Structure and Biology of the Intervertebral Disk in Health and Disease

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KEYWORDS

• Intervertebral disk • Degeneration • Extracellular matrix
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Low back pain is a leading debilitating condition that affects every population worldwide,[1] and can lead to diminished physical function, loss of wages, decreased quality of life, and psychological distress.[1–4] In fact, chronic low back pain may also lead to brain tissue destruction.[5–8] As a consequence, low back pain is one of the most common conditions for which to seek medical consultation and one of those preeminent for analgesic use in the United States.[3,4] Furthermore, the management of patients with low back pain can be a challenge, often requiring a multidisciplinary approach to treatment (see the article by Karppinen and colleagues elsewhere in this issue).[9–13]

Although low back pain is a multifactorial condition (eg, biopsychological, muscular, socioeconomic), intervertebral disk (IVD) degeneration has been indicated to be a strong etiologic factor (**Fig. 1**).[14–24] Intervertebral disk degeneration occurs in every population worldwide, mainly involving the lower lumbar segments (L4 to S1) where disk height narrowing also commonly occurs and generally affects almost all individuals by the sixth and seventh decade of life.[24,25] However, the development or, rather, severity of IVD degeneration is not linearly based on age; degenerative changes can be noted in young children and not yet be manifested in other adults.[19,24] Overall, the true prevalence of IVD degeneration in populations has yet to be determined, due to improper surveillance methods (ie, patient-based versus population-based), sampling issues, heterogeneity in the operational definition and imaging modalities in assessing the phenotype of disk changes, and an incomplete understanding of the risk-factor profile and its interaction effects that may affect degenerative changes and their manifestation in different age, gender, and ethnic groups.[14,15,26] Along these lines, the incidence rates of annular tears, disk bulging, and endplate defects/abnormalities are also not conclusive, and vary between studies.

The development of IVD degeneration is a complex, multifaceted condition. Various studies have suggested that, age, male gender, abnormal physical loading, trauma, infection, hormonal, overweight and obesity, altered metabolism, Schmorl's

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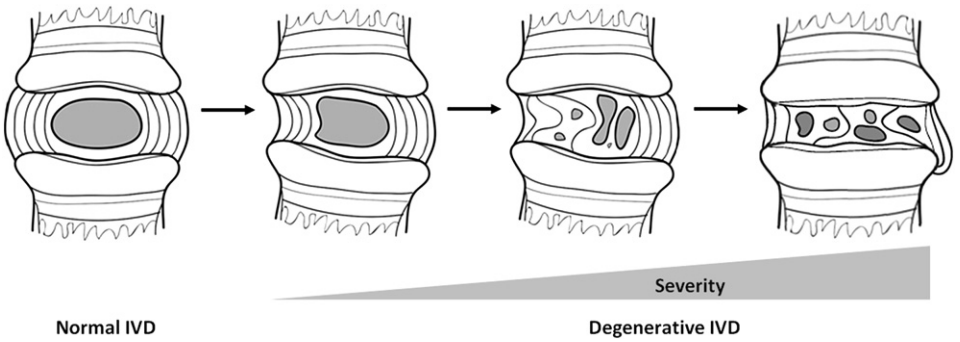

Fig. 1. Illustration of the different stages of intervertebral disk degeneration. Note the normal disk on the left and the progression of degenerative changes from left to right, which are characterized as chemical and structural changes of the disk (eg, dehydration of the nucleus pulposus, disruption of the annulus fibrosus, decreased disk height, and endplate changes). IVD, intervertebral disk.

nodes, cigarette smoking, and occupation are risk factors related to the development of IVD degeneration.[20,27–41] Several investigators have also noted that systemic conditions, such as atherosclerosis, may contribute to IVD degeneration, due to the "vascular insufficiency" provided to the vertebral body that may affect diffusion of metabolites and nutrients into the disk necessary to maintain a healthy environment.[42–46] Furthermore, it has been strongly suggested that IVD degeneration may be attributed to genetic factors. Familial aggregation studies have indicated that individuals with severe forms of IVD degeneration that are often symptomatic have family members with a history of disk-related problems, often seeking medical attention themselves.[47–51] Twin studies have also noted that more than 70% of variability of IVD degeneration may be attributed to genetics.[52–55] Moreover, observational cohort studies have identified specific genes that may play a role in the development of IVD degeneration, some of which may have a synergistic effect with environmental exposures and perhaps be age dependent (see the article by Kao and colleagues elsewhere in this issue).[56–59] As such, understanding the genetic epidemiology of IVD degeneration is imperative in comprehending the scope of the degenerative condition, why degenerative changes occur in certain individuals rather than others, and in developing a better understanding of the use of biological therapies for the prevention or regeneration of the disease process (see the articles by Sakai, Woods and colleagues, Leung and colleagues, and Bae and Masuda elsewhere in this issue). However, at a more basic level, understanding the structure and biology of the IVD in health and disease, in particular the developmental process, cellular origin, changes in the extracellular matrix (ECM) components, and maintenance in adult life, is essential.

INTERVERTEBRAL DISK

The IVD is a functional unit connecting the vertebral bodies of the spine. In humans there are 25 IVDs interposed from the axis to the sacrum. Each IVD consists of 3 structural components: a soft gelatinous nucleus pulposus (NP) in the center surrounded by a tough peripheral lamellar annulus fibrosus (AF), sandwiched between 2 cartilaginous endplates (EP) (**Fig. 2**). The components of the disk act synergistically, facilitating motions of the spine and acting as shock absorbers between vertebral bodies.[60–62]

Traditional concepts on the function of the disk relate to specific ECM proteins that assemble and interact to form the 3 distinct structures. While one can describe the NP, AF, and EP separately with distinct functions, the homeostasis of the IVD as a unit must have optimal function from all 3 structures. The impairment of one or more of these structures can lead to dire consequences with IVD degeneration. The ECM is produced and maintained by resident cells, and there are feedback mechanisms for cells to sense the ECM, while the ECM regulates extrinsic signals to cells for disk homeostasis.

DEVELOPMENT OF THE INTERVERTEBRAL DISK

The notochord is central to the development of IVD. The notochord is a rod-shaped midline structure of mesodermal origin found in chordate embryos during gastrulation, and represents a primitive axial skeleton.[63,64] As a structure that is recognized in all vertebrate embryos, its development has been well studied and described since the nineteenth century.[65] It is composed of cells derived from the organizer tissue at different stages of development.

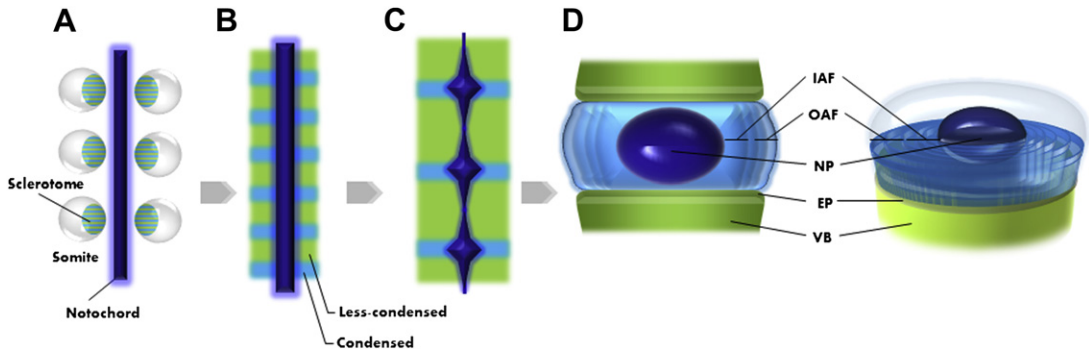

Fig. 2. Development of the intervertebral disk. (*A*) In early development, the notochord is localized adjacent to pairs of paraxial somites containing sclerotome cells. (*B*) Sclerotome cells migrate and condense around the notochord forming the perinotochordal sheath, with a metameric pattern of condensed and uncondensed regions (*C*) The notochord contracts within the developing vertebral bodies and expands within the future nucleus pulposus regions of the intervertebral disk. (*D*) Establishment of the basic structure of the intervertebral disk with formation of the cartilage endplate and the lamellar structures of the annulus fibrosus. EP, endplate; IAF, inner annulus fibrosus; NP, nucleus pulposus; OAF, outer annulus fibrosus; VB, vertebral body.

The contribution of the organizer to different regions of the notochord along the anterior-posterior axis is complex, but has been studied using cell-mapping tools and live time-lapse imaging.[66,67] In principle, the anterior notochord is formed by direct convergence of the anterior dispersed cells, the trunk notochord is formed by convergent extension of the node-derived cells, and the tail notochord is formed by posterior migration of the node-derived progenitors. The notochord is important not only as a signaling center but also as a structure that gives rise to the future NP.

In early gestation stages (30 days in human, 12 days in mouse), the notochord is located adjacent to the paraxial somites (see **Fig. 2**A) and can induce the differentiation of the ventral somatic derivatives into the sclerotome.[68] Sclerotome cells migrate and condense around the notochord, forming the perinotochordal sheath (see **Fig. 2**B). The cells form the unsegmented perichordal mesenchyme with a metameric pattern of condensed and uncondensed regions.[69] The condensed mesenchyme will give rise to the AF of the disk, whereas the uncondensed mesenchyme will form the future vertebral body, developed first as a continuous cartilaginous column forming around the notochord. As development progresses, the notochord is thought to be compressed or squeezed away from the cartilage anlagen regions of the vertebral bodies and expanded in the IVD anlagen regions (see **Fig. 2**C), giving rise to the future NP. In some instances remnants of the notochord can be detected within vertebral bodies, and are thought to be a possible origin of chordomas[69–71]; this would support the idea that notochordal cells may have progenitor properties. Although this notion remains controversial, cell-tracking analysis of notochordal cells in the mouse suggests this may be the case, with perhaps subsequent differentiation to mature NP cells.[70]

To date, no cell-tracking data are available to confirm the origin of the AF cells, but it is thought to originate from the somites. Little is known regarding the origin of the cells in the EP and its formation. It was first postulated that the NP is not derived from the side of the vertebral body but from undifferentiated cells, which accumulate in early development and develop into an organized structure under mechanical influences. This hypothesis was later refined to suggest induction from mechanical stimulus due to actions of compressive forces, torsion, and shear stresses occurring in the IVD, similar to the mechanical stresses that induce thickening and delamination of connective tissues (see the article by Inoue and Espinoza Orias elsewhere in this issue). Thus, the hyaline cartilage EP represents the interface between the vertebral body and the disk, and the annular epiphysis of the vertebral body develops in the marginal part of the EP. These descriptions of IVD development are derived from previous detailed anatomic analyses that need to be revisited using modern tools in molecular genetics, such as those available in the mouse.

STRUCTURAL ORGANIZATION OF THE INTERVERTEBRAL DISK

The AF is made up of concentric angle-ply layers that cross one another obliquely in space (see **Fig. 2**D). The AF is divided into inner and outer regions, which have distinct biochemical and

cellular composition as well as biomechanical properties. The outer AF is composed of fibroblastic cells, which produce type I collagen. The collagen fibrils form fibers, which run parallel within each lamella, organized into a ligamentous structure that inserts into the adjacent vertebral bodies.[72,73] Bundles of microfibrils are distributed within the interterritorial matrix and are colocalized with elastin fibers.[74] Cells in the inner AF are more chondrocyte-like, producing mainly type II collagen, and proteoglycans such as aggrecan. These changes give rise to a less fibrous and less organized structure compared with the outer AF.[75]

The vertebral EP is composed of two layers: an inner bony layer and an outer cartilaginous layer. The latter is a thin horizontal layer of articular cartilaginous structure, which interfaces the vertebral bodies and the IVDs. IVDs have limited nerve and blood vessel supply,[76,77] and the EPs act as the source and regulator of nutrient and oxygen diffusion from the vertebral bodies (see the article by Grunhagen and colleagues elsewhere in this issue).[78] The NP is an aggrecan-rich jellylike structure confined within the EP and AF. It is composed of chondrocyte-like cells producing polysaccharide/mucoprotein molecules, such as chondroitin sulfate, collagen, and elastin fibers. Aggrecan, with high anionic glycosaminoglycan content, is the major type of proteoglycan in the IVDs, providing the osmotic properties for compressive loading.[79] The specific matrix composition in each structural compartment supports the different mechanical role and cell signaling function of the disk cells.

The NP represents the center of the IVD. This region of the disk has attracted much attention, as it is thought to be where degeneration occurs with changes in cell morphology and ECM components, leading to reduced water content and narrowed disk height. In humans, studies have suggested that notochordal cells disappear after the establishment of the spinal column, and the cell population is gradually replaced by chondrocyte-like NP cells, whereas in mouse and other species notochordal cells are maintained in the NP.[80,81] There is also an apparent correlation between this maintenance of notochordal characteristics of cells in the NP and susceptibility to disk degeneration in the different species studied, including mouse, rat, rabbit, dog, sheep, and human. However, this is an area of controversy, as the "absence" of notochordal cells is based on morphologic and histologic studies.[82,83] Furthermore, notochordal-related molecular markers, such as cytokeratin types CK-8, CK-18, CK-19, and galectin-3, can be detected in adult human NP cells.[84–87] Thus, the precise fate of notochordal cells remains to be resolved.[70,87–91]

EXTRACELLULAR MATRIX AND IVD FUNCTION

The ECM in the IVD is a dynamic network of structural proteins that contributes to disk function, resisting mechanical loading and tensile force. While a key function of ECM is structural, one must also consider that the ECM provides the environment for cell maintenance and survival. In addition, the array of ECM components and the functionalities that they carry provide diverse interactions with soluble factors, such as growth factors, cytokines, morphogens, chemokines, and enzymes, modulating their interaction with or presentation to cells. The ECM is not an inert substance but continues to be produced and degraded in remodeling and repair processes. Throughout life, there are significant changes in the molecular composition and organization of the ECM network as part of development, growth, and aging.[92,93] Accelerated imbalance between anabolic and catabolic events within the IVD will affect the integrity of the matrix and disk function, leading to early IVD degeneration.[94–96]

In general, the major ECM components consist of collagens organized into various fibrillar networks providing the tensile strength required for specific tissue function.[97–99] The presence of elastin gives added elasticity to tissues.[100] Proteoglycans contain a small core protein, but have many highly negatively charged glycosaminoglycan (GAG) side chains, providing opportunities for interactions with other matrix molecules and soluble factors.[101,102] These GAG elements also attract cations with water retention properties, contributing to tissue hydration. Lastly, there is a huge array of structural glycoproteins, such as fibronectin,[103,104] laminins,[105] and tenasins.[106] These structural glycoproteins help to fine-tune tissue functionality as well as assist in the assembling and organization of the matrix. The ECM components and their role in IVD function are discussed here in relation to the specific IVD structures.

Annulus Fibrosus

The AF is a highly structured lamellar tissue, and can be subdivided into the outer annulus and inner annulus. In a healthy adult human disk, the outer annulus is made up of a series of 15 to 25 concentric lamellae with highly ordered collagen fibers oriented in sheets parallel with each lamella.[72,73] The outer annulus is composed of mainly type I collagen attributing to approximately 90% of the collagens in the IVD, together with smaller amounts of collagen types III, V, and VI.[107] Type III and V collagens can form heterotypic fibrils with type I

collagen, providing diversity to fibril properties, whereas type VI collagen molecules assemble into beaded filaments.[108] These collagen fibrils network with adjacent lamellae, working cooperatively with each other during dynamic loading.

Elastic fibers, which make up only 2% of the AF dry weight, is another organized ECM network that aligns parallel with the lamellae. The outer annulus consists of a higher elastin density and has a greater elastin colocalization with mircofibrils in comparison with the inner annulus.[74] Elastin is concentrated between the lamellae and is thought to function in protecting the disk from delamination, as well as help with the recovery of the lamellar structure after deformation under radial loads.[74]

Of interest, a translamellar bridging network (TLBN) has been identified within the AF,[109] where there is a network consisting of translamellar bridging fibers within the inter bundle space of an individual lamella, connecting fibers of the adjacent lamellae. The structural alignment of TLBN is suggested to enhance resistance toward radial, lifting, and torsional forces, and prevents the disjunction of lamellae under torsional force.[109]

Toward the inner AF, there is a transition to a type II collagen-enriched structure, with higher content of proteoglycans such as aggrecan, biglycans, and lumican, which results in a less organized fibrous structure.[75] The reason for this transition is not clear; perhaps there is a need for a progressive change of AF to establish a functional link between inner AF and the type II collagen–enriched NP. Postnatally, the boundary between the outer and the inner AF become less distinct, as does the interface between the inner AF and the NP with aging.

Nucleus Pulposus

As the NP enlarges with growth, it is filled with a soft cartilaginous-like matrix, but consists of very high levels of proteoglycans entrapped in a randomly orientated type II collagen fibrous network (**Fig. 3**). Like cartilage, there are also small amounts of type XI and IX collagens. Type XI collagens associate with type II collagens to form heterotypic collagen fibrils, whereas type IX collagen is a fibril-associated collagen that coats the surface of these cartilage-like collagen fibrils. There are unique interruptions within the triple helix of type IX collagen molecules, allowing bending of the triple helical molecules. The arrangement of type IX collagen on the fibril surface is such that some domains are projected away from the fibril for interaction with other matrix molecules, acting as a bridge between collagen fibrils and other matrix components. A role for type IX collagen in IVD integrity is implicated, as two of the type IX collagen genes (*COL9A2* and *COL9A3*) are associated with IVD degeneration.[110,111] It is significant that IVDs from patients with the risk Trp2 allele in the *COL9A2* gene are mechanically impaired, with

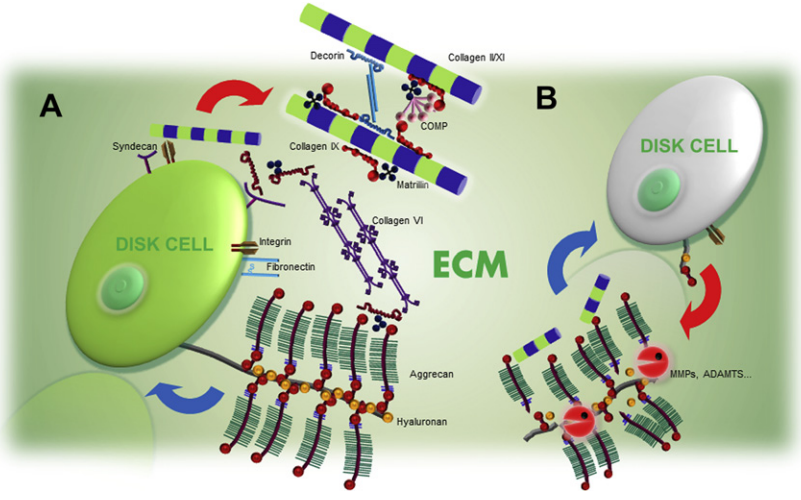

Fig. 3. Extracellular matrix components and environment in healthy and degenerated intervertebral disks. (*A*) Healthy disk cells producing the appropriate extracellular matrix (ECM) components for intervertebral disk function and interacting with the matrix components via specific receptors, such as integrin, responding to signals from the environment for tissue homeostasis. (*B*) Alteration in the disk cell environment with degrading extracellular matrix components altering the signals to disk cells, disrupting normal cell function and cell phenotype, with a negative impact on intervertebral disk function. ADAMTS, A Disintegrin And Metalloproteinase with Thrombospondin MotifS; COMP, Cartilage Oligomeric Matrix Protein; MMPs, matrix metalloproteins.

reduced water-retention property and resistance to compression.[56,57]

Chondroitin sulfate (CS) proteoglycan on the cell surface or ECM play important roles in the development and biological function of the IVD.[112] Similar to cartilage, the major CS proteoglycan in the ECM is aggrecan. Aggrecan has a relatively large core protein of about 2000 amino acids with distinct structural and functional regions. There are 3 globular domains (G1, G2, and G3). The first 2, G1 and G2, are localized toward the N-terminal region separated by a short interglobular domain (IGD). The third, the G3 domain, is localized near the C-terminal region of the core protein. Between the G2 and G3 domains are sites for the attachment of about 100 CS glycosaminoglycan side chains distributed along the CS1 and CS2 domains.[113] Nearer to the G2 domain are attachment sites for approximately 30 keratan sulfate (KS) glycosaminoglycan side chains that are short (22–30 disaccharide units) but highly variable.

The G1 domain mediates the interaction of aggrecan with hyaluronic acid (HA), and the interaction is stabilized by a small link protein that has properties similar to the G1 domain.[114,115] Up to 100 aggrecan molecules can be found on a single HA, resulting in a huge and highly charged aggregate with HA and other matrix molecules.[116]

The highly charged GAG chains attract and retain water in the NP, and produce a swelling pressure allowing resistance to compression from axial loading.[117] In human, the CS1 domain possesses a variable number of tandem repeats, and results in a variation of the length of the aggrecan core protein in different individuals.[118] It is suggested that individuals with a lower CS content are more susceptible to disk degeneration.[119,120] The G3 globular domain contains a C-type lectin motif, but no distinct carbohydrate binding has been identified. Recently, it has been shown that aggrecan via this domain can interact with matrix proteins containing EGF repeats, such as fibulins and tenasins. Fibulins are a family of secreted glycoproteins that interact with elastin and many other matrix proteins.[121] As such, the organization and assembly of the ECM can be established by the networking of aggrecan with other matrix proteins in the tissues.

Small leucine repeat proteins/proteoglycans (SLRP) are also present in the NP. The SLRPs include the small cartilage proteoglycans, such as fibromodulin, decorin, and lumican. These molecules have a central portion consisting of 10 or 11 repeats of approximately 25 amino acids with leucine residues at conserved sites. These SLRPs contain 1 or 2 keratan sulfate chains attached to the repeating units. The polysaccharides can directly interact with collagen and can serve to cross-bridge and cross-link collagen fibers, and regulate collagen fibril assembly. Decorin, via its core protein, can also bind to beaded filaments of type VI collagen at the N-terminal part of this collagen,[122] again acting as bridging molecules in the ECM. SLRPs can also bind growth factors, in particular transforming growth factor (TGF)-β, to regulate tissue homeostasis.[123]

Other SLRPs such as asporin and chondroadherin do not contain GAG side chains. Asporin also binds collagen via its leucine-rich repeat domain.[124] This molecule contains an N-terminal extension with a variable number of aspartic acid residues, and is a polymorphic region of the gene in the human population, ranging from 8 to 19 continuous aspartic acid (D) residues. It has been shown in Asian populations that individuals with the 14-repeat (D14) allele have a higher incidence of osteoarthritis and IVD degeneration, and is upregulated in cartilage of osteoarthritic patients and in patients with IVD degeneration.[125] Asporin also binds TGF-β, and the D14 variant was shown to bind TGF-β with a higher affinity than the common allele (D13) with 13 repeats.[126] A hypothesis is that asporin could regulate the availability of TGF-β and thus modulate the synthesis of matrix molecules. Chondroadherin does not have the N-terminal extension; however, like other SLRPs it interacts with collagen but also interacts with α2β1 integrin, a cell surface receptor by which cells sense their environment, and this interaction is thought to enhance matrix production.[127]

Cartilaginous Endplate

The biochemical composition of the cartilaginous EP is similar to the articular cartilage of joints. The ECM components described for the NP are also applicable to the EP. It must be emphasized that although many of the components found in the NP are cartilage ECM proteins, their relative amounts are very different, and thus differ in form and function. As in hyaline cartilage, this thin cartilage NP layer is composed of a network of randomly oriented collagen fibers within a gel of hydrated proteoglycans. At the junction with the inner annulus fibrosus the collagen network is more organized, oriented more horizontal and parallel to the vertebral bodies, with the collagen fibers running continually into the inner annulus. In cartilaginous endplate, the major proteoglycan is also aggrecan, but the relative level is lower than in the NP.[93,128] In cartilage, the length of the CS side chains appears to be longer and is higher in proportion relative to the KS side chains.

As a thin horizontal layer lying at the interface between the disk and the adjacent vertebral bodies, the NP acts as a selectively permeable barrier in which small and uncharged solutes can diffuse across readily, whereas the movement of anions or larger solutes is restricted.[78] However, permeability studies suggest that diffusion mainly occurs between the subchondral space and the central zone of the disk.[129] Type X collagen is normally found in hypertrophic cartilage undergoing mineralization. Its function in the ECM is not clear, but it is thought to have a role in cartilage mineralization.[130] Type X collagen is found in the central region of the cartilaginous endplate with aging,[131–133] and could be related to hypertrophic differentiation of chondrocytes and calcification within the endplate, impairing diffusion and thus nutritional supply to disk cells (see the article by Grunhagen and colleagues elsewhere in this issue).[41,134]

ECM HOMEOSTASIS AND DEGENERATIVE STATES

The ECM in the IVD undergoes extensive remodeling throughout development, growth, and aging. The balance between the process of matrix degradation, synthesis, and deposition determines the matrix composition in the IVD. This balance not only is critical for the quality and integrity of the matrix but also determines biological changes of disk cells in the control of differentiation, maintenance of cell phenotype, cell proliferation, and cell death. Conversely, maintenance of cell function and activity dictates the tolerance to physiologic stresses before a pathologic condition arises. A progressive imbalance and accumulative stress in cell function would manifest a degenerative phenomenon. The observation of loss of cellularity and altered disk cell activity or phenotype in the degenerated IVD is consistent with such a notion.

What causes disk degeneration is still not clear, but from the analysis of magnetic resonance imaging (MRI) studies, there are several structural abnormalities that can be considered. It is generally accepted that dehydration of the nucleus pulposus, as analyzed by MRI, is an indication of degeneration that progressively worsens, and can be associated with "tears" within the AF (high-intensity zones) or the cartilage endplate (ie, Schmorl's nodes). In some instances, the NP can herniate through a disrupted AF. These MRI changes are thought to be caused by failure of the tissue structures from alterations of the ECM. For example, the NP can lose its hydration property from a reduced proteoglycan content. The tears within the AF may occur via disruption of the organized collagen and/or elastin networks within the lamellae, or mineralization of the cartilaginous endplate affecting nutrition supply to the disk, causing early cellular senescence or cell death and impairing the capacity for tissue maintenance and repair.

Because of the similarity of the matrix components of the IVD to that of the cartilage in a joint, the lessons learnt from cartilage biology and pathology are frequently applied to the disk, bearing in mind the similarities and differences. Destruction of cartilage in osteoarthritis is used as a model system to look for similar occurrences in the disk.

Cytokines, Matrix Metalloproteinase, and Disk Degeneration

Cytokines are important in the biology and pathology of the IVD because of their potential role in regulating the integrity of connective tissues; they influence the synthesis and degradation of the ECM, ingrowth of nerves and blood vessels, and accumulation of macrophages that are characteristic of disk degeneration. These cytokines include tumor necrosis factor (TNF), TWEAK (TNF-like weak inducer of apoptosis), interleukin (IL)-1, IL-10, platelet-derived growth factor, vascular endothelial growth factor, insulin-like growth factor, TGF-β, endothelial growth factor (EGF), and fibroblast growth factor.[135–137] Whereas anabolic cytokines such as TGF-β can promote the synthesis of collagens and proteoglycans,[138,139] catabolic cytokines, such as TNF-α and IL-1, have received considerable attention because of their involvement in cartilage homeostasis and their ability to switch chondrocytes from an anabolic to a catabolic state.[140–142] TNF-α and IL-1 and their respective receptors are elevated in human degenerative IVD.[143,144] These proinflammatory cytokines can increase production of matrix-degradative enzymes, and enhance the breakdown of collagens and proteoglycans.[145,146] Thus, the expression or activity of a range of matrix metalloproteinases (MMPs) such as MMP-1, -3, -7, -9, -10 and -13,[146,147] as well as ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin MotifS)-4 and -5,[145,148] are increased in disk cells with age and degeneration. Significantly, the levels of some of these enzymes and their activities appear to correlate with the degree of degeneration.[144,149] ADAMTS-4 and -5 have high specificity for the cleavage of aggrecan and are also known as aggrecanase-1 and -2, respectively.

Degradation of type II collagen is initiated by cleavage of the triple helix at a specific MMP cleavage site,[150] whereas numerous MMPs and ADAMTSs may be involved in the degradation of aggrecan in response to cytokine stimulation.[151–153]

Given dehydration of the NP is a key feature of IVD degeneration, the degradation of aggrecan is a prime-candidate biological process in the initiation of degenerative changes. The enzymes and their kinetics for the cleavage of aggrecan have been intensively studied because of their involvement in osteoarthritis; however, their role in the degenerative process and how they cooperate are still largely unclear.

Studies have shown that MMPs in general have a lower efficiency for cleaving aggrecan within the IGD and CS2 regions compared with ADAMTS-4 and ADAMTS-5.[153,154] In vivo, activities of MMPs are regulated by the presence of tissue inhibitors of metalloproteinase (TIMP). In degenerated IVD, expression of the general MMP inhibitors, TIMP-1 and TIMP-2, are upregulated, whereas TIMP-3, a specific inhibitor for ADAMTS, remains relatively unchanged compared with the enhanced ADAMTS-4 and -5 expressions, suggesting ADAMTS enzyme activity may be an important factor in IVD degeneration. A recent study using a rabbit annular puncture model of IVD degeneration showed that suppressing ADAMTS-5 activity by siRNA injection reduces degradation within the NP, and improves MRI and histologic scores for IVD degeneration.[155] This result would be consistent with the finding that mice with genetic inactivation of the *Adamts-5* gene are more resistant to surgically induced osteoarthritis of knee joints.[156] A clear understanding of the degradative processes within the disk would be beneficial for the development of specific therapeutic targets.

As MMPs cleave matrix proteins at very specific sites, it is possible to follow the cleavage occurrences with neo-epitope antibodies that can recognize newly exposed N- or C-termini. This method was successfully applied to studies of aggrecan degradation from specific cleavage within the core protein.[157,158] Again, lessons from cartilage degradation suggest that cytokines that trigger the secretion of proteolytic enzymes may initially degrade aggrecan, followed by the release of other molecules such as COMP (Cartilage Oligomeric Matrix Protein) and fibromodulin, and progressively to the release of collagen fragments as the major type II collagen–containing fibrillar network is eroded away.[159] The MMP cleavage site for type II collagen is clearly defined, and neo-epitope antibodies are available for the specific detection and localization of these cleavage fragments.[92,160]

Extracellular Matrix Protein Changes in Disk Degeneration

In early stages of degeneration there are several changes in the pattern of matrix protein synthesis, reflecting an altered homeostasis. For instance, more type I collagen is found within the NP, whereas more type II collagen is detected in the outer annulus but less so in the endplates.[161] Although both type I and II collagens are fibrillar collagens, they assemble and organize into distinct fibrils of different size and supramolecular aggregates, and are not redundant in function. In fact, it could be detrimental for tissue function if these collagens are expressed ectopically.

The synthesis of proteoglycans is also altered with decreased aggrecan, increased versican, and other small leucine-rich repeat protein/proteoglycans, such as asporin, biglycan, and decorin, in human disk samples.[162–164] The relative proportion of GAGs also changes, from a chondroitin sulfate–enriched matrix to that of keratan sulfate, thus reducing the hydration property of the tissue.[165,166] This change is related to the higher content of sulfate of chondroitin sulfate GAGs being more negatively charged, able to attract more cations and contribute to the water-binding capacity. While the hydroxyl groups on disaccharide units of chondroitin sulfate GAG are differentially sulfated, contributing to structural heterogeneity, specific sulfation motif epitopes can be recognized by a variety of monoclonal antibodies, and have been used to study their pattern in development and postnatal growth[167] as well as degeneration of the IVD.[166,168] The findings are supportive of a significant role for chondroitin sulfate in IVD development and maintenance. In degeneration, an involvement in cellular reparatory processes was proposed.

With degeneration the cellular microenvironment becomes more hostile, with a high level of cytokines and a low level of oxygen and nutritional contents. Of interest, studies have demonstrated that cells in NP have the potential to repair the degenerated disk by generating more matrix molecules. For example, it was shown that the NP cells in degenerated IVD retain the ability to synthesize large aggrecan molecules with intact HA-binding regions.[169] It is also thought that in the early stages of IVD degeneration, disk cells attempt to restore normal function by synthesizing more water-attracting matrix proteins. However, it would be important to consider a reparatory process using more stringent criteria, perhaps from high-throughput global proteomic studies comparing proteins produced by IVD cells at different stages of development and degeneration.

As degeneration progresses the less hydrated and more fibrous NP fails to withstand the compressive loading, resulting in uneven distribution of forces to the surrounding AF. Additional stress is imposed on the AF, which will lead to

the formation of radial tears or bulge, or tears to the cartilaginous endplates.[170,171] This line of thinking is very much centered on dehydration of the NP. However, it is also possible that IVD degeneration could be initiated from the surrounding AF or the cartilaginous endplate. Indeed, there is evidence for changes in the endplate that alter nutritional supply to the nucleus, or changes in cell phenotype in the AF prior to dehydration of the NP. This predicament will only be resolved when researchers have good animal models for IVD degeneration, allowing detailed analysis of the sequence of molecular and cellular events.

DISK CELLS INTERACTING WITH THE ENVIRONMENT

In addition to structural support for tissue function, the ECM also provides information cues to inform cellular response. Disk cells are embedded in a sea of ECM. Cells sense their environment via cell surface receptors that directly interact with specific motifs or domains present within the matrix components. There are many cell surface receptors that can mediate cell-matrix interactions, of which integrins is a major class. Integrins function as heterodimers, consisting of one α and one β subunit, which combine to form 24 distinct integrin receptors.[172] Specific heterodimer combinations present cells with defined binding properties. On binding, specific downstream cellular effects are transduced, affecting cell fate, proliferation, and migration. Functions of integrins have been studied in many systems including cartilaginous tissues. For example, inactivation of the β1 integrin gene in chondrocytes affects the columnar structure of proliferating chondrocytes in the cartilage growth plate, critical for the linear growth of long bones.[173]

In human IVD, integrin subunits involved in the binding of collagens (α1, β1) and fibronectins (α5, αv, β1, β3, β5) have been detected in both the AF and NP.[174] The precise function of integrins in IVDs in not clear. However, an involvement in mechanotransduction has been suggested for a class of integrins that bind to the RGD (arginine-glycine-aspartic acid) sequence motif of the ligand.[175] This proposal would be consistent with the variations in expression profiles between the AF and NP[174]; tissues within the IVD with different mechanical properties. Of interest, cells from nondegenerated and degenerated IVD behaved differently in response to hydrostatic loading, suggesting altered mechanotransduction pathways.[175,176] This possibility would be consistent with the notion that cell-matrix interaction is impaired in degeneration, perhaps arising from an alteration in the ECM composition with a feedback loop that has a negative impact on cell function and phenotype.

CELL MAINTENANCE AND DISEASE STATES

Degenerative disease of the IVD is a disruption of homeostasis, contributed by deregulation of function or metabolism of cells in the system. Conversely, maintenance of cell function and activity dictates the tolerance to physiologic stresses before a pathologic condition arises. A progressive imbalance and accumulative stress would manifest a degenerative phenomenon. The observation of loss of cellularity and an altered disk cell phenotype in degenerated disk is consistent with such a notion.

Whereas IVD has few pain receptors except in the periphery of the disk [177,178] and may not be irritated until inflammation becomes moderate to severe, IVD degeneration may render the motion segments unstable under load, which results in tension and strain (see the article by Inoue and Espinoza Orias elsewhere in this issue).[179] The NP appears to be the first place of degeneration with observable changes, although it may not correspond to the primary site of defect. One working hypothesis points to the reduction of nutritional exchange through the ossification of endplate (see the article by Grunhagen and colleagues elsewhere in this issue). Additional theories include disk overload due to obesity or altered metabolism and/or introduction of low-grade inflammation brought on by fat cells and weight gain.[20,180] Insights from transgenic models imply that hyperactivity of muscles may also induce the degeneration.[181,182]

Association studies suggest that most cases of degeneration may be related to age-related processes together with multiple intrinsic and extrinsic components that accelerate the process; these include genetic factors and environmental stresses. Individuals who are genetically compromised in disk cell function may have abnormal adaptive response to stress and subsequently be more susceptible to IVD degeneration. Through studying disk microenvironments, it is becoming clear that disk cells have an extraordinary capacity to adapt to adverse microenvironments, including mechanical shear, tension in oxygen supply, nutrition and waste exchange, and osmotic pressure.

Intervertebral disks, especially on the lumbar levels, are subjected to high compressive load, which place excessive stress on disk cells.[183] Although disk cells may benefit from mechanical stimulation, excess load in the long term is thought

to be detrimental. Symptomatic subjects suffering from IVD degeneration may show signs of pain relief and disk height restoration after nonsurgical distraction,[184] and distraction devices may induce IVD regeneration in animal models, suggesting that mechanical stress may contribute to degeneration. However, disk cells have been shown to exhibit higher matrix anabolism and viability under a regime of dynamic and cyclic loading that mimics the loading in humans, implying that disk cells are in fact designed to adapt to physiologic mechanical stress.[185,186]

The IVD, especially the NP, suffers from hypoxia because of limited vascularization. However, like other cells in minimally vascularized tissues such as cartilage, disk cells are able to withstand low oxygen tension, in part by activating hypoxia-inducible factors (HIFs) to adjust their metabolic activities and protect from apoptosis. Reports show that low oxygen content appears not to impair disk cell metabolism or functionality in vitro,[187] indicating its limited effects on disk homeostasis. Nonetheless, recent study has shown that notochordal NP cells are more susceptible to oxygen deprivation than chondrocyte-like NP cells,[91] suggesting that loss of notochordal cells and hence IVD degeneration may indeed be linked to oxygen stress. HIF-1α regulates chondrocyte survival and production of aggrecan and collagen II.[188,189] In vitro studies have reported a similar function of HIF in NP cells,[190,191] suggesting disk cells are normally adapted to hypoxic conditions.

Albeit with low metabolic activity, disk cells also encounter low energy supply and high waste accumulation, again due to a lack of blood supply. This scenario includes low glucose and high lactic acid, and other metabolites.[192] How disk cells can cope with these aspects is still largely unclear, other than through the general exchange via surrounding vasculature and NP diffusion. A balanced waste production and removal is vital to maintain a minimal baseline level of stress for cells.[193] Inefficient waste removal and presence of cell corpses could induce inflammation, leading to a cascade of destructive events. It is thought that because of disk motion, exercise may prevent IVD degeneration through increased solute transport, reducing waste accumulation and boosting nutrient supply.[194]

Disk cells also live under a microenvironment of high osmotic pressure, established by the high hydrophilicity of the GAG chains in the aggrecan-rich matrix in the extracellular space. Thus, gradient of osmotic pressure will drive water into the disk cells. It is thought that disk cells may modulate the osmotic potential through the action of aquaporin-2 (a tonicity-sensitive water channel), TonEBP (Tonicity-responsive Enhancer Binding Protein), and acid-sensing ion channel 3 present on the membranes, regulating intracellular tonicity.[195,196] It has also been proposed that the vacuoles of NP contain ionic pumps, which have a function in regulating the cytoplasm tonicity under hypotonic stress.[197] It is not clear whether a deregulation in the antiosmotic pressure system may cause degeneration, but reports have shown that osmotic pressure has an impact on the disk cell proliferation, matrix production, and cellular response to cytokines.[198,199]

Disk cell apoptosis[200,201] has been associated with IVD degeneration. However, recent reports suggest that IVD degeneration is attributed to a loss of disk cell function rather than a loss of disk cells[202] owing to cell senescence.[203–205] Whether these cellular changes are related to the cause or consequence of IVD degeneration is not clear but is likely to be a combination of both, leading to a detrimental outcome. In degenerative disks, cells within the NP appear as clusters more characteristic of chondrocytes than NP cells. The origin and exact phenotype of these cell clusters is not clear. It is possible that their presence may reflect a compensation strategy of the disk to mount a self-repair process, albeit limited. These changes in disk cell activities, irrespective to how they are initiated, can result in presentation of factors associated with IVD degeneration. Cartilage matrix cannot replace the function of NP matrix. Proteoglycan to collagen ratio is a major parameter that differentiates NP from hyaline cartilage.[206] It is noteworthy that there is a gradual change in proteoglycan/collagen content in degenerative disks associated with a transformation from a gelatinous to cartilaginous structure in humans and various animal models.

The authors' previous population-based MRI study showed that 80% of the population by the age of 50 years will have lumbar IVD degeneration.[24] Such significant presentation implies that the degeneration is an inevitable age-related process. Strikingly, disk degeneration is also present in a large proportion of younger individuals between the age of 20 and 40 years.[24] Conversely, there are aged individuals who have no disk degeneration. Therefore, while there are risk factors that may contribute to early disk degeneration, there are also protective factors that prevent disk degeneration. Genetics have been shown to be a significant contributing factor, which is likely to be translated to cellular function to maintain disk homeostasis.

One important area that needs to be addressed is whether the disk has the ability for self repair,

and how this endogenous repair mechanism can be harnessed for therapeutic strategies; in the absence of such a repair mechanism, exogenous biological stimulus may be considered, for which cells and growth factors can be introduced to mount a repair (see the articles by Sakai, Woods and colleagues, Leung and colleagues, and Bae and Masuda elsewhere in this issue). Thus, the finding of a potential endogenous pool of progenitor cells in the IVD is exciting in that it may facilitate maintenance of homeostasis.[207,208] Better understanding of these progenitor cells, their source, and their maintenance would be of paramount importance. This understanding, together with a clearer understanding of the control of disk cell differentiation from progenitors as well as their applications in tissue engineering and cell therapy strategies, may hold the future for the management of IVD degeneration.

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