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Kimheak Sao
Thomas Jefferson University

Makarand V. Risbud
Thomas Jefferson University

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Review Article

Corresponding Author

Makarand V. Risbud

<https://orcid.org/0000-0003-3913-0649>

Department of Orthopaedic Surgery,
Thomas Jefferson University, 1025 Walnut
Street, Suite 501 College Bldg.,
Philadelphia, PA 19107, USA
Email: makarand.risbud@jefferson.edu

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Proteoglycan Dysfunction: A Common Link Between Intervertebral Disc Degeneration and Skeletal Dysplasia

Kimheak Sao^{1,2}, Makarand V. Risbud^{1,2}

¹Graduate Program in Cell Biology and Regenerative Medicine, Jefferson College of Life Sciences, Thomas Jefferson University, Philadelphia, PA, USA

²Department of Orthopaedic Surgery, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA

Proteoglycans through their sulfated glycosaminoglycans regulate cell-matrix signaling during tissue development, regeneration, and degeneration processes. Large extracellular proteoglycans such as aggrecan, versican, and perlecan are especially important for the structural integrity of the intervertebral disc and cartilage during development. In these tissues, proteoglycans are responsible for hydration, joint flexibility, and the absorption of mechanical loads. Loss or reduction of these molecules can lead to disc degeneration and skeletal dysplasia, evident from loss of disc height or defects in skeletal development respectively. In this review, we discuss the common proteoglycans found in the disc and cartilage and elaborate on various murine models and skeletal dysplasias in humans to highlight how their absence and/or aberrant expression causes accelerated disc degeneration and developmental defects.

Keywords: Intervertebral disc degeneration, Proteoglycan, Skeletal dysplasia, Nucleus pulposus

INTRODUCTION

Low back pain (LBP) is highly prevalent as age expectancy increases, and it is the leading cause of years lived with disabilities worldwide, affecting at least 600 million people globally.¹ A widely recognized contributor to chronic LBP is the degeneration of intervertebral disc (IVD). The discs are fibrocartilaginous tissues that lie between the vertebrae of the spinal column and provide flexibility to the spine and absorb the mechanical loads during diurnal events.² During aging, the mechanical properties of the disc are often compromised due to structural changes in the tissue. Disc and cartilage function depends significantly on the integrity and composition of the extracellular matrix (ECM) which consists of intricate and finely organized networks of collagens and proteoglycans (PGs).³ PGs through their sulfated glycosaminoglycans (GAGs) provide an osmotic mechanism to attract water molecules into the tissue essential

to accommodate compressive and tensile loads on the spine and joint tissues.⁴ Aging or genetic mutations in PG genes result in reduced levels or abnormalities which impair their biological functions. This review will focus on major PGs in the IVD and cartilaginous tissue and their contribution to disc pathologies and skeletal dysplasias.

PGs generally consist of a core protein covalently attached with one or more GAG chains joined through a tetrasaccharide bridge at a serine residue. These GAGs, typically long polysaccharides with repeating disaccharide structures, are categorized into 4 groups: chondroitin sulfate/dermatan sulfate (CS/DS), heparan sulfate (HS), keratan sulfate (KS), and hyaluronic acid (HA).⁵ The biosynthesis process of GAGs, specifically HS and CS, is tightly regulated in order to maintain their constant concentration within the tissue. Alteration in HS or CS GAG levels can affect tissue development, physiological, or pathological processes.^{6,7} Briefly, the process of GAG synthesis requires 5 uri-

dine diphosphate (UDP) derived activated sugars such as UDP-glucuronic acid (GlcA), UDP-*N*-acetylglucosamine (GlcNAc), UDP-xylose (Xyl), UDP-galactose (Gal), and UDP-*N*-acetylgalactosamine (GalNAc).⁵ These sugars are transported from the cytoplasm to the Golgi apparatus where a series of glycosyltransferases assemble the tetrasaccharide bridge. GAG synthesis begins when xylosyltransferases (encoded by XT1 or 2) add Xyl to a serine residue on the core protein, followed by galactosyltransferases I (B4GALT7) and II (B3GALT6) which add 2 Gal sugars. Next, a glucuronyltransferase (B3GAT3) adds GlcA to assemble a common tetrasaccharide bridge containing GlcA β 1-3Gal β 1-3Gal β 1-4Xyl β 1-*O*-Ser.^{5,8,9} The subsequent addition of GalNAc by GALNACT2 or GlcNAc by EXTL3 will then initiate the commitment for either CS or HS biosynthesis.⁸ The regulation of GAG biosynthesis in the context of disc biology is further discussed by Silagi et al. and others.^{9,10} All GAGs, except HA, undergo a sulfation process in the Golgi where sulfotransferases catalyze sulfate donor compound 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to modulate their sulfation profile.⁵ Alterations in sulfation profiles can modulate the critical physiological functions of PGs in developing tissues often leading to chondrodystrophies.¹¹ Based on the recent classification of genetic skeletal disorders, mutations of ion transporter-related or sulfation-related genes such as *SLC26A2*, *PAPSS2*, *IMPAD1*, *CHST3*, *SLC35B2*, *CHST14*, *DSE*, *CHST11*, *HS2ST1*, *SLC13A1* result in achondrogenesis, chondrodysplasia, Ehlers-Danlos syndrome, osteochondrodysplasia, brachydactyly, overlapping malformed digits and developmental delays, and disc degeneration.¹¹⁻¹³ Importantly, defects in matrix recycling by autophagy observed in lysosomal storage disorders are equally detrimental to skeletal tissues and during development.¹⁴

It is important to mention here that PGs were previously classified either by GAG type or PG size due to their heterogeneity. To simplify PG classification, Iozzo and Schaefer proposed to categorize mammalian PGs into 4 overarching classes based on their locations: extracellular, pericellular, cell surface, and intracellular.¹⁵ In the context of the joint, PGs are present on the cell surface and within the ECM of the growth plate, articular cartilages, and IVD. During development, these macromolecules can interact with constituent growth factors, cytokines, morphogens, and chemokines to influence cell adhesion, morphology, proliferation, migration, and differentiation.¹⁶ Major PGs, including aggrecan, versican, perlecan, and cell surface PGs such as glypicans and syndecans, particularly syndecan-4, and a few small leucine-rich proteoglycans (SLRP), such as biglycan and decorin, fibromodulin, and lubricin are abundant in the

articular cartilage and the disc to aid in tissue hydration, biomechanical function and cell signaling events (Fig. 1A and B). These will be discussed in detail below (Table 1).

STRUCTURE AND FUNCTION OF PROTEOGLYCANS IN MUSCULOSKELETAL PATHOLOGIES

1. Extracellular PGs

ACAN encodes for aggrecan, the most abundant PG in the IVD and joint cartilage. Aggrecan bears negatively charged KS and CS GAG side chains providing cartilage with its ability to bind water for hydration and withstand large compressive loads.¹⁷ Structurally, aggrecan consists of a core protein with 3 disulfide-linked globular regions (G1, G2, G3) with intervening extended regions between G2 and G3¹⁸ (Fig. 1A). Between the amino terminal G1 and G2 domains is the interglobular domain (IGD), a prominent proteolysis site that is thought to be involved in the physiological turnover of aggrecan.¹⁹ Following the G2 domain, the core protein is decorated with approximately 30 KS chains and 100 CS chains in the CS1 and CS2 domains; these domains are responsible for the water-binding property and its function as a structural PG. The G3 domain of aggrecan resides at the carboxy-terminal and is a complex region that is required for post-transcriptional processing.¹⁹ Aggrecan does not exist in isolation within the ECM but is instead composed of aggrecan supramolecular aggregates (Fig. 1B). These supramolecular aggregates form when multiple aggrecan molecules noncovalently attach to a link protein-bound HA filament.²⁰ Molecularly, the G3 domain is essential for sufficient modification of GAGs; without this, the CS-containing constructs are not secreted. In the developing skeleton, aggrecan expression is confined to chondrocytes and other cartilaginous tissues.²¹

The importance of aggrecan in cartilage was first illustrated in the embryonically lethal nanomelia affecting cartilage development in chickens²² and cartilage matrix deficiency (*cmd*) in mice resulting in cleft palate, dwarfism, abdominal compression, and respiratory failure after birth.²³⁻²⁵ Interestingly, heterozygous *cmd* mice appeared normal at birth, however, age-associated skeletal defects such as dwarfism and spinal misalignment began to show at 19-month of age.²⁶ The underlying mechanism in lethal nanomelia avian mutation is a transversion creating a premature stop codon, truncating part of CS2 and G3 domains and significantly reducing the steady-state level of aggrecan messenger RNA (mRNA).²⁷ The residual mRNA gets translated but is not secreted into the ECM,²⁸ thus

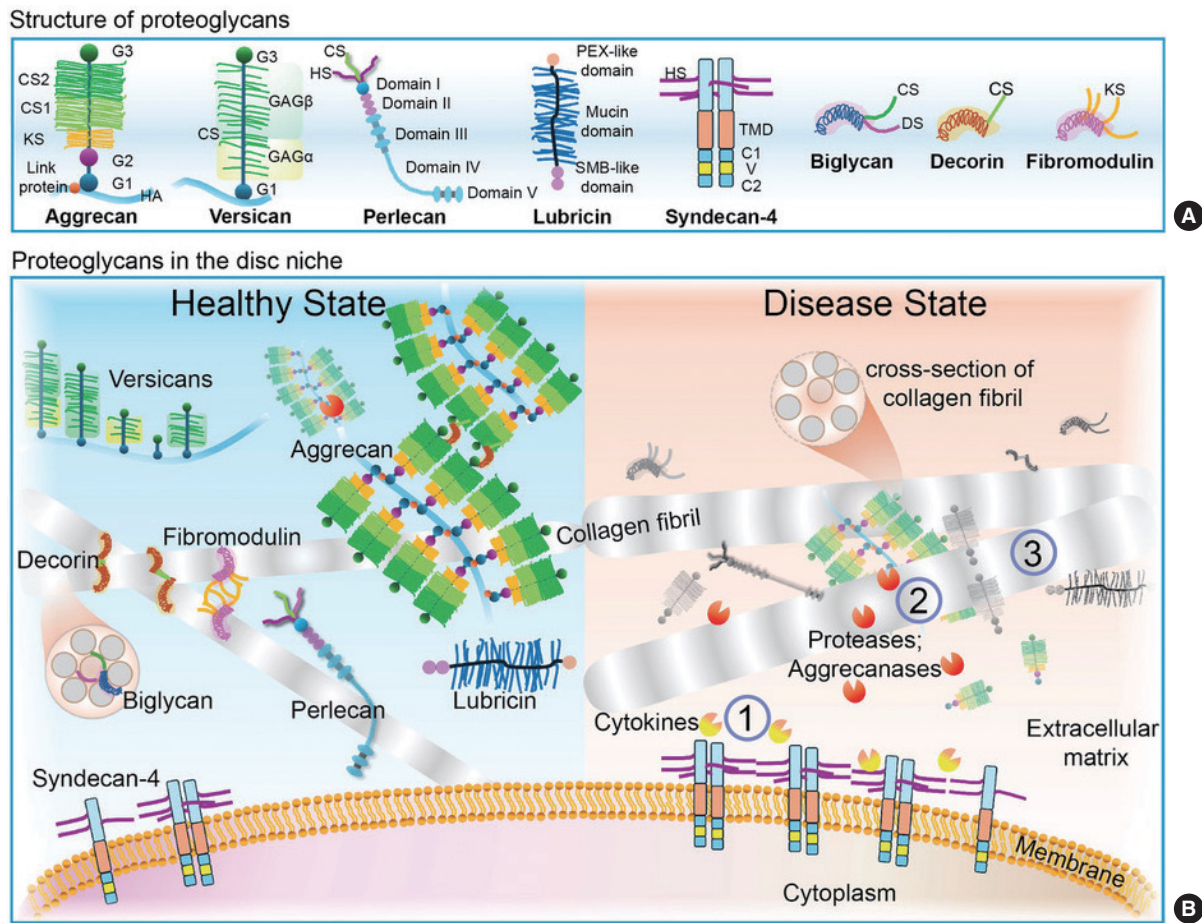


Fig. 1. Illustration of major proteoglycans and glycoproteins in the intervertebral disc in healthy versus disease state. (A) Illustrations showing the structures of aggrecan, versican, perlecan, lubricin, syndecan-4/SDC4, decorin, biglycan, and fibromodulin. (B) In healthy state, there is an abundance of large proteoglycans that maintain tissue hydration and collagen fibril integrity. In disease state or aging, (B1) there is an elevated level of cytokines including tumor necrosis factor- α , interleukin (IL)-1 β , and IL-6 which promote degenerative processes and expression of SDC4. (B2) Induction of SDC4 promotes a proteolytic event where (B3) ADAMTS-5 and matrix metalloproteinases cleave the disc extracellular matrix. ADAMTS-5, a disintegrin and metalloproteinase with thrombospondin motifs 5; HA, hyaluronic acid; HS, heparan sulfate; GAG, glycosaminoglycan; PEX, hemopexin; TMD, transmembrane domain; KS, heparan sulfate.

accumulating in the endoplasmic reticulum.²⁹ Similar to nanomia, cmd in mice also results in reduction of aggrecan mRNA expression and secretion of aggrecan into the ECM.²⁶ These detrimental effects foreshadowed the outcome of *ACAN* mutation in humans, discussed in a later section.

Versican, another large extracellular proteoglycan encoded by *VCAN*, is structurally related to aggrecan, possessing a terminal domain analogous to aggrecan's G1 and G3 regions, however it does not contain IGD, a G2 domain, and KS GAG attachment sites (Fig. 1A).¹⁵ To date, 5 isoforms of versican (V0–4) have been identified: the full-length versican (V0) and 3 splice variants that lack GAG α (V1), GAG β (V2), both GAG α and GAG β (V3), and portion of GAG β (V4).¹⁵ During development, full-

length versican is prominently expressed throughout the IVD.³⁰ As the disc matures, its expression decreases throughout but remains prominent between the lamellae of the annulus fibrosus (AF).^{30,31} Functionally, based on its structure and localization with elastin and fibulin-1, a glycoprotein that is incorporated into fibrillar ECM, versican is suggested to contribute to the organization of the disc ECM and provide structural support and resilience to mechanical forces.³¹ In cartilage development, the balance of versican expression is important in mediating local transforming growth factor beta (TGF- β) in chondrocyte differentiation and digit joint formation.³²⁻³⁴ Reductions in versican can compromise chondrogenesis and synovial joint development.³⁵ Mutations in the human versican gene result in the

Table 1. Animal models of proteoglycan dysfunction and mutations

Species	Gene	Disease model/ disorder	Mutation	Effect	Phenotype	References
Chicken	<i>Acan</i>	Nanomelia	Nonsense mutation	Transversion c.4537G > T: convert glutamate at 1513 to a stop codon, creating defect in CS2 and G3 domain. Truncation of aggrecan; accumulation in ER	Embryonically lethal, short-limb dwarfism, large, brachycephalic head, abnormal mandible, and maxilla	Li et al., ²⁸ 1993; Vertel et al., ²⁹ 1993
Mouse	<i>Acan</i>	Cartilage matrix deficiency (<i>cmd/cmd</i>)	7bp deletion in exon 5	Deletion of B subdomain of N-terminal globular G1 domain, which binds to HA Aggrecan, results in truncation and secretion of mature aggrecan product in the matrix	Dwarfism, enlarged abdomen, short snout, cleft palate, and protruding tongue, respiratory failure related to pulmonary hypoplasia	Watanabe et al., ²⁵ 1994
Mouse	<i>Acan</i>	Cartilage matrix deficiency (<i>cmd^{hbc}</i>)	Deletion of exons 2–18	Deletion of the globular domains G1, G2, and GAG regions KS, CS1, and C2 domains. Consequent in aggrecan truncation	Dwarfism, enlarged abdomen, short snout, cleft palate, and protruding tongue	Krueger Jr et al., ²⁴ 1999
Mouse	<i>Bgn</i>	<i>Bgn</i> -/0	Deletion of exon 2	Biglycan deficient	Thin dermis, low bone mass, larger irregular collagen fibril	Xu et al., ⁴⁶ 1998; Chen et al., ⁴⁵ 2002
Mouse	<i>Bgn</i> ; <i>Dcn</i>	<i>Bgn</i> -/0 <i>Dcn</i> -/-	Double deletion using <i>Bgn</i> -/0 and <i>Dcn</i> -/-	Biglycan and decorin deficient	Compounding effect with loose disorganized dermis, larger highly irregular collagen fibrils in tendons	Robinson et al., ⁵¹ 2017
Mouse	<i>Dcn</i>	<i>Dcn</i> -/-	Deletion of exon 2	Decorin deficient	Thin dermis, low bone mass, larger irregular collagen fibril	Danielson et al., ⁴⁸ 1997
Mouse	<i>Fmod</i>	<i>Fmod</i> -/-	Deletion of exon 2	Fibromodulin deficient	Abnormal tissue organization, thin collagen fibril diameter; abnormal dentin mineralization and alveolar bone formation	Gill et al., ⁵⁶ 2002; Ameye et al., ⁵⁹ 2002; Goldberg et al., ⁶¹ 2009
Mouse	<i>Hspg2</i>	<i>Hspg2</i> -/-	Deletion of exon 6	Mutant perlecan allele is transcribed, truncated protein could not be detected with a domain I-specific polyclonal antibody. Truncated form of domain I is not properly folded and is consequently degraded intracellularly as soon as it is translated	Homozygous mice died between E10–12 and perinatally. Embryos develop severe defect in cartilage, a tissue that lacks basement membranes. Reduced fibrillar collagen network shortened collagen fibers, and increased expression of cartilage ECM genes. Expansion of neuroepithelium, neuronal ectopias, and exencephaly	Costell et al., ⁸⁷ 1999
Mouse	<i>Hspg2</i>	<i>Hspg2</i> ^{C1532Y-Neo}	Substitution mutation	G-to-A substitution at <i>Hspg2</i> nucleotide 4595, change amino acid from C to Y at residue 1532. Disrupt disulfide bond formation within perlecan domain III, thus affect perlecan conformation	Reduction of perlecan. Altered matrix organization and stiffness as function of age and genotype. Short stature, impaired mineralization, misshapen bones, osteoarthritis-like joint dysplasias and myotonia. Associated with SJS disease in humans	Rodgers et al., ⁸⁹ 2007; Xu et al., ⁹⁰ 2016; Ocken et al., ⁹¹ 2020
Mouse	<i>Hspg2</i>	<i>Hspg2</i> ^{Δ3/Δ3}	Deletion of exon 3	Perlecan is devoid of ~20 kDa of domain I of its core protein and the GAG chains normally attached to this region; there was no detectable GAG substitution in domain V	GAGs accumulation in the nucleus pulposus, annulus fibrosus, and vertebral growth plates. Advanced chondrocyte hypertrophy and exostosis-like, ectopic bone formation at the cartilaginous endplate region	Shu et al., ⁹² 2019

(Continued)

Table 1. Animal models of proteoglycan dysfunction and mutations (Continued)

Species	Gene	Disease model/ disorder	Mutation	Effect	Phenotype	References
Mouse	<i>Prg4</i>	<i>Prg4</i> ^{-/-}	Deletion of exon 6	Removal of mucin-like domain; lubricin deficient	Accelerated synovial hyperplasia, disappearance of superficial zone chondrocytes. Increase articular surface friction, increase apparent torsional modulus in L1/2 disc, thinner AF	Rhee et al., ⁴¹ 2005; Teeple et al., ¹¹¹ 2015
Mouse	<i>Sdc4</i>	<i>Sdc4</i> ^{-/-}	Deletion of exon 2-part of 4	Deletion of ectodomain with three putative glycosaminoglycan attachment sites	Impaired wound healing and angiogenesis. Syndecan 4 regulates ADAMTS-5 and MMP3 under inflammatory milieu to degrade aggrecan. Essential for endochondral ossification to repair fracture healing. Exhibits smaller muscle fiber, defect in muscle regeneration after damage and myogenic satellite cell differentiation	Ishiguro et al., ⁷⁴ 2000; Echtermeyer et al., ⁷⁵ 2001; Cornelison et al., ⁷⁷ 2004; Bertrand et al., ⁷⁶ 2013; Rønning et al., ⁷⁸ 2020; De Rossi et al., ⁷⁶ 2021
Mouse	<i>Tnmd</i>	<i>Tnmd</i> ^{-/-}	Deletion of exon 1	Tenomodulin deficient	Advanced degenerative of the disc, increase hypertrophic-like chondrocytes, apoptosis, small collagen fibrils with low compressive stiffness. Abnormal collagen I cross-linking, inferior endurance running performance	Lin et al., ¹¹⁰ 2020; Docheva et al., ⁶³ 2005; Dex et al., ⁶⁴ 2017
Mouse	<i>Vcan</i>	<i>Prx1-Vcan</i>	Deletion of exon 2	Deletion of G1 domain; affect biosynthesis of versican	Distorted digits, tilted Joint surface and delayed cartilage development	Choocheep et al., ³³ 2010; Higuchi et al., ³⁴ 2021

ER, endoplasmic reticulum; HA, hyaluronic acid; GAG, glycosaminoglycan; ECM, extracellular matrix; SJS, Schwartz-Jampel syndrome; AF, annulus fibrosus; ADAMTS-5, a disintegrin and metalloproteinase with thrombospondin motifs 5; MMP3, matrix metalloproteinase-3.

dominantly inherited Wagner syndrome, caused by a base substitution mutation of *VCAN* at exon 8 that produces less V1 and more V2 and V3 isoforms, which is associated with progressive vision loss.³⁶

Encoded by *PRG4*, proteoglycan 4 or lubricin is a mucinous glycoprotein and an atypical proteoglycan that covers the cartilage and prevents it from cartilage damage. Its structure consists of 2 adhesive nonglycosylated subdomains flanking a heavily glycosylated and mucin-like central domain region. The amino terminus possesses a somatomedin-B-like domain while the carboxyl terminus has a hemopexin domain, both of which are responsible for mediation of cell-cell and cell-matrix interactions to promote cell attachment^{37,38} (Fig. 1A). The central domain mostly consists of GalNac, Gal, and NeuAc sugar groups O-linked at threonine residue, making it negatively charged and creating a strong repulsion via hydration forces.³⁷ Lubricin is found in the synovial joint, coated on the cartilage surface, and detected at markedly higher levels in the IVD compartments compared to other cartilage subtypes.^{39,40} Lubricin dele-

tion (*Prg4*^{-/-}) in mice by excision of exon 6, removing mucin-like domain, results in age-associated accelerated synovial hyperplasia, abnormal protein deposits on the cartilage surface, and disappearance of underlying superficial zone chondrocytes ultimately contributing to joint failure.⁴¹ Since lubricin is detected at a high level in the disc, its absence may be consequential to its integrity as discussed later.

Biglycan is a small ECM PG that is ubiquitously expressed in the articular regions, epiphyseal cartilage, vascular canals, and periosteum during development.⁴² It was reported to contribute to bone growth, muscle development and regeneration, and collagen fibril assembly. Biglycan contains 2 CS or DS GAG chains at serine-glycine attachments sites in the N-terminal region (Fig. 1A). It can bind to TGF- β ,⁴³ and modulate bone morphogenetic protein (BMP)-4 to influence osteoblast differentiation and maturation in bone development.⁴⁴ Located on the X chromosome region Xq28, genetic mutation of *BGN* affects males more than females. Early work on *Bgn* deficient male mice (*Bgn*^{0/0}) showed early onset of osteoporosis-like pheno-

type, skeletal abnormalities, and disc degeneration.⁴⁵⁻⁴⁷ Closely related to biglycan is the SLRP decorin, sharing >65% of its overall homology. It is encoded by *DCN* and only has one GAG chain (Fig. 1A). It is known for decorating along fibrillar collagen and regulates the association of collagen into proper fibrils protecting them from proteolysis¹⁵ (Fig. 1B). Deletion of decorin in mice (*Dcn*^{-/-}) results in fragile skin and tendons along with coarser and irregular fiber outlines without affecting bone mass.⁴⁸ In cartilage, decorin mediates matrix integrity and biomechanical functions by enhancing the linkages and assembly of aggrecan-aggrecan molecules and aggrecan-collagen II fibrils.⁴⁹ Since biglycan and decorin are highly homologous and co-expressed in tissues such as skin and bone, double deletion of biglycan and decorin (*Bgn*⁻⁰ *Dcn*^{-/-}) mice revealed compounding effects on skin fragility, severe osteopenia, and alteration of collagen fibril structure and mechanical properties of tendons.^{50,51} SLRPs fibromodulin and tenomodulin are also present in cartilage, tendon, and ligament ECM and contribute to the structural integrity of these tissues. Fibromodulin, encoded by *FMOD*, is a KSPG SLRP highly homologous with biglycan and decorin (Fig. 1A). It bears KS chains and binds to collagen I via residues located in leucine-rich region 11 in the convex surface of the protein core at a different region than decorin binding site.^{52,53} Specifically, it interacts with triple-helical type I and II collagens,⁵⁴ regulates fibrillogenesis and collagen fibril size,⁵⁵ and maintains long-term tissue integrity within the knee⁵⁶ (Fig. 1B). During collagen proteolysis, fibromodulin gets cleaved by a disintegrin and metalloproteinase with thrombospondin motifs 4 and 5 (ADAMTS-4 and-5) and matrix metalloproteinase-13 (MMP-13).^{57,58} Deletion of fibromodulin in mice (*Fmod*^{-/-}) results in abnormal tissue organization in the cross-sections of the tail, a larger proportion of thin collagen fibril diameter in the Achilles tendons, higher histological arthritis score, and abnormal dentin mineralization and alveolar bone formation.^{55,56,59-61} Similar to *Bgn*⁻⁰ *Dcn*^{-/-} double knockout, biglycan and fibromodulin double knockout (*Bgn*⁻⁰ *Fmod*^{-/-}) mice have impaired collagen fibrils in the tendons. This leads to gait impairment, increased ectopic tendon ossification due to increased use of leg joints, and severe premature osteoarthritis.⁵⁹ Furthermore, these mice develop accelerated temporomandibular osteoarthritis due to accelerated chondrogenesis secondary to reduced levels of sequestered TGF- β 1 in the ECM, leading to overcompensation of overactive TGF- β 1 signal transduction.⁶² Tenomodulin regulates tenocyte proliferation in tendons⁶³ and is required for proper collagen I cross-linking; deletion of tenomodulin (*Tnmd*^{-/-}) in mice resulted in abnormal collagen I

cross-linking and increased collagen fiber thickness and stiffness, leading to inferior endurance running performances.^{64,65}

2. Cell Surface PGs

Syndecans have a single-pass transmembrane protein core which includes an ectodomain bearing HS-chain or HS/CS chains, a transmembrane region, and an intracellular cytoplasmic domain. The cytoplasmic domain comprises of 2 conserved constant regions, C1 and C2, flanking a variable region, V1, that are responsible for syndecan-specific signaling⁶⁶⁻⁶⁹ (Fig. 1A). There are 4 syndecan family members: syndecan 1-4 (encoded by *SDC1-4*). *Sdc1* and *Sdc3* are the largest family members bearing 2 CS chains and several HS chains, while *Sdc2* and *Sdc4* are smaller and only bear HS chains.⁶⁷ All are intrinsically disordered and dynamic, enabling them to interact with numerous ligands including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor, BMP-2, and Indian hedgehog (IHH).^{66,70} While *Sdc1-3* are found in specific tissue types, *Sdc4* is ubiquitously expressed in most tissue types. *Sdc4* functions as a receptor or co-receptor to ligands strengthening the duration and intensity of downstream signaling. It can also form physical connections with the ECM and activate mechanosensory signaling to influence cytoskeletal reorganization during migration and the assembly and disassembly of integrin complexes at focal adhesion sites.^{66,71} During early embryo development of *Xenopus laevis*, gain- and loss-of-function experiments showed that the balance of *Sdc4* expression was crucial in regulating the convergent-extension movement in neural tube closure and neural crest-directed migration through the noncanonical Wnt pathway by its interaction with Frizzled 7 (*Fz7*) and Dishevelled (*Dsh*).⁷² Similar to *X. laevis*, *Sdc4* is expressed during murine development in the cranial neural folds on embryonic day 8–8.5 and is regulated by *Vangl2* during neural tube closure.⁷³ Unlike *X. laevis*, *Sdc4*-null mice have no obvious developmental defects besides delayed wound healing, impaired angiogenesis, and defects in muscle regeneration after damage and myogenic satellite cell differentiation.⁷⁴⁻⁷⁸ In recent years, we and others have reported that cytokines tumor necrosis factor alpha (TNF- α) and interleukin 1 beta (IL-1 β) induce *Sdc4* expression to mediate different musculoskeletal pathologies including rheumatoid arthritis, osteoarthritis (OA), and IVD degeneration.⁷⁹⁻⁸¹ Moreover, inhibition or blocking of *Sdc4* under inflammatory conditions prevents exacerbation of cartilage deterioration.⁷⁹

3. Pericellular Proteoglycan

HSPG2 encodes for the secreted perlecan, a large basement membrane HS proteoglycan presents in several ECM tissues including cartilage and the disc. The core protein of perlecan consists of 5 domains (domain I–V) with tandemly repeating modular motifs resembling “pearls-on-a-string,” 2 of which (domain I and V) possess HS GAG attachment sites⁸² (Fig. 1A). Perlecan expression has been well characterized in the early stages of embryogenesis in murine models. It appears as early as E10.5 in the heart, pericardium, blood vessels, and developing vertebral cartilage⁸³ and is highly expressed in cartilage primordia at E15.5.⁸⁴ Perlecan is expressed ubiquitously in muscle and bone marrow enabling homeostatic regulation of biological processes, such as the formation of cardiovascular tissue, localization of acetylcholine esterase to neuromuscular junctions,⁸⁵ and formation of bone.⁸⁶ Ablation of perlecan in mice (*Hspg2*^{-/-}) resulted in significant embryonic lethality due to pericardial hemorrhaging from defects in basement membranes and biomechanical deterioration of the contracting myocardium between E10–12.^{87,88} However, the few mice that survived birth died with skeletal dysplasia characterized by micromelia, narrow thorax, and craniofacial abnormalities, similar to skeletal defects of patients with lethal dyssegmental dysplasia, Silver-Handmaker type (DDSH).⁸⁸ Furthermore, perlecan-null cartilage exhibited reduced GAGs, disorganized collagen fibrils, diminished chondrocyte proliferation, and prehypertrophic zones; all of which contributed to abnormal skeletal phenotypes similar to those of patients with thanatophoric dysplasia type I, which is caused by activating mutations in *FGFR3*.⁸⁸ Since no homozygous knockout mice survived, hypomorphic *Hspg2*^{C1532Y-Neo} mice were generated to study nonlethal skeletal dysplasia mimicking Schwartz-Jampel syndrome (SJS) in humans.⁸⁹ These hypomorphic *Hspg2*^{C1532Y-Neo} mice have C1532Yneo mutation in domain III of perlecan, resulting in reduced perlecan expression, reduced cellular and ECM stiffness and defective pericellular matrix formation, potentially from impaired incorporation of newly synthesized ECM.^{89–91} Given that perlecan was also observed in developing vertebral cartilage, another perlecan murine model lacking exon 3 (*Hspg2*^{Δ3/Δ3}) was generated to study the role of *Hspg2* in the disc. Perlecan exon 3 encodes perlecan domain I, and its deletion in *Hspg2* exon 3 null mice resulted in a 22 kDa size reduction in the perlecan core protein.⁹² As a result, *Hspg2*^{Δ3/Δ3} mice had a higher GAG content in IVDs, advanced chondrocyte hypertrophy in the cartilage, and disorganization of the growth plate.⁹²

PROTEOGLYCAN-LINKED DISC PATHOLOGIES

The IVD is an avascular organ composed of a collagen-rich AF that encompasses the gelatinous PG-rich nucleus pulposus (NP) center; both compartments are anchored by superior and inferior endplates composed of a hyaline cartilaginous and bony endplate region. In young healthy disc tissue, the NP ECM is mainly composed of aggrecan and versican whose functions are to elevate water swelling potential and resist spinal compression. These PGs bear negatively charged CS side chains that draw in hydrated osmolytes, such as cations (Na⁺, Ca²⁺), ultimately creating a hyperosmotic environment.⁹ TonEBP/NFAT5 is the only known mammalian osmo-sensitive transcription factor that plays an important role in NP cell osmoadaptation and survival within hyperosmotic environment of the disc.^{93–95} AF, on the other hand, is composed of mainly collagens I and II to provide tensile strength. In degenerative disc pathologies under the aging or injury contexts, there is a marked loss of water-binding PGs and an increase of ECM remodeling that results in biochemical and biophysical changes in the disc.⁴ These changes include decreased PG synthesis and increased aggrecan proteolysis by ADAMTS-4 and-5 and MMPs.⁹⁶ In addition to these proteases, cytokines including TNF- α , IL-1 β , and IL-6 are important mediators of degenerative cascade under the inflammatory milieu.⁹⁷

Aggrecan is an essential component in the disc. As the disc ages, decreased aggrecan levels and functionality is observed. This is either due to a decrease in PG synthesis or an increase of PG degradation, shifting the balance from a proteoglycan-rich to collagen-rich profibrotic environment. In relatively healthy human NP tissue that correlated with gross morphologic Thompson grade of 2 (1 being the healthy and 5 being the most degenerated state), the GAG to hydroxyproline ratio was about 23:1; however, in the tissue with a Thompson grade of 4, the GAG to hydroxyproline ratio was reduced to about 5:1.^{98,99} As discussed earlier, *cmd* heterozygous mice cause spinal misalignment and movement problems with age due to impaired secretion or truncation of aggrecan.²⁶ To characterize the phenotypic and morphologic effects of aggrecan deletion on skeletal development, Lauing et al.¹⁰⁰ developed an *ACAN* mutation disease model in *cmd* mice (*cmd*^{bc}/*cmd*^{bc}) where the entire protein-coding sequence was deleted. This yielded severe skeletal defects in the limbs, ribcage, and vertebrae development with abnormal mRNA expression patterns of *Col10a1*, *Sox9*, *Ihh*, *Ptch1*, and *Fgfr3* in the growth plate. However, when homozygous *cmd*^{bc} was rescued with chick aggrecan transgene (*cmd*^{bc}/*cmd*^{bc};*Agc*l+),

skeletal defects were reversed by 20% in limbs and 80%, near-full reversal, in size and diameter of the ribcage and vertebrae.¹⁰⁰ This suggests that aggrecan has a major role in regulating key growth factors during development, especially in the development of axial skeletal structures like ribcage and vertebrae. Noteworthy, these detrimental effects likely foreshadowed the outcome of *ACAN* mutation in humans. While the *ACAN* gene mutation has not been specifically reported to cause human degenerative disc disease, heterozygous *ACAN* mutation is associated with both idiopathic short stature and accelerated bone aging which predisposes to early onset of lumbar disc degeneration or herniation.^{101,102}

As mentioned, loss of aggrecan in disc pathology may be due to increased matrix degradation. Barre et al.¹⁰³ reported a significant increase in *SDC4* mRNA expression in damaged human cartilage cultured primary monolayers compared to normal cartilage tissue, suggesting *SDC4* was a likely contributor to the disorganization of cartilage and the development of OA processes. The Risbud group was the first to show enriched *Sdc4* expression within the disc tissue¹⁰⁴ and the importance of HS synthesis in regulating matrix catabolism under an inflammatory milieu, mediating ADAMTS-5 activity to cleave aggrecan.⁸¹ Specifically, our findings showed that cytokines TNF- α and IL-1 β regulate *Sdc4* expression via the nuclear factor kappa B (NF- κ B)-p65/RelA dependent mechanism to activate ADAMTS-5⁸¹ and induce expression of MMP-3 through the mitogen-activated protein kinase–NF- κ B axis.¹⁰⁵ Importantly, analysis of degenerated human NP tissues showed a strong correlation between *SDC4*, ADAMTS-5, and cleaved aggrecan neopeptides.⁸¹ To investigate whether *Sdc4* deletion could delay the disc degeneration, we characterized the spinal phenotype of *Sdc4* null mice. Our findings show that *Sdc4* deletion results in early-onset osteopenia and alters biomechanical properties in the lumbar vertebrae due to the dysregulation of osteoclastogenesis. Congruent with this finding, a previous study demonstrated that the GAG-bearing ectodomains of syndecans 1–4 suppressed osteoclast differentiation.¹⁰⁶ Furthermore, discs of adult *Sdc4* knockout showed alterations in mature collagen cross-linking, CS content, and aggrecan turnover by aggrecanase. Interestingly, histological assessment of *Sdc4* knockout mice showed subtle cellular phenotypes with NP cells containing larger vacuoles and a thicker NP cell band. Importantly, the transcriptomic analysis suggested that *Sdc4* deletion downregulates genes associated with mitochondrial metabolism, autophagy, ER to Golgi protein processing, and HS biosynthesis and GAG degradation. These findings indicate that deletion of *Sdc4* reduces matrix

turnover which is speculated to be responsible for CS accumulation and GAG degradation in NP tissue.¹⁰⁷ Other investigators using *Has2*- and *Sulf1*-knockout mice have demonstrated the important of GAG function in PGs. Changes in hyaluronan synthesis and sulfation of GAG profiles can alter the structure and function of PGs. Specifically, *HAS2* and *SULF1* are important in HA synthesis and sulfation of GAGs, respectively, and their deletion resulted in defects of skeletal development, formation of IVDs,¹⁰⁸ and disc degeneration.¹⁰⁹

Collagens are also important structural molecules that provide structural integrity to the disc. Biglycan, tenomodulin, and lubricin are all key mediators in helping collagen maintain its organization, structure, and lubrication. *Bgn* deficient (*Bgn*^{0/0}) mice exhibited loss of notochordal cells at 6 months with advanced degenerative changes at 9 months. These changes were thought to have been caused by a loss of structural stability in collagens, leading to increased mechanical stress resulting in premature disc degeneration.⁴⁷ A study of *Tnmd*^{-/-} mice also showed advanced degenerative changes in the NP and inner AF of the disc, increased hypertrophic-like chondrocytes and apoptosis, decreased disc height index, and smaller collagen fibrils with lower compressive stiffness. In short, decreased proliferation compounded with compromised collagen biomechanical integrity led to defects in the tissue's ability to twist and withstand mechanical loads which subsequently increased the likelihood of degeneration and disc tears.¹¹⁰ Lubricin is another important glycoprotein critical in providing joint lubrication and in aiding the endurance to mechanical strain. Lubricin knockout (*Prg4*^{-/-}) mice were reported to have thinner AF, increased articular surface friction, early progressive surface damage, and increased apparent torsional modulus at L1/L2 disc¹¹¹; all of which highlight the functional importance of lubricin as a protective barrier in the joints.

PROTEOGLYCAN CORE PROTEIN DISORDERS-LINKED SKELETAL DYSPLASIAS

Since PGs are important in conferring mechanical, biochemical, and physical properties to tissues, mutations in genes encoding core proteins can detrimentally affect skeletal tissue development and integrity. Skeletal dysplasias noted in this review are linked to gene mutations in aggrecan (*ACAN*), biglycan (*BGN*) and perlecan (*HSPG2*)¹² (Table 2).

Table 2. Skeletal dysplasias caused by proteoglycan dysfunction and mutation

Species	Gene	Disease model/ disorder	Mutation	Effect	Phenotype	References
Human	<i>ACAN</i>	Spondyloepiphyseal dysplasia, Kimberley type (SEDK)	Frameshift mutation; autosomal dominant	A single-base-pair insertion, within the variable repeat region of exon 12, introduce a 212 amino acid frameshift followed by premature stop codon. Lack half of the CS1 domain, the complete CS2 domain, and the G3 domain, resulting in abnormally short aggrecan protein	Short stature and early development of osteoarthritis, especially in the knees, ankles, and hips. Variation of mutation also results in short stature with accelerated bone maturation, early onset of osteoarthritis, and craniofacial, limb, and vertebral abnormalities	Anderson et al., ¹¹⁴ 1990; Eyre et al., ¹¹⁵ 2005; Gleghorn et al., ¹¹⁶ 2005; Nilsson et al., ¹¹⁷ 2014; Kim et al., ¹¹⁸ 2022; Karatas et al., ¹¹⁹ 2023; Huang et al., ¹²⁰ 2023
Human	<i>ACAN</i>	Spondylo-epimetaphy-seal dysplasia (SEMD)	Missense mutation; autosomal dominant	Point mutation c.6799G > A p.Asp2267Asn amino acid substitution affected a highly conserved residue that contributes to the structure of the C-type lectin domain within the G3 domain of the protein, in part by coordinating binding of one of three calcium ions important for its structure	Heterogeneous group of disorders defined by the combination of vertebral, epiphyseal, and metaphyseal anomalies. Short stature, brachydactyly, distinct severe midface hypoplasia, short necks, barrel chests, lumbar lordosis, and macrocephaly	Tompson et al., ¹¹³ 2009; Fukuhara et al., ¹²² 2019; Stattin et al., ¹²¹ 2022
Human	<i>BGN</i>	X-linked SEMD	Spectrum of mutation: insertion/deletion, and nonsense	Mutation in <i>BGN</i> on chromosome Xq28. Point mutation c.439A > G (p.Lys147Glu) in Korean family; c.776G > T (p.Gly259Val) in the Italian family; and c.439A > G (p.Lys147Glu) mutation in the Indian patient	SEMDX were reported in three different ethnic background. Characterized by short stature with shortening of limbs, bowing of the legs, and lumbar lordosis	Camera et al., ¹²³ 1994; Cho et al., ¹²⁵ 2016; Meester et al., ¹²⁴ 2017
Human	<i>HSPG2</i>	Dyssegmental dysplasia, Silver-Handmaker type (DDSH)	Frameshift mutation; lethal autosomal recessive	89-bp duplication of exon 34 of <i>HSPG2</i> ; one unrelated case with 89-bp duplication compound with point mutation that results in skipping of entire exons 52 and 73. Homozygous 4-bp deletion in exon 31 causes frameshift mutation that results in truncation in the perlecan protein core	Diminished perlecan secretion. Dwarfism, anisodondyly and micromelia, small mouth, small chest, die shortly from respiratory insufficiency	Arikawa-Hirasawa et al., ¹²⁶ 2001; Rieubland et al., ¹²⁸ 2010; Ladhani et al., ¹²⁹ 2013
Human	<i>HSPG2</i>	Schwartz-Jampel syndrome (SJS)	Spectrum of mutations: insertion/deletion, missense, and nonsense. Autosomal recessive	Spectrum of mutations affecting perlecan domain II, III, IV, V. For example: Point mutation c.2746C > T on exon 22 from one parent results in premature stop codon (p.R916X), reducing perlecan mRNA level by 36.3%. Another case found c.1125C > G; p.Cys375Trp of <i>HSPG2</i> affect perlecan domain II	Continuous contractions of muscles throughout the body including the face, abnormal spinal curvature and shortening of the bone. Narrow eye openings (blepharophimosis) and pursed lips	Bauché et al., ¹³⁰ 2013; Lin et al., ¹³¹ 2021

1. Aggrecanopathies in Skeletal Dysplasia

In humans, *ACAN* mutations result in autosomal-dominant spondyloepiphyseal dysplasia Kimberley type (SEDK) and autosomal-recessive spondylo-epi-metaphyseal dysplasia (SEMD).^{112,113} Autosomal-dominant SEDK is a skeletal dysplasia characterized by a stocky short stature and early-onset progressive joint OA.

Anderson et al. first reported about SEDK identified in a multi-generational South African family of UK. white descent.¹¹⁴ Subsequently, Eyre et al.¹¹⁵ and Gleghorn et al.¹¹⁶ performed linkage studies and identified a novel locus on chromosome 15q26.1 where a single base-pair (bp) insertion introduced a frameshift of 212 amino acids that caused a premature stop codon in *ACAN*,

truncating aggrecan protein to lack half of the CS1 domain, the complete CS2 domain, and the G3 domain. Another autosomal-dominant mutation type led to short stature with accelerated bone maturation, early onset of OA, and craniofacial, limb, and vertebral abnormalities.¹¹⁷⁻¹²⁰

The autosomal-recessive SEMD was reported by Tompson et al.,¹¹³ describing 3 siblings with SEMD to have had extremely short stature, brachydactyly, distinct severe midface hypoplasia, short necks, barrel chests, lumbar lordosis, and macrocephaly. DNA sequence analysis of affected individuals revealed a missense mutation that predicted an amino acid substitution in the C-type lectin domain within the G3 domain,¹¹³ resulting in a reduction of aggrecan secretion.¹²¹ A recent case reported by Fukuhara et al.¹²² described an individual with SEMD caused by a heterozygous missense mutation in *ACAN*, however, the skeletal phenotype noted was much milder than the previous case, suggesting that mutations on different domains of *ACAN* can lead to different phenotypes.

2. BGN-Linked SEMD

Clinically, *BGN* mutation leads to X-linked SEMD¹²³ and Meester-Loeys Syndrome, a connective tissue-arterial aneurysms disorder.¹²⁴ Both disorders are characterized by skeletal dysplasia with short stature. To date, there are only 3 cases of X-linked SEMD (SEMDX) observed in an Italian, Korean, and Indian family.¹²⁵ This SEMDX disorder was caused by a missense mutation in *BGN* on chromosome Xq28 and phenotypically resulted in the shortening of limbs, bowing of the legs, and lumbar lordosis.

3. HSPG2-Linked Skeletal Dysplasia

The generation of perlecan deficient and hypomorphic mice was key to identifying HSPG2 mutations in human autosomal-recessive genetic diseases: DDSH and SJS.^{88,126,127} Clinically, DDSH is a rare autosomal-recessive skeletal dysplasia with anisodondyly and micromelia caused by perlecan truncation, resulting in diminished perlecan secretion. The mutation in patients was created by 89-bp duplication of exon 34 of *HSPG2* or a frameshift mutation that causes truncation in the perlecan protein core.^{92,126,128,129} SJS is also a rare autosomal-recessive disorder characterized by a spectrum of abnormal neuromuscular functions and skeletal dysplasia, such as continuous contractions of muscles throughout the body including the face, abnormal spinal curvature, and shortening of the bone.^{92,127,130,131} Patients with SJS survive and exhibit milder phenotypes compared to DDSH patients due to partially functional secreted

perlecan.¹²⁶ These phenotypes underscore the importance of perlecan in maintaining both cartilage integrity and muscle excitability.

CONCLUDING REMARKS

PGs are essential for proper skeletal development. Within the ECM, large PGs interact with growth factors and osmolytes to confer water-binding properties, tissue hydration, and bioavailability of growth factors, while smaller PGs regulate collagen fibril formation in the ECM. At the cell membrane, PGs can provide stabilization for ligand-receptor interactions, and potentiate signaling complexes that regulate growth factor sensitivity, cell migration, proliferation, and matrix adhesion. Studies have shown that gene mutations of major PGs such as aggrecan, perlecan, lubricin, biglycan, and tenomodulin result in disc degeneration and skeletal defects in murine models. These models described here could potentially be used to explore treatment modalities to restore ECM functionality during disc degeneration. In humans, proteoglycan gene mutations result in severe skeletal dysplasia, including lumbar disc herniation in some cases. Currently, there are no treatments for genetic skeletal disorders except corrective surgical procedures including osteotomy and spinal stenosis surgery.

Before we conclude, it is important to discuss a few emerging approaches to treat disc degeneration and restore the matrix function. During aging, senescent NP cells accumulate in the disc.¹³²⁻¹³⁴ Senescent cells are characterized by the senescence-associated secretory phenotype factors, particularly their secretion of proinflammatory ILs and several proteases including MMPs, ADAMTSs and serine protease HTRA1. To minimize age-associated disc degeneration, restore matrix function and to promote the reparative processes, senolytic drug cocktail of Dasatinib and Quercetin (D+Q) and pentosan polysulfate, a HS biomimetic, are under investigation as attractive options.^{135,136} The senolytic (D+Q) cocktail works by selectively inducing senescent cell apoptosis by targeting senescent cell antiapoptotic pathways.^{137,138} It should be noted that it is technically challenging to deliver biomimetics to inner disc compartments without causing structural alterations during delivery. In contrast, systemic administration of Dasatinib and Quercetin was found to be effective in murine models and is a viable therapeutic option in preventing age-associated disc degeneration and restoring matrix quality. Similarly, long-term systemic administration of a supplement called alpha-ketoglutaric acid was reported to attenuate inflammatory- and age-associated disc degeneration by

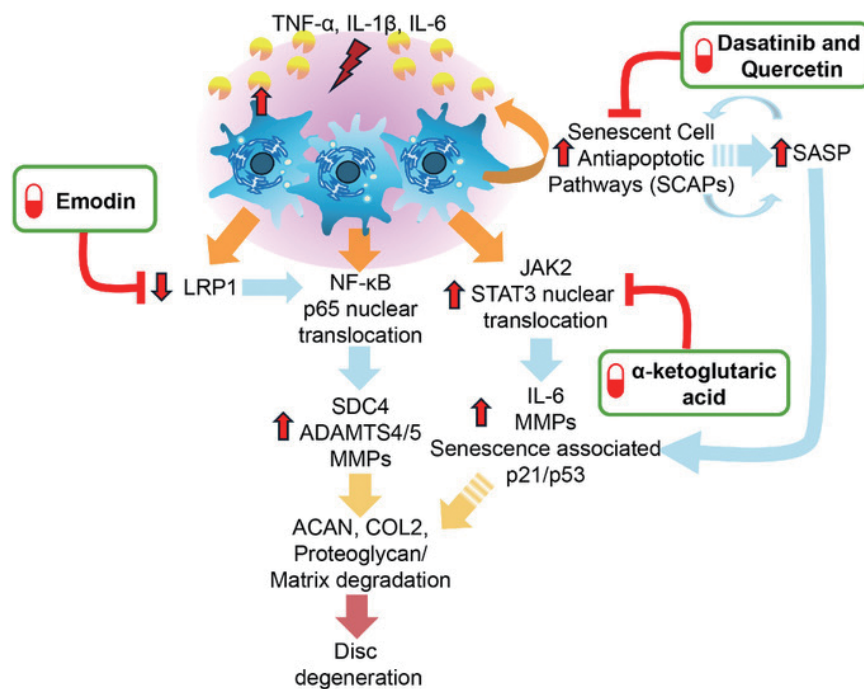


Fig. 2. A simplified illustration showing key pathways involved in disc degeneration. Increased levels of proinflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 due to injury or aging could (1) induce degradation of lipoprotein receptor-related protein 1 (LRP1); (2) nuclear factor kappa B (NF- κ B) mediated activation of p65 nuclear translocation, and subsequent promotion of SDC4-ADAMTS-5 proteolytic activity causing degrading of disc matrix; (3) activation of Janus tyrosine kinase 2/signal transducer and activator of the transcription 3 (JAK2/STAT3) signaling pathway elevating IL-6, matrix metalloproteinases (MMPs) levels, and senescence associated factors such as p21/p53; (4) increased expression of senescent cell antiapoptotic pathways (SCAPs) and senescence-associated secretory phenotype (SASP), preventing cells from secreting anabolic factors ultimately affecting tissue function. Several therapeutics such as Dasatinib and Quercetin (D+Q) senolytic drug, α -ketoglutaric acid, and Emodin in preclinical models are shown to delay or prevent degeneration by targeting SCAPs, JAK2/STAT3 signaling axis, and LRP1 signaling pathway, respectively.

suppressing catabolic IL-6 expression and preventing JAK2/STAT3 (Janus tyrosine kinase 2/signal transducer and activator of the transcription 3) phosphorylation involved in the degenerative process.¹³⁹ In the context of injury-induced disc degeneration, Emodin, a bioactive anthraquinone compound, has been proposed as a potential therapeutic to alleviate inflammation when injected into the injury site.^{140,141} This bioactive works by upregulating low-density lipoprotein receptor-related protein 1 to inhibit NF- κ B mediated degradation of MMPs and ADAMTS-5, ameliorating disc degeneration and preserving aggrecan expression and functionality *in vitro* and *in vivo*¹⁴⁰ (See Fig. 2 for summary of these potential therapeutics). Further clinical trials and controlled studies will be required to assess the efficacy of these therapies.

NOTES

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ORCID

Kimheak Sao: 0000-0002-9164-1529

Makarand V. Risbud: 0000-0003-3913-0649

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