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Review

Molecular mechanisms in intervertebral disc degeneration

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ABSTRACT

Low back pain is a chronic and expensive health problem that is the most common cause of restricted activity level in the population under 45 years of age. Among the structures forming the lumbar vertebra, pain is most often caused by the intervertebral disc. As a result of intervertebral disc degeneration, which usually starts in the 2nd decade, physiological load distribution is disrupted and pathological changes occur in the annulus fibrosis, facet joints and vertebrae. Nucleus pulposus herniation, which occurs as a result of ruptures occurring in the anulus fibrosis, and facet joint hypertrophy and arthrozis due to the change of the load distribution cause compression of the nerve roots. Disc degeneration, disc herniation and disc-induced pain develop due to all these degenerative changes along with vertebra endplate changes. Although conservative approaches and surgical approaches such as discectomy, segmental fusion and disc arthroplasty are widespread in the treatment of intervertebral disc degeneration; approaches to the regeneration of damaged tissue are very limited among the current treatment practices. In recent years, studies on whether disc degeneration can be reversed have been gaining importance in the literature. This increases the need for elucidation of the molecular basis of disc degeneration. On the other hand, a better understanding of the biological process involved in degeneration is the basis for producing therapeutic solutions. A better understanding of the degeneration process will enable the cellular and molecular-based treatments to be implemented in the future.

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1. Introduction

Low back pain is a chronic and expensive health problem that is common in society. 80% of adults have a history of being admitted to hospital with low back pain through their lives. Low back pain is the most common cause of restricted activity level in the population under 45 years of age. It is also the second most frequent complaint in hospital referrals and third most common reason of surgical operations [1].

There are many anatomical structures in the lumbar spine that cause pain, and this pain is often caused by the intervertebral disc. Previous studies have reported that symptomatic disc degeneration is the most common cause of pain. Also, it has been shown that degeneration in the lumbar intervertebral discs begun at earlier ages, mostly at the 2nd decade, compared to other tissues in the body.

Degeneration in intervertebral discs causes a decrease in physiological load absorption that leads to a change in load distribution. The change in load distribution causes pathological changes on the anulus fibrosis (AF), facet joints (FJ) and vertebrae. Formation of tears in the AF causes disruption of the structural integration of the intervertebral disc and central herniation of the nucleus pulposus (NP). Due to the changes in load distribution, hypertrophy and arthrosis are observed in facet joints. These changes result in pressure on nerve roots. Also, end plate changes in vertebrae, microfractures in trabecular bone and osteophytes

occur. As a result of these changes, disc degeneration, disc herniation and disc-originated pain appear [2].

Although conservative approaches and surgical approaches such as discectomy, segmental fusion and disc arthroplasty are widespread in the treatment of intervertebral disc degeneration; approaches to the regeneration of damaged tissue are very limited among the current treatment practices. In recent years, studies on whether disc degeneration can be reversed have been gaining importance in the literature. This increases the need for elucidation of the molecular basis of disc degeneration [3].

2. Normal intervertebral disc biology

The normal intervertebral disc is a complex structure with capillary beds originating from the cranial and caudal vertebrae, with NP at its center and AF at its periphery. AF and NP are structurally distinct from each other [3].

In NP, cells of notochordal origin are involved as predominants at birth. As age progresses, chondrocyte-like cells become dominant, similar to the AF inner region. Studies have showed that NP rich in notochordal cells that have reached the adult period degenerates later. This is why it is suggested that notochordal cells show stem cell characteristics [4]. The primary proteoglycan of NP is the molecule called 'aggregan' and is responsible for the high water content of NP.

A healthy IVD is avascular. In IVD, the cells are clustered around the end-plates, adapted to a low oxygen, slightly acidic environment. These cells are fed by capillaries that specialize between the bone and cartilage end-plate through diffusion. In the degenerated disc, diffusion capacity decreases and blood vessels disappear. As a result, the microenvironment becomes rich in lactic acid and shows a more acidic property. While extra-cellular matrix (ECM) production is reduced due to acidic microenvironment, enzymes in ECM degradation are not affected by this. At the end, the balance between construction and destruction deteriorates in the direction of destruction. Thus, degeneration is accelerated and macroscopic changes become visible [5]. Studies in the literature showed that blood circulation of intervertebral disc (IVD) is negatively affected by the metabolic diseases such as Gaucher disease and sickle cell anemia, nicotine consumption, vibration, atherosclerosis and the calcification and sclerosis in vertebral end-plates contribute to reduction of blood flow.

Non-myelinated nerves and free nerve endings that receive the sensation of a healthy IVD end at the outermost layer of AF. Laceration of AF due to the disc degeneration causes vascular structures and nerve endings to proceed into deep tissue, thus resulting in degenerative disc pain.

When the etiological factors that cause disc degeneration are examined, it could be said that environmental and genetic factors play an important role. Studies have shown that vibration, weight lifting and obesity, sedentary lifestyle, trauma and smoking are associated with disc degeneration. On the other hand, genetic factors account for 70% of the causes of disc degeneration [2].

3. Genetic factors in intervertebral disc degeneration

Studies based on kinship relationships and twin studies in the literature have shown that genetic factors are responsible for 75% of the susceptibility to intervertebral disk degeneration (IVDD). These studies have made apparent the need to investigate which genes are involved in genetic susceptibility. Recent studies have suggested that genes encoding collagen IX, vitamin D receptor, collagen I, aggrecan, and matrix metalloproteinase-3 (MMP-3) proteins might be associated with IVDD [2].

Collagen IX protein is involved in supporting the ECM structure of NP by covalently binding to collagen II. This protein is encoded by the col9a2 and COL9A3 genes. Genetic studies have shown that these genes are mutant in early and serious IVDD in Finns (COL9A2 and COL9A3) and Chinese (COL9A2). These mutations cause gln326trp change in the α2 chain of collagen IX (TRP 2 allele) and Arg103Trp change in the α3 chain (Trp3 allele). Both mutations lead to the omission of exon 3, resulting in disease-related phenotypes such as multiple epiphyseal dysplasia [6, 7].

The relationship between Vitamin D receptor (VDR) alleles 1 and 3 and IVDD degeneration was first described in Finns. These alleles have been observed to alter ECM functions by causing changes in the sulfation of glycosaminoglycans (GAG) [8]. In Chinese, the AA and Aa genotypes of the

VDR-Apa gene are associated with poor pain scores [9]. Collagen I is the dominant component of AF's ECM and provides resistance to tensile forces by forming a triple helix. It has been suggested that as a result of the G>T polymorphism altering the SP1 binding region, proportion of collagen I a1 sub-unit (COLIA1) gene have changed. Also it has been suggested that this change causes a weakening of mechanical strength and degeneration in AF [10]. The TT genotype of the collagen I α1 sub-unit gene (COLIA1) has been shown to be associated with IVDD in the Dutch, Finnish and Greek population [10-12]. Aggrecan is the primary proteoglycan found in the structure of IVD. The hydrophilic structure of aggrecan gives it high elastic deformability. High water content in MRI imaging of healthy IVD depends on this [2]. Variable numbers of tandem repeat (VNTR) polymorphism in the 12th exon, which codes the sulphate binding region of chondrite, of CS1 that is the sub-unit of Aggrecan core protein has been reported in the literature [13]. As a result of this polymorphism, the length of the aggrecan core protein changes. The Aggrecan ember protein is modified by GAGs and thus traps water. The shorter core protein binds less GAG and thus the water trapping capacity becomes altered [13, 14]. CS1 sub-unit VNTR polymorphisms are shown among the causes of early onset of IVDD seen in Japanese women. Degeneration has been reported to be more severe in patients with short VNTR [14]. In Turkish and Iranian society, short tandem repeat (STR) is a sign of bad prognosis, while in Finns, VNTRs are a sign of bad prognosis [2, 15, 16]. A common 5A/6A polymorphism associated with the promoter region of the MMP-3 gene has been reported. The 5A allele causes 2 times more activity than the 6A allele, and as a result of this polymorphism, MMP3 gene transcription increase leads to a predisposition to IVDD. Although the incidence of IVDD has been shown to increase due to 5A allele of the MMP3 gene in the older population in Japan, this increase has not been shown in the younger population [17]. In Chinese, the MMP3 5A allele increases the incidence of IVDD and creates a synergistic effect on people exposed to vibration due to their professions [9]. Videman *et al*. examined genes that may be associated with IVDD and suggested that aggrecan, collagen I, collagen IX, IL-1 and IL-18 might be associated with dehydration of IVD; while aggrecan, collagen XI and collagen IX might be associated with hernia formation and aggrecan might be associated with thinning of the IVD. On the other hand, there was no significant association between metalloproteinase families and disc degeneration [12]. The effect of aggrecan, a common member of all three categories, on disc degeneration will be addressed in the future.

4. Cellular senescence in IVDD

Senescence refers to irreversible inhibition of the cell cycle due to external stimuli or telomere uncapping. IVD begins to degenerate earlier than other organs in the body. In the literature, accumulations of damaged proteins, mitochondrial damage and dysfunction, telomere shortening, DNA damage and attrition in the cell's quality control mechanisms are reported to contribute to the ageing of the disc [18].

Aging cells characteristically form from aggregates. Cell sizes grow and flat or vacuolar phenotype is observed in aging cells. These cells wait in the G1 phase of the cell cycle and do not respond to mitogenic stimuli and they cannot replicate. The cell phenotype seen in aging cells, in which pro-inflammatory cytokines, matrix-degrading proteases, growth factors, and chemokines are abnormally secreted, is called senescence-associated secretory phenotype (SASP). Studies have revealed that SASP plays a role in degenerative diseases associated with aging. Major markers of senescence cells can be examined under 6 categories. These are the cell cycle regulators ($p16^{INK4A}$ and $p21^{Cip1}$ that in turn cause cell cycle to stop), SASP factors (IL-6 and IL-8) and monocyte chemoattractant protein-1 (Mcp-1), plasminogen-activated inhibitor-1 (Pai-1), senescence-associated β galaktosidoz (TU-ßgal) and telomere-associated and non-telomeric DNA damage foci [19].

Interest in studies evaluating the role of senescence in IVD degeneration has been growing steadily in recent years. Expression of $p16^{INK4A}$ in IVD was found to increase with age, and this increase in expression was correlated with the expression of proteins responsible for ECM destruction such as a disintegrin and metalloproteinase with Thrombospondin Motifs-5 (ADAMTS-5) and MMP-13 [20]. On the other hand, Gruber *et al*.'s

study showed an increase in SA-ßgal protein expression in high graded degenerate discs and decreased marking with Ki67 antibody [21]. It has been reported that the expression of inflammatory cytokines such as TNF-α, IL-1β, IL-17, IL-6 and COX-2 in these discs has increased and therefore they have an inflammatory microenvironment. This microenvironment causes ECM to move from anabolic process to catabolic process [22].

The mechanisms that cause senescence seen in IVD can be classified under 5 main categories: oxidative stress, genotoxic stress, mechanical stress, inflammatory stress and nutritional stress. The study of Hou *et al*. evaluating the effects of oxidative stress on rats showed that malondialdehyde (MDA), the secondary product of peroxidation of polyunsature fatty acid residues, was found to be higher in the disc tissue of elderly rats [23]. Advanced glycation end products (AGEs) such as carboxymethyllysine (CML) and pentosidine have been shown to be found in high amounts in aged human intervertebral discs [19]. Scharf *et al*.'s study in rats showed that oxidized aminoacids, which were more susceptible to destruction with MMP-1, MMP-2, MMP-9 and MMP-13 are present in excess amounts in the instervertebral disc tissue of elder rats [24]. An experimental study of the effects on oxidative stress in human NP cells showed increase in the expression of p53 and P21, suppression in expression and increase in fragmentation of aggrecan and collagen, as well as an increase of proinflammatory cytokines such as IL-6, IL-8, TNF-α and IL-1β in NP cells exposed to hydrogen peroxide. In addition to these, increase of matrix proteases such as MMP-1, MMP-2, MMP-9, MMP-13 and ADAMTS-4 and ADAMTS-5 has also been shown. As a result, all these changes lead to occurrence of senescence in cells [18]. The effects of genotoxic stress on senescence in IVD have been studied in animal experiment models. Increased $p16^{INK4A}$ expression, increased proteoglycan loss and increased MMP expression were observed in rats exposed to genotoxic stress-causing agents such as ionizing radiation and tobacco smoke. It has been shown by numerous studies that chronic abnormal mechanical overload reduces ECM synthesis, triggers the inflammatory process and causes catabolic reaction [25]. However, it has not yet been fully clarified how these effects make cells enter to senescence. The effects of inflammatory stress on disc degeneration have been investigated and it has been suggested that degeneration may be caused by both macrophages infiltrating the disc and by IL-6, IL-1β and TNF-α secreted by cells in the degenerate disc. Inflammatory cytokines cause increased expression of matrix metalloproteinase, while reducing the expression of anabolic ECM proteins such as aggrecan and collagen II. Studies have reported that, in line with these findings, the increase in inflammatory cytokines was both associated with more critical clinical symptoms and correlated with severity of disease. With aging and degeneration, the permeability of the vascular network in the vertebral end plates, which allows IVD to be fed through diffusion, is reduced. Atherosclerosis and smoking have been known to negatively affect the disc circulation. It is thought that the nutritional stress caused by IVD cells not being able to meet the nutritional support they need, provides the basis for IVDD by creating cellular senescence [19].

The results obtained from the examination of signaling pathways in the intervertebral disc senescence showed that the p53-$p21^{Cip1}$-Rb and $p16^{INK4a}$-RB signaling pathways involved in cell cycle and tumor suppression had critical roles in the induction of senescence. Activation of this signaling pathway in human and rat NP cells subjected to oxidative stress *in vitro* has been shown to induce senescence. On the other hand, it has been known that the canonical NF-kB pathway and MAPK signaling pathway are responsible for maintaining of cellular senescence and SASP. Accumulated oxidative stress and degeneration in cells increase the NF-kB transcription and thus NF-kB activation (PATIL 75-102). Increased activation both increases the expression of pro-inflammatory cytokines such as IL-6, IL-8 IL-1β and TNF-α, and triggers the degeneration process in the disc by activating matrix metalloproteinase such as MMP-1, -2, -9 and ADAMTS-5. The NF-kB signaling pathway is also the basic regulator of most of the SASP factors [26, 27]. The mitogen-activated protein kinase (MAPK) family is a family of proteins involved in the signal transduction pathway and is responsible for delivering responses to many extracellular signals. Extracellular signals

in mammals activate a number of major members of the MAPK family, primarily extracellular signal-regulated kinase (ERK), c-Jun NH2 terminal kinase (JNK), and P38 isoforms (p38MAPKs). These signaling pathways have been shown to induce senescence by affecting the local tissue homeostasis. The effects of the Wnt/B-Catenin pathway on degeneration in disc cells have also been investigated in the literature. Wang *et al.*'s study reported that the Wnt/B-Catenin pathway was activated during disc degeneration and that this pathway was probably regulated by lncRNA H19 [19]. Although the findings of this study indicate that this pathway may lead to the development of senescence phenotype in disc cells, further research is needed on the effects of this pathway. STIR 1 is a nicotinamide-dependent deacetylase that plays a role in many cellular functions such as differentiation, proliferation, apoptosis, and cell aging. Previous studies have evaluated the effects of STIR-1 on senescence and shown that expression of senescence in degenerate discs is severely reduced. Guo and colleagues reported that resvesterol applied to NP cells, which is isolated from human degenerate disc tissue, suppressed the apoptosis, $p16^{INK4A}$ and $p21^{Cip1}$ by activating dorsal-1 and also decreased expression of MMP13 and ADAMTS-5 [28]. With these findings in mind, it can be said that SIRT-1 suppresses cellular senescence and supports the healing of degenerated disc cells.

5. ECM in IVDD

IVD shows a heterogeneous structure consisting of NP, AF and vertebral end plates. While NP originates from the notochord, AF originates from the sclerotome, and accordingly their metabolism differs. Like other connective tissues IVD consists of collagen, proteoglycan, elastin, and glycoproteins. Unlike other tissues, these components are found fragmented in the environment and connective tissue is avascularized. Collagen accounts for 70% of the dry weight of IVD, and its concentration decreases moving from AF to NP. There are type 1 and type 2 collagen dominance in IVD. Proteoglycans in the structure of the IVD (PG) are divided into two groups according to their interactions with hyaluronic acid (HA). While aggrecan and versican form an aggregate by connecting to HA, small leucine-rich proteoglycans (SLRPs) and perlecan do not form aggreate. In NP the amount of glycosaminoglycan (GAG) is greater than AF and the basic GAG in a mature NP is the keratan sulfate. Elastin, on the other hand, supports the reconstruction of the deformed collagen. All variants of fibronectin from glycoproteins are found in the healthy and degenerate disc. Furthermore, lipids and catabolic enzymes and their specific inhibitors are other structural components of ECM [29].

Changing cell activity changes ECM composition and protein concentration. The degenerate disc loses water and becomes drier. The stress that the disc is subjected during the early stage of IVDD increases type-2 collagen secretion by increasing PDGF, IGF-1, and FGF expression (production). As the process progresses, the amount of Type-2 collagen and the collagen cross links weaken, and the amount of Type-1 and type-10 collagen begins to increase. As a result, fibrosis is seen in NP. In the late stage of IVDD, small leucin-rich proteoglycans (SLRPs) that are not forming aggregates such as biglycan, decorin and fibromodulin, and aggregate-forming proteoglycans such as aggrecan and versican decrease. As a result, the water-holding capacity of the disc decreases and the disc dries [2].

Aggrecan is the most glycolysis-capable member of the PG family. It consists of 3 globular regions, which are G1, G2 and G3. There is a short interglobular domain (IGD) between G1 and G2 regions; while there is a long GAG attachment region between the G2 and G3 regions, which shows interspecies variation. Keratan sulfate and chondritin sulfate bind to this region from different locations, and the sulfation patterns of them are different. The function of aggrecan in the disc is associated with core protein and GAG content. Due to aggrecan's osmotic properties, the disc swells and becomes resistant to compressive loads. Enough amount of aggrecan ensures the continuity of disc volume gained by water holding. Fibers of AF hold the NP with increased volume together by surrounding it. Lacerated AF fibers cannot withstand voluminous NP and herniation occurs. In healthy IVD, aggrecan is mostly found in NP. The concentration of aggrecan increases at the site of mechanical compression and when the compression is eliminated, the disc loses its water and returns to its original state. This condition is called diurnal rhythm and plays a very important role in feeding the disc. Aggrecan affects the

avascular and aneurally retention of the disc. High concentrations of aggrecans suppress neural and vascular tissue formation and prevent disc calcification. As a result, the amount, content, spread area and quality of aggrecan are vital for the normal tissue functioning. With aging, the distribution and amount of aggrecan in the disc changes. While aggrecan concentration decreases due to increased aggrecan degradation in NP, the remaining aggrecan molecules concentrate at the internal boundary of AF, trying to resist compressive charges in this region. Through the aging, aggrecan's interaction with HA decreases and gradually begins to fragment [29, 30].

When ECM production is disrupted in IVD, the catabolic enzymes become upregulated. Matrix metalloproteinases, cause the activation of latent enzymes in addition to direct matrix damage they create. In degenerative IVD, collagenases such as MMP-1, -8, -13; enzymes responsible for denature collagen destruction such as MMP-2, -9; and MMP-3, which is responsible for stromelysin-non-collagen matrix destruction become upregulated. The diversity and complexity of functions of the matrix metalloproteinase enzyme family can be evaluated based on the activity of MMP19. MMP-19 is an enzyme capable of destroying aggrecan, cartilage oligomeric protein (COMP), Type I collagen and Type IV collagen, but it is down regulated in the degenerative disc as opposed to the expected. MMP-19 shows its basic effect as extracellular. By stabilizing vascular structures, it keeps the disc avascular and allows IGF-1 to show its antiapoptotic and antimitotic effect. As a result of the down regulation of MMP-19, vascular ingrowth occurs and apoptosis increases. Aggrecanase and ADAMTS-4, which are members of the ADAMTS family, expression (production) are known to increase in IVD degeneration. These enzymes are responsible for disc turnovers in healthy disc tissue, while they are responsible for catabolic effect in degenerative discs. Cathepsins are proteases that are activated in acidic environment and it has been immunohistochemically shown that Catepsin D, Catepsin L and Catepsin G increase in the regions with focal degeneration in AF in IVDs [2].

6. Proinflammatory cytokine expression in IVDD

Cytokines are small proteins that are both byproducts and stimulants of the inflammatory process and show function as an intercellular signaling molecule. In addition, cytokines play a role in local and systemic response to inflammation and cause pain. IL-1 is a cytokine synthesized in discs and its activity in a healthy disc depends on the balance between activators and inhibitors of IL-1. Upregulated IL-1 in the degenerate disc causes inhibition of ECM production, increase of catabolic enzymes, increase of pro-inflammatory cytokines, and IVD cells to become introceptive to apoptosis. The nociceptive effects of TNF-α in IVD degeneration are more preliminary than catabolic effects similar to IL-1. Studies in the literature have shown that the herniated disc is responsible for the irritation of nerve roots and the progression of free nerve endings from external AF into the disc. In addition, the expression of IL-6 and IL-8 has also been studied, yet its potential effects on IVD degeneration are not fully known. As a result, cytokines are recognized as one of the current therapeutic targets due to their role in inflammation and nociceptive effects [2].

7. Apoptosis in IVDD

Reduction in IVD cellularity along with aging occurs with a mediation of apoptosis. It is known that lack of serum (nutrients), increase in nitric oxide levels and oxidative stress are the main factors that cause apoptosis. While activation of apoptosis occurs with BCL-2 suppression when the cell is subjected to stress, it could also be activated by the activation of death receptors (Fas receptor-CD95) [2].

Cellular and biochemical changes that occur as a result of degeneration in NP cells cause apoptosis in NP cells. Cartilage-specific ECM products are released in apoptosis-treated NP, resulting in loss of disc ECM.

In apoptosis seen in NP, the intrinsic pathway or extrinsic pathway may be used. The intrinsic pathway is basically controlled by Bcl-2, Caspase-3, collagen, and aggrecan, while the extrinsic pathway is regulated by the control of Fas-FasL levels. Bcl-2, the basic element of the intrinsic pathway, has been reported in the literature to

reduce the level of Caspase-3 mRNA during the IVD serum starvation, and to prevent apoptosis by increasing levels of Type 2 collagen and aggrecan expression. In addition, Bcl-2 inhibits IL-1β secretion by binding to nucleotide-binding domain and leucine-rich repeat containing protein-1 (NRLP-1) and shows anti-inflammatory action. In the case of oxidative stress, IL-1β has been shown to induce apoptosis by causing an increase in nitric oxide (NO) in NP cells and a decrease in proteoglycan levels, while hydrogen peroxide (H_2O_2) induces apoptosis by causing a decrease in aggrecan and type 2 collagen mRNA levels [31]. On the other hand, recent studies show that SIRT-1 increases the amount of aggrecan and type 2 collagen by activation of cartilage-specific ECM genes, and protects NP cells from apoptosis by increasing autophagia with the activation of the AKT signaling pathway. In IVD degeneration, NP cells undergo extrinsic pathway-mediated apoptosis by regulation of Fas-FasL levels. *In vitro* studies on NP cells obtained from humans have shown that FasL expression increases apoptosis and reduces angiogenesis by activating FADD (FAS-associated death domain) downstream and Caspase 3. Another study showed that notochordal cells in NP protect NP cells from IL-1β and FasL-mediated apoptosis by reducing Caspase 3 and Caspase 9 activity [32]. Apoptosis is controlled by miRNAs, and there are studies suggesting that abnormal expression of miRNAs is associated with IVD degeneration. In a study examining miRNA expression profiles, 28 miRNA were examined and in particular miRNA-155 was shown to be down regulated in degenerate NP cells. Studies suggest that miRNA-155 induces apoptosis *via* FasL [33, 34]. Recent studies show that miR-27A induces Akt and PI3K-mediated apoptosis *via* the PTEN signaling pathway. On the other hand, in an *in vitro* study using rabbit NP cells, it has been shown that activation of p38MAPK and JNK ½ signaling pathways induced apoptosis while activation of ERK1/2 signaling pathways was play a protective role against apoptosis [35].

AF plays a vital role in ensuring the structural support of the IVD. In apoptosis seen in AF, the intrinsic and extrinsic pathways are used, just as in NP. It has been reported that most AF cells synthesize anti-cytochrome C (anti sitC) and there was not anti-FasL synthesizing cell in the model of IVD degeneration based on mechanical overload in rabbits. This is an indication of the active use of the intrinsic pathway. Additionally it has been reported that Caspase 9 activity increased 24-48 hours after mechanical overload and cell proliferation was inhibited [36]. In another study, it was reported that cyclic loading increased NO secretion, resulting in increased endoplasmic reticulum (ER) stress markers such as CHOP, Grp7 and Caspase 12; mitochondrial depolarization and Caspase 9 activation [32]. There are only a few studies on the extrinsic pathway of apoptosis of AF cells in the literature. Rannou *et al*. in their *in vitro* study using rabbit AF cells, found that serum hunger increased Caspase 3 and Caspase 8 activity, but they did not observe cytosolic sitC secretion [37].

During IVD degeneration, apoptosis is known to occur in the end plates of the cartilage vertebrae and cell density is reduced. After mechanical loading in end plate chondrocytes of rats, phosphorylation of JNK, ERK and p38mapk is increased while sitC release and Caspase 9 and Caspase 3 activation are also increased. As a result, mechanical stress can be said to induce MAPK-mediated mitochondrial apoptosis in end plate cells [32].

8. Autophagy in IVDD

Studies have shown that autophagy is seen in both AF and NP in IVD. In an *in vitro* study on AF and NP cells obtained from rats, high-dose glucose administration was shown to induce autophagy by increasing expression of Beclin 1, LC3 and Atg3, 5, 7 and 12 [32].

Reactive oxygen products (ROS)-mediated autophagy and IVD degeneration have been reported to occur in rat NP cells subjected to compression. In this study, it was determined that the amount of Beclin 1 and LC3B-II increased and autophagosome formation was observed. In addition, Jiang *et al*. have showed that glucosamine in cells exposed to IL-1β and H_2O_2 was shown to protect NP cells by inducing mTOR-mediated autophagy. H_2O_2 is known to stimulate early autophagic response *via* the Erk/mTOR signaling pathway. In addition, the decrease in aggrecan levels in these cells is regressed and the apoptosis caused by IL-1ß is prevented [38]. On the other hand, Chen *et al*. in

their study on NP cells stimulated by hunger stress showed that hypoxia restricts ROS formation and NP cells survive due to increased autophagic ativity. In hypoxia, down regulation of ROS keeps autophagic activity at an appropriate level as a result of AMPK/mTOR inhibition *via* HIF-1a. Nutrient starvation increases the rates of LC-III/LC-I and Beclin-1/β-actin, resulting in an increased autophagosome formation [39].

It has been shown by studies in the literature that autophagy also occurs in AF cells. In AF cell culture obtained from rats, autophagy has been shown to occur in the case of starvation, while apoptosis is increased when autophagy is suppressed by 3-MA. Therefore, it can be said that autophagy in AF cells is protective against apoptosis and IVD degeneration [40].

IVD is fed by means of diffusion through blood vessels in the vertebral end plates. Studies show that autophagy is protective against calcification induced by the intermittent cyclic mechanical strain seen in vertebral end plates. In addition, Beclin and LC3 expression decreases severely as end plate chondrocyte activity decreases with aging [41].

9. MiRNAs in IVDD

Magnetic resonance imaging (MRI), a commonly used method for early diagnosis of IVD degeneration, is sensitive to changes in the water content, collagen concentration and orientation of the disc. Therefore, the diagnosis of decaying and late degenerative stages in IVD degeneration can be made by MRI, although it provides limited information in early diagnosis. In recent years, the role of miRNAs in early diagnosis of degeneration has been a subject of curiosity in the literature. Studies suggest that miR-155, miR-146, and miR-377 are present in the intervertebral disc, and that disruption in their regulation may be an early precursor to degeneration. miRNA array technologies and bioinformatics studies showed that 51 miRNA were dysregulated in NP in patients with IVD degeneration, while 10 miRNA expression was higher in NP than AF [42]. These results suggest to the researchers that the signaling patterns in AF and NP may be different. Expression of miRNAs affected in disc degeneration, can be roughly classified as increasing and decreasing miRNAs. Studies in the literature indicated that miR-155, miR-377, miR-146a, miR-93, miR-193a-3p and Mir-98, which play a regulatory role in anti-inflammatory and anti-catabolic processes, were down regulated. On the other hand, MiRNAs, which play a regulatory role in apoptosis and cell proliferation, such as miR-27a, mir-10b, miR-21, miR-184, miR-494, miR-100, have shown to be upregulated. Furthermore, studies showed that miRNAs played an active role in the development of NP cells and in the catabolism of major matrix components in the early stage of IVD degeneration. Although it has been reported that miRNAs evaluated in tissue and blood can be used in early diagnosis of IVD degeneration and manipulation of expression of miRNAs can be evaluated as therapeutic targets, more studies are needed on this subject [43].

10. Neural wingrowth in IVDD

Free nerve endings that show nociceptive properties in degenerated IVD run from tears in the outer anulus to the inner parts. In the healthy disc, aggrecan acts as an inhibitor and it forms the first point of resistance in AF. Vascular ingrowth, occurs simultaneously with neural ingrowth and also inhibits aggrecan vascular ingrowth [44]. Studies have shown that nerve growth factor (NGF) is released from vascular tissue and trk-A, which is a NGF receptor, is expressed in neural tissue. NGF has been reported to be found only in pain-causing degenerative discs. Brain-derived growth factor (BDGF) shows similar characteristics as NGF. It causes the continuity of sensory neurons in the disc and the formation of pain stimulation. Unlike NGF produced by vascular cells, BDGF is secreted by IVD cells and studies in the literature have shown that BDGF expression increases in degenerated discs [1, 2].

11. Molecular level treatment in IVDD

With a better understanding of the biological basis of IVD degeneration, interest in studies on treatments at the cellular and molecular level is increasing day by day.

Recent studies have focused on the application of cell niches and progenitor cells to degenerate discs, and cell-loaded biomaterials. During the aging process, progenitor cells cannot cluster again and

cause dysfunctional regeneration. NP progenitor cells (NPPCs) are key cells of the regeneration process that provide tissue homeostasis. These cells were firstly described by Sakai *et al*. Depletion of NPPCs has been shown to be associated with degeneration and aging. The AF-specific progenitor cells are the cells like bone marrow-originated mesenchymal stem cell. Studies on the use of these cell groups in cell-based treatments and tissue engineering are still continuing [1, 45].

It is important to increase the amount and quality of proteoglycan in degenerated IVD regeneration. SOX9 is an important transcription factor in Type 2 collagen synthesis. Therefore, whether genetic targeting of SOX9 could be a treatment method was evaluated. In this *in-vitro* study, IVD cells were transfected using adenovirus. In these cells, proliferation and proteoglycan synthesis have been shown to increase [5].

Cell-laden materials are a new area of research that has emerged in recent years in the treatment of degenerate IVD. It is theoretically believed that providing 3-D microenvironment will provide more effective regeneration. The choice of stem cells to be used in the IVD treatment is very important and scaffolds need to be resistant to mechanical loads. Mesenchymal stem cells (MSCs) originating from bone marrow and adipose tissue are often preferred. Studies have shown that MSCs and NP communicate bidirectionally in co-cultures. It has been reported that exosome-mediated communication has been established while providing anti-inflammatory and anti-catabolic effects with bioactive factor secretion. MSCs communicate with exosomes *via* miRNA-21 to protect NP cells from apoptosis and reduce degeneration. Fibrin, alginate, silk, gelatin, poly lactic-co-glycolic acid (PLGA), mucosa of the small intestine, hyaluran gel, and chitosan can be used as a scaffold. Also, injectable collagen hydrogels are used. These gels can be combined with MSC and cause minimal damage to the AF surrounding the NP during application. Recent studies have focused on the production of scaffolds biphasically in a way that mimics both the NP and the AF [5, 46].

Conclusion

The main goal to prevent IVD degeneration is to repair the abnormal microenvironment and restore the changing cell phenotype. Therefore, a better understanding of the biological process involved in degeneration is the basis for producing therapeutic solutions. Complex molecular interactions and the unique microenvironment of IVD are among the factors that complicate this solution. A better understanding of the degeneration process will enable the cellular and molecular-based treatments to be implemented in the future.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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