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SYMPOSIUM: CURRENT APPROACHES TO THE MANAGEMENT OF LUMBAR DISC HERNIATION

Molecular Basis of Intervertebral Disc Degeneration and Herniations: What Are the Important Translational Questions?

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Abstract

Background Intervertebral disc degeneration is a common condition with few inexpensive and effective modes of treatment, but current investigations seek to clarify the underlying process and offer new treatment options. It will be important for physicians to understand the molecular basis for the pathology and how it translates to developing clinical treatments for disc degeneration. In this review, we sought to summarize for clinicians what is known about the molecular processes that causes disc degeneration.

Results A healthy disc requires maintenance of a homeostatic environment, and when disrupted, a catabolic cascade of events occurs on a molecular level resulting in upregulation of proinflammatory cytokines, increased degradative enzymes, and a loss of matrix proteins. This promotes degenerative changes and occasional neurovascular ingrowth potentially contributing to the development of pain. Research demonstrates the molecular changes underlying the harmful effects of aging, smoking, and obesity seen clinically while demonstrating the variable influence of exercise. Finally, oral medications, supplements, biologic treatments, gene therapy, and stem cells hold great promise but require cautious application until their safety profiles are better outlined.

Conclusions Intervertebral disc degeneration occurs where there is a loss of homeostatic balance with a predominantly catabolic metabolic profile. A basic understanding of the molecular changes occurring in the degenerating disc is important for practicing clinicians because it may help them to inform patients to alter lifestyle choices, identify beneficial or harmful supplements, or offer new biologic, genetic, or stem cell therapies.

Introduction

Low back pain is a chronic, expensive, common medical problem. Over 80% of adults will report back pain at some point in their lives, and it is the most common cause of limited activity in people younger than 45 years of age [4]. Back pain is the second most frequent cause for visits to the hospital, fifth most common reason for admission to the hospital, and the third most common cause of surgical procedures [4, 91]. The economic effects of back pain are also measured in lost productivity because back pain is the most common cause of absence from work [22], and in the United States, total health expenditures by individuals with back pain is estimated to be approximately USD 91 billion [53]. Because of the high personal and economic burden of back pain, it is critical that treating physicians understand the basic physiology of the intervertebral disc and how the disruption in disc homeostatic balance can negatively

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affect patients during the course of intervertebral disc degeneration.

Physicians treating patients with spine-related disorders often face difficult questions from patients about the cause and anticipated course of pathology and treatment options. As such, it is important for physicians also to be able to understand the basic biology of intervertebral disc degeneration. Physicians who understand and successfully communicate the pathology behind changes in the disc may help patients experiencing pain and disability from herniated discs or severe disc degeneration. For example, knowledge of the associations between lifestyle habits and intervertebral disc degeneration can help physicians guide patients to make changes in social habits including diet, exercise, and substance use that could alter the course of spine pathology and prevent future disability. Additionally, many treatment plans are emerging that may alleviate back pain and spine pathology, including oral therapies, molecular protein-based therapies, and viral or stem cell use. Physicians will need to understand the scientific background of these studies to appropriately discuss their application as well as risk and benefits with patients.

We performed this review to provide answers from the basic science perspective to the questions we perceive to be most relevant to clinicians, because these topics are likely to become increasingly relevant in this era of novel biologic therapies.

How Is the Normal Disc Composed?

The intervertebral disc is primarily composed of cartilaginous vertebral endplates (EP), annulus fibrosus (AF), and nucleus pulposus (NP) [52]. The endplates are at the superior and inferior aspects of the disc. EPs are cartilaginous structures distinct from articular cartilage found elsewhere in the body [10, 42]. The AF is a thick, dense structure, which is divided into the outer and inner annulus. The outer annulus is composed of organized, collagenous concentric lamellae, which is primarily composed of fibroblast-like cells that produce mainly type I collagen. The inner annulus is more fibrocartilaginous and is composed of both type I and type II collagen. The AF experiences the tensile strain of the spine. Encased within the AF is the NP. Healthy NP is gelatinous and primarily made of proteoglycans in a loose network of type II collagen. Proteoglycans have a core protein with radiating glycosaminoglycan chains of keratin sulfate and chondroitin sulfate. The cumulative hydrophilic nature of these proteins provides the NP with hydrostatic properties allowing it to counteract compressive loading of the spine [94].

The NP is highly cellular with little proteoglycan during early development, but in the adult, the NP is rich in extracellular proteoglycans with few cells. In infancy, many of the cells in the NP are large vacuolated cells, which are believed to be of notochordal origin. The proportion of these notochordal cells is greatly diminished with the concomitant increase in the chondrocyte-like cell in adult NP tissue. Notochordal cells play an important role in stimulating glycosaminoglycan and proteoglycan synthesis by NP cells and may also serve as progenitor cells to preserve and control the number of NP cells [74]. As a result of these anabolic effects, loss of notochordal cells from aging or disease states has magnified effects with eventual changes overall disc function and composition [75].

How Are Changes of the Intervertebral Disc Classified?

Although various definitions of changes seen in the intervertebral disc exist, one commonly accepted classification of nomenclature is provided through collaborative efforts of a combined task force of the North American Spine Society, American Society of Spine Radiology, and American Society of Neuroradiology [20].

This group has classified degenerative and/or traumatic changes of the disc into a broad category including herniation and degeneration. Degeneration is classified as any fibrosis, narrowing of the disc height, bulging of the annulus, fissuring, defects in the EP, mucinous changes in the annular fibers, and osteophytes at the facet joints. Herniation is specifically defined as a "localized displacement of disc material beyond the limits of the intervertebral disc space" [20]. This classification encompasses all of the components of the disc (NP, AF, cartilage). The disc space is defined by the EP of the vertebral bodies and peripherally by the outer edge of the vertebral ring apophyses except osteophytes. Herniations are further classified as localized (< 25% disc circumference), broad-based (25%–50%), and circumferential (50%–100%) [20].

Why Do Discs Herniate?

Disc herniations can be a result of a sudden injury such as trauma or progressive degenerative changes as a result of the cumulative effect of repetitive stresses. Biomechanical studies have demonstrated that failure rarely occurs by axial compression alone and that flexion and torsion are also important contributors to the development of herniations in both acute trauma and degenerative changes [26, 40, 97].

In situations of chronic stress, a characteristic pattern of cellular changes occurs in the development of disc degeneration. The AF undergoes myxomatous degeneration and cyst formation, causing swelling of the AF fibers, leading to disruption and disorganization of fiber bundles [26, 40]. Similar to the AF, the NP also undergoes changes of dehydration, fibrosis, and necrosis. As a result of these degenerative changes, repetitive excessive loading can cause the NP material to herniate. Herniation of the NP content can occur through previously developed annular tears that have formed from the weakened AF, fissures in the EP, or disruptions at the EP junction [72]. Disc herniation can occur from both acute trauma and chronic degenerative changes with the latter as a result of catabolic changes over time, which leads to weakening of the disc structures.

If the Disc Is Aneural and Avascular, Why Does It Cause So Much Pain in Patients?

Studies disagree about whether and how the disc is able to cause pain, and as such, many investigations suggest possible connections. The young, healthy intervertebral disc is mainly avascular and aneural except for the outer one-third of the AF [56]. As aging occurs, disc matrix proteoglycan, a known inhibitor of vascular ingrowth, decreases and degenerative changes such as microfissures stimulate growth of innervated, vascularized granulation tissue, which propagates along fissures through the outer AF to the inner AF and NP [43, 70]. The vascularized granulation tissue permits the migration of macrophages and mast cells into the area as well. Collectively, the granulation tissue and the infiltrating cells express growth factors and proinflammatory cytokines, including fibroblast growth factor, transforming growth factor β1 (TGF-β1), interleukin (IL) 1β, and tumor necrosis factor α (TNF-α) [1, 43, 70].

The presence of macrophages and mast cells propagates the inflammatory cascade and may contribute to the development of pain. Macrophages increase the levels of multiple inflammatory mediators, especially IL-6 and IL-8, nitric oxide, TNF-α, and IL-1β [36]. The levels of these cytokines have correlated with pain intensity in patients, and persistent activation of sensory fibers upregulates nitric oxide synthase, thereby increasing the level of nitric oxide, suggesting a possible positive feedback loop of pain generation [39].

In addition to the development of vascularized granulation tissue, neuronal tissue develops. Degenerated disc cells secrete brain-derived growth factor, which promotes neuronal development [23]. The release of proinflammatory cytokines IL-1β and TNF-α from the surrounding tissues also upregulates nerve growth factor and expression of its receptors on the disc tissue [1]. Progressively small nerve fibers form along with the granulation tissue [70]. Nerve growth factor promotes the collateral sprouting of additional peripheral sensory nerves into the inner AF and the NP, increases nerve survival, and increases the action and sensitivity of nociceptive sensory neurons [1, 5, 15, 17, 24, 25, 46, 105].

Although no definitive mechanism has demonstrated a direct link between the intervertebral disc and pain, the changes in vascularization and neutralization of the disc that occur during the degenerative cascade suggest that there is a correlation between these changes and back pain (Fig. 1).

How Does Aging Have an Impact on Disc Degeneration and Herniations?

Aging and Oxidative Stress

With aging, there is progressive reduction in the number of vascular channels in the vertebral EP [9]. These channels are the primary source of diffusion of nutrients into and waste products out of the intervertebral disc. In addition to this loss of diffusion, aging increases the levels of proinflammatory cytokines such as IL-1, TNF-α, damaged proteins, mitochondrial dysfunction, DNA damage, and dysfunctional mechanisms of normal cell reparative mechanisms, all of which lead to disc matrix homeostatic imbalance [18, 21].

There is growing evidence that aging of the disc is related to damage from oxidative stress. Oxidative stress is a known driver of cellular senescence and apoptosis. Higher levels of oxidized proteins and transcription factors activated by oxidative stresses have been found in older discs compared with young discs [64]. Other evidence of age-related oxidative damage in the disc is the presence of advanced glycalation endproducts, which are molecules produced by nonenzymatic glycosylation and oxidation of proteins and lipids [6, 83]. The most common advanced glycalation endproducts in the disc are pentosidine and carboxymethyl-lysine. Pentosidine crosslinks collagen molecules and increases collagen stiffness as well as decreasing the synthesis of matrix proteins and proteoglycans. [6, 16]. Additionally, notochordal cells, the cells that persist in the NP, which are of notochordal origin, are greatly affected by oxidative stressors and activate both intrinsic and extrinsic pathways of apoptosis [38]. Without aging, there is reduced catabolic activity of NP cells and decreased NP cell number. The influences of oxidative stress on the intervertebral disc remain an active area of research and the ability to control or eliminate oxidative stress may lead to possible therapeutic or preventive treatments in the future [38].

Aging and Mechanical Changes

Changes in the AF and NP including dehydration, loss of organization, and fibrosis occur with aging and can lead to

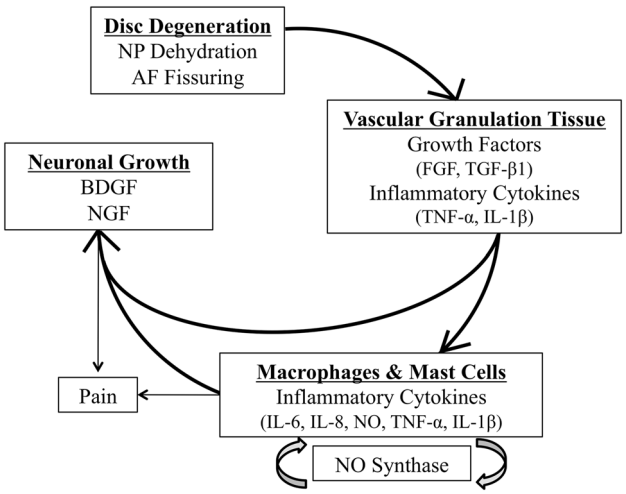

Fig. 1 A series of events occur during disc degeneration that are proposed to cause discogenic pain. FGF = fibroblast growth factor; BDGF = brain-derived growth factor; NGF = nerve growth factor; NO = nitric oxide.

lesions within the disc. Annular lesions are characterized by concentric tears, radial tears, and rim lesions. Concentric tears are crescent-shaped separations of the annular lamellae, whereas radial tears are irregular radial fissures that extend from the NP outward, and rim lesions are a separation of the outer annulus from the vertebral body [67]. Studies suggest different changes in mechanics from these individual lesions, which highlights the difficulty of testing because most spines have a combination of differing types of annular lesions [92, 93]. Despite the ongoing discussion about individual contributions of differing types of tears, the cumulative effect is a decreased ability of the disc to resist motion and an alteration in stress distribution in the disc, causing overloading on the surrounding structures. This alteration in disc mechanics has been demonstrated to have a substantial effect on disc mechanobiology with increased overall degenerative changes within the disc when these lesions occur, linking the alteration in mechanics and advancement of the disc degeneration [32].

How Do Environmental Factors Impact Disc Degeneration and Herniation?

Smoking

Smoking has also been demonstrated to have a profound impact on disc degeneration and herniation [8]. Smoking increases genes responsible for upregulation of proinflammatory stress responses in both the NP and AF [66]. Exposure to smoke extract also causes a dose-dependent toxicity in AF and NP cells. The disc is shifted to a catabolic profile with decreased expression of aggrecan, collagen, prostaglandin, and anticatabolic factors tissue inhibitor of matrix metalloproteinases and increasing activity of catabolic matrix metalloproteinases (MMPs) and the proinflammatory cytokine IL-1β [63, 66, 99, 102]. Smoking reduces production of aggrecan, a major proteoglycan component of disc matrix, and increases aggrecan cleavage, which diminishes the hydrostatic properties of aggrecan needed to counteract compressive forces [102]. These changes are demonstrated in overall histologic changes in the disc such as reduced cell proliferation, disruption in cell architecture, and disintegration of cells and matrix when compared with control cells [2].

Exercise

Maintenance of disc composition is a balance of catabolic and anabolic activity. In vitro, moderate physiologic loading of the spine produces a positive effect on the production of structural proteins and proteoglycans as well as slowing matrix degradation [28, 100]. The beneficial effects of moderate stresses associated with exercise appear to be related to magnitude and duration with the homeostatic effects being lost with long-duration and high-magnitude stresses that promote proinflammatory cytokines (IL-1β, TNF-α) and MMP production [85]. Similar findings have been shown clinically [54, 98]. Both laboratory and clinical evidence suggests that moderate activities promotes protective and reparative effects on the spine and may delay the development or progression of intervertebral disc disease.

Obesity

Obesity has wide-reaching effects on health, including disc degeneration and herniation. As body mass increases beyond a normal body mass index, disc degeneration increases linearly presumably as a result of the increased load on the intervertebral disc [50, 81, 90]. A peptide hormone, leptin, may be the link between disc degeneration and obesity because it is secreted primarily by adipose tissues and is a biomarker of obesity [14, 107]. Fibrocartilaginous tissues, including intervertebral disc cells, also secrete leptin and its functional receptor [27, 30]. Leptin increases MMP expression and promotes aberrant cell proliferation through the activation of multiple cytokine pathways and disruption of normal cytoskeletal organization by upregulating structural proteins F-actin, β-actin, and vimentin [48, 49, 108]. The resultant proliferation of abnormal NP cells is a possible mechanism for the

underlying detrimental influence of obesity on the development of intervertebral disc disease (Fig. 2).

Disc degeneration describes the result of a loss of overall homeostasis within the intervertebral disc, although there are numerous individual changes that interact with each other that culminate in catabolism of the disc. Changing mechanics of the disc, reactive oxygen, smoking, obesity, or extremes of exercise appear detrimental to disc homeostasis through various mechanisms. We presented age-related changes as well as environmental factors, which may or may not be modifiable by patients, but are areas of current investigation that may lead to prevention or possible treatments for disc degeneration in the future.

Are There Any Therapies to Improve the Natural History of Disc Degeneration?

Oral Treatments

There is high interest in potential oral treatments to slow down the natural history of disc degeneration. Conventional treatments such as nonsteroidal antiinflammatory drugs (NSAIDs) provide effective short-term back pain relief but do not alter the progression of disc degeneration [55, 76]. There is conflicting evidence about the effects of NSAIDs on intervertebral disc tissues. Previous in vitro studies suggest a decrease in matrix protein production, including collagen and proteoglycans, on exposure to NSAIDs [77, 87]. Conversely, others show cyclooxygenease-2 inhibitors permit sustained collagen and glycosaminoglycan synthesis and prevent the activation of inflammatory pathways in vitro and in vivo [68, 84, 89]. Further evidence needs to sufficiently evaluate the impact of NSAID treatment on intervertebral disc disease.

Use of oral supplements, including glucosamine, has gained popularity over the past several years. Glucosamine specifically is the most common natural supplement used by patients with low back pain; however, the evidence for glucosamine is mixed [7]. In vitro studies on AF and NP cells demonstrated no change on proliferation rate, an increase in glycosaminoglycan content, and decreased inflammatory mediators but decreased viability of AF cells [59, 101]. In vivo studies suggest a more negative effect of oral glucosamine supplementation on disc degeneration with findings of overall negative effect on disc matrix, including decreased type II collagen in AF cells and decreased aggrecan expression in both AF and NP cells [33]. Oral glucosamine supplementation also caused decreased proteoglycan content, greater disc degenerative histologic features, and decreased MRI index and MRI disc area compared with controls [33].

Omega-3 fatty acids are another oral supplement that has been alleged to help with back pain and disc degeneration. Omega-3 fatty acids are polyunsaturated essential fatty acids and act as an antiinflammatory medication [13]. Omega-3 fatty acid molecules are naturally released from injured cell membranes and competitively inhibit proinflammatory cytokines, including IL-1, IL-6, IL-12, and TNF-α [13]. Given their antiinflammatory mechanism of action, studies have compared omega-3 fatty acids with NSAIDs and found equivalent pain relief for chronic back pain with less side effects, including decreased bleeding risk [13, 57]. This is an evolving area of investigation that will continue to develop as the market for supplements continues to grow.

Protein-based Therapies

Protein-based therapies target a broad variety of molecules with either anabolic or anticatabolic effects on the disc. TNF-α is a potent proinflammatory cytokine that promotes proteoglycan metabolism and stimulates catabolic enzymes such as MMPs and aggrecanese in addition to other antianabolic, procatabolic changes in the disc. Because of its broad influence, inhibition of TNF-α may aid in restoring a homeostatic balance [60, 103]. Another potent proinflammatory cytokine is IL-1. The importance of IL-1 receptor antagonists was demonstrated by knockout mice that lacked the natural inhibitor of IL-1. These mice developed spinal abnormalities similar to those found in human disc degeneration, including loss of proteoglycans, lack of normal collagen structure, and upregulation of MMPs [71]. This suggests that supplementing native IL-1 receptor antagonist molecules might help prevent or slow disc degeneration. Despite some in vitro evidence supporting both TNF-α inhibitors and IL-1 receptor antagonists, in vivo and clinical effectiveness still needs to be demonstrated for intervertebral disc degeneration [45, 82].

Growth factors and cytokines are highly involved in degenerative disc disease and therefore are primary targets for biologic treatments. Growth factors such as the bone morphogenetic proteins (BMPs) are potent mitogens and belong to the TGF-β superfamily, which activates intracellular signal transduction proteins in the nucleus to initiate gene transcription [11]. BMP-2 and BMP-7 (osteogenic protein 1) act to increase proteoglycan production, specifically aggrecan and collagen types I and II in AF and NP cells [11, 37, 47]. Local injection of degenerative discs with BMP-2 and BMP-7 has been reported to maintain disc height and increase production of proteoglycans in both rat and rabbit models [3, 31, 34, 41, 58]. Despite successful demonstration of increased proteoglycan and collagen content, BMPs also cause ossification of the annulus and

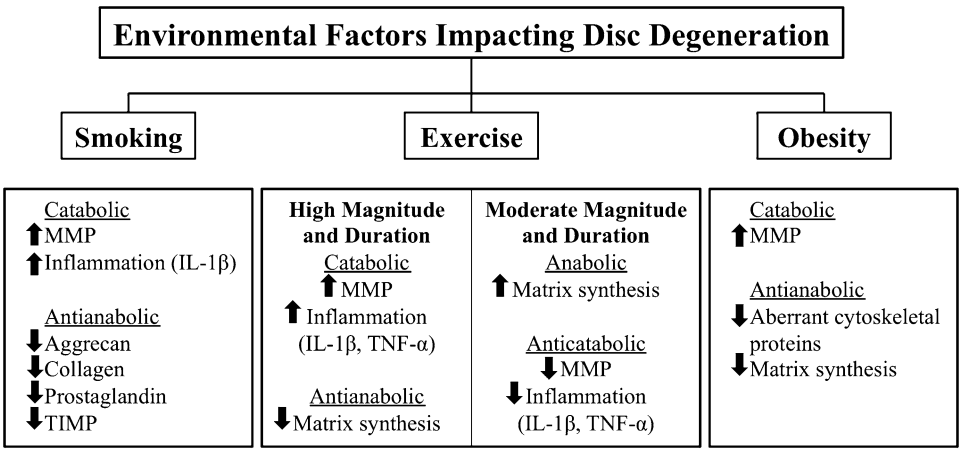

Fig. 2 Environmental factors impact disc degeneration by altering the homeostatic environment of the intervertebral disc. TIMP = tissue inhibitor of matrix metalloproteinases.

aberrant bone formation [29, 35]. Although successful at restoring healthy disc characteristics, further investigations regarding the safe application of this potent treatment must be conducted.

Gene-based Therapies

Although injection of biologic molecules into the disc allows direct delivery of a protein product, the relatively short half-lives of these proteins prevents long-term maintenance of their action, which would be required for a chronic disease such as disc degeneration. Gene therapy has the potential to deliver a recombinant gene of interest to the cells with the option of incorporating it into the cell's own DNA. This allows for long-term expression of a proanabolic or an anticatabolic therapeutic protein molecule. Many modalities of genetic transmission have been used, but as a result of their delivery efficiency, viral vectors are the modality most frequently used in intervertebral disc gene therapy research. Retroviruses, adenovirus, and adeno-associated viruses are a few of the different viruses used; each has its own risks and benefits, including the amount of genomic information that can be transmitted, immune response, and ability to integrate into the genome [40, 62, 69].

Gene therapies have been effective at slowing or reversing disc degeneration. In vivo transfection of intervertebral disc cells with an adenoviral vector containing the TGF-β1 gene resulted in increased TGF-β1 expression and proteoglycan synthesis [65]. Also, successful delivery of an adeno-associated virus encoded with genes for the proanabolic molecule BMP-2 and anticatabolic molecule tissue inhibitor of matrix metalloproteinases 1 showed decreased MRI, histologic, serum biochemical, and biomechanical evidence of disc degeneration over a 12-week period [41].

The ability to use a compound to induce or halt protein production from transduced genes offered a critical solution to control the potentially broad effects of overexpressed therapeutic gene products. Vectors can be developed containing a gene whose expression is initiated when the inducing agent such as tetracycline is delivered [96]. As such, with the removal of the induction agent, the protein production of the introduced gene ceases. The ability to control temporal expression of the therapeutic gene also allows for control and safe administration of these potentially powerful compounds [86].

Gene therapy is a promising field that would allow for long-term delivery of vital growth factors and cytokines to slow disc degeneration and potentially restore normal disc characteristics. While this technology is being refined, it will be critical to address the goals of identifying the optimal viral vector for delivery, minimizing inflammatory responses, maximizing gene transfection, and controlling subsequent gene expression to prevent complications.

Stem Cell Therapies

Stem cell therapies continue to develop in many fields including intervertebral disc degeneration. Several types of stem cells reported to have the potential to aid in repairing, delaying, or preventing intervertebral disc degeneration by repopulating the disc [19]. Stem cells differentiate into cells that create extracellular matrix and rebuild the disc and provide trophic effects such as upregulation of disc cell viability and inhibition of senescence and apoptosis [104].

Bone marrow-derived mesenchymal stem cells are relatively easy to obtain, are able to differentiate into

intervertebral disc cells, provide trophic support, and modulate the immune system [51]. These cells require cell-to-cell contact on three-dimensional spheres and growth factors such as TGF-β1 for development into NP-like cells [73, 88]. After differentiation, these cells can be placed on a gel scaffold or directly injected into the disc [73, 88]. Use of bone marrow-derived mesenchymal stem cells has demonstrated the ability to slow degeneration by preserving disc health as evaluated by MRI, histology, immunohistochemistry, and gene expression [44, 78–80]. These cells also demonstrated similar phenotypic activity to NP cells, including upregulation of aggrecan, versican, and type II collagen with suppression of the proinflammatory cytokines TNF-α and IL-1β [44]. This suggests that bone marrow stem cells may also enhance matrix homeostasis in the disc in addition to increasing the number of NP-like cells present.

Synovial stem cells demonstrate the highest expansion potential, highest colony-forming efficiency, and fastest growth kinetics of the various types of mesenchymal stem cells tested in vitro [106]. Synovial stem cells have a large chondrogenic effect by inducing collagen and aggrecan production and maintain intervertebral disc height in vivo [61]. These studies demonstrate the potential of synovial stem cells to contribute to the progression of stem cell technologies for intervertebral disc therapies.

Adipose-derived stem cells are easy to obtain and could potentially eliminate the need for cell expansion. In addition to the ease of access, adipose-derived cells have stable growth kinetics, wide differentiation potential, faster doubling time, and greater cellular matrix production than bone marrow-derived mesenchymal stem cells [12]. Additional trials are needed to assess the effectiveness and stability of adipose-derived stem cells in vivo and the safety of this technology.

Questions remain about the safety and large-scale application of this technology. Concerns persist regarding potential oncogenic transformation of stem cells or cell spillage with differentiation in unanticipated tissue locations leading to complications; this technology requires further studies to define its safe application [95].

Discussion

Disc degeneration occurs with age and involves a shift in the metabolic productivity of the intervertebral disc. The loss of homeostasis with upregulation of catabolic and antianabolic gene expression leads to decreased matrix components, weaker AF, and a dehydrated NP. These degenerative changes appear to increase the likelihood of disc herniation. Additionally, the degenerative and inflammatory changes occurring as the disc degenerates promote increased neural and vascular ingrowth into the disc, potentially accounting for the painful discomfort patients experience with disc degeneration.

As outlined, several factors impact disc degeneration and disc herniation, including aging, environmental and lifestyle choices such as smoking, exercise, and obesity. Current oral medications target the symptoms of disc degeneration but do not alter the cause and evolving evidence suggests harmful effects of some oral supplements; thus, additional research is needed. Developing medications such as TNF-α inhibitors and IL-1 receptor antagonists have the potential to offer therapeutic effects on disc disease but have thus far been relatively unsuccessful. BMPs are potent mitogens proven to positively impact disc degeneration in vitro and in vivo but require cautious clinical application because aberrant placement could have devastating neurologic complications as a result of the close proximity to the spinal cord. Additionally, the use of gene and stem cell therapies to maintain and potentially rebuild intervertebral disc materials holds great promise. Targeting, slowing, or even potentially reversing intervertebral disc degeneration could potentially aid in preventing disc herniation in addition to slowing the chronic issues associated with disc degeneration. Although advances are continually being made, additional research is crucial for safe application of these emerging technologies.

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