

# Characteristics of Small Leucine-rich Proteoglycans in the Intervertebral Disc Degeneration



Qingshen Wei<sup>1</sup> and Yuntao Zhang<sup>2\*</sup>

<sup>1</sup>Department of Orthopedics Surgery, Rizhao Traditionopnal Chinese Medicine Hospital, China

<sup>2</sup>School of Pharmaceutical Science, Jining Medical University, China

**Submission:** February 22, 2018; **Published:** March 07, 2018

**\*Corresponding author:** Yuntao Zhang, School of Pharmaceutical Science, Jining Medical University, Shandong, China, Tel: +86 633 2983688; Email: zhangyt16@gmail.com

## Abstract

The intervertebral disc (IVD) is important in the normal functioning of the spine. It is a cushion of fibrocartilage and the principal joint between two vertebrae in the spinal column and is responsible for spinal motion and load distribution. Small leucine-rich proteoglycans (SLRPs) are the major bioactive components of the extracellular matrix (ECM) of intervertebral disc and associated with fibrillogenesis, cellular growth and apoptosis and tissue remodelling. The most significant biochemical change to occur in disc degeneration is loss of proteoglycans (PGs). A more in-depth understanding of molecular basis of disc degeneration is essential to the design of therapeutic solutions to treat degenerative disc. This review focuses on the SLRP biochemical characteristics in the intervertebral disc degeneration.

**Keywords:** SLRPs; Proteoglycans; Glycosaminoglycans; Intervertebral discs; Degeneration

**Abbreviations:** SLRPs: Small Leucine-Rich Proteoglycans; PGs: Proteoglycans; GAGs: Glycosaminoglycans; KS: Keratan Sulfate; CS/DS: Chondroitin Sulfate/ Dermatan Sulfate; HS: Heparan Sulfate; IVD: Intervertebral Disc; ECM: Extracellular Matrix; AF: Annulus Fibrosus; NP: Nucleus Pulposus; LBP: Low Back Pain; PRELP: Proline/arginine-rich end Leucine-rich End Leucine-rich Repeat Protein; LRP: Leucine-Rich Repeats; MMPs: Matrix Metalloproteinases; TIMPs: Tissue Inhibitors of Metalloproteinases

## Introduction

The intervertebral discs (IVD) are partially movable joints that connect each of the vertebral bodies in the spine, functioning both to transfer loads and impart mobility [1]. Intervertebral discs are composed of an annulus fibrosus (AF) and a nucleus pulposus (NP). The AF is a strong radial tire-like structure made up of lamellae; concentric sheets of collagen fibers connected to the vertebral end plates. The extracellular matrix (ECM) of the central NP contains large quantities of the proteoglycans (PGs). Degeneration of the IVD is strongly implicated as a major cause of low back pain (LBP) [2,3]. Disc degeneration has been found to be associated with the loss of PGs function [4]. The etiology of disc degeneration has proven challenging to characterize because it is poorly defined and its progression is closely linked to aging [5]. Current knowledge of the principal pathogenesis resulting in this condition is limited.

Proteoglycans are macromolecules consisting of a protein core and glycosaminoglycans (GAGs) side-chains [6]. GAGs are unbranched carbohydrate chains of repeating disaccharide units. Since GAGs are negatively charged, they bind to other matrix molecules, cell adhesion molecules, and growth factors

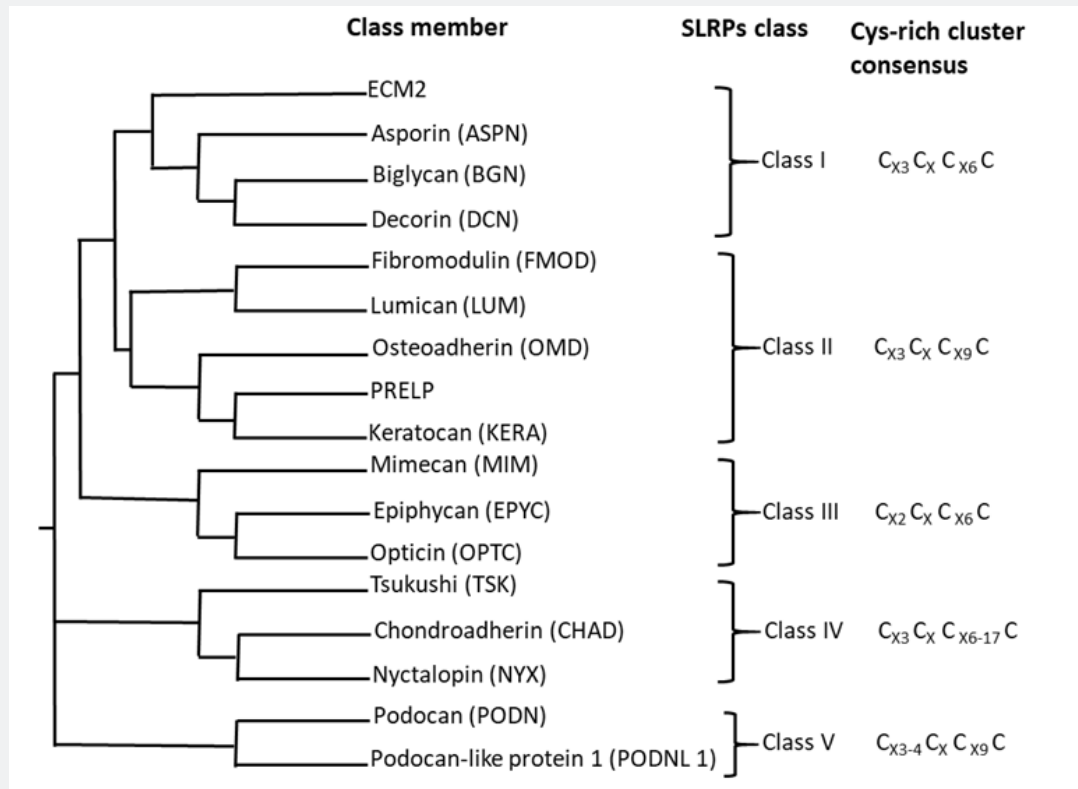
[7]. PGs can be divided into two classes: one class is the small leucine-rich proteoglycans (SLRPs) such as decorin, biglycan, fibromodulin, lumican, and mimican; another family consists of aggrecan, versican, brevican, and neurocan [6]. In this review, we discuss the biochemical characteristics of SLRPs in the intervertebral disc degeneration. Given the recent study that implicates SLRPs as the key components for IVD degeneration progression.

## SLRPs Classification

The class of SLRPs is a family of homologous proteoglycans harboring relatively small (36–42 kDa) protein cores harboring tandem leucine-rich repeats and undergoing post-translational modifications, including substitution with glycosaminoglycans (GAGs) side chains of various types [8, 9]. Originally, the SLRPs were grouped into three distinct classes based on nucleotide and protein sequence conservation, the organization of disulfide bonds at their N and C termini, and their genomic organization. More recently, the SLRPs gene family has expanded to encompass 18 genes classified into five distinct subfamilies by common structural and functional properties [10] (Figure 1). SLRPs are proteoglycans that have both protein cores and GAGs chains,

although non-canonical class IV and V SLRPs that do not contain any GAGs are also included in this family. The first class has a unique N-terminal Cys sequence. This includes decorin, biglycan and asporin, which are encoded by genes composed of eight exons with intron junctions in highly conserved positions [11]. The second class is comprised of five sub-members, including

fibromodulin, lumican, keratocan, proline/arginine-rich end leucinerich repeat protein (PRELP) and osteoadherin, which have an identical cysteine-rich region before the leucine-rich repeats (LRRs) [12, 13, 14]. This class of SLRPs is characterized by clusters of Tyr sulfate residues at their N-termini and contains primarily keratan sulfate chains and polylactosamine [15].



**Figure 1:** Classification and structural relationships of the SLRPs family. The consensus for the N-terminal Cys-rich cluster is shown next to the brackets.

ECM2: extracellular matrix protein 2; PRELP: proline/arginine-rich end leucine-rich repeat protein

The GAGs of SLRPs are differentially processed in development and aging, and are variable with regard to size, number, sulfation and epimerization in different tissues [16]. Through O-linked oligosaccharide, chondroitin sulfate/dermatan sulfate (CS/DS) chains are attached to core protein decorin [17,18]. In the case of decorin, a single CS/DS linkage site is present near the amino terminus of the core protein [19, 20], whereas lumican and keratocan possess four or five potential keratan sulfate (KS) attachment sites in the central leucine-rich repeat region of each core protein molecule [21,22,23], and mimecan has two potential KS attachment sites [24, 25]. Current molecular models of the corneal stroma suggest that these proteoglycan core proteins wrap themselves laterally around the collagen fibrils in a manner that folds their hydrophobic domains inside, against the collagen fibrils [26]. In contrast, the highly sulfated GAG chains (together with their associated water molecules of hydration) are thought to stick out laterally away from the sides of the collagen fibrils, forming an exterior hydrophilic shell. The thickness of that shell matches the thickness of the shell

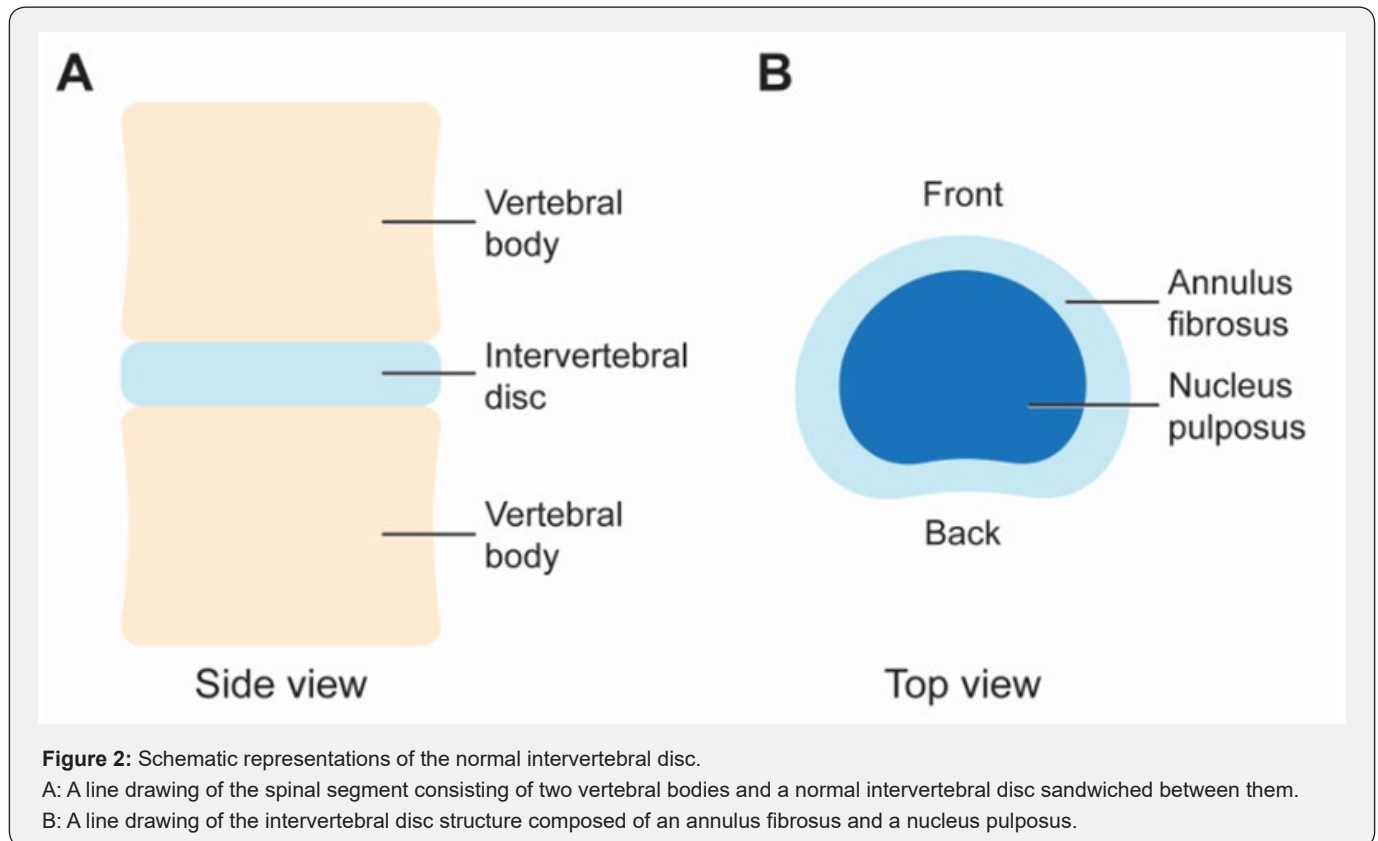
surrounding adjoining fibrils, producing a very precise center-to-center spacing between the collagen fibrils characteristic of the corneal stroma and necessary for its transparency [27]. Through this interaction with collagen (mostly with type I), PGs play important biological roles in collagen fibrillogenesis and matrix assembly.

### Structure of the Intervertebral Disc

The intervertebral discs lie between the vertebral bodies, linking them together (Figure 2). They are the main joints of the spinal column and occupy one-third of its height. Their major role is mechanical, as they constantly transmit loads arising from body weight and muscle activity through the spinal column. They provide flexibility to this, allowing bending, flexion and torsion. They are approximately 7–10mm thick and 4cm in diameter (anterior–posterior plane) in the lumbar region of the spine [2,5]. Intervertebral discs consist of an outer fibrous ring, the AF disci intervertebralis, which surrounds an inner gel-like center, the AP [5]. The AF is a strong radial tire-like structure made up

of lamellae; concentric sheets of collagen fibers connected to the vertebral end plates. The central NP contains large quantities

of the SLRPs and aggrecan, which aggregates along chains of hyaluronan [28].



The GAGs side chains of these PGs carry a fixed negative charge and generate an osmotic swelling pressure within an irregular meshwork of collagen II fibrils. Two thin endplates of hyaline cartilage extend superiorly and inferiorly over the inner AF and NP to interface with the vertebral bodies, and function to regulate nutrient diffusion between the disc and the vertebral bodies [29,30]. In the outer regions of the AF, collagen fibers anchor directly into the vertebral bone.

### Biological Roles of SLRPs in the Intervertebral Disc

It is now firmly established that specific SLRPs are functionally involved in intervertebral disc development and homeostasis. The SLRPs play important roles in the control of collagen fibrillogenesis, growth factor binding and sequestration or presentation and they can interact with signaling molecules controlling proliferation, differentiation and ECM synthesis and turnover [31]. Decorin regulates collagen fibrillogenesis, collagen degradation, cell growth and extracellular signaling in the ECM and connective tissue formation in skeletal muscle [32-34]. Fibromodulin and lumican are close homologues and play a role in the regulation of the assembly of collagen monomers into fibrils, which is important to the structural and mechanical integrity of connective tissues [35,36]. It has been reported that fibromodulin and lumican can influence collagen fibrillogenesis and hence fibril thickness [37].

Fibronectin is probably the most ubiquitous and best characterized of the adhesive glycoproteins. It plays a key role in matrix organization by interacting with integrins such as  $\alpha 5 \beta 1$  on cell surfaces, as well as ECM components such as collagen, fibrin and heparan sulfate (HS) PGs [38]. Many interactions between the cell and its surrounding ECM affecting cell adhesion, morphology and migration are modulated by glycoproteins (on cell surfaces and within the ECM). Normal disc function depends upon a balance between these activities. GAGs may also play an important role in regulating the development, growth and homeostasis of the disc through their ability to interact with soluble bioactive signaling molecules via sulfation motifs within their chain structure [39,40]. In general, the GAGs content of the disc is greatest within the NP, decreasing outwards towards the edges of the AF [41]. Sulfation confers a strong negative charge on the GAGs which allows them to bind water and provides viscoelastic properties to disc tissues [42]. The mature NP has the highest concentration of KS of any tissue and the KS isoform has a much larger chain length than equivalent isomers in other tissues [43,44].

### Changes of SLRPs in Intervertebral Disc Degeneration

The most significant biochemical change to occur in disc degeneration is loss of proteoglycans. With increasing age and degeneration, the disc changes in morphology, becoming more

and more disorganized. One of the major changes in ageing and disc degeneration is a decrease in fibromodulin in the adult NP and an increase in lumican in the AF during early juvenile development [45]. This may also involve structural changes, characterized by a loss of KS attachment [46]. Additionally, the fragmentation of fibromodulin is identified in the process of IVD degeneration and is the most extensively fragmented in the IVD [47]. Biglycan deficiency may be a possible mechanism of IVD degeneration, with disruption of the organization of collagen fibres and hence the ECM meshwork [48,49]. Fibromodulin is more abundant in the AF than in NP at all ages, and lumican is much more abundant in NP than in AF in the juvenile disc [46]. Keratocan has been identified in the IVD of patients with various disc disorders, in the forms of intact core protein and small fragments. Keratocan is either non-glycosylated or composed of monosulfated GAGs chains [50]. In addition, during maturation and ageing there is a steady increase in the ratio of KS to CS and an increase in the sulfation of the KS disaccharides. The concentration of CS/DS in the disc decreases with age and especially during the process of degeneration [51,52].

In addition, several matrix metalloproteinases (MMPs) have been identified in the IVD that appear to play a role in pathological degradation of the PGs in the ECM of the IVD [53]. Increased amounts of gelatinases (MMPs 2 and 9) [54], collagenases (MMPs 1, 8, 13) and stromelysin (MMP3) [55, 56] are found in more degenerate human IVD. Interestingly, the production of tissue inhibitors of metalloproteinases (TIMPs) and MMPs, or aggrecanases, appears to be linked; in more degenerate discs; increased MMP levels are accompanied by TIMP 1 [55] and TIMP 2 [56].

Furthermore, the loss of PGs in degenerate discs has a major effect on the disc's load-bearing behavior [57]. With loss of PGs, the osmotic pressure of the disc falls and the disc is less able to maintain hydration under load; degenerate discs have a lower water content than do normal age-matched discs, and when loaded they lose height and fluid more rapidly, and the discs tend to bulge [58]. Loss of PGs and matrix disorganization has other important mechanical effects; because of the subsequent loss of hydration, degenerated discs no longer behave hydrostatically under load [59]. With consequent loss of elasticity, the ligament will tend to bulge into the spinal canal, leading to spinal stenosis – an increasing problem as the population ages [5]. Moreover, lumbar disc herniation is one of the most common spinal degenerative disorders which may lead to LBP, radicular leg pain and disability.

### Conclusion and Perspectives

Degeneration of the intervertebral discs is a natural progression of the aging process. The most significant biochemical change to occur in disc degeneration is loss of proteoglycans. SLRPs plays a key role in mediating and keeping

the normal function of intervertebral disc, which may propose a potential of SLRPs-based therapies in disc regeneration and possibly the repair of other skeletal tissues.

### References

1. Raj PP (2008) Intervertebral disc: anatomy-physiology-pathophysiology-treatment. *Pain Pract* 8(1): 18-44.
2. Bogduk N (1991) The lumbar disc and low back pain. *Neurosurg Clin N Am* 2(4): 791-806.
3. Freemont AJ (2009) The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. *Rheumatology* 48(1): 5-10.
4. Roughley PJ, Alini M, Antoniou J (2002) The role of proteoglycans in aging, degeneration and repair of the intervertebral disc. *Biochem Soc Trans* 30(Pt 6): 869-874.
5. Urban JP, Roberts S (2003) Degeneration of the intervertebral disc. *Arthritis Res Ther* 5(3): 120-130.
6. Halper J (2014) Proteoglycans and diseases of soft tissues. *Adv Exp Med Biol* 802: 49-58.
7. Lima M, Rudd T, Yates E (2017) New Applications of Heparin and Other Glycosaminoglycans. *Molecules* 22(5): e749.
8. Iozzo RV, Murdoch AD (1996) Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity. *FASEB J* 10(5): 598-614.
9. Iozzo, RV (1997) The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth. *Crit Rev Biochem Mol Biol* 32(2): 141-174.
10. Nikitovic D, Aggelidakis J, Young MF, Iozzo RV, Karamanos NK, et al. (2012) The biology of small leucine-rich proteoglycans in bone pathophysiology. *J Biol Chem* 287(41): 33926-33933.
11. Fisher LW, Heegaard AM, Vetter U, Vogel W, Just W, et al. (1991) Human biglycan gene. Putative promoter, intron-exonjunctions, and chromosomal localization. *J Biol Chem* 266(22): 14371-14377.
12. Blochberger TC, Vergnes JP, Hempel J, Hassell JR (1992) cDNA to chick lumican (corneal keratan sulfate proteoglycan) reveals homology to the small interstitial proteoglycan gene family and expression in muscle and intestine. *J Biol Chem* 267(1): 347 -352.
13. Funderburgh JL, Funderburgh ML, Brown SJ, Vergnes JP, Hassell JR, et al. (1993) Sequence and structural implications of a bovine corneal keratan sulfate proteoglycan core protein. Protein 37B represents bovine lumican and proteins 37A and 25 are unique. *J Biol Chem* 268(16): 11874-11880.
14. Corpuz LM, Funderburgh JL, Funderburgh ML, Bottomley GS, Prakash S, et al. (1996) Molecular cloning and tissue distribution of keratocan. Bovine corneal keratan sulfate proteoglycan 37A. *J Biol Chem* 271(16): 9759 -9763.
15. Bengtsson E, Neame PJ, Heinegård D, Sommarin Y (1995) The primary structure of a basic leucine-rich repeat protein, PRELP, found in connective tissues. *J Biol Chem* 270(43): 25639-25644.
16. Funderburgh JL, Funderburgh ML, Mann MM, Conrad GW (1991) Arterial lumican. Properties of a corneal-type keratan sulfate proteoglycan from bovine aorta. *J Biol Chem* 266(36): 24773-24777.
17. Li W, Vergnes JP, Cornuet PK, Hassell JR (1992) cDNA clone to chick corneal chondroitin/dermatan sulfate proteoglycan reveals identity to decorin. *Arch Biochem Biophys* 296(1): 190-197.



18. Bianco P, Fisher LW, Young MF, Termine JD, Robey PG (1990) Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. *J Histochem Cytochem* 38(11): 1549-1563.
19. Kjellén L, Lindahl U (1991) Proteoglycans: structures and interactions. *Annu Rev Biochem* 60: 443-475.
20. Spiro RC, Freeze HH, Sampath D, Garcia JA (1991) Uncoupling of chondroitin sulfate glycosaminoglycan synthesis by brefeldin A. *J Cell Biol* 115(5): 1463-1473.
21. Corpuz LM, Dunlevy JR, Hassell JR, Conrad AH, Conrad GW (2000) Molecular cloning and relative tissue expression of decorin and lumican in embryonic quail cornea. *Matrix Biol* 19(7): 699-704.
22. Dunlevy JR, Neame PJ, Vergnes JP, Hassell JR (1998) Identification of the N-linked oligosaccharide sites in chick corneal lumican and keratocan that receive keratan sulfate. *J Biol Chem* 273(16): 9615-9621.
23. Corpuz LM, Dunlevy JR, Hassell JR, Conrad AH, Conrad GW (2000) Molecular cloning and relative tissue expression of keratocan and mimecan in embryonic quail cornea. *Matrix Biol* 19(7): 693-698.
24. Chen R, Jiang X, Sun D, Han G, Wang F, et al. (2009) Glycoproteomics analysis of human liver tissue by combination of multiple enzyme digestion and hydrazide chemistry. *J Proteome Res* 8(2): 651-661.
25. Iozzo RV (1999) The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. *J Biol Chem* 274(27):18843-18846.
26. Meek KM, Quantock AJ, Boote C, Liu CY, Kao WW (2003) An X-ray scattering investigation of corneal structure in keratocan-deficient mice. *Matrix Biol* 22(6): 467-475.
27. Christiansen DL, Huang EK, Silver FH (2000) Assembly of type I collagen: fusion of fibril subunits and the influence of fibril diameter on mechanical properties. *Matrix Biol* 19(5): 409-420.
28. Smith LJ, Nerurkar NJ, Choi KS, Harfe BD, Dawn M, et al. (2011) Degeneration and regeneration of the intervertebral disc: lessons from development. *Dis Model Mech* 4(1): 31-41.
29. Nerlich AG, Schaaf R, Walchli B, Boos N (2007) Temporo-spatial distribution of blood vessels in human lumbar intervertebral discs. *Eur Spine J* 16(4): 547-555.
30. Urban JP, Smith S, Fairbank JC (2004) Nutrition of the intervertebral disc. *Spine* 29(23): 2700-2709.
31. Hayes AJ, Isaacs MD, Hughes C, Caterson B, Ralphs JR (2011) Collagen fibrillogenesis in the development of the annulus fibrosus of the intervertebral disc. *Eur Cell Mater* 22: 226-241.
32. Reed CC, Iozzo RV (2002) The role of decorin in collagen fibrillogenesis and skin homeostasis. *Glycoconj J* 19(4-5): 249-255.
33. Seidler DG, Goldoni S, Agnew C, Cardi C, Thakur ML, et al. (2006) Decorin protein core inhibits in vivo cancer growth and metabolism by hindering epidermal growth factor receptor function and triggering apoptosis via caspase-3 activation. *J Biol Chem* 281(36): 26408-26418.
34. Reed CC, Iozzo RV (2002) The role of decorin in collagen fibrillogenesis and skin homeostasis. *Glycoconj J* 19(4-5): 249-255.
35. Svensson L, Närlid I, Oldberg A (2000) Fibromodulin and lumican bind to the same region on collagen type I fibrils. *FEBS* 470(2): 178-182.
36. Ezura Y, Chakravarti S, Oldberg A, Chervoneva I, Birk DE (2000) Differential expression of lumican and fibromodulin regulate collagen fibrillogenesis in developing mouse tendons. *J Cell Biol* 151(4): 779-787.
37. Goldberg M, Ono M, Septier D, Bonnefoix M, Kilts TM, et al. (2009) Fibromodulin-deficient mice reveal dual functions for fibromodulin in regulating dental tissue and alveolar bone formation. *Cells Tissues Organs* 189(1-4): 198-202.
38. Hynes RO, Yamada KM (1982) Fibronectins: multifunctional modular glycoproteins. *J Cell Biol* 95(2 Pt 1): 369-377.
39. Hayes AJ, Hughes CE, Ralphs JR, Caterson B (2011) Chondroitin sulphate sulphation motif expression in the ontogeny of the intervertebral disc. *Eur Cell Mater* 21: 1-14.
40. Caterson B (2012) Fell-Muir Lecture: chondroitin sulphate glycosaminoglycans: fun for some and confusion for others. *Int J Exp Pathol* 93(1): 1-10.
41. Bushell GR, Ghosh P, Taylor TF, Akeson WH (1977) Proteoglycan chemistry of the intervertebral disks. *Clin Orthop Relat Res* 129: 115-123.
42. Urban J, Maroudas A (1980) The chemistry of the intervertebral disc in relation to its physiological function. *Clin Rheum Dis* 6(1): 51-76.
43. Choi HU, Meyer K (1975) The structure of keratan sulphates from various sources. *Biochem J* 151(3): 543-553.
44. Heinegard D, Axelsson I, Inerot S (1979) Skeletal keratan sulfate from different tissues. Characterization and alkaline degradation. *Biochim Biophys Acta* 581(1): 122-127.
45. Sztrolovics R, Alini M, Mort JS, Roughley PJ (1999) Age-related changes in fibromodulin and lumican in human intervertebral discs. *Spine (Phila Pa 1976)* 24(17): 1765-1771.
46. Akhtar S, Davies JR, Caterson B (2005) Ultrastructural immunolocalization of elastin and keratan sulfate proteoglycan in normal and scoliotic lumbar disc. *Spine (Phila Pa 1976)* 30(15): 1762-1769.
47. Risbud MV, Guttapalli A, Tsai TT, Lee JY, Danielson KG, et al. (2007) Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. *Spine (Phila Pa 1976)* 32(23): 2537-2544.
48. Iozzo RV, Schaefer L (2010) Proteoglycans in health and disease: novel regulatory signaling mechanisms evoked by the small leucine-rich proteoglycans. *FEBS J* 277(19): 3864-3875.
49. Feng H, Danfelter M, Strömqvist B, Heinegård D (2006) Extracellular matrix in disc degeneration. *J Bone Joint Surg Am* 88 Suppl 2: 25-29.
50. Melrose J, Smith SM, Fuller ES, Young AA, Roughley PJ, et al. (2007) Biglycan and fibromodulin fragmentation correlates with temporal and spatial annular remodelling in experimentally injured ovine intervertebral discs. *Eur Spine J* 16(12): 2193-2205.
51. Oegema TR (1993) Biochemistry of the intervertebral disc. *Clin Sports Med* 12(3): 419-439.
52. Eyre DR (1979) Biochemistry of the intervertebral disc. *Int Rev Connect Tissue Res* 8: 227-291.
53. Liu J, Roughley PJ, Mort JS (1991) Identification of human intervertebral disc stromelysin and its involvement in matrix degradation. *J Orthop Res* 9(4): 568-575.
54. Crean JK, Roberts S, Jaffray DC, Eisenstein SM, Duance VC (1997) Matrix metalloproteinases in the human intervertebral disc: role in disc degeneration and scoliosis. *Spine (Phila Pa 1976)* 22(24): 2877-2884.
55. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, et al. (2000) Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. *Spine (Phila Pa 1976)* 25(23): 3005-3013.

56. Le Maitre CL, Freemont AJ, Hoyland JA (2004) Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol* 204(1): 47-54.
57. Lyons G, Eisenstein SM, Sweet MB (1981) Biochemical changes in intervertebral disc degeneration. *Biochim Biophys Acta* 673(4): 443-453.
58. Urban JPG, McMullin JF (1988) Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition and degeneration. *Spine* 13(2): 179-187.
59. Adams MA, McNally DS, Dolan P (1996) 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. *J Bone Joint Surg Br* 78(6): 965-972.



This work is licensed under Creative Commons Attribution 4.0 License  
DOI: [10.19080/APBIJ.2018.04.555640](https://doi.org/10.19080/APBIJ.2018.04.555640)

**Your next submission with Juniper Publishers  
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats ( Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

**Track the below URL for one-step submission**  
<https://juniperpublishers.com/online-submission.php>