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Molecular Mechanisms of Biological Aging in Intervertebral Discs

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Abstract

Advanced age is the greatest risk factor for the majority of human ailments, including spine-related chronic disability and back pain, which stem from age-associated intervertebral disc degeneration (IDD). Given the rapid global rise in the aging population, understanding the biology of intervertebral disc aging in order to develop effective therapeutic interventions to combat the adverse effects of aging on disc health is now imperative. Fortunately, recent advances in aging research have begun to shed light on the basic biological process of aging. Here we review some of these insights and organize the complex process of disc aging into three different phases to guide research efforts to understand the biology of disc aging. The objective of this review is to provide an overview of the current knowledge and the recent progress made to elucidate specific

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molecular mechanisms underlying disc aging. In particular, studies over the last few years have uncovered cellular senescence and genomic instability as important drivers of disc aging. Supporting evidence comes from DNA repair-deficient animal models that show increased disc cellular senescence and accelerated disc aging. Additionally, stress-induced senescent cells have now been well documented to secrete catabolic factors, which can negatively impact the physiology of neighboring cells and ECM. These along with other molecular drivers of aging are reviewed in depth to shed crucial insights into the underlying mechanisms of age-related disc degeneration. We also highlight molecular targets for novel therapies and emerging candidate therapeutics that may mitigate age-associated IDD.

Keywords

intervertebral disc; aging; oxidative damage; inflammation; cellular senescence; DNA repair

I. INTRODUCTION

Life expectancy has dramatically increased over the past century largely due to advances in medicine, control of infectious diseases, and improved nutrition. There were an estimated 0.5 billion people 65 years and older worldwide in 2010, and this number is projected reach a staggering 1.5 billion by 2050[1]. Emerging within the older population are numerous age-associated chronic diseases, including heart disease, cancer, and diabetes, which are the leading causes of death in developing countries[2]. Low back pain, which also increases with age, is the leading cause of physical disability[3,4]. Age-associated chronic diseases, including those of the musculoskeletal system, impose the greatest burden on global health presently and in the future.

One of the largest age-dependent chronic disorders is degeneration of the joints, resulting in enormous socioeconomic and health impacts. Intervertebral disc degeneration (IDD) and osteoarthritis and are the most common underlying causes of joint-related chronic disability and debilitating pain in the older adults[5–7]. Unfortunately, decreased mobility is a validated predictor of loss of independence and mortality in the elderly[8,9]. Preserving healthy joints, particularly intervertebral discs in the spine, is vital for maintaining mobility in old age[10]. Individuals over 60 years old are more likely to suffer from pain stemming from IDD. As such, there is now great impetus to understand healthy disc aging in order to preserve mobility and fitness in the elderly population.

Organismal aging results from time-dependent accumulation of molecular and cellular damage that leads to impaired tissue homeostasis and eventual physiological and functional decline. Numerous types of damage are implicated in driving aging: accumulation of damaged proteins, mitochondrial damage and dysfunction, telomere shortening, DNA damage, attrition of quality control mechanisms (autophagy, DNA repair, etc.), and the loss of tissue-specific progenitor cells and tissue regenerative capacity[11–14]. The consequences of these different types of damage have recently been categorized into key aging hallmarks: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell

exhaustion, and altered intercellular communication[15]. Remarkably little is known about the contribution of these aging hallmarks to disc degeneration.

The purpose of this perspective is to provide an overview of disc aging characteristics and to organize the biochemical cascade of disc aging into three phases: (1) accumulation of damage to biomolecules, (2) aberrant cellular response to damage, and (3) loss of biologic structure and function. This perspective will focus particularly on cell senescence, inflammation, and oxidative damage as underlying mechanisms driving disc aging. Other important hallmarks of aging that have not been explored in the disc will be also highlighted in order to stimulate research that identifies potential causes and therapies for age-associated IDD.

II. AGING CHARACTERISTICS OF INTERVERTEBRAL DISCS

A. Disc anatomy and composition

Intervertebral discs are polyaxial cartilaginous joints that function primarily to provide support and flexibility to the otherwise rigid spine[16]. Situated between two adjacent vertebrae, discs consist of an outer, fibrous annulus fibrosus (AF) that circumferentially encloses a central, gelatinous nucleus pulposus (NP). Discs are constrained within and connected to adjacent vertebral bodies by superior and inferior cartilaginous end plates (CEP). The AF is composed of highly organized lamellae of predominantly type I collagen fibrils with alternating fiber angles of approximately 30°. AF functions mainly to bear circumferential stresses required to restrain NP swelling and tensile forces generated during bending or twisting. Conversely, the NP contains loose randomly organized networks of collagen type II and elastin fibers that encase proteoglycan aggregates. NP functions mainly to counteract and distribute compressive loads with large swelling pressures[17]. Discs consist mostly of extracellular matrix (ECM) sparsely populated by cells that are fibrochondrocytic in AF and chondrocyte-like and notochordal in NP. Discs are predominantly avascular, aneural tissues that exchange nutrients and metabolites primarily by diffusion to and from micro-vessels in the CEP and outer AF[18]. The restricted transport and low cellularity of the discs limit repair and make the disc particularly susceptible to injury and the aging-associated accumulation of tissue damage.

B. Features of disc aging

Intervertebral discs appear to undergo age-related degenerative changes earlier in life than other tissues[19,20]. Based on studies of humans and different animal models (Table 1), these age-related changes include increased number and size of tissue fissures, the presence of granular debris, and neovascularization from the outer aspect of the annulus inwards[19]. The NP becomes more fibrous as its proteoglycan content and hydration diminish with age, leading to fissures and progressive loss of NP size and pressurization and overall disc height[21,22]. Age-related accumulation of oxidized matrix proteins transforms the clear, gelatinous NP in youth to yellow, fibrous tissue in older individuals due to deposition of a brown ‘age pigment’ known as lipofusin, which originates from the slow peroxidation of lipids[23] (Fig. 1). Ossification and thinning of the CEP, microfractures in the adjacent subchondral bone, bone sclerosis, and drastic reduction in the number of vascular channels

in the CEP are also found with increasing age[24]. Reduced CEP vascular flow might contribute to a further decreased in nutrient supply to the disc, accumulation of cellular waste products, and an increasingly acidic environment (pH 6.3–6.6) that together with other stress factors can negatively impact cell function[25]. As such, elucidating disc aging-induced mechanisms will provide new opportunities for ameliorating age-related IDD.

C. Distinguishing disc aging from disc degeneration

Disc aging can be distinguished from disc degeneration by several defining characteristics. First, disc degeneration refers to the structural and functional failure of the disc as a result of aberrant, pathological cellular and ECM changes[26]. Disc degeneration may be caused by genetic predisposition, injury, aging, and environmental factors such as smoking, or any combination thereof[27–33]. Unlike disc aging, disc degeneration is not exclusive to the older population, i.e., disc degeneration can be present in a younger person due to injury or faulty genetics[32–34]. Conversely, disc aging is systemic and occurs in all spinal discs of all older individuals. In other words, a degenerated disc, but never an aged disc, can be found among the other young healthy discs and body organs in a young individual. However, the specific differences between an aged disc and a degenerated disc have not been clearly defined as both appear to share a number of similar changes[19,20]. Importantly, future work to identify the features common to aging and degeneration that are most pathologic to disc function will be critical in guiding novel treatments.

D. Impact on disc aging by the surrounding spinal structures

Because aging is systemic, age-related changes in spinal structures can greatly impact disc health (Fig. 2). Osteoporosis and osteopenia are commonly observed in the aging spine, predisposing it to vertebral compression fractures[35] and correlating with increased IDD[36]. Moreover, age-dependent endplate thinning and fracture create abnormal stress distributions and injury propagation to the adjacent disc, which increases the risk of IDD[34,3738–40]. Modic changes in the vertebral body and/or endplate identified by MRI have been associated with aging along with loss of disc height and signal intensity[41]. Vertebral endplate sclerosis is predicted to reduce bulk fluid movement in to and out of the disc, which could limit ancillary nutrient transport[42,4344]. Together with reduced endplate pore density and size seen with aging, these changes could significantly impact disc nutrient supply and thus disc cell survival[18]. Age-related facet cartilage erosion can impose abnormal load on disc, altering local mechanobiological responses[45]. Aging of the posterior and anterior spinal ligaments, important dynamic stabilizers of the spine, alters their material properties (increased stiffness) which conceivably could also influence mechanical strain and stability of the discs[35,46]. Finally, age-driven changes in the spinal muscle, e.g., fatty deposit or infiltration, could also affect the overall stability of the spine and hence the biology of the disc[47]. In summary, disc aging is a complex systemic process that is intimately modulated by the interactions among the different aging spinal structures (Fig. 2).

III. BIOCHEMICAL CASCADE OF INTERVERTEBRAL DISC AGING

The biochemical process of disc aging can be organized into three distinct phases (Fig. 3A). First, there is damage to biomolecules such as DNA and proteins that results from exposure

to inflammatory and oxidative stress. Second, aberrant cellular responses to damage exacerbates tissue damage when the responses become dysregulated. Third, accumulated damage leads to loss of biologic structure and function of disc tissue, as discussed in details below. Aging of the other spinal structures (Fig. 2) probably follows this same biochemical cascade.

A. Phase I of Biochemical Cascade of Disc Aging: Biomolecular Damage

A solid body of research supports time-dependent accumulation of stochastic damage to biological macromolecules as a driver of aging[48]. Well documented in aged tissue are damaged proteins and genetic materials which lead to genomic instability, epigenetic alterations, and loss of proteostasis. Proteostasis refers to protein homeostasis regulated by a dynamic equilibrium of protein synthesis and degradation. ECM damage resulted from loss of proteostasis during the course of age-related IDD has been extensively documented. The role of DNA damage in disc aging has also been recently reported. However, epigenetic alterations in disc aging have not yet been explored.

Damage and Perturbation of ECM Integrity in Aged Disc—Accumulation of molecular damage in the ECM of the aging disc has been well recognized (Fig. 4A) and reviewed[49]. Disc proteoglycan aggregates, consisting primarily of aggrecan core protein, link protein, and hyaluronan encased within the collagenous fiber network, provide the osmotic properties that create swelling pressure necessary to counteract compressive loading. In the aged disc the majority of the aggrecan exists in a non-aggregated form and contains decreased glycosaminoglycan (GAG) chain length, which is thought to be derived from proteolytic damage[49]. Link protein levels decrease with age[50] while total disc hyaluronan levels increase with age, possibly as a response to its own proteolysis[51]. Versican is another major hyaluronan-binding proteoglycan in disc tissue which undergoes extensive degradative damage with aging[52]. The resulting non-aggregating proteoglycans may not have the same functional ability as that of intact aggregates, as their size, charge density, spatial rigidity and matrix interactions are diminished[49,51,53,54].

The disc also contains the small leucine-rich repeat family of proteoglycans (SLRPs), which are characterized by their interaction with collagen fibers. Biglycan and decorin, the dermatan sulfate proteoglycans which interact with collagen type VI and type I/II, respectively, lose their GAG content with age due to proteolytic damage[55]. The keratin sulfate-containing fibromodulin and lumicans are two other disc collagen fibril-associated SLRPs. Whereas fibromodulin abundance decreases with age and exists mostly in non-glycated form in adult discs, lumican increases in aged discs and exists as a glycoprotein throughout life[4956]. Since dermatan sulfate and keratin sulfate mediate intermolecular interaction, their age-related alterations most likely affects disc matrix structure. Age-related changes in other disc matrix constituents, e.g., elastin, fibronectin, have also been documented with unclear consequences on disc matrix organization and cell structure and function[4957].

Disc tissue contains three broad categories of fibrillar, fibril-associated, and pericellular collagens whose relative abundance changes with age. The disc collagen network consists

predominantly of Type I and II fibrillar collagen which accounts for approximately 80% of the total disc collagen. Type VI pericellular collagen accounts for 10–20% of the disc collagen[49]. Age-associated changes in disc collagen structure include proteolytic damage in fibrillar collagen as a result of dysregulated collagenase activity[58]. Damaged fibrillary collagen weakens the mechanical strength of disc tissue and leads to the formation of non-enzymatic crosslinks between basic amino acids of collagen and reducing sugars[59]. This results in advanced glycation end-products (AGEs) which elicit oxidative stress. AGEs are present throughout the disc and increase in abundance with age[60] and may impair collagen fibril formation[61,62]. In mice, oxidative stress was shown to increase loss of disc elasticity and alter the secondary and tertiary conformation of collagen molecules, which increases their susceptibility to cleavage by MMPs[63]. Hence age-related ECM molecular alterations can result in a decline in the structural integrity and biomechanical function of the disc.

DNA Damage in Aged Disc—Besides ECM damage, aged discs also exhibit cellular damage. In particular, damaged DNA, unlike damaged proteins or other macromolecules that generally can be degraded and replaced by new synthesis, is especially harmful and requires repair in order to maintain normal cellular function. Each cell in an organism is subjected to tens of thousands of DNA lesions each day due to the inherent chemical instability of DNA structure, metabolic byproducts, and environmental mutagens and genotoxins[11]. Despite elaborate repair mechanisms, cells still amass DNA damage over time. Inherited defects in genome maintenance mechanisms invariably lead to a variety of diseases characterized by accelerated aging of one or more organ systems[12]. For example, deficiency in humans of certain genes involved in repair of DNA damage, such as ERCC1-XPF, leads to dramatic progeroid, or accelerated aging syndrome[64]. Indeed, DNA repair-deficient *Ercc1*$^{-/\Delta}$ mice exhibit premature onset of key disc aging features, including loss of matrix proteoglycan, reduced disc height, and increased cellular senescence[28,65] (Table 1). DNA damage as a driver of disc aging is further supported by exposure studies of human and mice to genotoxic stress, including ionizing radiation and tobacco smoking, which in mice dramatically accelerated disc degenerative changes[66,6768].

Stressors Driving Disc Biomolecular Damage—Exogenous and endogenous stressors causing molecular damage in aging discs are thought to be predominantly oxidative and inflammatory in nature (Fig. 3A)[65,69]. Evidence of oxidative damage in aged disc include accumulation advanced glycation end products (AGEs), e.g., pentosidine and carboxymethyl-lysine, produced by nonenzymatic glucosylation and oxidation of proteins and lipids[6070]. Pentosidine, which cross-links collagen molecules, might play an important role in increased collagen stiffness and fragility to weaken cartilage biomechanics with old age[59,60,71]. Redox proteomic analysis also revealed oxidative post-translational modifications, e.g., protein carbonylation, in disc matrix isolated from aging mice; this change was associated with protein fragmentation and aggregation and increased disc stiffness[63].

The source of reactive oxygen species (ROS) driving oxidative damage includes free radicals generated from radiation, by-products of oxidative phosphorylation, cellular response to chronic inflammatory stress exposure, and decreased synthesis of ROS-scavenging

enzymes[72]. Although residing in a low oxygen tension environment, resident disc cells, especially AF cells, are capable of oxidative phosphorylation which can generate ROS. In addition, aged discs acquire fissures and associated neovascularization which exposure the otherwise hypoxic resident cells to higher oxygen tension and thus oxidative stress[73]. Increased ROS contribute to aging changes in cells and tissues by damaging proteins, lipids, and DNA. One key marker of protein oxidation is nitrotyrosine that is formed by the reaction of protein tyrosine residues with peroxynitrite (ONOO−)[74]. Peroxynitrite, formed by rapid reaction of nitric oxide (NO) with oxygen radical superoxide (O_2^-), is a potent cytotoxic damaging nitrating and oxidizing agent.

Intriguingly, hyperosmolality is recently demonstrated as a non-classical inducer of DNA damage. Hyperosmolality induces DNA double strand breaks, which activate the ATM-p53-p21WAF1 axis leading to the hypophosphorylation of the pRb protein and cell cycle arrest in the G_1 phase of the cell cycle[75]. NP cells are continuously exposed to hyperosmolality, up to 500mOsm/kg H_2O *in vivo* as compared with <300mOsm/kg H_2O in the majority of the other tissues[76]. Increased osmolality in NP cells was found to provoke chromatin changes and DNA damage[75]. It is still unclear what level of hyperosmolality is needed to overwhelm NP cell DNA repair capacity to introduce DNA damage.

Abnormal mechanical loading represents another major potential stressors that can promote disc tissue damage. Cohort analyses point to associations between long-term physical loading and loss of spinal mobility and disc height[77], and other age-associated IDD[78,7980]. In animal studies, rats with imposed upright stance for up to 11 months showed increased disc senescence, presumably due to altered magnitude and mode of disc loading[81]. Modest age-related IDD features in rat discs were observed following compressive overloading for eight weeks[82,83]. However, more studies are needed to establish role of abnormal mechanical loading in promoting disc degenerative changes, with or without age associations.

Last but not least, nutritional stress can also promote perturbation in disc tissue. The avascular nature of disc tissue results in an environment of low oxygen and glucose concentrations and high lactate concentration[84,85]. Despite low physiologic concentrations of oxygen[85] and glucose[84] and high concentrations of lactate (>10x plasma concentrations)[86], which acidify the inner disc environment, disc cells can remain viable and functional in this hostile environment. Yet low nutrition and pH are also the very factors that reduce the disc's resilience to additional nutritional and environmental stresses[87,88]; this is because disc nutrient supply barely hovers above the cellular requirements in the NP[89]. This precarious balance may expose disc cells to nutritional deprivation due age-related processes. For instance, disc cell death is initiated if glucose concentrations drop below critical thresholds (<0.5 mM) [90]. Acidic conditions (pH < 6.7) can also lower cell viability[91]. Low O_2 and pH conditions have been shown to diminish proteoglycan and collagen synthesis[86,92].

B. Phase II of Biochemical Cascade of Disc Aging: Aberrant Responses to Damage

In an attempt to repair damage, cellular responses may become dysregulated over time, which exacerbate cellular and ECM damage. Aberrant molecular signaling, abnormal

changes in cell fate, dysregulated nutrient sensing, and mitochondrial dysfunction all have been reported in non-disc tissues[15]. However, only abnormal alterations in cell fate (e.g., cellular senescence) and dysregulated signaling (e.g., NF-κB pathway) have been reported recently in studies on disc aging. These two areas of research are discussed below.

Cell Functional and Phenotypic Changes in Disc Aging—AF and NP differ in their developmental origin, with AF developing from the mesenchyme and the NP from the notochord. The cells in the outer AF are elongated and fibroblast-like, whereas the inner AF and NP are populated by more spheroidal, chondrocyte-like cells. While cell types in the outer AF appear to change little during lifetime, NP cell subpopulations undergo a substantial change whereby clusters of large vacuolated notochordal cells in young NP are replaced by smaller chondrocyte-like cells in older NP[93]. Cell density decreased from 0 to 16 years, and no significant variation occurred thereafter in human lumbar discs [94]. Cell density in normal, mature disc is approximately $4x10^6$ cells/cm^3 in the NP and $9x10^6$ cells/cm^3 in the AF [49]. During aging disc cells undergo a number of phenotypic changes, including a switch from an anabolic to a catabolic phenotype[95–99]. Cells isolated from aged discs exhibit reduced collagen and proteoglycan matrix anabolism[95–97,100]. Moreover, found in aged discs are elevated levels matrix proteoglycan degradative products and certain matrix metalloproteinases (MMPs), including MMP-3 and ADAMTS-5[10198,99102,103]. The pro-inflammatory cytokine TNF-α is also shown to be more highly expressed in older adult discs than young adult discs[104]. These observations suggest imbalanced matrix homeostatic phenotype as a consequence of age-associated changes in disc cells. Additionally, other cell phenotypic changes such as elevated necrosis, apoptosis, and senescence have been reported[105,106] (Fig. 4B). These phenotypic and functional changes are likely consequences of aberrant cellular responses to accumulated biomolecular damage in disc tissue. Such changes also likely contribute to loss of functional cells, leading to age-related depletion of disc matrix proteoglycan, tissue dehydration, and altered load distribution that may increase risk of injury[87,107].

The Role of Cellular Senescence in Disc Aging—Cell senescence, originally described as a process that limits cell proliferation[108,109], is an important mechanism for preventing the proliferation of potential cancer cells. This type of senescence is known as replicative senescence, characterized by cessation of cell proliferation due to critical shortening of telomere length after successive replicative cell cycles. Another type of cellular senescence was discovered relatively recently, which was termed, “stress-induced premature senescence (SIPS)”. SIPS is formed as a result of accumulate genomic and mitochondrial DNA damage. SIPS cells also acquire a senescence associated secretory phenotype (SASP), a unique feature in which they secrete high amounts of numerous inflammatory cytokines and matrix proteinases, which can have profound catabolic effects on neighboring cells and ECM[110,111]. Because senescent cells accumulate in various tissues and organs with aging[112], SASP is currently believed to disrupt tissue structure and function and promote aging[113]. This theory is supported by a seminal study demonstrating that clearance of senescent cells delays aging-associated disorders[114].

An increased number of senescent cells were observed in both aged and degenerated discs[105,115–118], as measured by increased expression of senescent markers, including senescence-associated β-galactosidase (SA-βgal), $p16^{INK4A}$, and decreased telomere length. Thus, cellular senescence is a potential driver of both disc degeneration and disc aging, possibly through the SASP mechanism that promotes pathologic disc matrix catabolism. Evidence supporting this idea originally came from a study showing a positive correlation between the senescent marker $p16^{INK4A}$ and matrix proteases expression (MMP-13 and ADAMTs-5) in human disc tissue, implicating senescent disc cells as a source of these catabolic enzymes[105]. *In vitro* cell culture studies using H_2O_2 to simulate oxidative DNA damage that induces SIPS also revealed an altered, catabolic phenotype[119]. These H_2O_2-induced senescent disc cells exhibited SASP, as characterized by their secretion of high levels of MMPs and pro-inflammatory cytokines. These senescent disc cells also showed growth arrest and perturbed matrix homeostasis, i.e., reduced matrix synthesis capacity (anabolism) and increased matrix degradation (catabolism)[120]. However, to determine the causative role of cellular senescence in driving disc aging, genetic and pharmacological *in vivo* strategies are needed to study the effects of ablation of senescent cells on age-associated IDD.

Possible causes of disc cellular senescence—DNA damage is the underlying cause of cellular senescence, but how cells become senescent in disc tissue is not fully understood. Current evidence supports the existence of both replicative senescence and SIPS in discs. Telomere length shortening, a marker of replicative senescence, is observed in aged and degenerated human disc tissue, as is increased $p16^{Ink4a}$ immunopositivity, a marker of SIPS[105,121]. Elevated cellular senescence was observed in discs of DNA repair-deficient $Ercc1^{-/\Delta}$ mice[28] as well as in genotoxin-exposed mice [66,67], suggesting that DNA damage is a key driver of disc cellular senescence. Other potential sources of oxidative DNA damage include oxidative stress induced by inflammation and high glucose-induced oxidative stress, e.g., in diabetes[122]. IL-1, a predominant cytokine implicated in the pathogenesis of disc degeneration[123,124], has been suggested to promote SIPS in NP cells. Indeed, spontaneous age-related IDD with associated senescent phenotype is seen in an IL-1Ra knockout mouse model [125]. Finally, activation of WNT/β-catenin signaling was also reported to promote cellular senescence in rat disc cells[126], but it is unclear in disc tissue how this signaling is influenced by oxidative or inflammatory stress that drives senescence. In summary, disc senescence phenotype appears to be specific aberrant cell response to DNA damage which can cause further tissue perturbation and damage during the course of disc aging (Fig. 3A).

Aberrant Molecular Signaling in Disc Aging—Biomolecular damage, e.g., DNA damage, can initiate aberrant signaling cascade, which then, if left unchecked, acts to cause further molecular damage. It is now well known that in addition to environmental factors and behavior traits, genetics greatly influences lifespan. Most longevity genes identified starting from the early 1980s in various models (worms, fruit flies, mice, monkeys … etc.) implicate one of three major signaling pathways in cells: insulin/IGF-1, sirtuins, or mTOR[13,127,128]. These three pathways regulate a variety of cellular processes, including cell growth, cell proliferation and survival, protein synthesis, and transcription mechanisms. The roles of these signaling pathways in disc aging, however, have not been explored although it is

known that Insulin-like growth factor-1 (IGF-1) induces disc anabolic activity[129] while SIRT1 is expressed by human NP cells and acts to suppress NP cell matrix metabolism and proliferation[130]. However, signaling other than these three pathways have been reported to influence age-related IDD, including NF-κB, MAPK, and HIF-1α signaling which are known to be involved in stress responses and SIPS, which are discussed below.

NFkB signaling in age-related disc degeneration—NF-κB signaling is central to the cellular response to inflammation, stress and damage. NF-κB is comprised of a family of structurally related transcription factors, which in mammals consists of five protein subunits, RelA or p65, c-Rel, RelB, p50 and p52. NF-κB exists as a homodimer or a heterodimer, with the p50–p65 heterodimer being the most common form which controls the expression of the majority of NF-κB-regulated genes[131]. Chronic activation of NF-κB has been linked to tissue aging and many age-related degenerative diseases, including musculoskeletal disorders such as muscular dystrophy, osteoarthritis, and osteoporosis[132,133134].

NF-κB is also implicated age-associated IDD[135]. In disc tissue, NF-κB activity was shown to correlate with accumulated oxidative stress and increase with age and degeneration[70]. Systemic inhibition of NF-κB activity by pharmacologic and genetic means has been shown to ameliorate age-associated IDD in a mouse model of accelerated aging[136]. Most other studies focus on the role of NF-κB in mediating degenerative and inflammatory disc disease. Increased NF-κB activity is found in degenerative discs[135]. Compared to asymptomatic discs, symptomatic discs have higher levels of pro-inflammatory cytokines that are considered typical NF-κB target genes, e.g. TNF-α, IL-1β, IL-6 and IL-8[104,123,124,137]. Together, these findings support the role of dysregulated NF-κB chronic activation in promoting IDD and age-related IDD.

MAPK signaling in disc biology and disc aging—Mitogen-Activated Protein Kinases (MAPKs) are a family of signal transduction pathways, allowing the cells to respond to multiple extracellular inputs, such as hormones, growth factors, inflammatory cytokines, and environmental stresses such as ionizing radiation or osmotic stress[138,139]. In mammals, these diverse signals activate at least three major subfamilies of MAPKs, the extracellular signal-regulated kinases (ERK), c-Jun NH2-terminal kinases (JNKs), and p38 isoforms (p38MAPKs)[140,141]. Activation of MEK/ERK and JNK are involved in the induction of cellular senescence[142143]. On the other hand, p38 MAPK activation is a marker of senescence and plays a vital role in establishing SASP which probably affects local tissue homeostasis[144]. Consistent with *in vitro* data, up-regulated p38 MAPK expression has been reported in senescent AF cells isolated by laser capture microdissection[145].

Multiple components of the catabolic machinery (e.g. MMPs, ADAMTSs, COX-2, PGE2, iNOS, etc) are regulated by MAPK family members. Many of these catabolic genes are also regulated by NF-κB signaling, highlighting the cross-talks between the components of MAPK and NF-κB pathways[135]. In disc tissue, the major pro-inflammatory cytokines, i.e., IL-1β and TNF-α, activate ERK and/or p38 and consequently catabolic molecules such as ADAMTs-4, MMP-3 or syndecan-4 [146–149]. Inhibition of MAPK activation by specific synthetic compounds or naturally occurring molecules such as glucosamine prevent these

processes, indicating that MAPK regulation may represent a promising tool to mitigate disc degeneration and aging[147,150].

Increased disc cell proliferation and formation of cell clusters is a characteristic feature of disc degeneration[151]. This is thought to be partly due the over-expression of growth factors and their receptors[152]. Growth factors such as PDGF, IGF-I or bFGF stimulate cell proliferation via ERK activation[153,154], indicating an additional role of MAPK in disc degeneration. Conversely, hyperosmotic conditions inhibit ERK, arrest cell cycle in the G_2/M phase via p38 MAPK activation and restrain the mitogenic effect of growth factors, indicating that the reduced osmolality prevailing in the degenerated disc may boost cell proliferation[155]. Whether disc aging involves these same signaling pathways as they are related to cell proliferation and clustering awaits further investigation.

HIF-1α signaling in disc biology and disc aging—For any meaningful discussion of aging of disc at the molecular level, it is important to consider the unique niche and physiology of the cells of NP and inner AF. NP resides in a hypoxic and hyperosmotic environment[156,157] to which they have adapted a novel hypoxia signaling governed by the activities of the transcription factors hypoxia-inducible factor-1α and -2α (HIF-1/2). Unlike other cell types in which HIF-1α protein stability and activity are enhanced under hypoxia and abolished under normoxia, NP cells constitutively express both HIF-1α and HIF-2α, even under normoxic conditions[158]. In fact, NP cells are either partially or wholly refractory to propyl-hydroxylases (PHD)-dependent degradation[159,160]. Similarly, Factor Inhibiting HIF-1 (FIH-1) does not control HIF-1α transcriptional activity in NP cells[161] as typically seen in other cell types. The constitutive HIF-1 expression has important metabolic consequences for NP cells which are obligate glycolytic and rely very little on aerobic respiration even when oxygen is abundant [162]. In addition to this metabolic adaptation, HIFs promote survival and function of NP cells through upregulation of crucial genes including, aggrecan[162], galectin-3[163], β-1,3-glucuronyltransferase 1[164], and VEGF-A[165]. In fact HIF-1 is indispensable for NP cell survival as demonstrated by conditional knockout of HIF-1α in mouse notochord by Foxa2-Cre which results in smaller, non-vacuolated cells in the NP at E15.5 and massive apoptotic cell death in the NP at birth[166]. Thus, the important role of HIF signaling in physiological adaptation and function of NP cells suggests that its dysregulation may contribute to cellular aging and degeneration.

The role of HIF signaling in organismal aging was recently elucidated. Dysregulated increase in HIF-1α activities due to age-dependent decline of nuclear NAD^+ was found to interfere with the coordination between nuclear and mitochondrial activities, resulting in mitochondrial dysfunction and accelerated aging[167]. However, other studies suggested a role of HIF-1 in suppressing cellular senescence[168,169]. These conflicting results require further investigation in order to elucidate how dysregulated HIF signaling contributes to disc aging, given that robust and stabilized HIF-1α expression is vital for NP cell survival and function[162]. One possible mechanism involves cross-talk between HIF and NF-kB pathways under pathological conditions[170,171]. TNF-α controls expression of PHD2 and 3 through NF-κB pathway, and both PHDs in turn serve to control p65/RelA of NF-κB transactivation. Importantly, PHDs partially control a broader TNF-dependent inflammatory response by promoting expression of several cytokines and chemokines[170,171]. More studies are needed

to detangle the complex interaction and possible dysregulation between HIF and NF-kB pathways that might predispose disc tissue to accelerated aging.

C. Phase III of Biochemical Cascade of Disc Aging: Loss of Biologic Structure and Function

Loss of disc biologic structure and function (phase III) is an end consequence of time-dependent accumulation of damage resulting from biomolecular changes (phase I) and associated aberrant signaling (phase II) (Fig. 3A). Damage-induced apoptosis, cellular senescence, and pathologic alterations in matrix metabolism all likely contribute to the loss of disc functional cells, stem cell pool[172–175], and ECM structural integrity (Fig. 4). These changes are expected to alter ECM-cell communication and eventually the normal physiology and biomechanical function of the disc (Fig. 3A).

Loss of disc ECM integrity and altered cell mechanobiology—Loss of disc matrix proteoglycans decreases the negatively charged ionic environment, reducing hyperosmotic loading ionic flux[176]. Loss of hydration alters fluid pressurization and fluid flows in the disc[177]. Age-related changes to the solid matrix, such as increased non-enzymatic collagen cross-linking[59178], protein glycation[179], or protein denaturation[180] can increase structural stiffness [180] and alter disc strains[181]. Moreover, AF damage increases with age[182], which can alter tensile stiffness and annular local strains[183184]. These changes perturb the cellular microenvironment and compromise normal disc function.

Cell clustering and interactions with the pericellular matrix also appear to change with age[185], which suggest altered cellular mechanical properties[186]. Indeed, disc cells from young and middle-aged bovine respond differently to the same compression regimes, with older cells showing diminished capacity for matrix repair[187]. Similarly, AF cells from mature and aged pigs subjected to loading exhibit differences in anabolic and catabolic gene expression, further suggesting changes to cells or mechanotransduction with aging[188]. Cells isolated from injury-induced degenerative discs also demonstrated differences in response to mechanical loading[189], but the effect of physiologic aging was not evaluated.

Mechanical features of aging discs: loss of physiological biomechanics—The complex structure of the disc allows multiaxial motion while maintaining stability[190,191]. NP and AF interact to support compressive loading and facilitate segmental motion. Compression increases swelling pressure in the NP[192193] which is constrained by the surrounding, distensible AF and the adjacent CEPs[194]. In distraction, the AF limits vertebral body motion[181]. The organized, concentric lamellae of the AF resist torsion through circumferential tensile stress in its collagen and elastin fibers[195–197]. The physiological function of the spine thus depends on these integrated interactions among the different disc regions[198,199]. A number of mechanical features specific to aging have been identified within the disc. Hydration levels within the NP decrease in aging, which, along with GAG depletion[200], reduces swelling pressure in the NP[192201] and increases the shear modulus of NP with age[202]. On the other hand, hydration in the AF remains relatively unchanged with aging[192], and annular tensile properties change only modestly with aging[183,203]. The net

result of these age-related changes, is a loss of elasticity and increased stiffness[63180], which overlaps with mechanical changes in non-age related disc degeneration.

POTENTIAL THERAPIES TO DELAY AGE-ASSOCIATED IDD

The events within disc aging cascade present potential therapeutic targets for treating or delaying age-related IDD (Fig. 3B). In theory, treatments that reduce free radical production may ameliorate macromolecular damage. Indeed, accelerated aging *Ercc1*$^{-/\Delta}$ mice treated with systemic administration of XJB-5-131, a mitochondria-targeted ROS scavenger, resulted in improved disc GAG content and proteoglycan synthesis[204]. This demonstrates that mitochondria-derived ROS drives aging-related IDD and that radical scavengers may play a promising role in slowing disc aging. Anti-oxidants such as curcumin and resveratrol have also been reported to be therapeutic for treating IDD, as have a number of other anti-inflammatory agents such as NSAIDS and IL-1Ra[205–210]. Whether these same agents are therapeutic against age-related IDD await further research.

Minimizing the aberrant damage responses that exacerbate tissue damage is another strategy for treating age-related IDD. One promising candidate is the NF-κB pathway whose chronic activation has been closely linked to age-related diseases. Previous studies demonstrated that blocking NF-κB activity pharmacologically and genetically in the *Ercc1*$^{-/\Delta}$ rodent model of accelerated aging delayed the onset of age-dependent disc proteoglycan loss and other degenerative changes[136,211]. Moreover, intra-discal injection of ‘naked’ NF-κB decoy oligonucleotides proved effective in partially restoring disc height in an animal model of IDD[212], indicating that dysregulated activation of NF-κB is involved in disc matrix loss.

Other potential strategies involve the use of protein-, gene-, and cell-based therapy to counter age-related changes to the matrix and cells, respectively[213–215] (Fig. 3B). The goal of most gene therapy approaches is to replace loss of disc ECM via increased matrix synthesis or to inhibit catabolic factors that degrade the matrix[216]. Cell-based therapy frequently aims to restore loss of functional disc cells with possible anti-inflammatory effects[217,218]. These interventions aim at either preventing damage, ensuring optimal cellular response to damage, or restoring tissue loss associated with aging. Indeed, such strategies exist and have been extensively investigated in the context of disc degeneration. To be effective, however, therapeutic interventions need to target the early phases of the disc aging cascade prior to the occurrence of functional failure.

CONCLUSIONS AND PERSPECTIVES

A large number of studies dating back to the early 1950s describe various age-related disc degenerative changes. However, the molecular mechanisms which initiate and mediate disc aging are still poorly understood. Recent advances in the aging research field are beginning to reveal important insights into the mechanisms of organismal aging. Based on these insights, we proposed to organize the complex multi-step process of disc aging into three distinct phases to guide future research: (1) biomolecular damage, (2) aberrant damage responses, and (3) loss of biologic structure and function. While genomic instability, cellular senescence, and dysregulated NF-κB signaling have been recently uncovered in disc aging,

research is still lagging to elucidate the roles of other important aging hallmarks such as mitochondrial dysfunction, stem cell exhaustion, altered intercellular communication, and epigenetic alterations in disc tissue. The unique disc biological niche, i.e., mechanically loaded, nutrient-poor, acidic and hypoxic environment, offers an excellent opportunity to discover novel disc aging mechanisms that might be distinct from those driving aging in other tissues. Importantly, disc aging is a systemic process that does not occur in isolation and is likely influenced by the aging processes of neighboring spinal structures (Fig. 2), circulating factors, and remote body tissues and organs. Hence, aging research of the whole spine, not just the disc, is an important, immediate future direction. The broad, multi-tiered approach is necessary to provide the basic information needed for the development of effective therapies and interventions to delay the onset of age-related spinal disorders. This is imperative given aging is a big risk factor for IDD-associated chronic pain and disability, the prevalence of which will undoubtedly be amplified with the growing aging population.

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LIST OF ABBREVIATIONS

AF	Annulus Fibrosus
ADAMTS	A Disintegrin And Metalloproteinase with Thrombospondin Motifs
AGES	Advanced Glycation End products
CEP	Cartilaginous Endplate
ECM	Extracellular Matrix
GAG	Glycosaminoglycan
IDD	Intervertebral Disc Degeneration
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
NP	Nucleus Pulposus
ROS	Radical Oxygen Species
SA-βgal	Senescence Associated Beta Galactosidase
SASP	Senescence Associated Secretory Phenotype
SIPS	Stress-induced Premature Senescence
SLRP	Small Leucine-rich Repeat family of Proteoglycans

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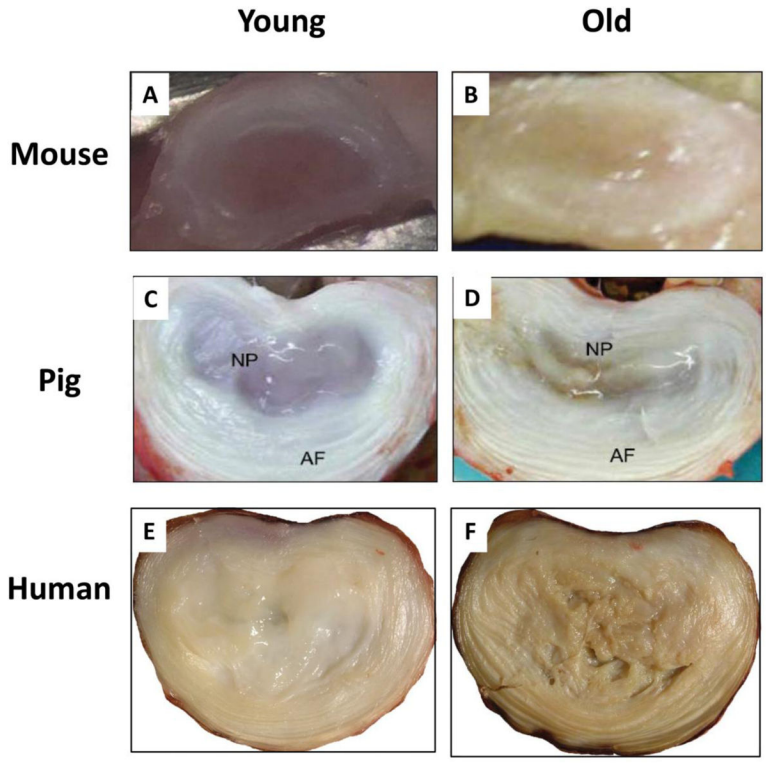

Figure 1. Gross features of the aged mammalian discs

Axial sections of young and aged lumbar discs of 3 month- vs. 24 month-old mice (A, B), 3 week- vs. 3 year-old pigs (C, D) [219], and 16 year- vs. 55 year-old humans (E,F, courtesy of Dr. Ian Stokes), are shown. Old discs exhibit an overall loss of hydration, loss of demarcation between the AF and NP boundary, and tissue discoloration (old disc more yellowish). Average lumbar disc cross-sectional diameters are approximately 2–3mm for mice, 24–30 mm for pigs, and 45–55mm for humans [220].

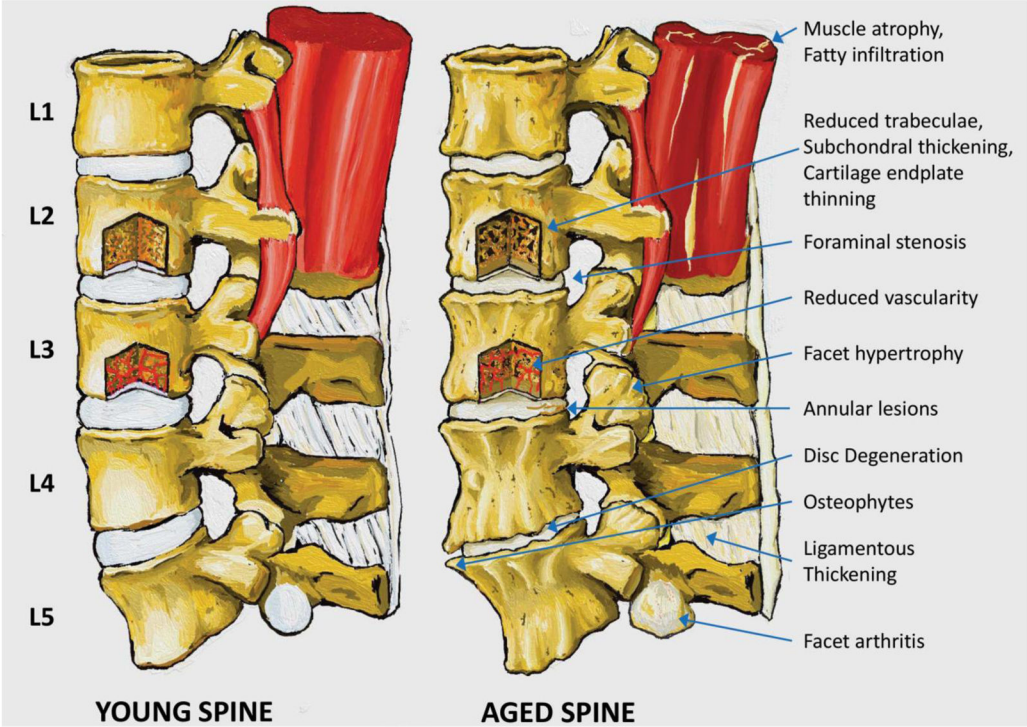

Figure 2. Gross features of the aging spine

Young and aged lumbar spines are visually compared to illustrate the wide range of tissues and processes involved in aging of the spine. Muscle atrophy and fatty infiltration is evident at L1-2 in the aged spine. 25Similarly, a window into L3 depicts reduced vascularity and fewer capillaries reaching the endplate. Foraminal stenosis is shown at L2-3 (arrow), L3-4, and L4-5, and facet hypertrophy is evident at L3-4 (arrow) and L4-5. An annular lesion is present in the posterior portion of the L3-4 disc. Disc degeneration with evident loss of disc height and prominent anterior osteophytes occur at L4-5. Ligamentous thickening is indicated in the interspinous and supraspinous ligament; thickening of the ligamentum flavum occurs with aging but is not observable in the sagittal view. Finally, facet cartilage arthritis is revealed on the inferior facet of L5.

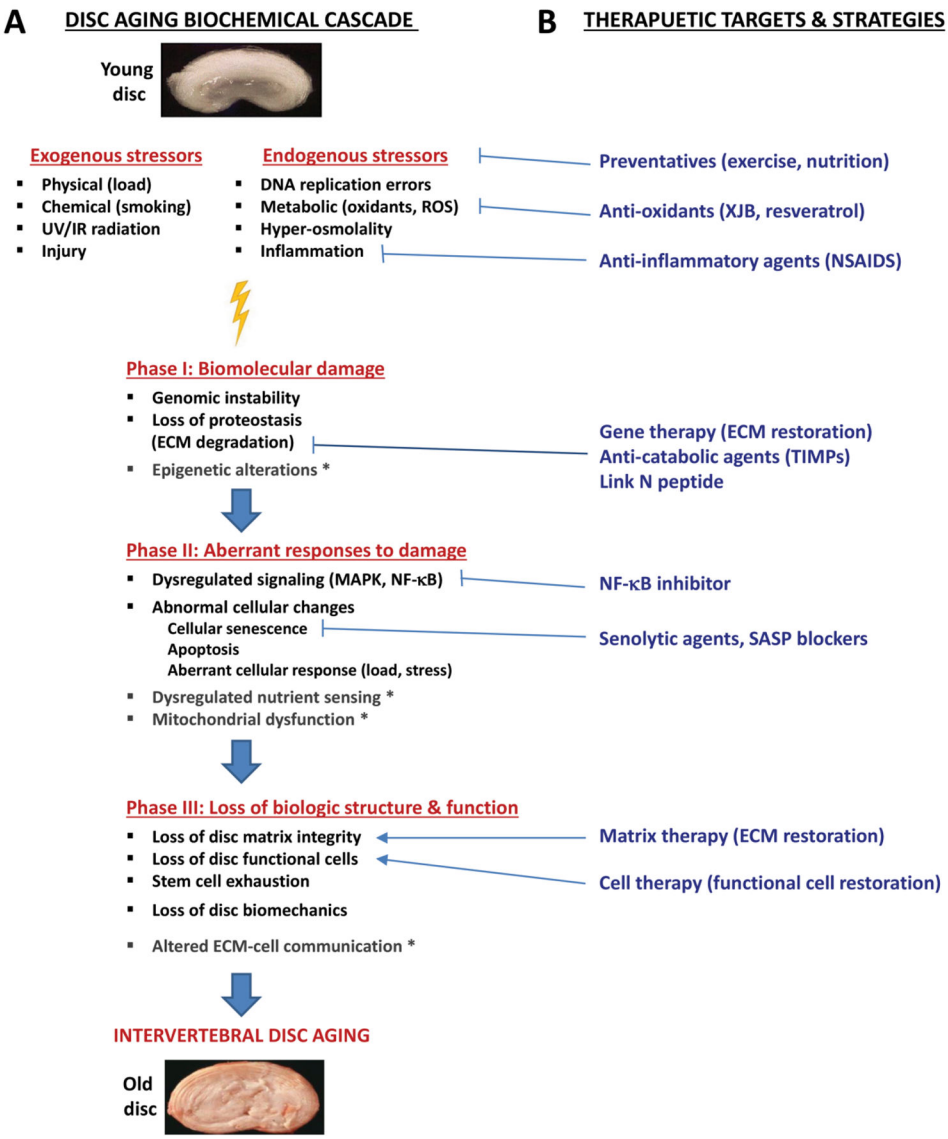

Figure 3. Proposed biochemical cascade in disc aging process and potential therapeutic targets

(A) With aging, there is time-dependent accumulation of biomolecular damage (Phase I), most importantly, DNA damage, in the disc due to exogenous and endogenous factors. Cellular responses to accumulated damage over time become dysregulated (Phase II) leading to more damage and eventual loss of disc biologic structure and function (Phase III). This results in degenerative changes observed in aged discs. * Observed in other tissues but not yet investigated in disc tissue. **(B)** Potential therapeutic targets to delay or ameliorate age-related degeneration. Oxidative and inflammatory stress can be reduced with anti-oxidants and anti-inflammatory drugs. Reducing chronic activation of NF-κB signaling by pharmacologic intervention may be efficacious in delaying age-related degeneration. Moreover, removal of senescent cells or blocking formation of SASP could potentially mitigate disc matrix catabolism. Finally, protein-, gene- and cell-based therapy could also conceivably delay or help restore age-related loss of disc matrix and functional cells.

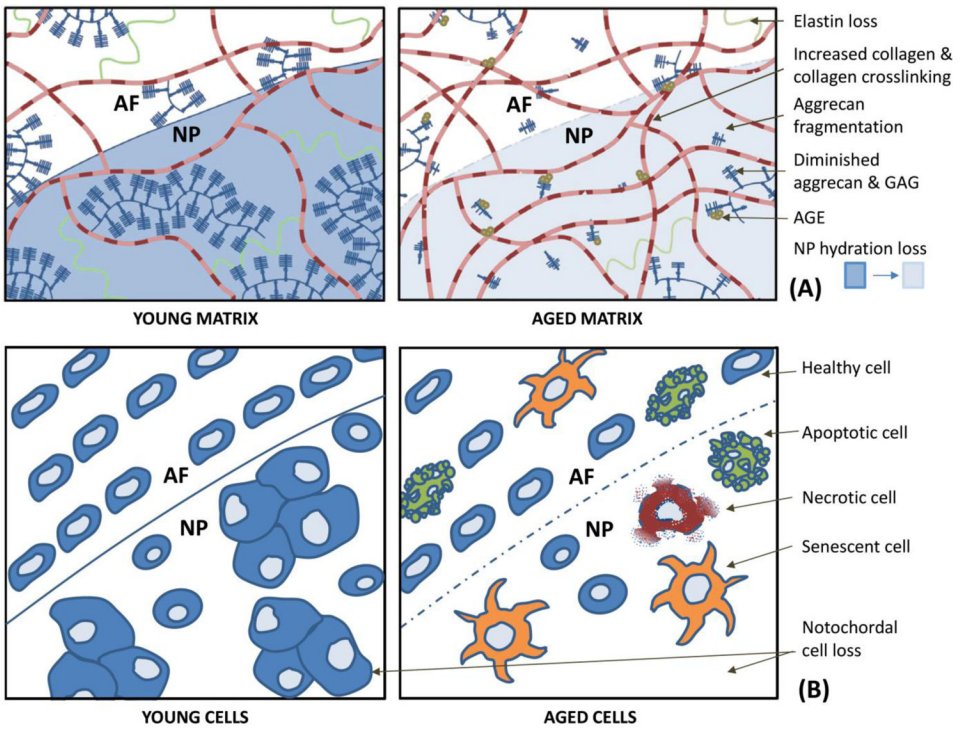

Figure 4. Molecular and cellular features of the aging disc

Young and aged extracellular matrix (A) and cells (B) are schematically compared to summarize important changes that occur during disc aging. **Panel A,** young matrix is rich in elastin (green, coiled fiber), aggregated aggrecan (dark blue, bottle-brush aggregate), and collagen fibers (banded fibers). Aged matrix shows loss of elastin, increased collagen and collagen crosslinking, fragmented aggregan, diminished GAG quality, reduced aggregan aggregates, increased accumulation of advanced glycation end-products (AGEs) along with lower hydration[49]. **Panel B,** young AF cells are elongated fibrochondrocytes and NP cells are a mixture of large, clustering, notochordal cells and smaller, chondrocyte-like cells. Aged cells show reduced cellularity, loss of notchordal cells, and incidence of senescence, apoptosis, and necrosis.

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Table 1

Animal models used to investigate age-associated intervertebral disc degeneration

Mouse models

Gene symbol	Gene name/description	Protein function	Model	Age-related disc degenerative changes	Ref
Ercc1	*Excision repair cross-complementing rodent repair 1*	DNA repair	M	Systemic *ERCC1*-deficiency accelerated age-dependent disc degeneration	28
n/a	*Senescence-accelerated mouse (SAM)*	Gene not yet identified	Derived from AKR/J	Amyloid deposition in disc AF, articular cartilage	221
Dmd^{Mdx}	*X-chromosome-linked muscular Dystrophy*	Dystrophin-skeletal muscle integrity	M	Age-related loss of disc proteoglycan	222
Cnn2	*Connective tissue growth factor* (CTGF or CCN2)	Matricellular protein involved in cellular adhesion, migration, ECM synthesis	TG	Notochord-specific *CCN2* deletion accelerated age-dependent disc degeneration	223
Bgn	*Biglycan*	Small leucine repeat proteoglycans ECM	TG	Age-related early onset of disc degeneration	224
Ky	*Kyphoscoliosis peptidase*	Cytoskeleton-associated protease required for normal muscle growth	ARM	Degenerative changes in cervical and thoracic discs; kyphosis	225
Skt	*Sickle tail*	Linked to Danforth's short tail locus	TG	Abnormal development of the intervertebral disc	226
n/a	*Danforth's short tail locus (Sd)*	Gene not yet identified	M, SD	Aberrant patterns of vertebrae and disc development	227
C57Bl6	*Wildtype mice*	Natural aging mice		Natural aging-related disc degeneration	228

Other animal models

Animals	Scientific name	Key features & analysis methods	Age-related disc degenerative changes	Ref
Hamster	*Cricetinae*	Chinese hamsters prone to development of spontaneous diabetes. Spines of age 10–33 months histologically analyzed	Aging changes in intervertebral discs Spondylosis was present at an earlier age in diabetic than in nondiabetic hamster.	229
Rat	*Rattus*	Male Fisher 344 rats. μCT of spines of age 3, 12, 18, 30 months	Age-associated morphometric and degenerative disc changes	230
Sand rat	*Psammomys obesus*	Spontaneous development of diabetes. Age 1–46 months analyzed radiographically and histologically	Age-related and diabetes-related spontaneous development of lumbar disc degeneration	231

Other animal models

Animals	Scientific name	Key features & analysis methods	Age-related disc degenerative changes	Ref
Rabbit	*Oryctolagus cuniculus*	New Zealand White rabbits. Age 1–30 months studied by MRI, histology, gene expression	Natural aging-related disc degeneration	232
Pig	*Sus scrofa domesticus*	Porcine discs of age 2–3 weeks, 6–9 month, 2–3 years analyzed by histology, GAG assay, cell density assay, gene expression	Disc MMP-1 increased with age. GAG and collagen I, II, aggrecan decreased with age.	219
Dog	*Canis lupus familiaris*	Discs of chondrodystrophic (CD) and nonchondrodystrophic(NCD) dogs of age 1–7 years were analyzed by histology, GAG, and MMP activity	CD dogs showed early age onset of disc degenerative changes compared to NDC dogs.	233
Sheep	*Ovis aries*	Discs of new-born, 3, 12, and >36 months were analyzed mechanically and microscopically to assess nucleus pulposus-endplate integration	Rapid increase in NP-EP insertion nodes between birth and 3 months, after which this integration remained constant.	234
Alpaca	*Vicugna pacos*	Young (2–6 years) and older (>10 years) alpaca underwent MRI evaluation to detect cervical spine degeneration	No cervical disc degeneration in young alpacas. Increased disc degeneration incidence and severity at lower cervical levels in older alpacas	235
Rhesus Macaques	*Macaca mulatta* (Macaques monkeys)	Longitudinal study of macques 11–32 years of age to assess disc space narrowing (DSN) by radiography.	Age-associated disc space narrowing, osteophytosis, increased disc tissue stiffness	236

Genetic models: *Mutation* (M), *Transgenic* (TG), *Autosomal recessive mutation* (ARM), *Truncation mutation* (TM). N/A: not applicable.