

Molecular Mechanisms of Intervertebral Disc Degeneration

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Abstract:

Intervertebral disc degeneration is a well-known cause of disability, the result of which includes neck and back pain with associated mobility limitations. The purpose of this article is to provide an overview of the known molecular mechanisms through which intervertebral disc degeneration occurs as a result of complex interactions of exogenous and endogenous stressors. This review will focus on some of the identified molecular changes leading to the deterioration of the extracellular matrix of both the annulus fibrosus and nucleus pulposus. In addition, we will provide a summation of our current knowledge supporting the role of associated DNA and intracellular damage, cellular senescence's catabolic effects, oxidative stress, and the cell's inappropriate response to damage in contributing to intervertebral disc degeneration. Our current understanding of the molecular mechanisms through which intervertebral disc degeneration occurs provides us with abundant insight into how physical and chemical changes exacerbate the degenerative process of the entire spine. Furthermore, we will describe some of the related molecular targets and therapies that may contribute to intervertebral repair and regeneration.

Keywords:

Intervertebral disc, nucleus pulposus, annulus fibrosus, degeneration, cellular senescence, oxidative stress, inflammation, DNA damage

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Introduction

Over the past century, life expectancy has continued to increase, with an estimated 0.5 billion people worldwide aged 65 or older, and is projected to reach 1.5 billion people by 2050¹. It is well documented that low back and neck pain increase with age, and are the 1st and 4th leading causes of disability, respectively²⁻⁴. Back pain is one of the most common orthopaedic conditions, and it accounts for 149 million missed worked days annually with an estimated loss of \$90 billion in the United States, secondary to related disability⁵. Specifically, intervertebral disc degeneration (IDD) plays a substantial role in the generation of back pain, involving more than 50% of all cases⁶⁻⁸. In the elderly, decreased mobility is a confirmed predictor of loss of independence and increase in mortality. This makes the preservation and understanding the degeneration of healthy joints, especially intervertebral discs (IVDs), vital in our aging population⁹⁻¹¹.

IDD refers specifically to the functional as well as the structural failure of the disc related to its cellular pathogenesis and extracellular matrix (ECM) modifications¹². Causes of disc degeneration include aging, injury, genetics, and en-

vironmental factors such as smoking, or a combination¹³⁻¹⁹. IDD is not exclusive to the older population, although the aged population and cases with degenerated disc appear to share similar changes¹⁸⁻²². The IVD is one of the first tissues to undergo degeneration in adults, with an average onset in the second decade of life and is known to be influenced by a combination of genetic, biological, aging, and physical chemical changes^{20,21,23}. The exact pathophysiology of degeneration has not yet been completely delineated, but this review will focus on some of the known cellular changes and molecular pathways leading to IDD.

Characteristics of IVD

IVD are situated between two cartilaginous end plates of adjacent vertebra of the spine. They help in constructing a polyaxial cartilaginous joint that allows for flexibility, while also providing support^{24,25}. The IVD is made of two major components: an annulus fibrosus (AF) and a nucleus pulposus (NP). The AF is composed of highly organized rings known as lamella, rich in collagen type I that surround the NP. These collagen fibers of the AF arranged in alternating

angles of approximately 30-60 degrees serve to restrain the NP's circumferential stress during bending and twisting, while preventing lateral displacement and collapse of the NP^{24,26}. The NP that is centrally located, is composed of approximately 80% water caused by the osmotic gradient of proteoglycans, primarily aggrecan, which consists of highly anionic glycosaminoglycan side chains of chondroitin and keratan sulfate. The NP also contains a loose and randomly arranged framework of sparse collagen type II and elastin fibers, which in combination with the proteoglycan, serves as a primary shock absorber defusing compressive stresses into the end plates and the annulus fibrosus^{24,27}. In addition, the cartilage end plates, composed primarily of cells with a morphology similar to that of chondrocytes that produce a hyaline matrix, serve as an interface between the adjacent vertebral body and IVD²⁸. As discs are predominantly avascular, the cartilaginous end plates are particularly important for nutrient and metabolite delivery to the disc by diffusion through its rich blood supply. This tenuous perfusion and low cellularity makes the disc susceptible to injury, in addition to the limited repair and accumulation of dysfunctional tissue^{29,30}.

Degenerative changes lead to an altered spinal mechanical function with loss of elasticity and an increased stiffness³¹. Maintaining stability in the multiaxial motion of the spine is a complex interaction between all components of the spine working together counteracting compressive and tensile stress³²⁻³⁹. Degenerative changes of the spine that exacerbate the IDD process include facet cartilage erosion, weakening of supportive ligaments such as posterior and anterior spinal ligaments, and physiological muscle atrophy such as fatty infiltration⁴⁰⁻⁴³. Cells isolated from annular-puncture induced degenerative discs have shown unfavorable altered responses to mechanical loading and exaggerated response to inflammatory stimulus⁴⁴. Studies in rats have indicated an increased IDD when they are imposed to an upright stance, which presumably alters the disc loading⁴⁵. Other analyses have also made associations with long-term loading leading to loss of spinal mobility and decrease in disc height⁴⁶⁻⁴⁹. This loss of anatomical structure is the end result of damage-induced apoptosis, cellular senescence, and pathologic alterations to metabolism with the subsequent loss of standard cellular function and ECM structural support⁵⁰⁻⁵³.

It is known that the ECM of the NP and AF are vital to the biomechanical function and physiological state of the IVD^{54,55}. The ECM of the NP contains only approximately 1% cells, based on its total volume. These cells produce structural matrix proteins, cytokines, growth factors, and proteases. All these products serve to maintain the balance between ECM production and degradation, while preserving biomechanical function^{54,55}. Progressive proteoglycan loss with coinciding changes of low oxygen tension, free radical formation, changes in pH, and increased activity of aberrant proteolytic enzymes, leads to loss of disc height and compressive resistance. These changes result in a progressive disruption of the spinal motion leading to spinal instability

and mechanical stress, compounding the degenerative process^{56,57}.

Pathology of IDD

IDD has been defined by the loss of biologic structural support and function through the accumulation of degenerated molecules leading to inappropriate cellular reactions that exacerbate the pathogenesis⁵⁸. These changes lead to a fibrous disc with dehydration of the NP, loss of disc height, and accumulation of granular debris. In addition, there is neovascularization in the peripheral AF and an increase in the number and size of fissures^{20,59,60}. The cartilaginous end plates also undergo ossification and thinning with subsequent microfractures, bone sclerosis, and reduction in the blood supply. The reduction in perfusion followed by a decrease in nutrient supply and accumulation of cellular waste, all lead to an increase in the acidic environment that can negatively affect cell function⁶¹⁻⁶⁵. The molecular mechanisms contributing to IDD that have been elaborated below result in cellular dysfunction through the accumulation of damaged proteins, altered intercellular communication, deregulated nutrient sensing, mitochondrial dysfunction, DNA damage, and interruption of tissue regenerative capacity with loss of progenitor cells (Fig. 1)⁶⁶⁻⁷⁰.

Degeneration of ECM in the NP

Typical ECM molecules in NP are proteoglycan aggregates, consisting of core protein, link protein, sulfated glycosaminoglycan (S-GAG), and hyaluronan, all encased within a collagenous fiber network. This ECM provides higher osmotic pressure to create swelling pressure capable of withstanding compressive loading stresses⁷¹. As discs degenerate and simultaneously dehydrate, the majority of the matrix evolves with decreased amount of GAG, aggrecan and elastin, and increased amounts of collagen and collagen crosslinking, fragmented aggrecan, and advanced glycation end productions (AGEs)⁷³. This decreased amount of GAG chain lengths and link protein levels, while hyaluronan levels increase, are all thought to be a result of proteolytic and glycolytic damage⁷¹⁻⁷³. Versican is another hyaluronan-binding protein that undergoes deterioration with the degeneration of discs. These non-aggregating proteoglycans appear to have a reduced functional ability compared to intact aggregates based on their size, charge density, spatial rigidity, and matrix interactions⁷¹⁻⁷⁶. Syndecan-4 is a transmembrane heparan sulfate proteoglycan that also plays a significant role in IDD through intracellular signaling⁷⁷⁻⁷⁹. Specifically, dysregulated activities of syndecan-4 mediate matrix and aggrecan degradation by a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) function and matrix metalloproteinases (MMPs) expression e.g., MMP3⁷⁷⁻⁷⁹. The small leucine-rich repeat family of proteoglycans (SLRPs) typically play a significant role in structural support of the ECM. But with degeneration, SLRPs ap-

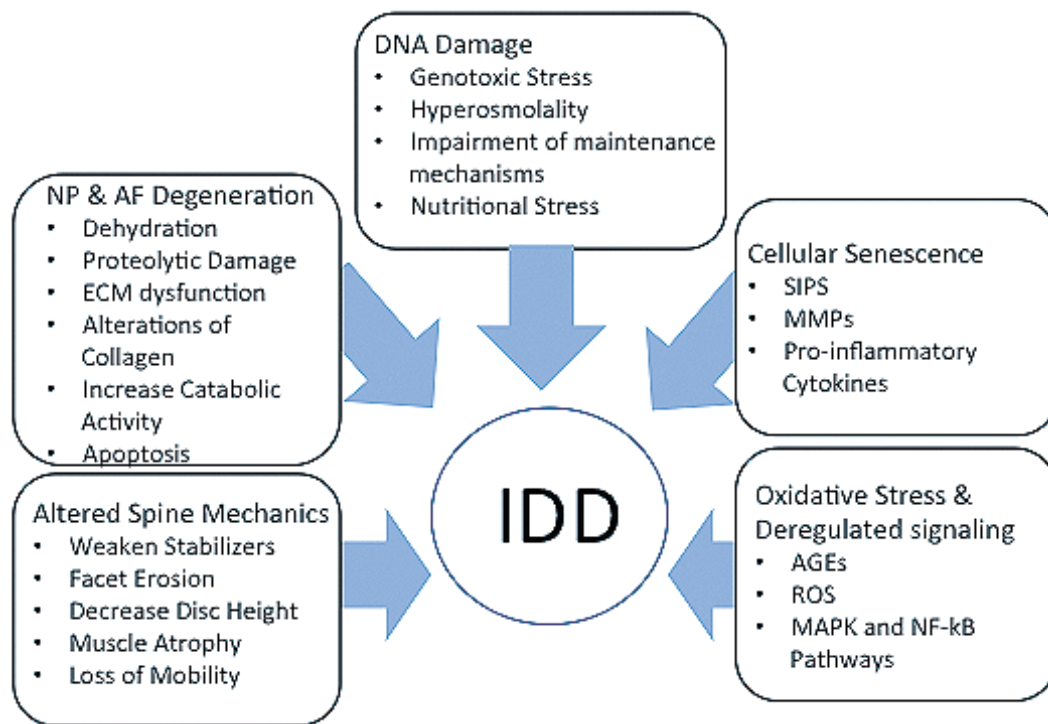


Figure 1. Factors Contributing to Intervertebral Disc Degeneration.

Demonstration of the multifactorial contributions to Intervertebral Disc Degeneration (IDD) including alterations of spine mechanics, Nucleus Pulposus (NP) & Annulus Fibrosus (AF) degeneration, DNA damage, Cellular Senescence and Oxidative Stress & Deregulated signaling, Extracellular Matrix (ECM), Stress-induced Premature Senescence (SIPS), Matrix Metalloproteinases (MMPs), Advanced Glycation End Products (AGEs), and Reactive Oxygen Species (ROS).

pear to undergo proteolytic damage leading to a loss of their GAG content, negatively affecting the structural support of the ECM⁷¹. Specifically, biglycan and decorin, dermatan sulfate proteoglycans, interact with collagen type IV and type I/II respectively⁷¹. Others include disc collagen fibril associated SLRPs, feratin sulfate-containing fibromodulin and lumicans, which decrease and increase with age respectively, are thought to have an effect on disc matrix structure^{71,80}. As mentioned above, discs contain a rich collagen network containing 80% of Type I and II, and 10-20% of type IV⁷¹. This collagen structure also undergoes proteolytic damage from dysregulated collagenase activity, resulting in weakened mechanical strength and the formation of non-enzymatic crosslinks between basic amino acids of collagen and reducing sugars^{81,82}. As elaborated below, this proteolytic damage results in increased oxidative stress through AGEs, which increase throughout the disc with degeneration impairing collagen fibril formation^{83,84}. To further support this concept, oxidative stress has been shown to decrease disc elasticity and alter the formation of secondary and tertiary structure of collagen molecules in mice models^{31,83-85}. This increases their susceptibility to cleavage by MMPs, resulting in a deterioration of the biomechanical strength and structural integrity³¹.

Degeneration of ECM in the AF

Although the exact mechanism is not completely deline-

ated, it is assumed that mechanical load, oxidative stress, genetics, inflammation, and DNA damage also contribute to a rupture of the AF through apoptosis and altered integrity with herniation of the NP^{86,87}. Homeostasis in ECM, through regulation of catabolic and anabolic functions, have been shown to play an essential role in maintaining the integrity of the AF structure⁸⁸. Specifically, with disc degeneration, this homeostasis is disrupted with excessive matrix catabolic activity^{89,90}. Two apoptotic pathways in mammalian cells, the mitochondrial pathway and Fas/FasLigand pathway have both been shown to be involved in AF cell apoptosis regulation⁹¹⁻⁹³. Islet amyloid polypeptide (IAPP), identical in composition to amyloid protein, participates in the regulation of glucose metabolism, reactive oxygen species, apoptosis, and inflammation⁹⁴⁻⁹⁸. It has been shown that the expression of IAPP, the calcitonin receptor, and receptor activity modifying protein decrease in AF cells with IDD⁹⁹. This decrease in IAPP induces an increase in reactive oxygen species and intracellular calcium concentration along with a decrease in MMPs, all leading to cellular death⁹⁹. Human samples, mouse models, and AF culture experiments have demonstrated that mechanical overload-induced IDD is mediated through the mitochondrial apoptotic pathway in AF cells⁹². In addition, abnormal loading on intervertebral disc resulted in a thickening and stiffening of collagen fibrils of the AF at the microscale and alteration of the collagen fibrils at the nanoscale. These changes likely lead to a change in the me-

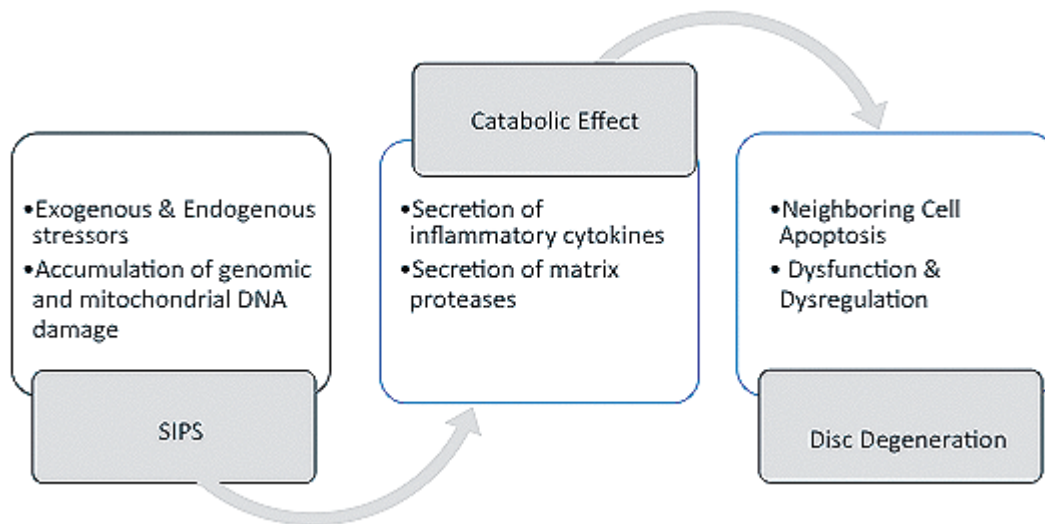


Figure 2. Stress-Induced Premature Senescence Catabolic Effects. Illustrating the stepwise formation and subsequent catabolic effects of stress-induced premature senescence (SIPS).

chanical and physiological homeostasis¹⁰⁰.

DNA Damage

Proteins and other macromolecules can be degraded and replaced; however, this is not the case with DNA⁶⁶. DNA requires repair mechanisms, and despite these rigorous mechanisms, cells still accumulate damaged DNA over time. It has been shown that inherited defects in genome maintenance mechanisms can lead to a variety of diseases with accelerated degeneration⁶⁷. For example, DNA repair-deficient *Ercc1/D* mice exhibit early onset of key IDD features, including loss of matrix proteoglycan, reduction in disc height, and increased cellular senescence^{57,69}. DNA damage as a cause of IDD is further supported by exposure to genotoxic stress in humans and mice, including ionizing radiation and tobacco smoking that accelerate disc degeneration¹⁰¹⁻¹⁰⁴.

Hyperosmolality has also been shown to induce DNA damage, through activation of the ATM/p53/p21^{WAF1} pathway leading to the hypophosphorylation of the pRb protein and cell cycle arrest in the G1 phase of the cell cycle¹⁰⁵. An increase in osmolality has been shown to lead to chromatin changes and DNA damage¹⁰⁶. Although it is still unclear what level of hyperosmolality in NP is needed to induce DNA damage, cells of the NP are exposed to hyperosmolality levels up to 500 mOsm/kg H₂O *in vivo* compared to < 300 mOsm/kg H₂O in the majority of other tissues¹⁰⁵.

Nutritional stress is another factor that can affect disc tissue leading to degeneration and cellular damage. Because discs are mostly avascular, the nutritional environment resides narrowly above the cellular requirements in the NP with low oxygen and glucose¹⁰⁷⁻¹⁰⁹. In addition, the disc maintains an acidic environment with high concentrations of lactate¹¹⁰. It has been shown that low O₂ and low pH conditions can cause DNA damage and reduce proteoglycan and

collagen synthesis¹¹¹. This extreme environment puts the disc at risk to any additional stresses^{112,113}.

Cellular Senescence

Cellular senescence, an important mechanism for the limiting of proliferation of potential cancer cells, has been described by cessation of cell proliferation due to the critical shortening of telomere length following successive replicative cell cycles^{114,115}. Another type of cell senescence, stress-induced premature senescence (SIPS), results from accumulation of genomic and mitochondrial DNA damage. In addition, SIPS cells also acquire a senescence associated secretory phenotype leading to high amounts of secreted inflammatory cytokines and matrix proteinases causing a catabolic effect on neighboring cells and on the ECM, promoting degeneration (Fig. 2)¹¹⁶⁻¹²⁰. This pathologic disc matrix process is further supported by the observed amount of senescent cells which have been measured by an increased expression of senescent markers including senescence-associated β-galactosidase, p16^{INK4A}, and decreased telomere length. In addition, p16^{INK4A} has been shown to have a positive correlation with the expression of matrix metalloproteases e.g., MMP-13 and ADAMTs-5¹²¹⁻¹²⁵. Further supporting DNA impairment can lead to disc cellular senescence is the observation of elevated cellular senescence in the disc of DNA repair-deficient *Ercc1/D* genotoxin exposed mice^{14,101,102}. Also, further supporting SIPS as a potential cause of disc degeneration are *in vitro* cell culture studies using H₂O₂ to simulate oxidative stress leading to DNA damage with a transformed, catabolic phenotype¹²⁶⁻¹²⁸. This leads to increased matrix degradation with high levels of MMPs and pro-inflammatory cytokines, with IL-1 as a predominant cytokine in the pathogenesis of IDD¹²⁶⁻¹²⁸.

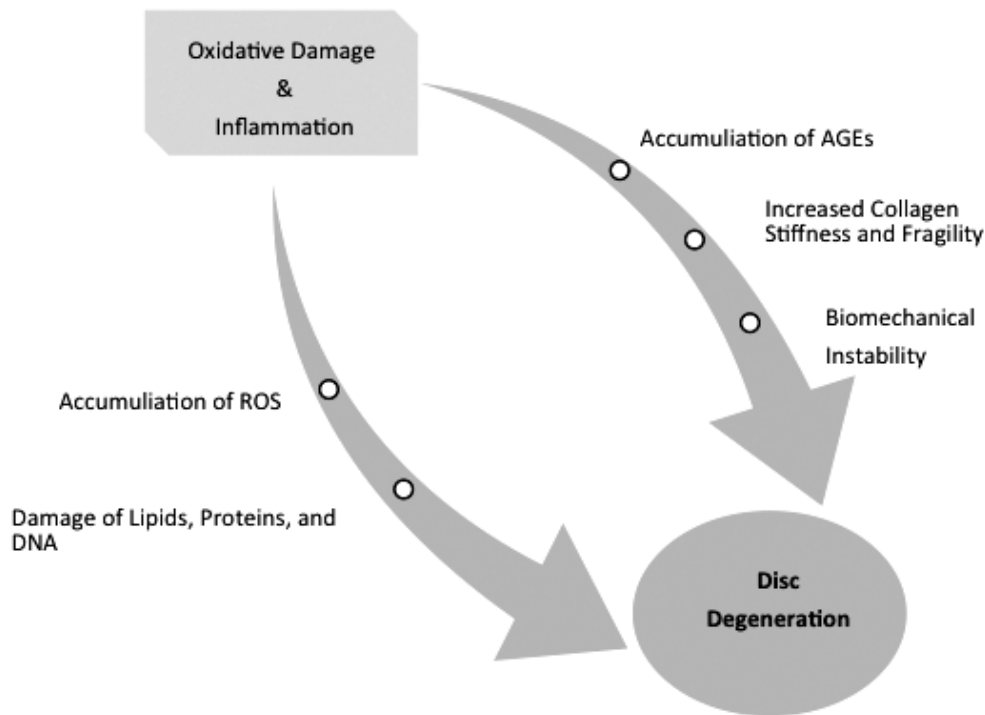


Figure 3. Pathways Contributing to Intervertebral Disc Degeneration. Demonstrating two pathways - inflammation and oxidation - that contribute to intervertebral disc degeneration. Reactive Oxygen Species (ROS), Advanced Glycation End Productions (AGEs).

Oxidative Stress and Deregulated Signaling

Oxidative inflammation is thought to be one of the main causes of molecular damage through exogenous and endogenous stressors (Fig. 3)^{103,104}. The advanced glycation end productions (AGEs) including pentosidine and carboxymethyllysine, further support oxidative damage as a source of molecular damage^{80,129}. In the example of pentosidine, which cross links collagen molecules, there is evidence to suggest that it may play a role in increased collagen stiffness and fragility affecting the biomechanics^{80,82,129,130}. Also, oxidative post-translational modifications, *i.e.* protein carboxylation, have also shown an increase in protein fragmentation and aggregation leading to increased disc stiffness in a mice model²¹. Although disc cells reside in relative low oxygen tension environments, reactive oxygen species (ROS) are still generated through oxidative phosphorylation¹³¹. As disc degeneration and AF develop fissures with associated neovascularization, this leads to an increase in oxygen tension on otherwise hypoxic cells, further supporting the oxidative stress on the disc environment¹³¹.

ROS is detrimental to the structural and functional homeostasis of the disc by causing the damage of lipids, DNA, and proteins¹³². Hydrogen peroxide, identified in human NP tissue, along with peroxisomes detected in AF cells *in vitro*, further support discs cells as ROS generators^{133,134}. With mitochondrion dysfunction as a known source of excessive ROS production, the role of mitochondrion-dependent ROS has been described in various disc cells including human and rat NP and AF cells¹³⁵⁻¹³⁹. And more specifically, exces-

sive ROS production has been reported in degenerative discs of rats¹⁴⁰. Several studies have shown hydrogen peroxide to down regulate the expression of collagen type II and aggrecan in both human and rat disc cells.^{126,141-145} In addition, pro-inflammatory cytokines leading to ROS overproduction have been shown to suppress matrix synthesis and increase the expression of matrix degradation proteases in human and rat disc cells^{126,137,141,142,145,146}. ROS is also known to form positive feedback loops that enhance ROS production in disc cells^{126,136,141,145-150}.

Inappropriate and deregulated signaling (*e.g.* NF- κ B and MAPK pathway) in addition to abnormal variations in cell fate (*e.g.*, cellular senescence), dysregulated nutrient sensing, and mitochondrial dysfunction has also been reported in deteriorating tissues including IDD⁷⁰. Elevated levels of the pro-inflammatory cytokine TNF- α , matrix proteoglycan degradative products, MMPs-including MMP-3, ADAMTS-5, have also been reported in degenerative disc suggesting an imbalance of matrix homeostasis¹⁵¹⁻¹⁵⁴. Other phenotypic and functional changes that likely result from irregular responses to damage include depletion of disc matrix proteoglycan, tissue dehydration, and altered disc load distribution. These distorted conditions lead to elevated necrosis, apoptosis, and senescence^{112,121,157,158}.

Potential sources of oxidative stress and DNA damage include inflammation and high glucose induced stress, as in diabetes¹⁵⁹. NF- κ B signaling is known to play a critical role in a cell's response to inflammation and damage, and an increase in its activity has been connected to IDD with accumulated oxidative stress^{129,139,159,160}. Pharmacologic and genetic

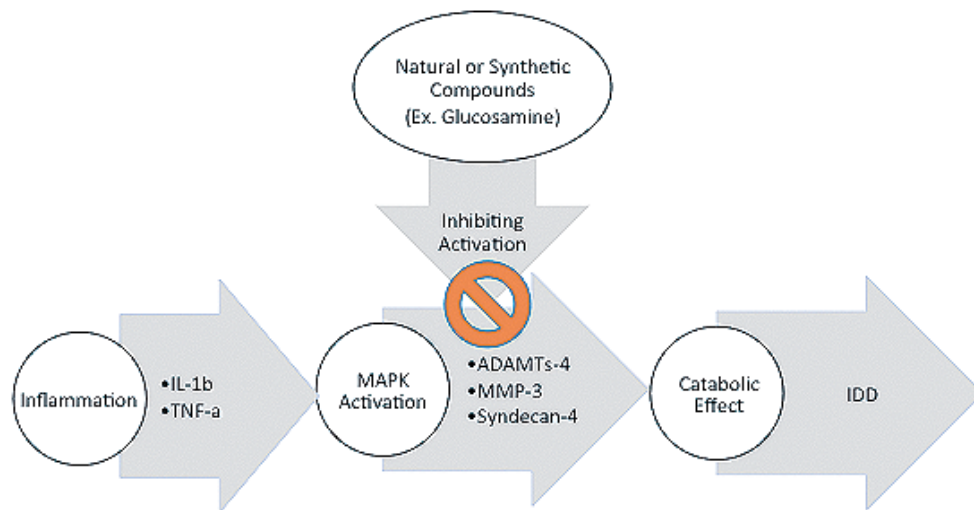


Figure 4. Activation of MAPKs Pathway.

Illustrating one of the roles that inflammation and MAPK pathway play in contributing to intervertebral disc degeneration (IDD), and a possible method of hindering IDD by preventing the activation of the MAPK pathway.

systemic inhibition of NF- κ B activity has been shown to reduce associated IDD in a mouse model¹⁴¹). Symptomatic discs have been shown to have higher levels of pro-inflammatory cytokines, TNF- α , IL1 β , IL-6, and IL-8, which are considered to be associated with the NF- κ B pathway^{127,128,156,162,163}). Mitogen-Activated Protein Kinases (MAPKs), a family of signal transduction pathways, allow cells to respond to extracellular inputs, including inflammatory cytokines and environmental stress^{164,165}). Specifically, the expression of p38 MAPK, a subfamily of MAPK, has been described in senescent AF cells¹⁶⁶). Many components of the catabolic process (ex. MMPs, ADAMTs, COX-2, PEGE2, iNOS, etc.) are dually regulated by MAPK and NF- κ B, showing the overlap between the two signaling pathways¹⁵⁵). As mentioned above, pro-inflammatory cytokines (IL-1 β and TNF- α) activate MAPK pathways leading to catabolic molecules such as ADAMTs-4, MMP-3, and syndecan-4^{146,167-169}). This relationship may represent a tool to ease disc degeneration by blocking MAPK activation either with synthetic or natural compounds such as glucosamine (Fig. 4)^{146,170}). Another characteristic feature of disc degeneration is the formation of cell proliferation clusters in damaged areas, which is thought to be partly due to the overexpression of growth factor and receptors^{171,172}). Indicating another role of MAPK in disc degeneration is the fact that growth factors such as PDGF, IGF-1, and bFGF stimulate cell proliferation through extracellular signal-related kinases, another subfamily of MAPKs^{173,174}).

Conclusion

The distinctive disc function that maintains stability and motion of a mechanically loaded structure in nutrient-poor, acidic, and hypoxic environment, offers an extraordinary opportunity to discover novel disc degeneration mechanisms.

As noted above, disc degeneration is a systemic process that does not occur in isolation and is influenced by the degeneration of adjacent spinal structures and systemic factors. Therefore, degeneration research of the whole spine is an imperative for the future direction of spine degeneration and regeneration research. And more specifically, therapeutic interventions will need to target the early phases of disc and spine degeneration prior to functional failure. Several recent studies have strengthened our understanding of intervertebral repair and regeneration. The *in vivo* performance of an acellular disc-like angle ply structure that mimics the native intervertebral disc was studied in a rat tail model, and it was noteworthy that native cells were able to infiltrate the angle ply structure while promoting tissue formation and restoring some biomechanical behaviors¹⁷⁵). Mesenchymal stem cell injections, another way to induce regeneration, have been shown to increase the amounts of GAG accumulation when injected early into injured rat intervertebral discs¹⁷⁶). Inorganic polysphates have been shown to promote proteoglycan accumulation in NP cells, even under hypoxic conditions¹⁷⁷). In addition, bone morphogenetic protein 2 (BMP-2) and BMP-7 have been shown to stimulate the NP to produce aggrecan and collagen II *in vitro* and in organ culture models¹⁷⁸). And as mentioned above, NF- κ B inhibition results in the largest reduction of IL-1 β , a pro-inflammatory catabolic cytokine¹⁷⁹). With disc degeneration being a significant risk factor for associated pain and disability, the prevalence will continue to rise with our growing and aging population. This should help stimulate further research to develop safe and effective treatments for intervertebral degeneration and pathology.

Conflicts of Interest: The authors declare that there are no relevant conflicts of interest.

Author Contributions: Sean M. Rider wrote and prepared the manuscript. All authors have read, reviewed, and approved the article.

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