Current Knowledge and Future Therapeutic Prospects in Symptomatic Intervertebral Disc Degeneration

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Intervertebral disc (IVD) degeneration is the main source of intractable lower back pain, and symptomatic IVD degeneration could be due to different degeneration mechanisms. In this article, we describe the molecular basis of symptomatic IVD degenerative disc diseases (DDDs), emphasizing the role of degeneration, inflammation, angiogenesis, and extracellular matrix (ECM) regulation during this process. In symptomatic DDD, pro-inflammatory mediators modulate catabolic reactions, resulting in changes in ECM homeostasis and, finally, neural/vascular ingrowth-related chronic intractable discogenic pain. In ECM homeostasis, anabolic protein-regulating genes show reduced expression and changes in ECM production, while matrix metalloproteinase gene expression increases and results in aggressive ECM degradation. The resultant loss of normal IVD viscoelasticity and a concomitant change in ECM composition are key mechanisms in DDDs. During inflammation, a macrophage-related cascade is represented by the secretion of high levels of pro-inflammatory cytokines, which induce inflammation. Aberrant angiogenesis is considered a key initiative pathologic step in symptomatic DDD. In reflection of angiogenesis, vascular endothelial growth factor expression is regulated by hypoxia-inducible factor-1 in the hypoxic conditions of IVDs. Furthermore, IVD cells undergoing degeneration potentially enhance neovascularization by secreting large amounts of angiogenic cytokines, which penetrate the IVD from the outer annulus fibrosus, extending deep into the outer part of the nucleus pulposus. Based on current knowledge, a multi-disciplinary approach is needed in all aspects of spinal research, starting from basic research to clinical applications, as this will provide information regarding treatments for DDDs and discogenic pain.

Key Words: Symptomatic intervertebral disc degeneration, pro-inflammatory cytokines, angiogenesis, nerve ingrowth, treatment of DDD

INTRODUCTION

Practicing spinal specialists encounter many people suffering from lower back pain (LBP) on a daily basis. LBP is experienced by over two-thirds of the general population at least once in their lifetime[1] and is a potential socioeconomic burden.[2,3] Several anatomic structures of the lumbar spine contribute to LBP,

including the paravertebral muscles, various ligaments, nerves, intervertebral discs (IVD), adjacent vertebral bodies, and bilateral facet joints.[4-6] Among these, IVDs are the primary sources of intractable LBP and represent a definitive morphologic change induced by aging or mechanical stress. These changes may be asymptomatic and subclinical in some cases; however, in most cases, annulus injury/inflammation are major factors resulting in discogenic chronic LBP.[6] Although pathomechanisms remains unknown, research has suggested possible molecular mechanisms of how IVDs act as pain sources, followed by degenerative changes, inflammatory reactions, and subsequent processes.

Following natural aging processes, all aspects of the human body undergo gradual degeneration, including the spine and IVDs, resulting in degenerative disc diseases (DDDs). The effects of aging on IVDs result in various biomechanical changes and associated molecular and structural changes.[7] These degenerative changes start from a young age, often even from the

2nd or 3rd decades, which is much earlier than any other of our body's systems.[2,8] The global population is aging, with more older people, resulting in a significantly increased number of older patients suffering from spinal degeneration and accompanying LBP. Therefore, a thorough understanding of the basic pathomechanisms of DDDs is essential. In all fields of spinal research, much effort has been made to contribute to our existing knowledge of the possible biomechanisms and apply this knowledge to clinical aspects. The current research aims were to bring laboratory-based findings to the clinical field, in a so-called "Bench to Bed" effort.[3]

This review article provides a gross narrative review of the molecular basis of symptomatic DDDs, emphasizing the role of inflammation, angiogenesis, and extracellular matrix (ECM) regulation therein. We also review molecular, cell-level signaling during degeneration and briefly review future perspectives in IVD biology.

THE ANATOMY AND STRUCTURAL CHARACTERISTICS OF IVDS

IVDs are cartilaginous structures lying between two adjacent vertebral bodies and primarily function as a bearing and transmitting structure for vertical weight. IVDs also act as buffers for the load descending caudally and allow flexibility while providing stability to the spine.[9,10] Structurally, IVDs are composed of three anatomical components: the inner nucleus pulp-

osus (NP), the outer annulus fibrosus (AF), and the endplate surrounding the central fibrocartilaginous structures (Fig. 1). Healthy NP is a hydrophilic, high-pressured structure containing a large volume of collagen II and elastin fibers.[11,12] Due to these characteristics, the NP contains a large proportion of water (up to 80% in healthy NPs) and can act as a pressure/weight absorbing structure compensating for compressive loads transmitted via the AF and peripheral endplates.[9,13] In terms of morphology, the NP is made of chondrocytes, and the ECM surrounding these cells is rich in aggrecan, which contributes to the NPs high water content and gel-like nature, and generates high osmolar pressures.[14,15] The AF is comprised of dozens of concentric ring-like layers (lamellae) rich in collagen I. These lamellae form a unique structure within the AF by aligning diagonally to the axis of the spine and alternating their directions to provide the maximal tensile force of the AF structure. Thanks to this unique structure, the AF can restrain the inner NP, which has a high osmolar pressure, especially during motion activities of the IVDs, such as flexion/extension or bending/twisting.[16] Cells in the AF are fibroblastic, unlike the NP, and their elongated cellular shape contributes to the collagen I-rich ECM alignment,[2,17] probably providing the basic structure for the lamellae in the AF. These different characteristics of the NP and AF give the IVD its distinctive viscoelasticity and its functional role as a weight-bearing structure. Finally, the surrounding endplate is a cartilaginous, thin-layered structure at the periphery that forms an interface between two adjacent vertebral segments and acts as a channel for nutrition and oxygen
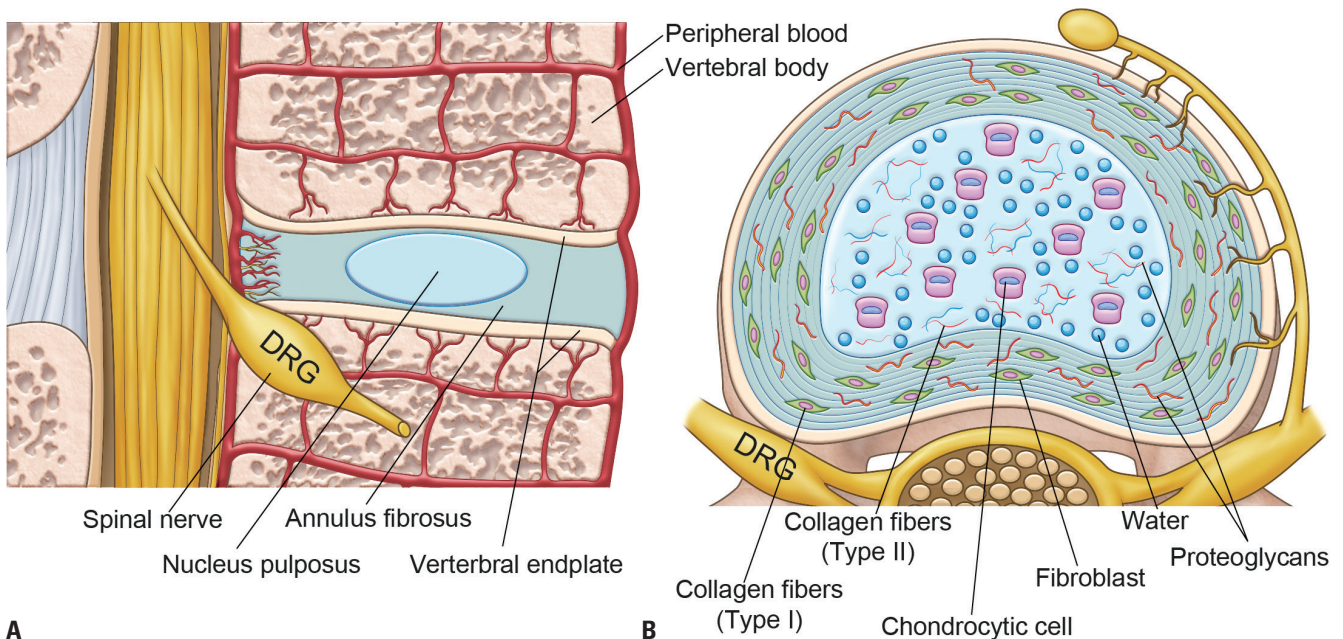
Fig. 1. Anatomy and structural characteristics of the IVD. (A) IVDs are composed of outer AF and inner NP, and is surrounded by upper and lower endplates, which serve as channels for nutrition and oxygen supply. Spinal nerve structures, including the DRG, juxtapose the IVD. (B) The dominant cellular components of NP are chondrocytes, and they are surrounded by abundant ECM with high water content. The AF is composed of fibroblasts surrounded by collagen I rich ECM forming lamella structure. IVD, intervertebral disc; AF, annulus fibrosus; NP, nucleus pulposus; DRG, dorsal root ganglion; ECM, extracellular matrix.

supply.[10,18] IVDs are avascular when healthy and obtain their nutrition or oxygen by diffusion through the endplates. As IVDs are hypoxic and contain sparse numbers of cells, mostly composed of ECM, this unique tissue is more vulnerable to degenerative changes than any other tissue in our bodies.

THE BIOMOLECULAR ASPECT OF IVD DEGENERATION

To view degeneration in an accurate context, the normal aging process as it advances with age must be considered.[18,19] IVD aging and degeneration result from molecular configurations of the NP and AF, structural changes in IVD architecture, and subsequent loss of its typical biomechanical characteristics (Fig. 2). In normal aging, inflammatory reactions play important roles in phenotypic changes in IVD cells, alongside a deterioration in ECM composition. The significance of inflammation in discogenic pain has been demonstrated previously in clinical and laboratory-based evidence and is discussed in later sections. Over the last several decades, extensive research has aimed to discover the molecular biological basis of inflammatory reactions in DDDs, which has improved our understanding of these diseases.

Laboratory findings from in vivo and in vitro DDD models have consistently shown elevated expression of pro-inflammatory molecules. In initial research on IVDs in the 1990s and 2000s, interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha were key molecules of interest. IL-1 beta is a member of the IL-1 cytokine family, which acts as an intracellular/extracellular signaling molecule stimulating various inflammatory signaling pathways and resulting in the production of pro-inflammatory cytokines, such as IL-6, -8, and COX-2.[20,21] TNF-alpha was initially discovered due to its role in tumor cellular biology; its role in inflammation by binding to TNF receptors was discovered later. The pathomechanism of LBP is well described by a strong association between these cytokines and IVD degeneration. Degenerated IVDs or DDD models express significantly high levels of TNF-alpha and IL-1 beta.[21-23] Therefore, both these molecules are frequently used as IVD inflammatory stimulators in laboratory research. While IL-1 beta and TNF-alpha were initially thought to be secreted by inflammatory cells recruited to IVDs during degeneration, further studies in the 2000s discovered that disc cells also secrete these inflammatory cytokines.[6,24-28]

Human AF and NPs exposed to IL-1 beta show markedly elevated secretion of IL-6 and -8 and upregulated levels of pro-inflammatory markers, such as TNF-alpha,[6,25,29-32] indicating the role of IL-1 beta in increasing the inflammatory responses within IVDs. IL-1 beta also modulates and stimulates ECM degradation during inflammation, resulting in the progress of IVD degeneration. ECM degradation induces and enhances
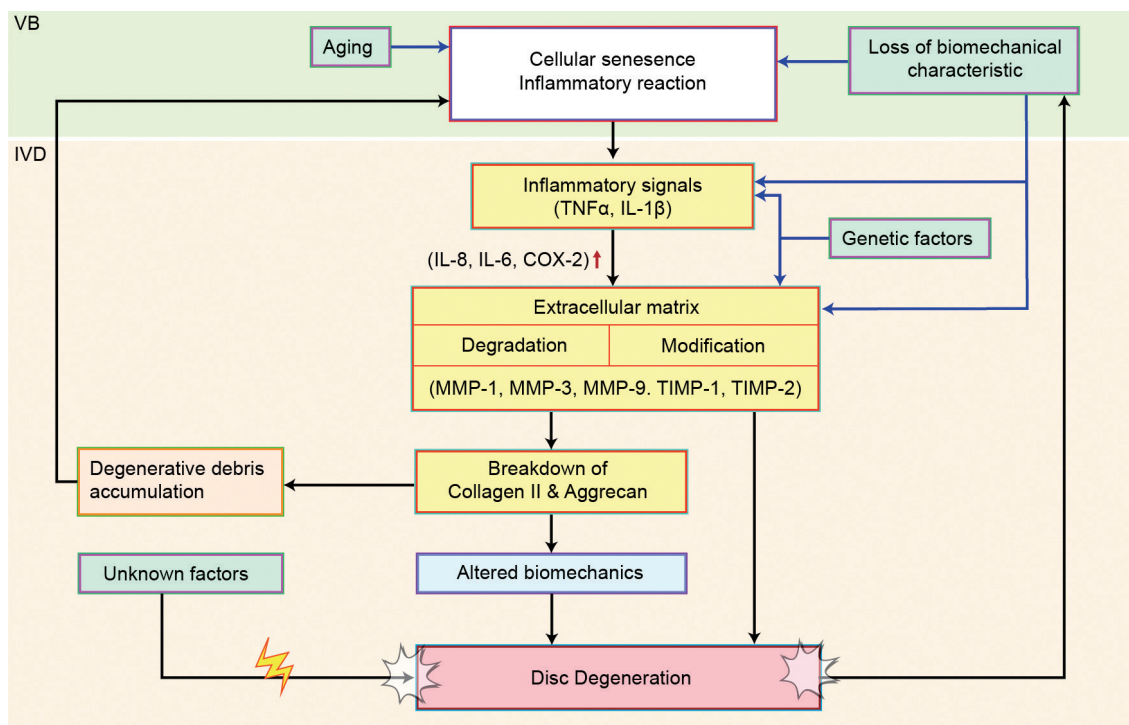
Fig. 2. Pathomechanism of disc degeneration. IL-1 beta and TNF-alpha are the key molecules that initiate and modulate inflammatory signaling pathways, which consequently lead to IVD degeneration. Resultant simultaneous ECM homeostasis dysregulation occurs in a cyclic manner. IL, interleukin; TNF, tumor necrosis factor; COX, cyclooxygenase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; VB, vertebral body; IVD, intervertebral disc; ECM, extracellular matrix.

matrix metalloproteinase (MMP)-1 and -3 production in human IVDs,[33-36] leading to major ECM homeostasis dysregulation in DDDs. In addition to these key roles in IVD degeneration, IL-1 beta also regulates IVD cell proliferation and accelerates IVD cellular aging and apoptosis, suggesting its broad involvement in IVD physiology.[30] TNF-alpha exhibits a similar role to IL-1 beta in IVDs. TNF-alpha also activates inflammatory reactions in IVDs, and various pro-inflammatory cytokines are simultaneously secreted by immune cells and disc cells, with the resultant progression of DDD or the production of clinical pain.[36-38] Increasing evidence suggests that even transient exposure to cytokines can induce this inflammatory cascade. Short-term TNF-alpha stimulation has been shown to result in irreversible long-term catabolic effects,[39] and macrophages that secrete pro-inflammatory cytokines could also activate significant inflammation in IVD cells.[36] A wide range of inflammatory reactions occur within IVDs following simultaneous stimulation and secretion of these cytokines, and this phenomenon triggers an irreversible cascade of pathogenic responses, including anatomic changes (nerve/vessel ingrowth), ECM dysregulation, and pain generation.[22,40-42] In summary, inflammatory mediators, as indicated by IL-1 beta and TNF-alpha activity, modulate catabolic reactions within IVDs and result in changes in ECM homeostasis and the occurrence of neural/vascular ingrowth-related chronic intractable discogenic LBP.

EXTRACELLULAR MATRIX REGULATION IN DDD

Intracellular and intercellular signaling pathways play critical roles in the development of IVD degeneration. However, at the extracellular level, changes in the normal homeostasis of the ECM are important components involved in DDD. Despite the ECM of IVDs containing only a small number of cells, its biophysiological function is critical. As active changes occur during the IVD degeneration process, consequent changes in the ECM composition or concentration occur because the ECM can no longer maintain normal balance.[43,44] In normal IVDs, ECM production and simultaneous expression of degradative enzymes control ECM homeostasis. As the synthesis of major collagens that comprise the inner NP, including collagen II and aggrecan, decreases, IVDs gradually lose their distinctive phenotypic characteristics, anatomical structure, and function.[2,45] In addition to decreased synthesis of the ECM, a more significant molecular change following the active progression of DDD is increased degradative enzyme production within the IVD. Upregulation of ECM degradation enzymes is enhanced under the catabolic conditions of the IVD, resulting in synergetic production of inflammatory cytokines, leading to further ECM degradation.[35,43] In degenerated discs, the production of MMPs -1, -3, -8, and -13 is significantly enhanced, whereas these MMPs are hardly detected in healthy IVDs. These

MMPs have been shown to be directly and indirectly associated with IVD degeneration processes through the activation or stimulation of latent enzymes.[44-47] The activated MMPs are not limited to collagens but also include non-collagen proteins. The expression of these specific MMPs is regulated via inhibitory MMPs, such as tissue inhibitors of metalloproteinase (TIMP)-1 and -2.[24,36]

The action of MMPs and TIMPs within the IVD is important, as these enzymes can degrade most ECM peptides and play decisive roles in the inflammation degeneration pathway (Fig. 3). MMP-1 plays a key role in IVDs as it cleaves collagen I and II, which are rich in the NPs and AFs, respectively. MMP-3 denatures collagens and is a key MMP showing significant expression changes in DDDs.[18,48] TIMPs regulating MMP-1 and -3 expression are expressed at low levels in healthy IVDs; however, during degeneration/inflammation of the IVD, TIMP-1 and -2 expression increases alongside subsequent upregulation of MMPs.[28,35] Kwon, et al.[36] confirmed this change in ECM regulation and identified that specific patterns differ under different oxygen conditions. This catabolic change representing dysregulation of ECM homeostasis appears to be more significant under higher oxygen conditions than hypoxic conditions, indicating that a change in the normal hypoxic condition of IVDs is associated with active degeneration or inflammation.[36,49]

Besides the chemical reactions that follow degeneration or inflammation, persistent and repetitive mechanical stress, such as a large axial load on the IVDs, can also result in deleterious shifts in ECM homeostasis. Research has shown that anabolic protein-regulating genes undergo decreased expression and changes in ECM production, while MMP gene expression increases, resulting in aggressive ECM degradation.[50,51] Hence, the resultant loss of normal IVD viscoelasticity and the concomitant change in ECM composition represent key mechanisms in DDDs and the initial step of discogenic back pain.

DEVELOPMENT OF SYMPTOMATIC DDD

Previous research has focused greatly on the molecular biochemical cascade of disc aging or DDDs. However, patients seek clinical advice due to symptomatic back pain, not degenerative changes. In the mid-2000s, research groups, including Kim, et al.,[6] began to focus on the roles of IVD molecular inflammatory pathways in discogenic back pain. Symptomatic DDDs result from pre-existing injuries to IVDs and the subsequent response of the AF following those injuries. As an injury occurs, starting from the outer layer of the AF, abundant macrophages are recruited from blood vessels near the AF to the site, resulting in a cascade of inflammatory reactions.[4,6] This macrophage-related inflammatory cascade is represented by the secretion of high levels of pro-inflammatory cytokines, inducing inflammation itself and simultaneous aberrant angiogenesis (Fig. 4). Pain-related IL-6 cytokines are also secreted

in high concentrations, resulting in symptomatic LBP. An association between symptomatic back pain and pain-related pro-inflammatory cytokines is well known. Serum protein and mRNA levels of IL-6, -8, and TNF-alpha were significantly higher in patients with severe back pain than patients with lesser pain intensity.[52-54] Of these cytokines, IL-6 is strongly associated with chronic back pain, regulating the expression of high-sensitive C-reactive protein, a systemic marker for in vivo inflamma-
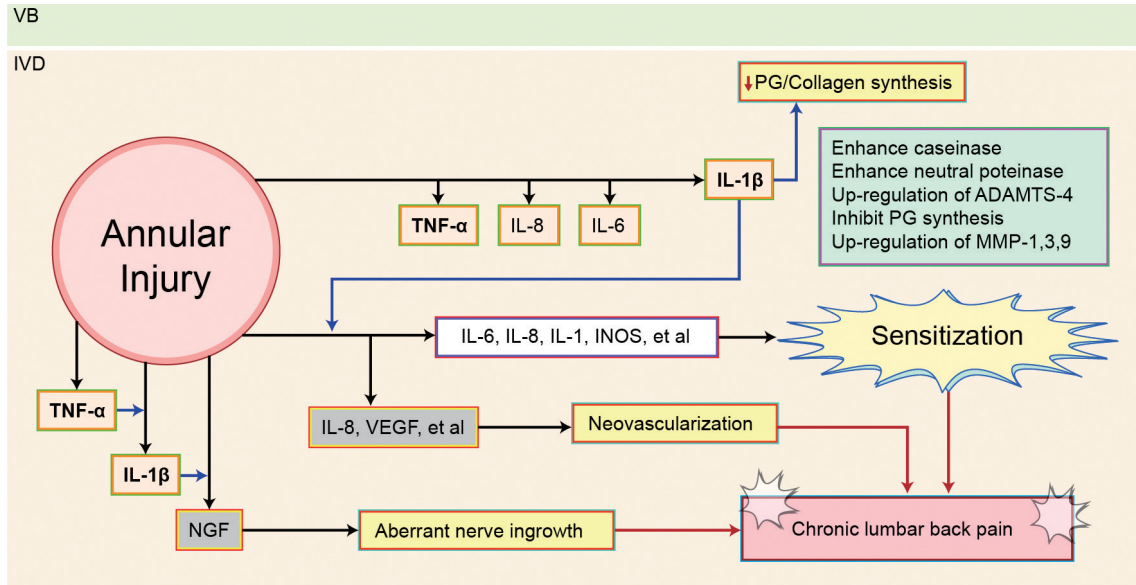
Fig. 3. Inflammatory response in IVD following annular injury. An inflammatory response is initiated following annular injury, which also causes pain sensitization. The inflammatory response is followed by dysregulation of extracellular matrix homeostasis, neovascularization, and aberrant nerve ingrowth, all of which are related to chronic lumbar back pain. VB, vertebral body; IVD, intervertebral disc; IL, interleukin; TNF, tumor necrosis factor; INOS, inducible nitric oxide synthase; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; PG, proteoglycan; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; MMP; matrix metalloproteinase.
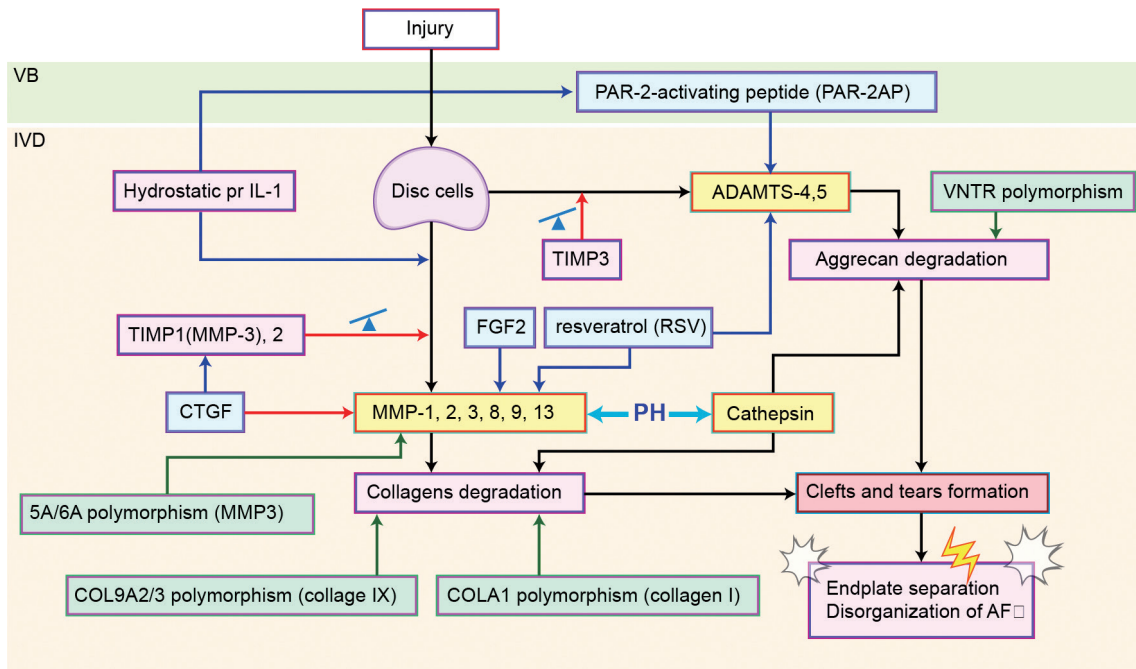
Fig. 4. Regulation of extracellular matrix in degenerative IVD. Injury to the IVD elicits up-regulation of MMPs and TIMPs, which is responsible for collagen and aggrecan degradation, causing clefts and tears formation in IVD. Subsequently, they result in degeneration of IVDs. PAR, protease-activated receptors; IL, interleukin; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; FGF, fibroblast growth factor; VNTR, variable number tandem repeats; CTGF, connective tissue growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; IVD, intervertebral disc; AF, annulus fibrosus.

tion.[55,56] The significance of IL-6 in painful inflammatory IVDs has been established in in vivo, in vitro, and clinical studies: gene expression and cytokine expression of IL-6 and IL-1 beta were observed in animal models by inflammatory stimulation of the IVDs.[57-59] In vitro studies have also shown consistent results supporting the significant role of IL-6 in pain and IVD degeneration.[5,6,60] In a clinical prospective comparative cohort study, intradiscal tocilizumab (an IL-6 receptor antibody) exerted a short-term analgesic effect in patients with discogenic LPB,[61] and further clinical studies also demonstrated the correlation between pain-related outcomes and IL-6.[62]

Another key pro-inflammatory cytokine contributing to IVD-related symptomatic pain is TNF-alpha. TNF-alpha influences the catabolic response and has a significant influence on nociceptive pain generation.[63,64] As the recruitment of macrophages in injured IVDs occurs, many pro-inflammatory markers are secreted, including IL-1, -6, and TNF-alpha, and pain is generated under these circumstances. IVD injuries are directly related to increased production of TNF-alpha, which is secreted from NPs, AFs, and non-resident inflammatory cells, such as macrophages.[40,65] Although several clinical trials have targeted TNF-alpha-induced pain generation by anti-TNF-alpha agents, such as etanercept, these have yet to show promising anti-inflammatory results in lowering significant discogenic pain.[66,67] However, based on previous research on TNF-alpha and its association with discogenic pain, it is expected that proper dosing will enable its future use as a pain modulator.

Mitogen-activated protein kinases (MAPKs) are a group of protein kinases that modulate cellular signaling pathways. MAPKs are associated with various cell responses to external stimuli, including inter- and extracellular signaling, inflammatory reactions, and other environmental inputs, such as radiation or mechanical stress.[68,69] As the inflammatory reaction progresses in IVDs, enhanced expression of pro-inflammatory cytokines stimulates MAPKs, and these are associated with symptomatic DDD.[5,70] MAPK inhibitors suppress the production of pain-modulating cytokines, such as IL-6 and -8, under inflammatory stimulation to IVDs, suggesting their key role in discogenic back pain. Park, et al.[5] showed that p38 MAPK regulates IL-6 production during inflammatory responses of IVDs and that Jun N-terminal kinase and extracellular signal-regulated kinase 1/2 modulate pro-inflammatory reactions, indicating the key roles of these pathways in symptomatic DDDs.

Accompanying the multi-directional pathologic pathways generating pain in IVDs, there is unexpected neural ingrowth into the AFs inner surface, which results in increased pain in the IVD. The IVD is initially an avascular and aneural structure, and nerve ingrowth occurs following the upregulation of pro-inflammatory cytokines in degenerated IVDs.[28,64,71,72] Animal model studies, human cadaveric studies, and patient specimen histologic/chemical analyses have consistently shown sensory nerve ingrowth into inner AFs and NPs.[71-74] Alongside this anatomic change that induces discogenic back pain, nerve

growth factor (NGF) also plays a key chemical role by inducing nerve growth. By directly binding and acting on pain-related cellular receptors, such as tyrosine kinase A or p75 neurotrophin, increased secretion of NGF enhances the pain generating pathway, resulting in discogenic pain.[23] Additional to the nerve ingrowth, the dorsal root ganglion (DRG), which is a cluster of sensory neurons that act as sensory signal modulators and transducers, also plays as a critical part in development of DDD. Various pain related proinflammatory chemicals, including brain-derived neurotrophic factor and neuropeptide substance P, are upregulated and eventually generate nociception and pain signals.[75,76] This phenomenon contributes to painful degenerative changes of the discs and various presentations of sensory symptoms accompanying DDD. DDD again brings an alteration to the environment of the DRG, such as increased perineural pressure, ischemia, or acidosis, completing a cycle of DDD-mediated DRG neuropathy.[77]

All of the various discogenic pain-related pro-inflammatory cytokines, growth factors, and enzymes are of particular interest in spinal research because understanding the established pathways of these molecules in pain production can lead to the discovery of possible therapeutic targets for symptomatic IVDs.

ANGIOGENESIS IS A CRITICAL STEP IN SYMPTOMATIC DDD

The degeneration of IVDs has recently emerged as an area of interest for investigating the role of angiogenesis in the development of inflammation. While nerve ingrowth into the inner surface of AFs occurs during active inflammation and degeneration, blood vessels also grow into the IVDs in symptomatic DDDs. Urban and Roberts[50] described the IVD as "The largest avascular organ in the body," suggesting that IVDs do not contain blood vessels and form a hypoxic environment when healthy. IVDs are adapted to this unique microenvironment without a direct oxygen supply; therefore, the ingrowth of blood vessels and nerves indicates an aberrant change to the normal hypoxic microenvironment.[71,78,79] Thus, angiogenesis is considered a key initial pathologic step of DDD and LBP by inducing changes to normal homeostasis. Vascular ingrowth into IVDs is also seen in the histopathologic evaluation of degenerated or painful discs. Histology findings have reported vascularized granulation tissue containing blood vessels penetrating the IVD from the outer AF and extending deep into the outer part of the NP.[4]

Vascular endothelial growth factor (VEGF) is associated with the regulation of endothelial cell proliferation, which is regulated by hypoxia-inducible factor-1 (HIF-1).[80] Activation and expression of HIF-1 is dependent on oxygen concentrations, hence VEGF expression representing the neovascularization activity in IVDs may also be dependent on hypoxic conditions. IVD cells undergoing degeneration potentially enhance

neovascularization by secreting large amounts of angiogenic cytokines, such as VEGF and IL-6, and -8 (Fig. 5).[36,37,78] The exact mechanisms and whether IVD neovascularization or degeneration is the cause or the consequence remain unclear. Notwithstanding, the role of oxygen-level responsive protein VEGF-related neovascularization has been reported previously: high VEGF expression is seen in painful discs or degenerated IVDs.[36,81-83] Kwon, et al. described AF and NP's reactions to inflammatory stimulation under different oxygen conditions. Under hypoxic conditions, which is the normal environment for the inner part of the IVDs, VEGF expression is initiated and regulated by HIF-1 regulation and acts as the critical step initiating vascular ingrowth.[36,80] IL-8 expression exhibits a similar response to inflammatory stimulation under hypoxic conditions and, as IL-8 is a pro-inflammatory cytokine associated with aberrant angiogenesis,[84] it is clear that IL-8 plays a role in DDDs or symptomatic LBP. Conversely, IL-6 production under hypoxic conditions is significantly less than that under normoxic conditions after inflammatory stimulation, indicating the importance of angiogenesis and discogenic LBP in IVD degeneration.[36,85]

Following degeneration, blood vessels and nerve endings co-migrate into IVDs and show synergetic effects. NGF stimulates sensory nerve ingrowth into IVDs and acts as a chemostimulant for endothelial cell proliferation, resulting in active blood vessel formation in the discs.[86] IVDs and proliferating vascular endothelial cells also produce NGF and contribute to simultaneous intradiscal nerve-end ingrowth, promoting discogenic pains via DRGs near IVDs.[87] Neural tissue ingrowth and endothelial proliferation reportedly share an intracellular pathway, simultaneously stimulating both neural cells and vascular cells, finally leading to a possible cascade in the development of DDDs.[88]

THERAPEUTIC PROSPECTS FOR IVD

Current research is targeting possible therapeutic or prognostic approaches that modulate the inflammatory response mediating the degenerative cascade in IVDs. Currently, treatments are primarily aimed at controlling the existing degenerated IVDs by systemic medication or focal injections into the inflamed disc or surgically eliminating the IVD tissue directly. Instead of being fundamental treatments, these therapies are conservative approaches. Due to the lack of treatment options, current research is focused on finding and developing new therapies.

Initial trials have injected anti-inflammatory protein solutions directly into the IVDs, aiming to stop or reverse the inflammatory cascade and regenerating the degenerated IVD. Cao, et al.[89] performed various trials of protein intradiscal injections and showed varying IVD responses to the treatments. Specific proteins, such as bone or cartilage growth factors and bone morphogenic proteins, have shown some positive results in clinical trials and animal studies.[90-92] However, due to counter complications or adverse effects and the relatively short-acting time within the discs, these trials require improvement, including developing better delivery systems if they are to be considered routine treatments. Gene therapies or cell-based therapies are also actively being investigated by several groups.[93,94] Genes inserted or edited are delivered into IVD cells via classic methods, such as virus vectors, or by high-end techniques, such as gene editing tools [clustered regularly interspaced short palindromic repeats (CRISPR)]. Gene therapy provides the
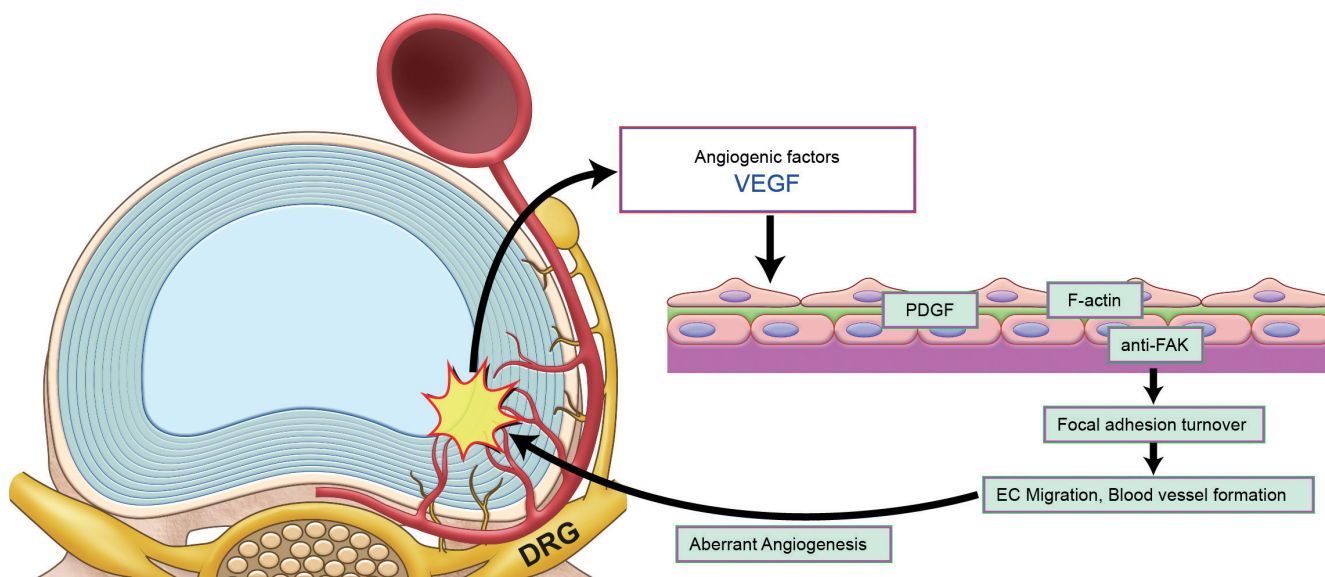
Fig. 5. Angiogenesis in symptomatic degenerative DDD. Inflammatory stimulation on the IVD are followed by elevated expression of VEGF, which relates to unexpected aberrant angiogenesis in IVD. DDD, degenerative disc disease; IVD, intervertebral disc; DRG, dorsal root ganglion; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; EC, extracellular; FAK, focal adhesion kinase.

possibility to manipulate the local pathologic pathway of inflammation and, hence, to be able to modulate the degenerative cascade itself.[95] Potential target genes include transcription factors for disintegrin, MMPs, TIMPs, or chondrocyte-specific transcription factors,[96-98] and key inflammatory reaction-related genes, such as IL-1.[99] Cell-based therapies are also effective as the cells interact with resident IVD cells, and stem cell injections are the most widely performed trials to date. Stem cell injection has an advantage over gene therapies as the stem cells can interact directly with IVD cells and contribute to regeneration.[46,100] Mesenchymal stem cells can regenerate injured tissue by secreting relevant cytokines and differentiating into correct cell phenotypes, including IVDs.[101,102] Nonetheless, both gene and cell-based therapies face obstacles. The possible systemic responses to these trials are not fully understood, and it is impossible to control or regulate the complex DDD cascade by interfering with a few target molecules. Furthermore, extensive studies are needed, as we do not fully understand the detailed interactions and possible counter-reactions to these novel treatments on IVDs.

Kim, et al.[34] investigated non-invasive direct stimulation to IVD cells using biphasic electrical currents or photobiomodulation and produced promising results, showing significantly suppressed expression of inflammatory mediators.[103] Although these trials are attractive, considering the non-invasive nature of energy transmission to IVDs, there remain some obstacles to obtaining a safe and effective method for delivering stimulus to the IVD, which lies in the center of the vertebral axis. As our knowledge of IVD biology and degeneration increases, more therapeutic candidates will become available.

Recently, several groups of researchers have focused on the regenerative or anti-degenerative effect of senolytic drugs. The use of senolytics has its basis on the fact that a significant number of senescent cells are present within the IVD under degeneration process. Cellular senescence is a phenomenon characterized by an alteration in the cell cycle that is triggered by aging cells, and this particular phenomenon is also found in degenerated discs.[104,105] Therefore, applying senolytics is based on the concept that apoptosis of IVD cells will be selectively induced only in senescent cells by inhibiting the significant pro-survival pathomechanism of old cells, finally leading the entire tissue into anti-senescent states. Novais, et al.[106] reported preserved viability, phenotypic characteristics, and ECM composition in IVD cells, along with a significant prevention against senescence progression, in mice DDD models after using a combination of senolytics, Dasatinib, and Quercetin. Shao, et al.[107] reported similar anti-degenerative effects for Quercetin alone in in vitro research and discovered that these effects in IVDs are potentially regulated via the NF-kB axis pathway. These early studies on the effect of senolytics for DDD suggest that senolytic agents can be potential solutions for active DDDs by mitigating symptomatic progression of DDDs.

Table 1 presents summary of various therapeutic perspects.

Table 1. Summary of Therapeutic Perspectives

Therapeutics	Mechanisms
Intradiscal injections	
Corticosteroids[89]	Anti-inflammation
OP-1[90]	Restoration of disc height by stimulating the synthesis of proteoglycans and collagen
BMP and growth factors[91]	
Gene therapy	
Virus-mediated[93]	- Introduction of IL-1 receptor antagonist cDNA - Transfer of TGF-β1, TGF-β3, CTGF, and TIMP-1 genes
Non-virus-mediated[93,94]	- Microbubble-enhanced ultrasound technique for introduction of plasmid DNA - Polyplex micelle delivering miRNA and MMP-cleavable peptide cross-linkers - RNA interference via gene silencing (e.g. ADAMTS-5 siRNA and caspase-3 siRNA) - mTOR/RAPTOR siRNA for the regulation of autophagy
CRISPR-Cas9[94]	Downregulation of TNFR1 and IL1R1 expression
Stem cell therapy[101,102]	
	Mesenchymal stem/stromal cells activation leads to IL-1 receptor antagonist expression, secretion of TNF-α stimulated gene/protein 6 reducing NF-κB signaling, and secretion of PGE2, which induce macrophages to secrete IL-10
Non-invasive direct stimulation	
Photobiomodulation[103]	Administration of various wavelengths and energy densities to modulate biological molecules, such as ECM modifying enzymes
Senolytics[106]	
Dasatinib and Quercetin	A Src/tyrosine kinase inhibitor and a flavonoid that binds to BCL-2 modulate growth factors and cell cycle proteins

OP-1, osteogenic protein-1; BMP, bone morphogenic protein; IL, interleukin; TGF, transforming growth factor; CTGF, connective tissue growth factor; TIMP, tissue inhibitor of metalloproteinase; miRNA, microRNA; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; siRNA, small interfering RNA; TNFR, tumor necrosis factor receptor; ECM, extracellular matrix.

CONCLUSIONS

Following decades of research aimed at understanding the complex pathomechanisms of IVD degeneration and discogenic pain, our understanding of the age-related disease is better than ever before. However, much remains undiscovered. Thorough discussion and understanding of diseases are essential to find and design possible therapeutic strategies. Based on the extent of current knowledge, further research is needed to clarify the detailed mechanisms of degeneration and to develop possible treatment modalities. A multi-disciplinary approach that considers the entirety of spinal research is needed, from basic research to clinical applications. Worldwide efforts of numerous research groups, academic organizations, and international cooperation among IVD specialists will bring advances in basic spine research in the future. This multi-modal effort will lead us to novel treatments for DDDs and discogenic pain.

AUTHOR CONTRIBUTIONS

Conceptualization: all authors. Data curation: all authors. Formal analysis: all authors. Funding acquisition: all authors. Investigation: all authors. Methodology: all authors. Project administration: Joo Han Kim and Woo-Keun Kwon. Resources: Joo Han Kim and Woo-Keun Kwon. Software: all authors. Supervision: Joo Han Kim and Woo-Keun Kwon. Validation: all authors. Visualization: all authors. Writing—original draft: Joo Han Kim and Woo-Keun Kwon. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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REFERENCES

1. Andersson GB. Epidemiological features of chronic low-back pain. Lancet 1999;354:581-5.
2. Kepler CK, Ponnappan RK, Tannoury CA, Risbud MV, Anderson DG. The molecular basis of intervertebral disc degeneration. Spine J 2013;13:318-30.
3. Kim JH. Bench to bed (B2B) project; intervertebral disc degeneration in collaboration with biospine. Neurospine 2020;17:1-2.
4. Peng B, Hao J, Hou S, Wu W, Jiang D, Fu X, et al. Possible pathogenesis of painful intervertebral disc degeneration. Spine (Phila Pa 1976) 2006;31:560-6.
5. Park JJ, Moon HJ, Park JH, Kwon TH, Park YK, Kim JH. Induction of proinflammatory cytokine production in intervertebral disc cells by macrophage-like THP-1 cells requires mitogen-activated protein kinase activity. J Neurosurg Spine 2016;24:167-75.
6. Kim JH, Studer RK, Sowa GA, Vo NV, Kang JD. Activated macrophage-like THP-1 cells modulate anulus fibrosus cell production of inflammatory mediators in response to cytokines. Spine (Phila Pa 1976) 2008;33:2253-9.
7. Fehlings MG, Tetreault L, Nater A, Choma T, Harrop J, Mroz T, et al. The aging of the global population: the changing epidemiology of disease and spinal disorders. Neurosurgery 2015;77 Suppl 4:S1-5.
8. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo award in basic science. Spine (Phila Pa 1976) 2002;27:2631-44.
9. Shapiro IM, Vresilovic EJ, Risbud MV. Is the spinal motion segment a diarthrodial polyaxial joint: what a nice nucleus like you doing in a joint like this? Bone 2012;50:771-6.
10. Rider SM, Mizuno S, Kang JD. Molecular mechanisms of intervertebral disc degeneration. Spine Surg Relat Res 2019;3:1-11.
11. Purmessur D, Cornejo MC, Cho SK, Hecht AC, Iatridis JC. Notochordal cell-derived therapeutic strategies for discogenic back pain. Global Spine J 2013;3:201-18.
12. Dowdell J, Erwin M, Choma T, Vaccaro A, Iatridis J, Cho SK. Intervertebral disk degeneration and repair. Neurosurgery 2017; 80:S46-54.
13. Jahnke MR, McDevitt CA. Proteoglycans of the human intervertebral disc. Electrophoretic heterogeneity of the aggregating proteoglycans of the nucleus pulposus. Biochem J 1988;251:347-56.
14. Samartzis D, Cheung KM. Lumbar intervertebral disk degeneration. Orthop Clin North Am 2011;42:xi-xii.
15. Walker MH, Anderson DG. Molecular basis of intervertebral disc degeneration. Spine J 2004;4:S158-66.
16. Ambard D, Cherblanc F. Mechanical behavior of annulus fibrosus: a microstructural model of fibers reorientation. Ann Biomed Eng 2009;37:2256-65.
17. Buckwalter JA. Aging and degeneration of the human intervertebral disc. Spine (Phila Pa 1976) 1995;20:1307-14.
18. Roughley PJ. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. Spine (Phila Pa 1976) 2004;29:2691-9.
19. Antoniou J, Steffen T, Nelson F, Winterbottom N, Hollander AP, Poole RA, et al. The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration. J Clin Invest 1996;98:996-1003.
20. Weber A, Wasiliew P, Kracht M. Interleukin-1beta (IL-1beta) processing pathway. Sci Signal 2010;3:cm2.
21. Gabay E, Wolf G, Shavit Y, Yirmiya R, Tal M. Chronic blockade of interleukin-1 (IL-1) prevents and attenuates neuropathic pain behavior and spontaneous ectopic neuronal activity following nerve injury. Eur J Pain 2011;15:242-8.
22. Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T. Inflammatory cytokines in the herniated disc of the lumbar spine. Spine (Phila Pa 1976) 1996;21:218-24.
23. Ohtori S, Inoue G, Miyagi M, Takahashi K. Pathomechanisms of discogenic low back pain in humans and animal models. Spine J 2015;15:1347-55.
24. Le Maitre CL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. J Pathol 2004;204:47-54.
25. Kim JH, Studer RK, Vo NV, Sowa GA, Kang JD. p38 MAPK inhibition selectively mitigates inflammatory mediators and VEGF production in AF cells co-cultured with activated macrophage-like THP-1 cells. Osteoarthritis Cartilage 2009;17:1662-9.
26. Le Maitre CL, Richardson SM, Baird P, Freemont AJ, Hoyland JA. Expression of receptors for putative anabolic growth factors in human intervertebral disc: implications for repair and regeneration of the disc. J Pathol 2005;207:445-52.
27. Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration.

Arthritis Res Ther 2005;7:R732-45.

28. Bachmeier BE, Nerlich A, Mittermaier N, Weiler C, Lumenta C, Wuertz K, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. Eur Spine J 2009;18:1573-86.

29. Studer RK, Vo N, Sowa G, Ondeck C, Kang J. Human nucleus pulposus cells react to IL-6: independent actions and amplification of response to IL-1 and TNF-α. Spine (Phila Pa 1976) 2011;36: 593-9.

30. Wang Y, Che M, Xin J, Zheng Z, Li J, Zhang S. The role of IL-1β and TNF-α in intervertebral disc degeneration. Biomed Pharmacother 2020;131:110660.

31. Wang K, Chen T, Ying X, Zhang Z, Shao Z, Lin J, et al. Ligustilide alleviated IL-1β induced apoptosis and extracellular matrix degradation of nucleus pulposus cells and attenuates intervertebral disc degeneration in vivo. Int Immunopharmacol 2019;69:398-407.

32. Jin H, Wang Q, Wu J, Han X, Qian T, Zhang Z, et al. Baicalein inhibits the IL-1β-induced inflammatory response in nucleus pulposus cells and attenuates disc degeneration in vivo. Inflammatio 2019;42:1032-44.

33. Shi C, Wu L, Lin W, Cai Y, Zhang Y, Hu B, et al. MiR-202-3p regulates interleukin-1β-induced expression of matrix metalloproteinase 1 in human nucleus pulposus. Gene 2019;687:156-65.

34. Kim JH, Choi H, Suh MJ, Shin JH, Hwang MH, Lee HM. Effect of biphasic electrical current stimulation on IL-1β-stimulated annulus fibrosus cells using in vitro microcurrent generating chamber system. Spine (Phila Pa 1976) 2013;38:E1368-76.

35. Kim JH, Park JH, Moon HJ, Kwon TH, Park YK. Matrix degradative enzymes and their inhibitors during annular inflammation: initial step of symptomatic intervertebral disc degeneration. J Korean Neurosurg Soc 2014;55:237-43.

36. Kwon WK, Moon HJ, Kwon TH, Park YK, Kim JH. The role of hypoxia in angiogenesis and extracellular matrix regulation of intervertebral disc cells during inflammatory reactions. Neurosurgery 2017;81:867-75.

37. Moon HJ, Yurube T, Lozito TP, Pohl P, Hartman RA, Sowa GA, et al. Effects of secreted factors in culture medium of annulus fibrosus cells on microvascular endothelial cells: elucidating the possible pathomechanisms of matrix degradation and nerve ingrowth in disc degeneration. Osteoarthritis Cartilage 2014;22: 344-54.

38. Kepler CK, Markova DZ, Hilibrand AS, Vaccaro AR, Risbud MV, Albert TJ, et al. Substance P stimulates production of inflammatory cytokines in human disc cells. Spine (Phila Pa 1976) 2013; 38:E1291-9.

39. Purmessur D, Walter BA, Roughley PJ, Laudier DM, Hecht AC, Iatridis J. A role for TNFα in intervertebral disc degeneration: a non-recoverable catabolic shift. Biochem Biophys Res Commun 2013;433:151-6.

40. Burke JG, Watson RW, McCormack D, Dowling FE, Walsh MG, Fitzpatrick JM. Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. J Bone Joint Surg Br 2002;84:196-201.

41. Navone SE, Marfia G, Giannoni A, Beretta M, Guarnaccia L, Gualtierotti R, et al. Inflammatory mediators and signalling pathways controlling intervertebral disc degeneration. Histol Histopathol 2017;32:523-42.

42. Shamji MF, Setton LA, Jarvis W, So S, Chen J, Jing L, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. Arthritis Rheum 2010; 62:1974-82.

43. Sakai D, Grad S. Advancing the cellular and molecular therapy for intervertebral disc disease. Adv Drug Deliv Rev 2015;84:159-71.

44. Crean JK, Roberts S, Jaffray DC, Eisenstein SM, Duance VC. Matrix metalloproteinases in the human intervertebral disc: role in disc degeneration and scoliosis. Spine (Phila Pa 1976) 1997;22: 2877-84.

45. Kozaci LD, Guner A, Oktay G, Guner G. Alterations in biochemical components of extracellular matrix in intervertebral disc herniation: role of MMP-2 and TIMP-2 in type II collagen loss. Cell Biochem Funct 2006;24:431-6.

46. Gruber HE, Ingram JA, Hanley EN Jr. Immunolocalization of MMP-19 in the human intervertebral disc: implications for disc aging and degeneration. Biotech Histochem 2005;80:157-62.

47. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine (Phila Pa 1976) 2000;25:3005-13.

48. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE award competition in basic science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. Eur Spine J 2002;11:308-20.

49. Mayer JE, Iatridis JC, Chan D, Qureshi SA, Gottesman O, Hecht AC. Genetic polymorphisms associated with intervertebral disc degeneration. Spine J 2013;13:299-317.

50. Urban JP, Roberts S. Degeneration of the intervertebral disc. Arthritis Res Ther 2003;5:120-30.

51. Neidlinger-Wilke C, Würtz K, Urban JP, Börm W, Arand M, Ignatius A, et al. Regulation of gene expression in intervertebral disc cells by low and high hydrostatic pressure. Eur Spine J 2006;15 Suppl 3:S372-8.

52. Licciardone JC, Kearns CM, Hodge LM, Bergamini MV. Associations of cytokine concentrations with key osteopathic lesions and clinical outcomes in patients with nonspecific chronic low back pain: results from the osteopathic trial. J Am Osteopath Assoc 2012;112:596-605.

53. Uçeyler N, Rogausch JP, Toyka KV, Sommer C. Differential expression of cytokines in painful and painless neuropathies. Neurology 2007;69:42-9.

54. Wang K, Bao JP, Yang S, Hong X, Liu L, Xie XH, et al. A cohort study comparing the serum levels of pro- or anti-inflammatory cytokines in patients with lumbar radicular pain and healthy subjects. Eur Spine J 2016;25:1428-34.

55. Gebhardt K, Brenner H, Stürmer T, Raum E, Richter W, Schiltenwolf M, et al. The course of high-sensitive C-reactive protein in correlation with pain and clinical function in patients with acute lumbosciatic pain and chronic low back pain - a 6 months prospective longitudinal study. Eur J Pain 2006;10:711-9.

56. Khan AN, Jacobsen HE, Khan J, Filippi CG, Levine M, Lehman RA Jr, et al. Inflammatory biomarkers of low back pain and disc degeneration: a review. Ann N Y Acad Sci 2017;1410:68-84.

57. Jung WW, Kim HS, Shon JR, Lee M, Lee SH, Sul D, et al. Intervertebral disc degeneration-induced expression of pain-related molecules: glial cell-derived neurotropic factor as a key factor. J Neurosurg Anesthesiol 2011;23:329-34.

58. Rothman SM, Huang Z, Lee KE, Weisshaar CL, Winkelstein BA. Cytokine mRNA expression in painful radiculopathy. J Pain 2009; 10:90-9.

59. Bianchi M, Franchi S, Ferrario P, Sotgiu ML, Sacerdote P. Effects of the bisphosphonate ibandronate on hyperalgesia, substance P, and cytokine levels in a rat model of persistent inflammatory pain. Eur J Pain 2008;12:284-92.

60. Moon HJ, Kim JH, Lee HS, Chotai S, Kang JD, Suh JK, et al. Annulus fibrosus cells interact with neuron-like cells to modulate

production of growth factors and cytokines in symptomatic disc degeneration. Spine (Phila Pa 1976) 2012;37:2-9.

61. Sainoh T, Orita S, Miyagi M, Inoue G, Yamauchi K, Suzuki M, et al. Single intradiscal injection of the interleukin-6 receptor antibody tocilizumab provides short-term relief of discogenic low back pain; prospective comparative cohort study. J Orthop Sci 2016;21:2-6.

62. Koerner JD, Markova DZ, Schroeder GD, Antonacci CL, Mendelis J, Vaccaro AR, et al. Correlation of early outcomes and intradiscal interleukin-6 expression in lumbar fusion patients. Neurospine 2020;17:36-41.

63. Igarashi T, Kikuchi S, Shubayev V, Myers RR. 2000 Volvo award winner in basic science studies: exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Molecular, histologic, and behavioral comparisons in rats. Spine (Phila Pa 1976) 2000;25:2975-80.

64. Freemont AJ. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology (Oxford) 2009;48:5-10.

65. Yoshida M, Nakamura T, Sei A, Kikuchi T, Takagi K, Matsukawa A. Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. Spine (Phila Pa 1976) 2005;30:55-61.

66. Cohen SP, Wenzell D, Hurley RW, Kurihara C, Buckenmaier CC 3rd, Griffith S, et al. A double-blind, placebo-controlled, dose-response pilot study evaluating intradiscal etanercept in patients with chronic discogenic low back pain or lumbosacral radiculopathy. Anesthesiology 2007;107:99-105.

67. Tobinick EL, Britschgi-Davoodifar S. Perispinal TNF-alpha inhibition for discogenic pain. Swiss Med Wkly 2003;133:170-7.

68. Huang P, Han J, Hui L. MAPK signaling in inflammation-associated cancer development. Protein Cell 2010;1:218-26.

69. Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev 2012;92:689-737.

70. Gruber HE, Hoelscher GL, Ingram JA, Zinchenko N, Hanley EN Jr. Senescent vs. non-senescent cells in the human annulus in vivo: cell harvest with laser capture microdissection and gene expression studies with microarray analysis. BMC Biotechnol 2010;10:5.

71. Freemont AJ, Watkins A, Le Maitre C, Baird P, Jeziorska M, Knight MT, et al. Nerve growth factor expression and innervation of the painful intervertebral disc. J Pathol 2002;197:286-92.

72. Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O'Brien J, Jayson MI. Nerve ingrowth into diseased intervertebral disc in chronic back pain. Lancet 1997;350:178-81.

73. Inoue G, Ohtori S, Aoki Y, Ozawa T, Doya H, Saito T, et al. Exposure of the nucleus pulposus to the outside of the anulus fibrosus induces nerve injury and regeneration of the afferent fibers innervating the lumbar intervertebral discs in rats. Spine (Phila Pa 1976) 2006;31:1433-8.

74. Yamauchi K, Inoue G, Koshi T, Yamashita M, Ito T, Suzuki M, et al. Nerve growth factor of cultured medium extracted from human degenerative nucleus pulposus promotes sensory nerve growth and induces substance P in vitro. Spine (Phila Pa 1976) 2009;34:2263-9.

75. Ma J, Stefanoska D, Grad S, Alini M, Peroglio M. Direct and intervertebral disc-mediated sensitization of dorsal root ganglion neurons by hypoxia and low pH. Neurospine 2020;17:42-59.

76. Xiao WH, Bennett GJ. Persistent low-frequency spontaneous discharge in A-fiber and C-fiber primary afferent neurons during an inflammatory pain condition. Anesthesiology 2007;107:813-21.

77. Kim YS, Anderson M, Park K, Zheng Q, Agarwal A, Gong C, et al. Coupled activation of primary sensory neurons contributes to chronic pain. Neuron 2016;91:1085-96.

78. Ohba T, Haro H, Ando T, Wako M, Suenaga F, Aso Y, et al. TNF-alpha-induced NF-kappaB signaling reverses age-related declines in VEGF induction and angiogenic activity in intervertebral disc tissues. J Orthop Res 2009;27:229-35.

79. Karamouzian S, Eskandary H, Faramarzee M, Saba M, Safizade H, Ghadipasha M, et al. Frequency of lumbar intervertebral disc calcification and angiogenesis, and their correlation with clinical, surgical, and magnetic resonance imaging findings. Spine (Phila Pa 1976) 2010;35:881-6.

80. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling-in control of vascular function. Nat Rev Mol Cell Biol 2006;7:359-71.

81. Kokubo Y, Uchida K, Kobayashi S, Yayama T, Sato R, Nakajima H, et al. Herniated and spondylotic intervertebral discs of the human cervical spine: histological and immunohistological findings in 500 en bloc surgical samples. Laboratory investigation. J Neurosurg Spine 2008;9:285-95.

82. Agrawal A, Gajghate S, Smith H, Anderson DG, Albert TJ, Shapiro IM, et al. Cited2 modulates hypoxia-inducible factor-dependent expression of vascular endothelial growth factor in nucleus pulposus cells of the rat intervertebral disc. Arthritis Rheum 2008;58:3798-808.

83. Fujita N, Imai J, Suzuki T, Yamada M, Ninomiya K, Miyamoto K, et al. Vascular endothelial growth factor-A is a survival factor for nucleus pulposus cells in the intervertebral disc. Biochem Biophys Res Commun 2008;372:367-72.

84. Kim JH, Moon HJ, Lee JH, Kim JH, Kwon TH, Park YK. Rabbit notochordal cells modulate the expression of inflammatory mediators by human annulus fibrosus cells cocultured with activated macrophage-like THP-1 cells. Spine (Phila Pa 1976) 2012;37:1856-64.

85. Kwon WK, Moon HJ, Kwon TH, Park YK, Kim JH. Influence of rabbit notochordal cells on symptomatic intervertebral disc degeneration: anti-angiogenic capacity on human endothelial cell proliferation under hypoxia. Osteoarthritis Cartilage 2017;25:1738-46.

86. Nico B, Mangieri D, Benagiano V, Crivellato E, Ribatti D. Nerve growth factor as an angiogenic factor. Microvasc Res 2008;75:135-41.

87. Wuertz K, Haglund L. Inflammatory mediators in intervertebral disk degeneration and discogenic pain. Global Spine J 2013;3:175-84.

88. Santos PM, Winterowd JG, Allen GG, Bothwell MA, Rubel EW. Nerve growth factor: increased angiogenesis without improved nerve regeneration. Otolaryngol Head Neck Surg 1991;105:12-25.

89. Cao P, Jiang L, Zhuang C, Yang Y, Zhang Z, Chen W, et al. Intradiscal injection therapy for degenerative chronic discogenic low back pain with end plate Modic changes. Spine J 2011;11:100-6.

90. Masuda K, Imai Y, Okuma M, Muehleman C, Nakagawa K, Akeda K, et al. Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. Spine (Phila Pa 1976) 2006;31:742-54.

91. Imai Y, Okuma M, An HS, Nakagawa K, Yamada M, Muehleman C, et al. Restoration of disc height loss by recombinant human osteogenic protein-1 injection into intervertebral discs undergoing degeneration induced by an intradiscal injection of chondroitinase ABC. Spine (Phila Pa 1976) 2007;32:1197-205.

92. Moriguchi Y, Alimi M, Khair T, Manolarakis G, Berlin C, Bonas-

sar LJ, et al. Biological treatment approaches for degenerative disk disease: a literature review of in vivo animal and clinical data. Global Spine J 2016;6:497-518.

93. Takeoka Y, Yurube T, Nishida K. Gene therapy approach for intervertebral disc degeneration: an update. Neurospine 2020;17:3-14.

94. Han IB. Moving forward: gene therapy for intervertebral disc degeneration. Neurospine 2020;17:17-8.

95. Vadalà G, Sowa GA, Kang JD. Gene therapy for disc degeneration. Expert Opin Biol Ther 2007;7:185-96.

96. Paul R, Haydon RC, Cheng H, Ishikawa A, Nenadovich N, Jiang W, et al. Potential use of Sox9 gene therapy for intervertebral degenerative disc disease. Spine (Phila Pa 1976) 2003;28:755-63.

97. Nishida K, Doita M, Takada T, Kakutani K, Miyamoto H, Shimomura T, et al. Sustained transgene expression in intervertebral disc cells in vivo mediated by microbubble-enhanced ultrasound gene therapy. Spine (Phila Pa 1976) 2006;31:1415-9.

98. Nishida K, Suzuki T, Kakutani K, Yurube T, Maeno K, Kurosaka M, et al. Gene therapy approach for disc degeneration and associated spinal disorders. Eur Spine J 2008;17 Suppl 4:459-66.

99. Wehling P, Schulitz KP, Robbins PD, Evans CH, Reinecke JA. Transfer of genes to chondrocytic cells of the lumbar spine. Proposal for a treatment strategy of spinal disorders by local gene therapy. Spine (Phila Pa 1976) 1997;22:1092-7.

100. Sakai D, Andersson GB. Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. Nat Rev Rheumatol 2015; 11:243-56.

101. Vadalà G, Sowa G, Hubert M, Gilbertson LG, Denaro V, Kang JD. Mesenchymal stem cells injection in degenerated intervertebral disc: cell leakage may induce osteophyte formation. J Tissue Eng Regen Med 2012;6:348-55.

102. Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther 2012;20:14-20.

103. Hwang MH, Son HG, Lee JW, Yoo CM, Shin JH, Nam HG, et al. Photobiomodulation of extracellular matrix enzymes in human nucleus pulposus cells as a potential treatment for intervertebral disk degeneration. Sci Rep 2018;8:11654.

104. Vo N, Seo HY, Robinson A, Sowa G, Bentley D, Taylor L, et al. Accelerated aging of intervertebral discs in a mouse model of progeria. J Orthop Res 2010;28:1600-7.

105. He S, Sharpless NE. Senescence in health and disease. Cell 2017; 169:1000-11.

106. Novais EJ, Tran VA, Johnston SN, Darris KR, Roupas AJ, Sessions GA, et al. Long-term treatment with senolytic drugs Dasatinib and Quercetin ameliorates age-dependent intervertebral disc degeneration in mice. Nat Commun 2021;12:5213.

107. Shao Z, Wang B, Shi Y, Xie C, Huang C, Chen B, et al. Senolytic agent Quercetin ameliorates intervertebral disc degeneration via the Nrf2/NF-κB axis. Osteoarthritis Cartilage 2021;29:413-22.