apparent that versican levels were indeed higher in the nucleus than the annulus. In addition, both disc regions possessed greater amounts of versican than articular cartilage. Other experiments also demonstrated that detection of the disc versican was considerably decreased on the immunoblots if the keratanase treatment was omitted, indicating that the versican G1 region can be substituted with keratan sulfate.

Results - Analysis of message heterogeneity was carried out directly on extracts of intervertebral disc so that any changes in gene expression associated with cell isolation or culture could be avoided. The work revealed that in both the adult annulus fibrosus and nucleus pulposus, only the V1 form of versican message, possessing exon 8 but not exon 7, could be detected. Analysis at the protein level identified multiple forms of versican, all possessing G1 domains, in the nucleus. Sizes ranged from that of an isolated G1 domain to the intact core protein, and there was no major change in the pattern of structural heterogeneity with age. A similar range of structural heterogeneity was also observed in both the anterior and posterior regions of the annulus. In the annulus there was a trend towards lower detection of versican in the older adult specimens. When extracts of more mature adult intervertebral disc were analyzed under equivalent conditions, it was apparent that versican levels were indeed higher in the nucleus than the annulus. In addition, both disc regions possessed greater amounts of versican than articular cartilage. Other experiments also demonstrated that detection of the disc versican was considerably decreased on the immunoblots if the keratanase treatment was omitted, indicating that the versican G1 region can be substituted with keratan sulfate.

Discussion - The adult human intervertebral disc appears to express only the V1 isoform of versican in all regions. This is similar to articular cartilage, where at all postnatal ages only the V1 isoform of versican has been detected. In common with aggrecan, the disc versican appears to be extensively modified by proteolysis within the extracellular matrix, ultimately resulting in the accumulation of isolated G1 domains. Also in common with aggrecan, the versican G1 domain can be substituted with keratan sulfate. This is somewhat surprising as versican is commonly considered to possess only chondroitin sulfate. Presumably, the keratan sulfate occupies a site that is compatible with N-linked or O-linked oligosaccharide synthesis. At present it is not clear whether matrix metalloproteinases or aggrecanase result in the accumulation of isolated G1 domains. It is also unclear whether the levels versican in the disc exceeds that in articular cartilage at all ages, as in the latter tissue versican levels appear to be most abundant in the young juvenile. It is interesting to speculate that the increased presence of versican in the adult nucleus may act as a compensatory mechanism to counteract the age-related decrease in aggrecan the precedes disc degeneration. At present, however, it is unclear whether versican is able to fulfill the compression resisting function normally associated with aggrecan.

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