LOCALIZATION AND DISTRIBUTION OF CARTILAGE OLIGOMETRIC MATRIX PROTEIN IN SPINE

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Introduction  Cartilage oligomeric matrix protein (COMP) is an important structural component of extracellular matrix [1]. Previous reports have shown that it is primarily localized to articular cartilage, with presence also found in tendon, ligament, and synovium. Its importance is suggested by its association with several pathological conditions. COMP distribution and degradation rates are altered in human rheumatoid and osteoarthritic cartilage [2]. Altered levels of COMP and its degradation products in serum and synovial fluid have been proposed to be used as markers for cartilage destruction in osteoarthritis and rheumatoid arthritis [2,3]. The importance of COMP to cartilage structure and function is further underscored by findings that COMP mutations lead to human skeletal dysplasias, pseudoachondroplasia, and multiple epiphyseal dysplasia, including Fairbanks and Ribbing types [4,5]. These dysplasias are due to mutations in residues in the type 3 calcium-binding repeats and COOH-terminal globular region of COMP which lead to the misfolding of the protein [6]. Patient’s spine also show characteristic platyspondyly [4,5], especially in childhood, suggesting that COMP plays a role in maintaining normal spine structure and function. However, the distribution and role of COMP in spine has not been investigated. The purpose of this study is to investigate the distribution of COMP in normal spine with emphasis on intervertebral disc localization.

Materials and Methods: Sprague-Dawley male rats, 9 months old, 500 gms each, were used in this study. Intervertebral discs (IVD) from rat tail as well as lumbar spine were dissected from the surrounding tissues. Total RNA was isolated and reverse transcribed followed by PCR amplification. IVD was also homogenized and extracted with 4 M Guanidine HCl, and extracted proteins were separated by SDS polycrylamide gel electrophoresis, transferred to a piece of nitrocellulose membrane, followed by immunodetection with F8 polyclonal antibodies recognizing COMP [6]. Spine samples for histology and immunohistochemistry were fixed in 10% formalin for 24 hours before IVD was dissected out and paraffin embedded. 5 μm cross sections were cut and immunostained with COMP antibody F8 using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) followed by hematoxylin counterstaining.

Results: Protein extraction from IVD, when separated on SDS-PAGE and immunoblotted with F8 polyclonal antibodies against COMP, showed the presence of intact COMP which comigrated with the positive control of recombinant human COMP (Figure 1). Since COMP has been shown to exist in serum, which through circulation may reach the IVD, we further confirmed that COMP was produced by IVD in situ using RT-PCR (Figure 2). COMP was detected in IVD along with the other extracellular matrix proteins including collagen type II and aggrecan (Figure 2 left panel). COMP message was detected in IVDs from both lumbar spine as well as tail (Figure 2 right panel).

Immunohistochemistry further localized COMP to IVD from both the lumbar spine and tail (Figure 3). COMP was found in both the annulus fibrosus and nucleus pulposus. COMP antibody stained strongly the annulus fibrosus of both tail and lumbar spine, with obvious lamellae patterns, which were more obviously observed with distinct layers in the tail IVD (Figure 3 B, C, D and F). The nucleus pulposus also stained markedly with COMP in the extracellular matrix between the cells (Figure 3 B, C, E and G). In sagittal sections of lumbar spine and tail, COMP was localized to the endplates and subchondral bone of the spine (data not shown).

Discussion: We have shown the presence of COMP in the IVD from both the tail and lumbar spine of the rat, using RT-PCR, immunoblot, as well as immunohistochemistry methods. RT-PCR with IVD indicates that COMP can be produced by the cells in IVD in situ. Immunohistochemistry data have shown that COMP is present in the annulus fibrosus as well as the nucleus pulposus of IVD. The laminae pattern of staining indicates that COMP may play a role in maintaining the normal structural stiffness of the IVD. The localization and distribution of COMP in IVD also provides structural basis for why mutations in COMP leading to pseudoachondroplasia and multiple epiphyseal dysplasia also affect patient spine. Our data also suggest that COMP, which has been indicated in articular cartilage degeneration, may also play a role in maintaining normal disc function and disc degeneration. Further studies will be needed to study the role COMP plays in various spine pathological conditions.