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Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration

Nam V. Vo, PhD[a], **Robert A. Hartman, MS**[a,b], **Takashi Yurube, MD, PhD**[a,c], **Lloydine J. Jacobs, MD**[a], **Gwendolyn A. Sowa, MD, PhD**[a,b], and **James D. Kang, MD**[a,*]

[a]Department of Orthopaedic Surgery, Ferguson Laboratory for Orthopaedic Research, University of Pittsburgh, E1641 Biomedical Science Tower, 200 Lothrop St, Pittsburgh, PA 15261, USA

[b]Department of Physical Medicine and Rehabilitation, University of Pittsburgh, 3471 5th Ave., Suite 202, Pittsburgh, PA 15213, USA

[c]Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Abstract

BACKGROUND CONTEXT—Destruction of extracellular matrix (ECM) leads to intervertebral disc degeneration (IDD), which underlies many spine-related disorders. Matrix metalloproteinases (MMPs), and disintegrins and metalloproteinases with thrombospondin motifs (ADAMTSs) are believed to be the major proteolytic enzymes responsible for ECM degradation in the intervertebral disc (IVD).

PURPOSE—To summarize the current literature on gene expression and regulation of MMPs, ADAMTSs, and tissue inhibitors of metalloproteinases (TIMPs) in IVD aging and IDD.

METHODS—A comprehensive literature review of gene expression of MMP, ADAMTS, and TIMP in human IDD and reported studies on regulatory factors controlling their expressions and activities in both human and animal model systems.

RESULTS—Upregulation of specific MMPs (MMP-1, -2, -3, -7, -8, -10, and -13) and ADAMTS (ADAMTS-1, -4, and -15) were reported in human degenerated IVDs. However, it is still unclear from conflicting published studies whether the expression of ADAMTS-5, the predominant aggrecanase, is increased with IDD. Tissue inhibitors of metalloproteinase-3 is downregulated, whereas TIMP-1 is upregulated in human degenerated IVDs relative to nondegenerated IVDs. Numerous studies indicate that the expression levels of MMP and ADAMTS are modulated by a combination of many factors, including mechanical, inflammatory, and oxidative stress, some of which are mediated in part through the p38 mitogen-activated protein kinase pathway. Genetic

*Corresponding author. Department of Orthopaedic Surgery, Ferguson Laboratory for Orthopaedic Research, University of Pittsburgh, E1641 Biomedical Science Tower, 200 Lothrop St, Pittsburgh, PA 15261, USA. Tel: (412) 648-1090; fax: (412) 383-5307. kangjd@upmc.edu.

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predisposition also plays an important role in determining gene expression of MMP-1, -2, -3, and -9.

CONCLUSIONS—Upregulation of MMP and ADAMTS expression and enzymatic activity is implicated in disc ECM destruction, leading to the development of IDD. Future IDD therapeutics depends on identifying specific MMPs and ADAMTSs whose dysregulation result in pathological proteolysis of disc ECM.

Keywords

Intervertebral disc degeneration; Extracellular matrix; MMPs; ADAMTS; Aging

Introduction

Intervertebral disc degeneration (IDD) underlies most musculoskeletal disorders of the spine that result in economic loss and individual distress owing to associated pain, morbidity, and physical disability. Intervertebral disc degeneration-related conditions are among the most common causes of disability among all workers aged 18 to 64 years [1]. In particular, back pain, which includes conditions related to the cervical and lumbar spine, is the most common reason for a doctor's visit among US citizens today [2].

The fibrocartilaginous intervertebral disc (IVD) is situated between the adjacent cartilaginous end plates. The gel-like core nucleus pulposus (NP) of the IVD functions as a shock absorber that dissipates compressive forces outward from its center to the surrounding annulus fibrosus (AF) and the adjacent vertebral bodies. The AF consists of approximately 20 concentric rings (lamellae) of highly organized collagen Type I fibers orientated approximately at 60° to the vertical axis of the spine and run parallel within each lamella but perpendicular between adjacent lamellae allowing for maximal tensile strength [3]. The AF maintains the pressurization of NP under compressive loads.

The major molecular components of the IVD are water, collagen, proteoglycans, and elastin. The NP consists primarily of proteoglycan matrix and water held together by an irregular network of collagen Type II and elastin fibers. The primary proteoglycan in the NP is aggrecan, which contains a large number of highly anionic glycosaminoglycan (GAG) side chains (ie, chondroitin sulfate and keratan sulfate), allowing the NP to imbibe water essential for counteracting compressive loads. The AF has the highest collagen content, with collagen Type I being more predominant in the outer layers [4]. Collagen Type II is the highest in the NP and decreases toward the periphery of the IVD. The IVD contains other collagens and proteoglycans as well as many other matrix constituents in different abundances depending on the age and stage of degeneration of the IVD [5].

Intervertebral disc degeneration is generally believed to be a consequence of increased catabolism of the extracellular matrix (ECM). Intervertebral disc degeneration is associated with proteolytic degradation of ECM macromolecules, leading to large structural changes. These changes include disorganization of the AF, dehydration and fibrosis of the NP, and calcification of the cartilaginous end plates. One mechanism proposed the changes leading to IDD initiation within the NP [6]. Loss of NP proteoglycan matrix coupled with an increased concentration of nonaggregating proteoglycan breakdown products diminish the hygroscopic properties of ECM, resulting in decreased water content and swelling pressure. These changes in the NP result in abnormal load in AF, resulting in further ECM breakdown and ultimately leading to structural failure seen in IDD.

Intervertebral disc degeneration research over the last decade has placed greater emphasis on understanding the mechanism of ECM degradation, particularly focusing on the regulation

of gene expression and activity of matrix metalloproteinases (MMPs), disintegrins and metalloproteinases with thrombospondin motifs (ADAMTSs), and tissue inhibitors of metalloproteinases (TIMPs). Gene and protein expression of numerous metalloproteinases have been studied in human IVDs as well as in experimental animal models. Excellent review articles have documented catabolic changes in the ECM of degenerative IVDs [7,8], roles of inflammatory mediators in these changes [9], and mechanical regulation of MMPs in the progression of IDD [10]. However, there is no up-to-date literature that systematically and thoroughly reviews the expression profile of MMPs, ADAMTSs, and TIMPs in IDD. The regulation of metalloproteinase expression in the IVD cells and their role in IDD are also still incompletely understood and inadequately described. The objectives of this review are to provide an overview of the current knowledge about the gene expression profile of MMPs, ADAMTSs, and TIMPs in nondegenerated versus aged and degenerated IVDs; to review the reported regulation of gene expression and enzymatic activity of these metalloproteinases by key physiologic effectors, such as mechanical, inflammatory, oxidative stress, and genetic predisposition; and to highlight the importance of how dysregulation of certain key metalloproteinases and their inhibitors can contribute to ECM degradation and therefore could be useful therapeutic targets for treating IDD.

Biology of MMPs, ADAMTSs, and TIMPs

Matrix metalloproteinases and ADAMTSs are proposed to be the major catabolic enzymes in the IVD. Matrix metalloproteinases belong to a large family of zinc-dependent proteinases [11–13]. At present, there are 24 MMPs identified in humans, including two genetic duplications of MMP-23 [11]. Matrix metalloproteinases are traditionally categorized into six groups based on substrate specificity, protein structure, and subcellular localization: collagenases, gelatinases, stromelysins, matrilysins (minimal domain), membrane-type MMPs, and other types [11]. Collagenases (MMP-1, -8, -13, and -18) primarily act on fibrillar collagen. Gelatinases (MMP-2 and -9) digest the denatured collagens, gelatins, and laminin. Stromelysins (MMP-3, -10, and -11) proteolyze a variety of substrates, including proteoglycans, gelatins, collagens, and some pro-MMPs. Matrilysins (MMP-7 and -26) digest different ECM components, including aggrecan, as well as growth factors and cytokines. Membrane-type MMPs (MMP-14–17, -24, and -25) are localized to plasma membranes, possess cytoplasmic domains that influence intracellular signaling pathways, and may activate other MMPs [14]. The rest of the MMPs (MMP-12, -19, -20, -21, -23, -27, and -28) are also implicated in tissue matrix homeostasis and repair, but their substrate specificities are not well defined. Matrix metalloproteinases have been traditionally known to be the primary mediators of ECM degradation, thereby permitting normal remodeling and contributing to pathological tissue destruction, but recently they have been shown to act on nonmatrix substrates important for many other biological activities [15]. Matrix metalloproteinases are usually secreted in inactive proforms and require activation, which involve a number of regulatory activator proteins, many of which are still unknown. In addition, MMP enzymatic activity is also modulated by TIMPs. This multilevel regulation ensures proper temporal and spatial enzymatic activity of various MMPs for growth, tissue repair, and remodeling. It is generally believed that dysregulation of MMP expression and activity is responsible for pathological matrix catabolism found in many diseases such as IDD and osteoarthritis (OA).

Disintegrins and metalloproteinases with thrombospondin motifs are a more recently discovered family of metalloproteinases that bind to components of the ECM [16]. Approximately 20 different ADAMTSs belonging to four categories are reported based on their structural and functional analyses: hyalectanase (ADAMTS-1, -4, -5, -8, -9, -15, and -20), von Willebrand factor (ADAMTS-13), procollagen *N*-peptidase (ADAMTS-2, -3, and -14), and a fourth group with unknown function (ADAMTS-6, -7, -10, -12, -16, -17, -18,

and -19). The ADAMTS-1, -4, -5, -8, -9, -15, -16, and -20 have aggrecanolytic properties [17,18]. Because of their high efficiency in cleaving aggrecan, ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) are classified as the major aggrecanases [19,20]. Like MMPs, ADAMTSs are secreted in a proform and regulated by subsequent processing of the protein.

Catabolic activities of MMPs and ADAMTSs on IVD matrix are balanced by the inhibitory actions of TIMPs. There are four TIMP subtypes currently known, each with limited specificity for different metalloproteinases. TIMPs bind MMPs in 1:1 stoichiometry [11]. Tissue inhibitors of metalloproteinase-1 and -2 inhibit MMPs through noncovalent interaction with active MMPs. Aggrecanase activity from bovine cartilage can be inhibited by TIMP-1, but only under very high doses (>210 nM TIMP-1) [21]. Disintegrins and metalloproteinases with thrombospondin motifs-1 are inhibited by TIMP-2, but only under very high TIMP-2 concentration (500 nM) [22]. Tissue inhibitors of metalloproteinase-3 is tightly bound to the ECM by interactions with sulfated GAGs, and unlike TIMP-1, it is a potent inhibitor of ADAMTS-4 and -5 [23,24].

Disc MMP gene expression

MMPs expressed in nondegenerated IVDs are likely involved in normal tissue repair and remodeling. In young and preadolescent IVDs, MMP expression appears to be minimal but increase in adult IVDs [25], where MMP-1, -2, -3, -7, -10, and -19 have been detected by immunohistochemical analysis [25–29]. However, Le Maitre et al. [27] did not observe MMP-3 and MMP-13 expression in normal adult IVDs, so the physiological role of these MMPs in humans remains unclear. Similar to protein expression, mRNA expression for most MMPs is low in nondegenerated IVDs [30].

Increased expression of different MMPs has been observed in human degenerative IVD tissue [25,27,29–32]. Crean et al. [31] reported significant correlation between elevated MMP-2 and -9 levels and degenerative IVD grade. Roberts et al. [29] observed the largest increase with degeneration in MMP-3 followed by MMP-7 using immunohistochemistry. Weiler et al. [25] showed that MMP-1, -2, and -3 protein expression correlated highly with IVD histomorphological degenerative findings. Bachmeier et al. [30] reported strong correlation between histological degeneration grade and MMP-3 and to a lesser extent MMP-7 mRNA and protein in symptomatic human lumbar IVDs. On the other hand, MMP-8 was not highly expressed, but it increased consistently throughout degeneration. Le Maitre et al. [27,32] showed that the MMP-7 and -13 proteins were most highly expressed in the degenerated inner NP and AF. MMP-1 and -3 significantly increased only in severely degenerated NPs, whereas MMP-7 and -13 increased in earlier stages of NP degeneration. Of note, MMP-13 was consistently significantly upregulated in early degeneration but does not appear to vary with the higher grades of degeneration [27]. Gruber et al. [33] reported MMP-28 as a constitutively expressed MMP in human IVD tissue, which is present in matrix of more degenerated discs, but another recent study showed no positive correlation between MMP-28 expression and IDD severity grade [34]. Although these observations implicate MMP-1, -2, -3, -7, -8, -9, -10, -12, -13, and -14 in the development of IDD, there is no direct evidence as to which MMP(s) play the most pivotal role in ECM degradation of the IVD. These immunodetection studies simply indicate the presence of these MMPs without differentiating the inactive pro-MMPs from the active forms. More important, the presence of MMP protein do not necessarily equate to enzymatic activity and matrix breakdown. Table 1 summarizes MMP expression in human IVD tissue.

Disc ADAMTS gene expression

Similar to articular cartilage, loss of aggrecan is an early critical event in the degenerative cascade in IVD tissue [35,36]. Because of their efficient proteolysis of aggrecan, Sandy [37] argued that ADAMTS-mediated aggrecanolysis is much more destructive in causing GAG loss and cartilage degradation than that mediated by MMPs. In fact, aggrecan degradation in normal and OA cartilage involves primary cleavage by aggrecanases and not by MMPs [38]. However, normal disc aggrecanase expression is replaced by MMP expression in a mechanically induced degeneration model [39], highlighting the unresolved complexity of aggrecan breakdown and differences between cartilage and disc matrix catabolism.

The presence of ADAMTS-1, -4, -5, -9, and -15 mRNA and protein in normal human IVDs suggest that these aggrecanases may play a role in normal physiologic function of IVD [27,40]. ADAMTS mRNA levels do not appear to vary between cells of the NP and AF, but protein expression seems to be higher in the NP and inner AF than in the outer AF [40]. ADAMTS-1, -4, -5, and -15 mRNA expression increases significantly with IDD [27,40,41]; ADAMTS-4 level increases more in the AF than the NP of degenerated samples [40]. ADAMTS-1, -4, -5, -9, and -15 protein expression increases in the NP and inner AF with a significantly higher amount of ADAMTS-4 in the NP than in the inner AF [27,40,41]. Meanwhile, Patel et al. [42] compared protein expression in mildly and severely degenerated tissues and observed the increase in ADAMTS-4 but not ADAMTS-5 with degeneration in the NP and AF. Therefore, compared with MMPs, ADAMTS expression profile in IDD is still unclear and controversial. Studies using knockout mice suggest that ADAMTS-5 is the primary aggrecanase driving cartilage destruction in OA [43,44]. Conversely, a recent study investigating aggrecanase- and MMP-mediated aggrecan degradation in rats suggests that normal patterns of aggrecanase activity decrease and MMP-mediated degradation increases in disc degeneration [39]. Thus, further investigations are needed in IVD research to elucidate the role of ADAMTS-5 and other ADAMTSs. Table 2 summarizes ADAMTS expression in human IVD tissue.

Disc TIMP gene expression

Three of the four known subtypes of TIMPs are reported to be expressed in the IVD: TIMP-1, -2, and -3. These TIMPs are present at low levels in nondegenerated IVDs [27]. Tissue inhibitor of metalloproteinase-3 is expressed in nondegenerated IVD tissue [27,40] at higher levels than TIMP-1 and -2 [27]. In IDD, TIMP-1 and -2 mRNA and protein expression increases along with MMPs [30]. In human degenerated and herniated IVDs, TIMP-1 and -2 expression levels correlated moderately with MMP-1, whereas TIMP-1 expression correlated with MMP-2. No significant correlation between TIMP-1 and -2 and MMP-3, -7, -8, -9, and -13 were observed [30]. There is a greater increase in TIMP-1 than TIMP-2 between apparently normal aged and degenerated human IVDs [29]. Thus, although it appears that these TIMPs are upregulated in degeneration to counter the catabolic effect of MMPs, the regulatory mechanisms controlling TIMPs is not well understood. TIMP-3 expression appears to be regulated differently from TIMP-1 and -2 in IDD. Tissue inhibitor of metalloproteinase-3 is not upregulated in degenerated human IVD specimens [27,40] in which ADAMTSs were elevated. This imbalance suggests a dysregulation of aggrecanase activity in human degenerative IVDs, which may be a key contributor to IDD. Table 3 summarizes TIMP expression in human IVD tissue.

Proteolytic activity of disc MMPs and ADAMTSs

The relative importance of MMPs and ADAMTSs in ECM catabolism has long been controversial. MMP- and ADAMTS-generated aggrecan fragments have been detected in human IVD samples [40,42,45]. These aggrecan fragments were detected more frequently in degenerated than in nondegenerated IVDs [40]. However, MMP-generated aggrecan product

was reported to increase with aging but stay unchanged with degeneration grade, whereas ADAMTS-generated aggrecan fragments decreased with aging [45]. In addition, the level of ADAMTS-generated proteolytic aggrecan fragment was unchanged during disc degeneration [42]. A greater amount of MMP- than ADAMTS-generated aggrecan fragments in degenerated IVDs was reported [29]. Further studies are needed to verify if aggrecanase-mediated aggrecan proteolysis precedes that of MMP during the progression of disc degeneration.

Regulation of gene expression of disc MMPs, ADAMTSs, and TIMPs

In IVD, expression of MMPs, ADAMTSs, and TIMPs and their activity are modulated by aging [46] and various stimuli and stressors [47]: mechanical, inflammatory, oxidative stress, as well as environmental factors, such as tobacco smoking and genetic predisposition (Table 4). Furthermore, depending on the type and stage of IDD diseases, these stressors are thought to act in various combinations to initiate and propagate the metalloproteinase-mediated ECM degradation, leading to the eventual structural failure of IVD. For instance, physical injuries or excessive mechanical strain have been reported to induce catabolic cascades and matrix breakdown in IVD [39,48]. However, it is often not possible to identify a specific event that initiates disc matrix catabolism because many other contributing factors, that is, aging, smoking, genetic predisposition, and so on are involved.

Regulation by mechanical stimuli

Intervertebral disc function mechanically to support axial compression and facilitate six degrees-of-freedom motion. Basic research has shown that mechanical stimulation of the IVD influences metalloproteinase expression and activity. Catabolic responses depend on the magnitude, frequency, and duration of mechanical stimulation as well as by the specific region of IVD.

Nucleus pulposus

Magnitude of compressive loading appears to induce a threshold effect on catabolic gene expression. Invitro, higher magnitudes of compression (2.0 or 4.0 MPa) resulted in decreased MMP-3 gene expression compared with lower (0.7 MPa) magnitudes [49]. Similarly, moderate dynamic ex vivo loading (peak: 1.0 MPa) increased MMP-3 gene expression, whereas higher compression (peak: 2.5 MPa) decreased the expression [50]. Dynamically loaded discs in vivo at a moderate loading level (1.0 MPa) increased MMP-3, -13, and ADAMTS-4 gene expression, whereas low loading level (0.2 MPa) did not have any significant effect [51]. Thus, the data demonstrate that relative gene expression of metalloproteases in the NP is exquisitely sensitive to differences in the magnitude of the applied load.

Frequency and duration also modulate MMP expression. In dynamically loaded rat tails, NP gene expression for MMP-3 and -13, but not ADAMTS-4, were sensitive to frequency at 1.0 MPa. In contrast, the AF was insensitive to frequency changes [51]. More studies are needed to clarify the effect of frequency on catabolic and anticatabolic gene expression. Duration of mechanical stimulation is also a powerful regulator of ECM catabolism. In vitro, NP cells exposed to prolonged compression (24 hours) demonstrated increase in mRNA expression compared with cells experiencing shorter (4 hours) duration of compression [49]. MacLean et al. [52] illustrated that MMP-3, -13, and ADAMTS-4 gene expression peaked at 2 hours of dynamic loading, with MMP-13 similar to control values after longer (4 hours) duration of loading. Similarly, Hsieh and Lotz [53] noted significant increase in MMP-2 activation after 4 days of static compression in mouse tails, although there was no change in MMP-2 mRNA expression after longer (7 days) duration. In addition, long-term loading effects on MMPs have been investigated in rat tails. Sustained (56 days) static compression increased

MMP-3 gene expression after 7 days and elicited increasing amount of protein throughout the length of the study [54]. Meanwhile, long-term dynamic loading had little effect at 2 or 8 weeks on catabolic gene expression. Mechanical stimulation at 8 weeks upregulated TIMP-1 and ADAMTS-4, but changes were small [55]. These data demonstrate that MMPs and TIMPs demonstrate differential regulation in response to changes in duration of loading. Recent mounting evidence with different loading regimens has revealed that dynamic compression stimulates anabolism, whereas static compression accelerates catabolism and induces IDD [56].

Annulus fibrosus

The AF is also sensitive to magnitude, frequency, and duration of mechanical stimulation. Similar to the NP, higher magnitude of dynamic compression in vivo increased AF transcription of MMP-3, -13, and ADAMTS-4 [51]. This has also been observed in vitro, as AF cells exposed to moderate level of tensile strain (6%) demonstrated decreased MMP-3 gene expression and enzymatic activity compared with unstimulated cells, whereas high level of tensile strain stimulated increased MMP-3 activity despite a persistent decrease in gene expression [57]. Although MMP-1 gene expression was not affected as significantly in these experiments, MMP-1 enzymatic activity did increase with increasing magnitude of tensile strain. In addition, under inflammatory condition, increased TIMP-1 expression was noted at low magnitude and decreased expression was noted at high magnitude. Examined in whole organ, static asymmetric ex vivo loading at 0.2 MPa elicited small but significant increase in MMP-1 and TIMP-1 gene expression in the convex side of bovine tails, which is experiencing constant tension [58]. Also in the AF, duration of mechanical stimulation has a significant effect on the matrix biology. In vitro, the beneficial effect of decreasing MMP expression in AF cells exposed to 4 hours of tensile strain was lost after prolonged (24 hours) tensile strain [57]. A short-term in vivo loading study showed increased gene expression for MMP-3, -13, and ADAMTS-4 after 0.5 to 2 and 4 hours of dynamic loading [52]. Dynamic loading performed in vivo by Wuertz et al. [55] showed trends toward decreased MMP expression with 2 weeks of short duration (1.5 hours per day) loading compared with long duration (8 hours per day) loading, although these changes did not reach statistical significance.

To explore the more complex motions expected to be seen physiologically, recent studies have explored the effect of torsion on IVD mechanobiology with emphasis on AF responses. An in vivo rat tail model of dynamic (1.0 Hz) loading shows increased ADAMTS-4 gene expression in the AF at the highest rotation angle (30°), no significant change in MMP-13 and -3 at any angle, and a decrease in MMP-12 at lower rotation angles [59]. Chan et al. [60] applied a smaller range of rotation angles at 0.1 Hz to ex vivo bovine tails. Catabolic gene expression tended to increase but did not reach statistical significance. Interestingly, MMP-13 gene expression seemed to increase with increasing angle in the AF.

Regulation by inflammatory and oxidative stress

Inflammatory pathways are linked with IDD. Increased levels of tumor necrosis factor (TNF)-α [61,62] and interleukin (IL)-1α [63], IL-1β [62,63], and IL-6 are frequently detected in degenerative, nonherniated human IVDs. Weiler et al. [61] showed that TNF-α expression is closely correlated with aging and histological degeneration grades. In experimental animal models of IDD, the AF puncture model is known to induce degenerative responses to acute inflammation with injury [48,64]. IL-1β and IL-8 production in p38 mitogen-activated protein kinase (MAPK)-activated cells were observed around the stab wound [48]. Injured discs show progressive upregulation of IL-1β and MMP-3, and downregulation of TIMP-1 with increasing IDD severity [65]. Similarly, annular laceration greatly induced MMP-1, -9, and -13 mRNA expression [66]. In addition,

not only were IL-1 levels increased in IDD but also the use of IL-1 receptor antagonist inhibited ECM degradation and reduced the number of MMP-3 and ADAMTS-4 immunopositive cells [67]. These in vivo findings support in vitro studies, which show that the proinflammatory mediators upregulate MMP expression. Both TNF-α and IL-1β have been shown in in vitro studies to influence gene expression of MMP-3, TIMP-1, and ECM structural genes, as well as the catabolic mediators, such as nitric oxide, IL-6, and prostaglandin E2 [68,69]. IL-1 stimulation is reported to upregulate the expression of MMP-3, -13, and ADAMTS-4 and downregulate the expression of ECM genes [63]. Gene microarray analysis revealed the greatest induction of MMP-1, -3, and -12 among the measured MMPs stimulated by TNF-α in human IVD cells (Vo et al., unpublished data). Studer et al. [68,69] reported that TNF-α and IL-1β treatment of human and rabbit NP cells reduced the TIMP-1:MMP-3 protein ratio and downregulated expression of aggrecan and collagen types I and II; inhibition of p38 MAPK activation mitigates these inflammatory effects. These data indicate that IVD cellular response to TNF-α and IL-1β is mediated via p38 MAPK activity and its inhibition represents a potential candidate for molecular therapy to treat IDD.

There is growing evidence for oxidative damage as a key contributor to ECM degeneration in the IVD. Immunomorphological analysis revealed higher level of the marker of oxidative protein damage carboxymethyl-lysine in degenerated IVDs from aged compared with young patients. Similarly, nuclear factor kappa-light-chain-enhancer of activated B cells, a transcription factor activated in response to cellular stress including oxidative stress, was increased in aged IVDs [70]. The source of reactive oxygen species (ROS) driving oxidative damage includes free radicals generated from radiation, byproducts of oxidative phosphorylation, as well as from cellular response to proinflammatory cytokines. Reactive oxygen species have been shown to be produced by chondrocytes in response to stimulation by numerous cytokines and growth factors, including TNF-α, IL-1, and transforming growth factor-beta, as well as by integrin stimulation with fibronectin fragments [71–73]. IL-1-dependent production of ROS has also been associated with chondrocyte DNA damage [74]. Although cells in IVD tissue reside in an environment with a low oxygen tension, they do consume oxygen and therefore exhibit aerobic metabolism [75]. In addition, aged IVD tissue accumulates fissures, resulting in neovascularization and exposure of the otherwise hypoxic resident cells to high oxygen tension and oxidative stress. Studies exposing human NP cells to normoxia (20% O_2) revealed increased expression of MMP-1 and -3 compared with those grown at hypoxia (5% O_2), suggesting that oxidative stress can upregulate MMPs in the IVD (Vo et al., unpublished data). In addition, IVD cells exposed to tobacco smoke extract, which contains many oxidants, also upregulate various MMPs and ADAMTS and suppress TIMP expression [76].

Influence of expression of MMPs by genetic predisposition

Growing evidence suggests that genetic predispostion plays a vital role in the development and progression of IDD [77,78]. IVD tissue degradation has been shown to be enhanced by polymorphism in the vitamin D receptor [79], factors involved in matrix integrity [80–82], and catabolism [83–86]. Focusing on genes involved in ECM catabolism, Takahashi et al. [83] showed a correlation of a 5A/6A polymorphism in the promoter region of the MMP-3 gene with greater incidence of lumbar IDD in elderly Japanese subjects. Matrix metalloproteinase-2-1306C/T polymorphism has been frequently detected in young Chinese population with lumbar IDD [84]. Similarly, a -1607 polymorphism in the promoter region of MMP-1 was associated with lumbar IDD in a southern Chinese population; the association became stronger in subjects older than 40 years [85]. A higher frequency of a -1562C/T polymorphism in the MMP-9 promoter region was discovered in young northern Chinese men with lumbar IDD compared with healthy controls. An association between the

CC/TT MMP-9 genotype and magnetic resonance evidence of severe IDD was also observed [86]. Although the practical implications in a more heterogeneous population with a broad set of IVD-related pathologies remain unclear, it is evident that genetic variation in the promoter region of MMPs may contribute to the development of IDD.

Implications for future treatment of IDD

Current treatment of IDD is restricted primarily to symptom management rather than treatment of underlying causes. Novel strategies to delay or halt the characteristic ECM loss in degeneration aim to shift the balance of matrix homeostasis by increasing anabolism or decreasing catabolism. Early intervention to minimize catabolism is favorable because attempts to promote anabolism in the IVD cells in a degenerative, nutrient-deprived, mechanically altered environment may prove challenging [87–89]. Inhibition of MMPs and ADAMTSs has demonstrated efficacy and therapeutic potential in slowing the progression of OA [68,90–92]. Because of the essential role of these enzymes throughout the body's connective tissue, inhibition of MMPs and ADAMTSs or stimulation of TIMPs must be tissue specific and carefully localized to the IVD. Yet, delivering therapeutic agents to the avascular IVD must overcome the likely unintended consequences of intradiscal injection puncture [93,94]. Thus, systemically delivered agents may be more attractive, but are met with the barrier of reaching sufficient intradiscal concentration to have an effect. Therapeutic agents also face the challenges of limited nutrition, low acidity, and altered mechanics in degenerative IVD environment. Significant questions still remain regarding the timing and delivery of agents aimed at limiting ECM catabolism. As tissue remodeling and turnover is critical for maintenance of structural integrity and healing of injury even in mature tissue, any attempt to limit MMP activity must keep this delicate balance in mind. In addition, in approaching therapeutic consideration, treatment of the patients' symptoms must be paramount, and it is unclear to date how expression of these catabolic enzymes affects pain and function. Addressing these questions will improve fundamental understanding of MMP, ADAMTS, and TIMP enzymes in IDD and provide a clearer picture of where and how to intervene to improve treatment of individuals suffering from IDD-related disorders.

Conclusions

Matrix metalloproteinases and aggrecanases appear to be the primary mediators of ECM degradation in IDD. Clarifying gene regulation of these metalloproteinases has recently been a central subject of IVD research. Stimuli from mechanical strain, inflammatory and oxidative stress can independently or jointly modulate the expression and activity of specific subsets of MMPs and ADAMTSs. Understanding the mechanisms of how certain MMPs and ADAMTSs are dysregulated, which result in IVD matrix destruction, is important for future development of therapeutics to treat IDD.

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Table 1

Expression of MMPs in human intervertebral discs

Gene symbol	Gene title (alternative names)	Matrix [11–13] substrates	Other substrates [13,15] (resultant effectors)	Expression in nondegenerated human discs (references)	Increased expression in degenerated human discs (references)
Minimal domain					
MMP-7	(Matrilysin, uterine)	PGs, Lam, Fib, F/F, Ent, Ten, Vit, Gel, Col 3-5, 9-11	Dec (bioavailable TGF-β), Pro-TNF-α (bioavailable TNF-α)	Yes [29,32]	Yes [29,32]
			Pro-MMP-2 and -7 (MMP-2 and -7)		
Collagenases					
MMP-1	(Interstitial collagenase)	Col 1-3, 7, 10, Gel	Perlecan (bioavailable FGF), IGFBP-2, -3 (bioavailable IGF)	No [25,29]	Yes [25]
		Gel, Ent, Agc, Ten	a-Act (inactive serpin)		
MMP-8	(Neutrophil collagenase)	Same as MMP-1	a-PI (inactive serpin)	Yes [29,30]	Yes [29,30]
			Pro-MMP-8 (MMP-8)		
MMP-13	(Collagenase 3)	Same as MMP-1	a-Act (inactive serpin)	No [27]	Yes [27]
Stromelysins					
MMP-3	(Stromelysin 1, progelatinase)	Same as MMP-7	Dec (bioavailable TGF-β), Pro-TNF-α (bioavailable TNF-α)	No [25]	Yes [25,27,30]
			Perlecan (bioavailable FGF), IGFBP-2, 3 (bioavailable IGF)	Yes [27,29]	
			Pro-IL1-B (IL-1β), a-Act (inactive serpin)		
			Pro-MMP-1, -3, -7, -8, -9, and -13 (MMP-1, -3, -7, -8, -9, and -13)		
MMP-10	(Stromelysin 2)	Same as MMP-7	Pro-MMP-1, -8, and -10 (MMP-1, -8, and -10)	Yes [28]	Yes [28]
Gelatinases					
MMP-2	(Gelatinase A)	Agc, Gel, Ela, Fib, Lam	Pro-TGF-B2 (TGF-β2), Pro- IL-1B (active IL-1β),	Yes [25,31]	Yes [25,30,31]
			IGFBP-3/5 (bioavailable IGF), Pro-TNF -α(TNF-α)		
		Vit, Col-1, -4, -5, -7, -10, and -11	FGF-R1 (bioactive FGF-R1 ectodomain)		
			Pro-MMP-1, -2, and -13 (MMP-1, -2, and -13)		
MMP-9	(Gelatinase B)	Same as MMP-2	Unknown (bioavailable VEGF), Pro-IL-1B (IL-1β)	No [25]	No [25]
			Pro-TGF-B2 (TGF-β2), Pro-TNF-α (TNF-α)	Yes [29,31]	Yes [29–31]
Other					

Gene symbol	Gene title (alternative names)	Matrix [11–13] substrates	Other substrates [13,15] (resultant effectors)	Expression in nondegenerated human discs (references)	Increased expression in degenerated human discs (references)
MMP-28	(Epilysin)	Casein	Unknown	Yes [33,34]	No [33,34]

a-Act, α1-actichymotrypsin; Gel, gelatin; Vit, vitronectin; F/F, fibrin/fibrinogen; EN, entactin; TE, tenascin; Ela, elastin; a-PI, α1-proteinase inhibitor; Fib, fibronectin; Col, collagen; PG, proteoglycan; Lam, laminin; Agc, aggrecan; Dec, decorin; MMP, matrix metalloproteinase; TGF, transforming growth factor; TNF, tumor necrosis factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IL, interleukin; VEGF, vascular endothelial growth factor.

Table 2

Expression of agrecanases in human intervertebral discs

Gene symbol	Gene title [16,95] (alternative names)	Matrix [16,95] substrates	Expression in nondegenerated human discs (references)	Increased expression in degenerated human discs (references)
ADAMTS-1	(Aggrecanase-3, METH-1)	Agc, Ver V1	Yes [40]	Yes [40]
ADAMTS-4	(Aggrecanase-1, KIAA0688)	Agc, Brv, Ver V1	Yes [27,40,42]	Yes [27,40,42]
		Fmd, Dec		
		Trf, COMP		
ADAMTS-5	(Aggrecanase-2, ADAMTS-11)	Agc	Yes [40–42]	Yes [40]
				No [42]
ADAMTS-9	(KIAA1312)	Agc, Ver	Yes [40]	No [40]
ADAMTS-15		Agc	Yes [40]	Yes [40]

Fmd, fibromodulin; Ver, versican; Dec, decorin; Agc, aggrecan; Brv, brevican; Trf, transferin; COMP, cartilage oligomeric matrix protein; ADAMTS, disintegrins and metalloproteinases with thrombospondin motif.

Table 3

Expression of TIMPs in human intervertebral discs

Gene symbol	Enzyme targets (reference)	Other substrates (reference)	Expression in nondegenerated human discs (references)	Increased expression in degenerated human discs (references)
TIMP-1	Strong: MMP-1, -3, -7, -9 [96]	proMMP-9 [97]	Yes [29]	Yes [27,29,30]
	Weak: MMP-14 [97]		No [27]	
TIMP-2	Strong: MMP-2, -14 [96]	proMMP-2 [97]	Yes [27,29]	Yes [29,30]
	Strong for all MMPs [98]			No [27]
TIMP-3	ADAMTS-1, -4, and -5 [98]	TNF-α-converting enzyme [98]	Yes [27,40]	No [27,40]
		proMMP-2, -9 [97]		

TIMP, tissue inhibitor of metalloproteinase; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.

Table 4

Regulation of gene expression of disc MMPs, ADAMTSs, and TIMPs

Stressor	Species	Model	Region	Outcome	Condition & response (references)
Mechanical					
MMP-1	Rabbit	Cells	AF	mRNA, activity	T: mag (+) [57,58], freq (+) [57], dur (+) [57]
			NP	mRNA	HP: mag (+) [99]
MMP-2	Mouse	Tissue	AF+NP	Activity	Cmp: dur (+) [53]
MMP-3	Human, bovine, rabbit, rat	Tissue, cells	AF	mRNA	Cmp: mag (+) [50,51], freq (+) [51], dur (++) [52]
				mRNA, activity	T: mag (+), freq (+), dur (+) [57]
			NP	mRNA, protein	Cmp: mag (++) [51], freq (++) [51], dur (+) [52,53]
				mRNA	HP: mag (+) [99,100]
MMP-13	Ovine, rat	Tissue, cells	AF	mRNA	Cmp: mag (+) [51], freq (+) [51], dur (+) [52]
			NP	mRNA	Cmp: mag (+) [51], freq (+) [51], dur (+) [52]
				mRNA	HP: mag (+) [100]
ADAMTS-4	Ovine, rat	Tissue	NP	mRNA	Cmp: mag (+) [51], freq (+) [51], dur (+) [52,55]
			AF	mRNA	Cmp: mag (+) [51], freq (+) [51], dur (+) [52]
TIMP-1	Human, rat	Tissue, cells	AF	mRNA	T: mag (+) [57,58], freq (+) [57], dur (+) [57]
			NP	mRNA	Comp: dur (+) [55]
				mRNA	HP: mag (+) [99]
Inflammatory					
MMP-1	Rabbit	Tissue	AF+NP	mRNA	Annular laceration [66], TNF-α in vitro *
MMP-3	Human, rabbit	Tissue, cells	AF, NP	mRNA, protein	Annular puncture [65], TNF-α & IL-1β in vitro [68,69]
					IL-1β in vitro [63], TNF-α *
MMP-7	Human	Cells	NP	mRNA	TNF-α in vitro *
MMP-9	Rabbit	Tissue	AF+NP	mRNA	Annular laceration [66]
MMP-10	Human	Cells	NP	mRNA	TNF-α in vitro *
MMP-12	Human	Cells	NP	mRNA	TNF-α in vitro *
MMP-13	Human, rabbit	Tissue, cells	AF, NP	mRNA	Annular laceration [66], IL-1β in vitro [63]
MMP-28	Human	Cells	AF, NP	mRNA	TNF-α, IL-1β, LPS in vitro [34]
ADAMTS-4	Human	Cells	AF, NP	mRNA	IL-1β in vitro [63]
TIMP-1	Human, rabbit	Tissue, cells	NP	mRNA, protein	Annular puncture [65], TNF-α & IL-1β in vitro [68,69]
TIMP-3	Human	Cells	NP	mRNA, protein	TNF-α in vitro *
Oxidative					
MMP-1	Human	Cells	NP	mRNA	20% vs. 5% O_2 †
MMP-3	Human	Cells	NP	mRNA	20% vs. 5% O_2 †

Cmp, compression; Mag, magnitude; (+), <10 fold; HP, hydrostatic pressure; Freq, frequency; (++), 10–100-fold; T, tensile stretch; Dur, duration; MMP, matrix metalloproteinase; ADAMTS, disintegrins and metalloproteinases with thrombospondin motif; TIMP, tissue inhibitor of metalloproteinase; AF, annulus fibrosus; NP, nucleus pulposus; IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide.

* Vo N., unpublished data.

[†]Nasto L, Ngo K, Sowa G, Kang J, Vo N. Role of oxidative stress on matrix proteoglycan homeostasis in intervertebral disc. Manuscript in preparation.