

Review Article

The molecular basis of intervertebral disc degeneration

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Abstract

BACKGROUND: Intervertebral disc (IVD) degeneration remains a clinically important condition for which treatment is costly and relatively ineffective. The molecular basis of degenerative disc disease has been an intense focus of research recently, which has greatly increased our understanding of the biology underlying this process.

PURPOSE: To review the current understanding of the molecular basis of disc degeneration.

STUDY DESIGN: Review article.

METHODS: A literature review was performed to identify recent investigations and current knowledge regarding the molecular basis of IVD degeneration.

RESULTS: The unique structural requirements and biochemical properties of the disc contribute to its propensity toward degeneration. Mounting evidence suggests that genetic factors account for up to 75% of individual susceptibility to IVD degeneration, far more than the environmental factors such as occupational exposure or smoking that were previously suspected to figure prominently in this process. Decreased extracellular matrix production, increased production of degradative enzymes, and increased expression of inflammatory cytokines contribute to the loss of structural integrity and accelerate IVD degeneration. Neurovascular ingrowth occurs, in part, because of the changing degenerative phenotype.

CONCLUSIONS: A detailed understanding of the biology of IVD degeneration is essential to the design of therapeutic solutions to treat degenerative discs. Although significant advances have been made in explaining the biologic mediators of disc degeneration, the inhospitable biochemical environment of the IVD remains a challenging environment for biological therapies. © 2013 Elsevier Inc. All rights reserved.

Keywords: Disc degeneration; Biology of disc degeneration; Molecular basis of disc degeneration; Cytokine expression; Painful disc degeneration

Introduction

Low back pain (LBP) is one of the most common musculoskeletal complaints, estimated to trigger between 2.8%

[1] and 5% [2] of health-care visits in the United States and an even higher percentage in young patients with fewer chronic medical conditions. The overall cost of LBP exceeds \$100 billion/year in the United States alone, when considering both direct costs and indirect costs, such as lost wages and productivity [3].

Although there are numerous potential pain generators in the lumbar spine, symptomatic disc degeneration is thought to be a significant contributor to LBP [4,5] and accounts for more than 25% of lumbar fusion surgery performed in the United States [2]. Lumbar spine disc degeneration begins earlier in life than degeneration of any other connective tissue in the human body, often by the second decade [6–9]. As degeneration progresses, the intervertebral disc (IVD) becomes less able to efficiently absorb physiological loads, resulting in load transfer to

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adjacent vertebral bodies leading to end plate changes, osteophyte formation, and trabecular microfractures [10]. Increased loading is also borne by the facet complex [7] leading to arthrosis, hypertrophy, and possible neural impingement. Degenerative fissures in the lamellae of the annulus fibrosis (AF) coalesce [11], leading to a lack of structural integrity, which may allow herniation of the central nucleus pulposus (NP) material. This constellation of morphological changes often occur without associated symptoms as demonstrated by the high incidence of degenerative changes in the asymptomatic population [12] but may cause disc-related pain in some patients. Degenerative processes also likely contribute to susceptibility to disc herniation although the relationship between disc degeneration, discogenic pain, and disc herniation is incompletely understood from a clinical perspective. At present, other than ablative techniques that remove a symptomatic disc and reconstruct the segment through either a surgical fusion or disc arthroplasty, no treatments exist to restore or regenerate the damaged tissue. The clinical results of spinal fusion and disc arthroplasty remain suboptimal [13,14].

It is unclear whether disc degeneration can be reversed or halted once started. Puncture of the AF with a needle induces a seemingly minor injury but one that has been shown in numerous animal models to progress to obvious degenerative changes in quadruped animals that normally do not develop disc degeneration [15–19]. The inability of the disc to repair itself after such an injury has been capitalized on by scientists searching for a reproducible animal model of human disc degeneration [20]. Similarly, iatrogenic disc injury in humans after discography has been demonstrated to result in disc degeneration [21,22], a fact that raises questions regarding the feasibility of therapeutic intervention after the onset of symptomatic disc degeneration.

The molecular basis of degenerative disc disease has been an intense focus of research recently, which has greatly increased our understanding of the biology underlying this process. Alterations in IVD production of extracellular matrix (ECM), inflammatory cytokines, and degradative enzymes occur in a stepwise cascade leading to the end stage morphological changes evident on routine clinical imaging studies. The development of alternative biological treatment modalities for disc repair or regeneration will require a detailed understanding of these biological processes to reverse or halt the progression of degenerative

changes within the native disc, if components of disc degeneration are found to directly contribute to associated symptoms. The objectives of this broad narrative review are to describe the biology of the normal IVD, potential explanations of the genetic basis for disc degeneration, and the characteristic molecular, structural, and cellular changes that occur during disc degeneration.

Identification of articles

Relevant, recent research on the molecular basis of IVD pathobiology was identified via a search performed by the first author and reviewed by all coauthors using the Pubmed database. Included articles were limited to the English language. Search terms included the following: intervertebral disc degeneration, molecular basis of disc degeneration, ECM degradation, gene polymorphism, genetics of disc degeneration and combinations of these terms. Each term above was used with the word disk substituted for disc. Articles were reviewed to identify those that discussed genes postulated to play an important role in IVD degeneration and had a previously established role in normal or degenerative disc biology.

Biology of normal IVD

The IVD is part of an anatomic unit that includes the NP located centrally, the AF located peripherally, and the cartilaginous end plates with their associated capillary beds both cranially and caudally. A summary of structural differences between AF and NP is found in Table 1. The healthy AF comprises concentric layers of predominantly type I collagen fibrils, which serve as a boundary containing the inner NP. The AF layers become less well-organized, incorporate an increasing proportion of type II collagen, and increase in water content moving from superficial layers inwards. The orientation of collagen fibers in the AF layers is not uniform; fibers are aligned at approximately 30° to the longitudinal axis of the spine alternating their direction with each concentric layer, a feature that provides optimal tensile strength for containment of the NP [23]. Although there is initially a distinct demarcation between the AF and NP, this distinction disappears early in life as IVD degeneration begins [10]. The NP, in contrast, is more isotropic than the AF with an amorphous arrangement of type II collagen. The

Table 1
Differences between normal AF and normal NP

Feature	AF	NP
Cell shape	Elongated, fibroblast-like	Rounded, chondrocyte-like
Dominant collagen type	Collagen I	Collagen II
Proteoglycan content	Low (~25%)	High (~70%)
ECM water content	Low	High
Biomechanical role	Tensile force to contain NP	Resists axial compression
Primary form of degradation	Loses structural integrity	Loses proteoglycan and water content

AF, annulus fibrosis; NP, nucleus pulposus.

hydrophilic nature of the NP is responsible for a high swelling pressure. This property gives the IVD its characteristic viscoelasticity and compressive strength, which typically exceeds that of the adjacent bony end plates [24]. In a healthy IVD, the swelling pressure of the NP and the tensile strength of the AF are balanced and determine the intervertebral height while allowing for the conversion of NP axial compression into AF hoop stresses [25].

Cells in the outer layers of the AF are fibroblast-like with elongated nuclei that are aligned with the collagen fiber rows. Peripheral AF ECM contains mostly type I collagen with a relatively low proteoglycan and water content [7]. Moving centrally within the AF, cells become more rounded and assume a chondrocyte-like phenotype as the ECM becomes higher in type II collagen and proteoglycan [7]. Overall, collagen content in the AF comprises approximately 60% of dry weight whereas proteoglycans account for approximately 25% [7]. Other collagen types are present in smaller amounts, including type XI collagen, which is important in the assembly of type II collagen fibers and type IX collagen, which forms crosslinks between the adjacent collagen fibrils.

The cell population in the NP varies considerably from that of the outer AF. Cells in the NP at birth are predominantly of notochordal origin. However, with growth, the NP region of the disc assumes a significant majority of chondrocyte-like rounded cells, similar to those found in the inner layers of the AF during adolescence [26]. Some cells that maintain a notochordal appearance may survive into adulthood and play a role in delaying the onset of degenerative changes within the disc [27]. The ECM in the NP contains more type II collagen and a significantly higher proteoglycan concentration compared with the AF. Aggrecan, the most common proteoglycan, makes up as much as 50% of NP dry weight and is responsible for the

high water content of the disc via its net negative charge and the cations it attracts to the ECM [24].

Although degenerative conditions are associated with vascular ingrowth [28], healthy IVDs are avascular. Intervertebral disc cells have adapted to function in this oxygen-deprived slightly acidic environment [29] and cluster near the end plates where specialized capillary beds between the bony and cartilaginous end plate components provide nutrition via diffusion [30]. With aging, the diffusional capacity of the end plate decreases as blood vessels are lost [9], leading to a microenvironment that becomes increasingly acidic through the buildup of lactic acid. This decreases the ability of IVD cells to produce ECM but does not inhibit the production of degradative enzymes. These nutritional changes are believed to tip the balance toward accelerated degeneration by degrading the ECM of the disc, ultimately resulting in the macroscopic changes described above [31].

The disc nutritional supply is tenuous and several factors can affect the diffusion of nutrients through the end plate or disc, leading to the onset of the stressed disc microenvironment. Blood flow through the capillary beds adjacent to the end plate can be altered by environmental factors including exposure to nicotine or vibration [32–35]. Systemic diseases may also alter the delivery of blood to the end plate capillary beds and retard disc nutrition, including atherosclerosis [36,37] or conditions that affect the microvasculature, such as sickle cell anemia and Gaucher disease [38]. Similarly, end plate sclerosis or calcification of the cartilaginous end plate has been implicated in some cases of disc degeneration as the cause of impaired nutrient diffusion [39] (Fig. 1).

Innervation of the normal disc is limited because nerve fibers do not normally penetrate deeper than the outermost layers of the AF [40–44]. The predominant nerve supply to the outer annulus involves small, nonmyelinated, free nerve

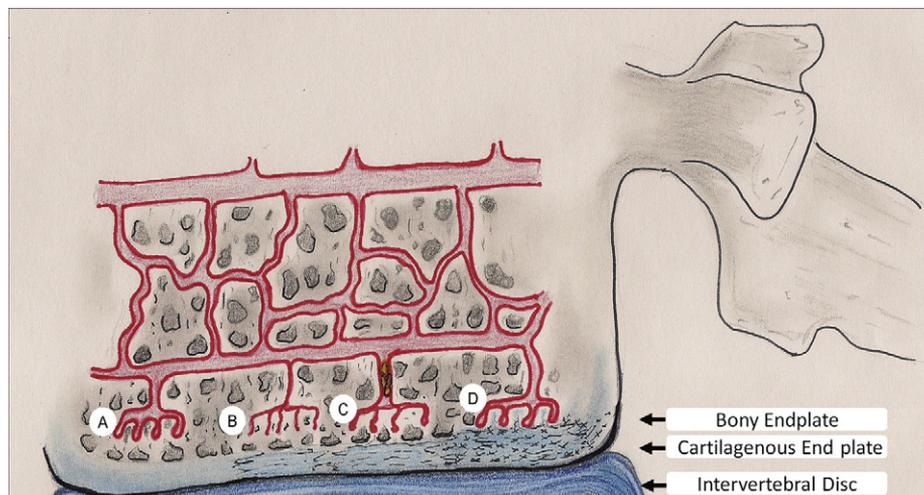


Fig. 1. Schematic of conditions that can compromise end plate vasculature supply: A: Normal state; B: Vasoconstrictive processes, such as nicotine exposure, which narrow the terminal capillary beds; C: Vaso-occlusive processes, such as atherosclerosis, also narrow capillary lumen via an obstructive process; and D: End plate sclerosis, which prevents normal diffusion into the disc.

endings that transmit to the sinuvertebral nerve, a branch of the ventral ramus. Following the formation of annular fissures, vascular tissue and nerve endings penetrate deeper into the disc and may play a role in the transmission of pain from the degenerative disc [28,45]. The vertebral end plate has also been implicated as a potential source of pain. Patients with radiographic evidence of advanced disc degeneration and chronic back pain demonstrated an abundance of nociceptive nerve endings compared with patients without back pain [46].

Etiology of disc degeneration

Disc degeneration and associated degenerative entities such as disc herniation has been attributed to many different factors, both environmental and genetic. Occupational exposures such as vibration [47–50], mechanical influences such as heavy lifting and weight [32], lifestyle factors such as lack of exercise [50], and the use of non-Swedish and non-Japanese cars [51] have been blamed for contributing to IVD degeneration and herniation. Injuries related to lifting or trauma [49,52–54] and tobacco use [32,51,54,55] have often been described as associated factors. Although the etiology is likely multifactorial, the importance of genetic factors has become evident in recent years and probably accounts for greater than 70% of an individual's risk of degenerative disc disease with smaller contributions from environmental factors [56–58].

Genetic influences

The influence of genetics in the development of IVD degeneration is well-established. Matsui et al. [59] found an increased severity of radiographic markers of disc degeneration when comparing patients with and without a first order relative who had undergone lumbar spine surgery for disc herniation. Postacchini et al. [60] and Bijkerk et al. [56] both reported rates of discogenic back pain in relatives of patients with back pain that were significantly higher

than in nonrelated controls, estimating that approximately 75% of the overall variance between the nonrelated individuals was accounted for by genetic variance [56]. A number of twin studies have also contributed to our understanding of the genetic contribution to IVD degeneration. A series of investigations on a Finnish twin cohort [58,61,62] has demonstrated hereditary associations with degenerative disc changes in the lumbar spine that far outweighed the effects of environmental factors on the development of IVD degeneration. Overall, these studies have estimated that genetic factors account for up to three-quarters of susceptibility to lumbar disc degeneration whereas environmental factors, such as occupational vibration exposure and smoking, contribute the balance.

These studies describe the relative importance of genetics as a risk factor for developing IVD degeneration but do not provide an insight into the genes that may be responsible. Recently, several genes have been identified that may play a role in the degeneration of IVD and have been subsequently studied in various different human populations. Such discoveries often occur via a candidate gene approach in which investigations into a process are focused on target genes with a known related biological function. A listing of relatively well-characterized specific genes proposed to play a role in IVD degeneration that have been studied in various ethnic populations is presented in Table 2. These discoveries have advanced our understanding of the etiology of IVD degeneration but much of the research performed to date has not yet been conducted on a scale large enough to allow generalization of findings to more heterogeneous populations. Furthermore, it must be emphasized this is only a partial listing of candidate genes, which seem particularly promising and which already have well-described roles within the IVD.

Collagen IX

Type IX collagen is covalently bound to type II collagen, presumably as a supporting structure for the NP ECM

Table 2
Genes associated with IVD degeneration studied in human populations*

Protein	Likely IVD function	Alleles with variants	Association with IVD degeneration and populations studied
Collagen IX	ECM support	COL9A2	Associated with IVD degeneration across two ethnic populations (Finns and Chinese)
	ECM support	COL9A3	Strongly associated with IVD degeneration in Finns; absent in Chinese study population
Vitamin D receptor	Alters GAG structure	TaqI	Strong association across three study populations (Finns, Chinese, and Japanese)
	Alters GAG structure	Apa	Associated with IVD degeneration in Chinese population and not yet investigated in others
Collagen I Aggrecan	Primary AF support	COL1A1	Associated with IVD degeneration in three European populations
	NP osmotic gradient	CS1	Inconsistent: associated with IVD degeneration in some studies, not associated in other studies
MMP-3	ECM degradation	5A/6A	5A allele associated with IVD degeneration in two Asian populations

IVD, intervertebral disc; ECM, extracellular matrix; GAG, glycosaminoglycan; AF, annulus fibrosus; NP, nucleus pulposus; MMP, matrix metalloproteinase.

* This list is not comprehensive.

[63,64]. The potential role of type IX collagen (encoded by genes COL9A2 and COL9A3) in disc degeneration was first suggested by mutations in a Finnish population associated with early and severe IVD degeneration [65–67]. Subsequent characterization in a population from China similarly demonstrated an increased risk of IVD degeneration with an odds ratio of 2.4 for the COL9A2 allele [68]. The COL9A3 allele, which triples the risk of severe disc degeneration in Finns versus individuals with other COL9A3 alleles [66], was absent in the Chinese population. Although the role of type IX collagen is not fully characterized, the alleles investigated in these studies demonstrate an association with the degenerative disc disease across genetically diverse human populations.

Vitamin D receptor

The role of the vitamin D receptor (VDR) in bone metabolism is well-characterized, and variant alleles have been implicated in disease states, such as vitamin D-resistant rickets [69] and osteoarthritis [70,71]. An association between VDR alleles and disc degeneration was first reported in a Finnish population [72] and likely affect the disc via alterations in the sulfation of glycosaminoglycans and related changes in ECM function. Associations have been described between polymorphisms of the VDR gene and lower scores on qualitative and quantitative scoring systems that assess severity of discogenic pain [73]. Videman et al. [72] determined that approximately 15% of the inter-individual variability because of genetic/familial causes could be explained by VDR allelic variation. Studies in Japanese [74] and Chinese [75] populations supported this association between VDR polymorphisms and degenerative disc disease. The Chinese study, which associated allelic variants with NP signal changes on magnetic resonance imaging (MRI), established an odds ratio of 2.6 for the likelihood of disc degeneration in their cohort, which increased to almost six in a young subgroup [75]. Recently, a VDR allele has been identified in a Chinese population that imparts an increased risk for IVD degeneration in a synergistic manner with occupational exposure to twisting and bending activities [76]. These associations between variant VDR alleles and disc degeneration across multiple diverse genetic populations make a strong case for the role of this gene in the pathophysiology of disc degeneration.

Collagen I

As described above, type I collagen is the predominant component of the AF ECM, comprising approximately 70% of the dry weight of the outer annulus and providing tensile strength to resist the compressive load imparted onto the functional disc unit. Based on the functional importance of collagen in AF function, collagen I polymorphisms have been investigated as potential contributors to disc degeneration. Type I collagen forms a triple-helix, and

polymorphisms that alter transcription factor binding sites may alter the type I collagen subtype ratios in vivo, decreasing the strength of IVD collagen and predisposing to early degeneration. Pluijm et al. [77] investigated the relationship between collagen allele variation and both osteoporosis and degenerative disc disease in a population of elderly Dutch patients. Although they found no relationships with osteoporosis, they reported a polymorphism of type I collagen (TT genotype of COL1A1, the gene for the α -1 subunit), which was associated with IVD degeneration based on a radiographic scoring system with an odds ratio of 3.6. Reports on associations with disc degeneration from Finnish [67] and Greek populations [78] have supported this finding.

Aggrecan

Aggrecan is the primary proteoglycan found in the IVD and is vital to the normal function of the disc; the hydrophilic nature of proteoglycans provides elastic deformability and gives a healthy disc its characteristic high water signal on MRI. A subunit of the aggrecan core protein known as CS1 demonstrates polymorphisms in the number of times a repeated sequence contained within the gene occurs [79]. Although not been proven yet in vivo, these changes in the core subunit are suspected to affect the ability of the subunit to bind glycosaminoglycans, a function essential to trap water within the IVD. Clinically, this polymorphism was first associated with early-onset disc degeneration in a female Japanese population [80]. This first study and subsequent studies from Iran [81] and Turkey [82] reported that lower numbers of tandem repeats were associated with more severe disc degeneration. This finding was accompanied by the explanation that a smaller number of repeats reduced the disc's ability to trap water and would therefore accelerate disc degeneration. This appealing explanation is challenged, however, by a subsequent investigation [83] that also found an association between an aggrecan polymorphism and IVD degeneration in a Finnish population. In contrast to the Japanese study [80], Solovieva et al. [83] found disc degeneration was associated with a moderate number of tandem repeats and that patients with either greater or fewer number of repeats had lower rates of disc degeneration. A fourth study [84] found no association between the same aggrecan subunit polymorphisms and IVD degeneration, creating a considerably more confusing picture which will require further investigation to resolve.

Matrix metalloproteinase 3

Because of their roles as degradative enzymes that act on the ECM in degenerative discs, genes encoding metalloproteinases were suspected to play a role in the genetic susceptibility to IVD degeneration. The matrix metalloproteinase 3 (MMP3) gene contains a common polymorphism in which the promoter associated with one allele (5A) has

twice as much activity as the promoter associated with the second allele (6A) [85,86], suggesting that the 5A phenotype could be associated with higher protein expression and IVD degeneration because of higher transcription rates. Indeed, the 5A allele was found to be associated with higher incidence of lumbar IVD degeneration in an elderly Japanese population based on radiographic criteria [87]. The same study failed to show any association between the 5A allele and IVD degeneration in a younger Japanese subgroup. Subsequently, the 5A allele was studied in a larger population of patients from China and was associated with an increased likelihood of disc degeneration with an odds ratio of 1.96 [76]. Similar to the findings relating to VDR alleles, this study reported synergistic increases in disc degeneration in patients with the 5A allele and occupations requiring frequent bending and twisting or chronic vibration exposure.

Videman et al. [67] recently described associations between a large number of candidate genes and several parameters associated with IVD degeneration in 588 patients from the Twin Spine Study, a Finnish cohort. The imaging parameters included in this study were loss of water signal on MRI, disc space narrowing, and dorsal disc bulges. The analysis of 25 candidate genes not only found associations between some of the genes described above but also investigated a number of other candidate genes. Associations were reported between IVD desiccation and the presence of particular alleles of aggrecan, type I collagen, type IX collagen, type XI collagen, interleukin (IL)-1, and IL-18. Associations were reported between IVD disc bulging and alleles for aggrecan, type IX collagen, and type XI collagen and an aggrecan allele was found to be associated with IVD narrowing. No associations were found between metalloproteinase alleles and disc degeneration.

Cell senescence

Cellular senescence was originally defined as the point at which a cell stops dividing [88,89]. Reasons for the inability to replicate can fall into one of the two categories: replicative senescence (RS) or stress-induced premature senescence (SIPS). The loss of telomeres, repetitive sequences at chromosome ends, is responsible for RS. During DNA replication, some of the terminal sequence of each chromosome is lost with each subsequent round of replication. Telomeres ensure that the portion lost is a noncoding sequence not vital to cellular function. Once the safety zone provided by a telomere is used up, coding regions of the chromosome will no longer be protected and may be lost during replication. If enough damage accumulates such that the cell cannot make repairs to the important interrupted sequences, the cell stops replicating but remains alive and metabolically active. The cellular mechanisms explaining SIPS are more straightforward and involve the accumulation of irreparable DNA damage caused by oxygen radical damage from insults such as mechanical injury [90] and

inflammatory cytokines release [91], which disrupt cellular function and replication. Although senescence is an inevitable part of cellular aging both in vivo and in vitro, studies relying on a senescence-related enzyme (senescence-associated- β -galactosidase) and direct measurement of telomere length indicate the process is accelerated in degenerative discs [92–95] compared with age-matched non-degenerative discs. The known inhospitable biomechanical and biochemical environment of degenerative IVDs suggests that SIPS and not RS is likely responsible for accelerated cellular senescence.

Decreased matrix production

Over time, changes in cellular activity may occur, altering the composition and concentration of ECM proteins and proteoglycans [96–103]. A significant contributor to disc desiccation and fibrosis is the decreased ability of IVD cells to maintain a normal extracellular phenotype, despite the upregulation of mitogenic factors such as platelet-derived growth factor [104,105], insulinlike growth factor-I (IGF-I) [106,107], and basic fibroblast growth factor [108,109] in the early stages of degeneration. The synthesis of type II and type IX collagen normally peaks early in life but falls off at an accelerating rate in degenerating discs after an initial transient compensatory increase in type II collagen production. The decrease in matrix synthesis is accompanied by a declining number of collagen cross-links, a factor that may synergistically weaken the NP structural integrity [110]. Conversely, type I and type X collagen expressions increase with advancing degeneration, resulting in NP fibrosis [111] and a loss of a distinct border between the AF and NP. The expression of aggrecan and versican decreases with disc degeneration although the exact role of versican in the IVD is not well-defined. Similarly, the expression of smaller proteoglycans, such as biglycan and decorin, which likely function in ECM assembly and repair, as well as fibromodulin decrease with more advanced degeneration [102].

Increased degradative enzyme production

In association with a declining ability to produce ECM, cells in degenerative IVDs upregulate degradative enzymes. Matrix metalloproteinases are extracellular zinc-dependent proteinases that are produced in a latent form and require activation, often by other MMPs to become active. The expression of MMPs has been extensively studied in degenerative IVDs because of the importance of MMP in regulating ECM turnover in biologic systems. In addition to direct matrix damage, MMPs also indirectly contribute to disc degeneration via activation of latent enzymes. MMPs 1, 8, and 13 (collagenases); MMPs 2 and 9 (degrade denatured collagen); and MMP 3 (stromelysin, which degrades non-collagen matrix proteins) are all upregulated in the degenerative IVD [112–118], suggesting that each plays a role

in ECM degradation. The complexity and diversity of function of this enzyme family is evident in the actions of MMP19. Although MMP19 is capable of cleaving aggrecan, cartilage oligomeric matrix protein (COMP), types I and IV collagen, and fibronectin, similar to other MMPs, it appears to be downregulated, not upregulated in degenerative discs [117]. Its degradative actions are likely secondary to other extracellular functions: it prevents the stabilization of capillary structures to keep healthy discs avascular and it allows IGF-1 to exert its antiapoptotic and mitogenic effects on cells by sequestering an inactivating IGF-binding protein [119]. Decreased MMP19 expression would favor a degenerative phenotype by allowing vascular ingrowth and higher rates of apoptosis.

Upregulation of aggrecanase, as certain members of the ADAMTs (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) enzyme family are known, is seen in degenerative discs [120]. Hatano et al. [120] suggested that ADAMTS-4 is involved in the disintegration and regression of herniated discs, although their study also found expression in discs with simple protrusions that had not progressed to herniation. Le Maitre et al. [121] and Pocket et al. [122] have reported expression in healthy disc tissue and an increased expression in the setting of IVD degeneration, suggesting that these enzymes may be active constitutively, possibly facilitating normal matrix turnover but playing a role in matrix degradation seen during disc degeneration.

Cathepsins are proteases that are active in acidic environments. Cathepsins D and L have been shown, using immunohistochemistry, to localize to regions of IVDs that demonstrate focal degeneration [123], and act predominantly in the AF. Similarly, Cathepsin G activity is present in degenerative but not control discs [124]. Although MMPs have garnered more attention for their association with the degenerative ECM in IVDs, cathepsins function ideally in this milieu as their peak enzymatic activity coincides with the slightly acidic environment of the degenerating disc.

Proinflammatory cytokine expression

Cytokines are small proteins that function within and between cells as signaling molecules and serve multiple functions in the body, including serving as a link between tissue injury and local or systemic signs of inflammation and pain. Cytokines are a byproduct of and stimulant of the inflammatory cascade but do not directly degrade the IVD in the same manner as MMPs, instead they act indirectly by promoting the production of inflammatory substances by the disc cells.

Interleukin-1 is the prototypic inflammatory cytokine expressed in the disc. It contributes to a degenerative IVD phenotype by inhibiting the production of ECM [125–127], increasing production of degradative enzymes

[127–129], establishing a positive feedback loop for further cytokine production [126,129], and sensitizing IVD cells to other apoptosis triggers, although it does not trigger apoptosis on its own [111,130]. Interleukin-1 is also expressed in the normal disc and is controlled through an elegant balance of an activating receptor (IL-1RI) and an inhibiting receptor (IL-1Ra), a mechanism that becomes unbalanced in the setting of disc degeneration [127].

Although tissue necrosis factor- α (TNF- α) influences catabolic pathways in a manner similar to IL-1 [131,132], this effect is likely clinically less important than its nociceptive role [23]. The link between TNF- α and pain was initially established in the spine based on the irritating effect of herniated discs on adjacent nerve roots [133,134], which was later demonstrated to be partially mediated by TNF- α [135]. The upregulation of TNF- α in the degenerative IVD [116,136,137] may contribute to the associated pain seen in some patients, especially following the ingrowth of free nerve endings into fissures in the outer annulus [9,28,45,138].

Expression profiles of other cytokines including IL-6 and IL-8 have also been investigated. Studies have reported the expression of these cytokines in degenerative discs [136,139–142], but less is known regarding the potential role of these cytokines in contributing to an inflammatory or degenerative environment.

Cytokines make an attractive therapeutic target. Although a degenerative phenotype on MRI may be asymptomatic as demonstrated by the cross-sectional studies of asymptomatic patients [12,143] with high rates of degenerative IVD changes, the established role of cytokines in nociception may present a therapeutic target that could directly improve symptoms in affected patients.

Apoptosis

Cellularity of the IVD declines with normal aging [9,144,145], in part, because of the increased rates of apoptosis or programmed cell death (PCD) [9,146,147]. Although some increase in PCD is commensurate with normal aging, entry into PCD appears to occur at a higher rate for cells in degenerative discs.

Programmed cell death in degenerative IVDs is likely secondary to biomechanical and biochemical triggers [148,149], although the methods used to determine PCD rates have been called into question because of the wide disparity in estimates of apoptosis rate [150]. Triggers for cell entry into apoptosis include many of the same mechanisms discussed above for senescence, such as mechanical causes [151–154], and a host of biochemical stimuli, including serum/nutrient deprivation [155,156], nitric oxide exposure [148], and oxidative stress [157].

On a cellular level, PCD is activated by one of two signaling pathways [158]. The first involves binding of a ligand to one of the cell membrane death receptors such as the Fas receptor or CD95. Ligand binding results in the activation

of intracellular caspases, which cleave proteins within the cytoplasm and nucleus, disrupting the essential cellular machinery and leading to cell death. Another pathway for the activation of PCD occurs via suppression of Bcl-2, an apoptosis regulating protein, which then allows the release of mitochondrial signaling proteins into the cytoplasm, triggering caspase activation.

Neural ingrowth

Although healthy IVDs are aneural and avascular, neurovascular ingrowth into degenerative discs has been consistently noted on histologic analysis [9,28,45,138] along fissures in the outer annulus (Fig. 2). These free nerve endings show evidence of a nociceptive nerve phenotype based on the expression of substance P [28], a pain mediator. In the healthy IVD, intact and abundant aggrecan inhibits nerve ingrowth as shown in experiments that demonstrated differential neurite growth when cultured with either normal aggrecan or aggrecan deglycosylated to resemble aggrecan in a degenerative IVD [159]. Aggrecan from the AF, the first line of defense against nerve ingrowth, was more inhibitory to nerve growth than aggrecan isolated from the NP. Vascular ingrowth typically accompanies neural ingrowth and endothelial cells have been shown to interact with aggrecan in a similar manner as neurites. Thus, aggrecan from healthy IVDs inhibits endothelial growth whereas deglycosylated aggrecan is less inhibitory, implicating ECM degradation in the pathogenesis of neurovascular ingrowth [160]. Vascular tissue expresses nerve growth factor (NGF) in vessels that accompany ingrowing nerves. In turn, nerve tissue expresses *trk-A*, a high affinity receptor for NGF, which is associated with pathways encouraging neurite growth and survival [45]. Nerve growth factor was found to be expressed only in blood vessels in painful degenerative IVDs although vascular ingrowth occurs in both painful and nonpainful degeneration, suggesting a close association with the development of associated symptoms [45]. Another mitogenic factor that appears to encourage neuronal ingrowth into the IVD is brain-derived growth factor (BDGF). Brain-derived growth factor has a similar role to NGF in the differentiation and survival of sensory neurons and is associated with fibers that transmit painful stimuli. Although NGF is produced by vascular cells, BDGF is produced by IVD cells and its expression has been shown to be correlated with increasing degeneration of the IVD [161,162].

Conclusion

A detailed understanding of the biology of IVD degeneration on a molecular level is essential for the design of therapeutic solutions to treat degenerative discs. The complexity and interconnectedness of the various molecular pathways, which contribute to IVD degeneration and the relatively inhospitable biochemical environment within

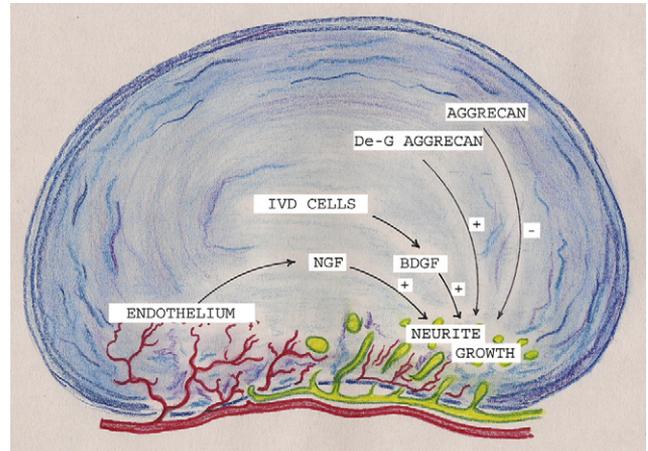


Fig. 2. Schematic of factors that are stimulatory (+) or inhibitory (–) for neural ingrowth into the posterior intervertebral disc (IVD). Stimulatory factors include nerve growth factor (NGF), brain-derived growth factor (BDGF), and de-glycosylated aggrecan (“De-G Aggrecan”). Normal aggrecan inhibits neural ingrowth.

the IVD make this task difficult. Development of biological diagnostic tests that can identify patients with markers suggesting they will have painful progression of IVD degeneration would be valuable, especially given the frequent disconnect between radiographic appearance of degenerative changes and associated symptoms [12]. Molecules with known roles in inflammatory and nociceptive cascades are attractive targets for therapeutic intervention and for use as diagnostic markers to identify the stage of the process and the likely presence of symptoms from a given disc.

Several potential avenues for the development of biologic therapies have been identified, although the unique and hostile environment of the IVD makes the implementation of a biological strategy challenging (Table 3) and distinguishing who will benefit from treatment of degenerative discs presents a tremendous challenge on its own. Direct injection of growth factors, which boost ECM production could upregulate IVD metabolic activity by slowing or reversing the degradation of structural proteins. This strategy is complicated by relatively rapid degradation of proteins shortening any in vivo effect such a treatment would have or requiring serial invasive treatments.

Some of the myriad of intercellular and intracellular signaling pathways that play roles in the degeneration and inflammation associated with IVD degeneration may be amenable to pharmacologic disruption. This strategy has been successful in blocking actions of key mediators of other diseases, such as rheumatoid arthritis. Blockade of inflammatory and pain mediators could provide symptom relief without the need to address degenerative IVD morphology. Effective delivery of therapeutic proteins may be limited by the avascular nature of the disc in contrast to other parts of the body like joint synovium, where blood-based delivery of proteins to block the action of

Table 3
Biochemical characteristics of the disc that pose challenges for biological therapy

IVD Characteristic	Treatment strategy affected	Effect on potential biological therapies
Poor cellular nutrition	Cell implantation	Avascular IVD prevents delivery of nutrients, worse with end plate sclerosis/calcification, atherosclerosis
Poor metabolic waste clearance	Cell implantation	Increase in cell number will increase lactic acid production, slow clearance secondary to avascular IVD
Acidic	Decrease ECM degradation Cell implantation	Acidity does not affect MMP activity but enhances activity of cathepsins Acidic environment decreases ECM production by disc cells, both native and implanted
Avascular	Increase ECM synthesis	As above and cannot use intravascular delivery of agents to increase ECM, decrease cytokine production

IVD, intervertebral disc; ECM, extracellular matrix; MMP, matrix metalloproteinase.

TNF has been used to successfully treat rheumatoid arthritis.

A more elegant manner of stimulating IVD cellular activity is to introduce the relevant genes into IVD cells using gene therapy. Such strategies are intellectually appealing, but the development of gene therapy as a viable human treatment strategy is still in the early stages of development. Although this strategy has been reported with success in vivo using animal models [17,163,164], much more work is necessary to explore its viability for the treatment of human disease, especially given the proximity of the disc to the central nervous system and well-publicized past complications of gene therapy in human clinical trials.

Decreased IVD cellularity and the related loss of matrix proteins and biomechanical properties could be addressed with direct cell implantation using pluripotent cells or differentiated cells such as disc cells or cells with a disc-like phenotype like chondrocytes. Although a rat model has demonstrated success in the long-term viability of transplanted cells [165], differences between the milieu of the rat and human IVD may limit the translation of this success to treatment of humans. A clinical trial exploring the harvest of disc cells at the time of discectomy, in vitro cell expansion, and reimplantation via injection is currently underway in Europe [166], although some questions regarding the methodology of the trial have been raised [167]. The problem cellular transplantation hopes to address may also be its downfall as a viable treatment strategy—the degenerative IVD is not a welcoming environment. Intervertebral disc degeneration leads to declining nutrient and oxygen diffusion and availability, decreasing pH, and increased rates of cellular apoptosis, factors that will present as challenges to the success and long-term viability of many potential treatments.

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