

Review Article

Cell Senescence: A Nonnegligible Cell State under Survival Stress in Pathology of Intervertebral Disc Degeneration

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The intervertebral disc degeneration (IDD) with increasing aging mainly manifests as low back pain (LBP) accompanied with a loss of physical ability. These pathological processes can be preliminarily interpreted as a series of changes at cellular level. In addition to cell death, disc cells enter into the stagnation with dysfunction and deteriorate tissue microenvironment in degenerative discs, which is recognized as cell senescence. During aging, many intrinsic and extrinsic factors have been proved to have strong connections with these cellular senescence phenomena. Growing evidences of these connections require us to gather up critical cues from potential risk factors to pathogenesis and relative interventions for retarding cell senescence and attenuating degenerative changes. In this paper, we try to clarify another important cell state apart from cell death in IDD and discuss senescence-associated changes in cells and extracellular microenvironment. Then, we emphasize the role of oxidative stress and epigenomic perturbations in linking risk factors to cell senescence in the onset of IDD. Further, we summarize the current interventions targeting senescent cells that may exert the benefits of antidegeneration in IDD.

1. Introduction

A systematic analysis for the Global Burden of Disease Study 2017 showed that low back pain was the top cause of years lived with disability (YLD) counts from 1990 to 2017 [1]. Concerning the relation with potential loss of functional status in the work force, disc degenerative diseases, as the major cause of low back pain, have posed huge burden on the global health care systems and economies [2, 3]. Ascribed to the life-span extension and the growth of aging population worldwide, the incidence of IDD will progressively and steadily increase and exacerbate the problem above. Multiple studies support the notion that degenerative discs always accelerate cellular senescence which may precipitate the pathology of IDD [4]. Disc cells undergo not only apoptosis but also dys-

function in IDD in an age-related manner. The latter, an abnormal cell state, plays a crucial role in matrix homeostasis imbalance. New pharmacological strategies focus on the elimination or reverse of senescent cells in degenerative discs for the prevention and treatment of IDD [5, 6].

As a fundamental cause of aging, cell senescence has been proved in all major chronic diseases in the cardiovascular system, nervous system, and especially musculoskeletal system and in chronic tumor [7–10]. However, the initial factors triggering disc cell senescence are exceptionally complex. Among the perspectives proposed in recent years to explain the emerging senescent cells in degenerative discs, one states that inner age-related stress and external microenvironment-derived stimuli both act as a promoter of cellular senescence to accelerate IDD [11]. Since the IDD is involved in multiple

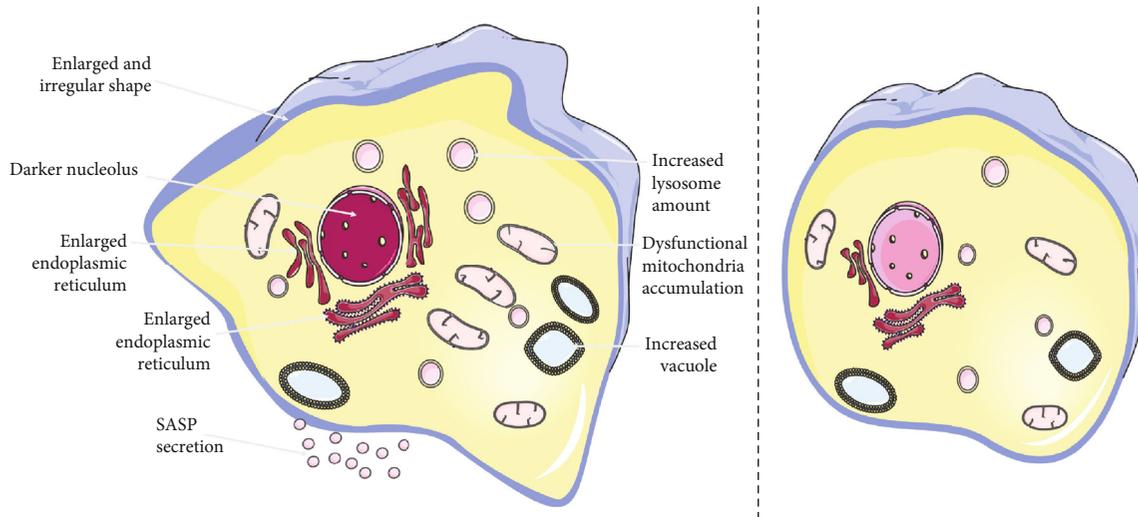


FIGURE 1: Morphological differences between senescent cells and normal cells. There are characteristic morphological alterations emerging in senescent cells compared with normal cells, such as increased but irregular cell size, darker nuclear chromatin, increased lysosome amount, dysfunctional mitochondrial accumulation, enlarged endoplasmic reticulum, increased vacuole, and secreting SASP. These features can be meaningful tools for identifying senescent cells and determining the senescent process.

risk factors, mechanisms underlying these stressors that induce adaptive cell state changes have not been fully clarified. Finding critical intermediators from intricate cues seems to be conducive to inhibit cell senescence at the beginning of IDD.

In this review, we introduce the features of cell senescence and emphasize that it occurs as a general stress response. And we illustrate the impact of senescence on IDD. The role of oxidative stress and epigenetics linking multiple risk factors to cell senescence is summarized. Finally, we discuss relative therapeutic strategies in IDD. Hopefully, the brief introduction could primarily establish a link between cellular survival stress and IDD from a perspective of cell senescence.

2. Cell Senescence

2.1. The Features of Cell Senescence. Cell senescence is characterized by a cell state of proliferating arrest and secreting senescence-associated secretory phenotype (SASP) [12]. Cell cycle arrest plays a bilateral role in pathophysiological processes. On the one hand, it inhibits cell division and blocks the tissue renewal. On the other hand, it also prevents a further proliferation of harmful cells undergoing senescence [13]. Underlying cell cycle arrest, various molecular signals and pathways organize a complex network to exert effects [14]. All of them eventually converge on the p53/p21/retinoblastoma (RB) and p16/RB pathways to prevent senescent cell proliferation [14, 15]. Telomere shortening and stressor induction lead to replicative-related senescence (RS) and stress-induced premature senescence (SIPS) with respective mechanisms [16]. Previous investigations proved that these two major senescence phenotypes were involved in most chronic diseases.

As another major characteristic of senescent cells, SASP contains secretions of proinflammatory factors, chemokines,

cytokines, protein enzymes, and other bioactive factors [17]. In mechanism, some certain pathways including nuclear factor kappa-B (NF- κ B), cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING), and mammalian target of rapamycin (mTOR) have been proved to control SASP [18, 19]. Meanwhile, epigenetic perturbations may regulate SASP at the transcriptional level [18]. This specific phenotype mediates cell-to-cell and cell-to-matrix signals to trigger immune-related clearance, inflammation formation, cell reprogramming, and microenvironment remodelling, which are involved in a series of diseases [17, 20–22]. Similar to senescence-associated markers like senescence-associated β -galactosidase (SA- β -gal), several major SASP components are used to indicate cell senescence [18, 23, 24]. However, the secretory factors are always variable due to their heterogeneity and nonspecificity. Therefore, increasing studies based on the diversity of SASP are carried out in recent years.

In addition to cell cycle arrest and SASP, there are multiple changes of cellular components and morphology emerging in senescent cells [18] (Figure 1). These traits can be meaningful tools for distinguishing senescent cells and determining senescence process [4, 25–27]. However, cellular senescence is actually weakly defined due to the diversity of senescent biomarker expression in various subtypes. Recently, Ogrodnik et al. proposed a new model coordinating cell expansion and cell cycle arrest to screen senescent cells, which provided us a novel idea [28]. Meanwhile, increasing senescence subtypes and hallmarks are found in different cell types [18]. Exploring novel biomarkers with specifically differential expression in various senescence types is of great significance, which has become the focus of current researches [29, 30]. Although the standard of identification is not fully unified, antagonizing the progression of cell senescence has been a potential strategy in approaches to blunt chronic diseases.

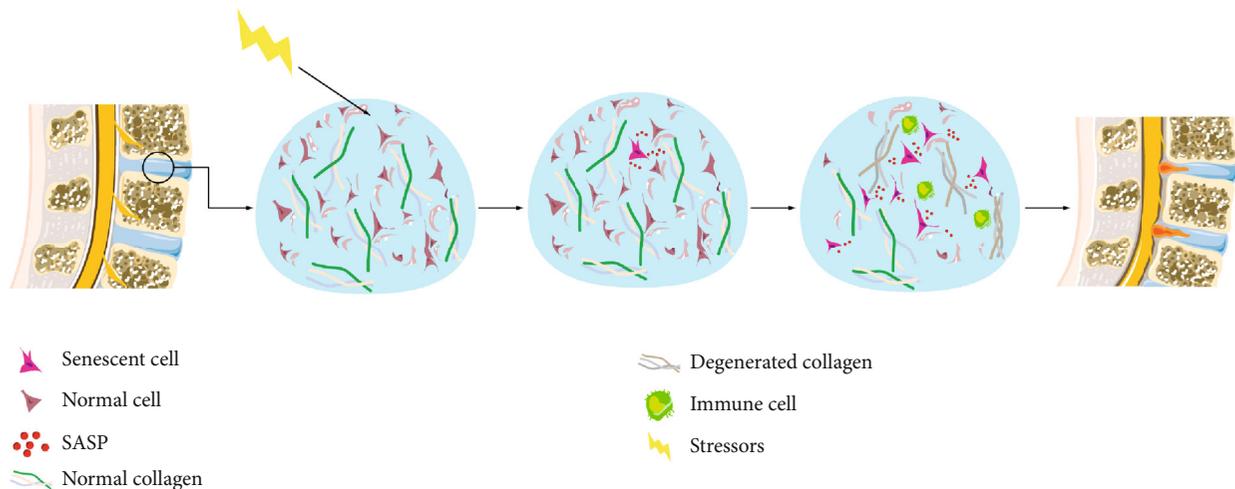


FIGURE 2: The role of cell senescence in the pathogenesis of IDD. Cell senescence occurs in response to intrinsic and extrinsic stressors in intervertebral discs. The primary senescent disc cells cause chronic inflammations, immune cell recruitment, and ECM degeneration through SASP. The deterioration of tissue microenvironment and persistent stress cause the accumulation of senescent cells which further accelerates tissue remodeling and eventually leads to IDD.

2.2. Cell Senescence Occurs under Various Stressors. Cell senescence has been proved in majority of cell types as a response to various stressors [31]. The telomere exhaustion was firstly proved to cause cell senescence during cell division [32]. In addition to deoxyribonucleic acid (DNA) replication-associated stress, cells also face many other stressors from the inner nucleus such as oncogene activation [33]. For instance, the expression of oncogenic *ras* transforms rodent cells to a permanent G1 arrest by upregulating p53 and p16 [33]. The cell cycle arrest prevents the original tumorigenesis. Moreover, under the stress of radiotherapy [34] or chemotherapy [35] without a fatal dose, cells suffer DNA damage and are driven into the premature senescence instead of apoptosis to seek survival. Moreover, under stressful microenvironment, epigenomic perturbations also regulate senescence process. The modification of global or local chromatin alters specific gene expression to trigger cellular senescence independent of the DNA damage response (DDR) [36].

Meanwhile, cells need to withstand stress from the extra nucleus. As a cellular response to tissue damage, cells at the damage site undergo transient senescence. Senescent fibroblasts and endothelial cells secrete platelet-derived growth factor AA (PDGF-AA) to induce myfibroblast differentiation at the site of injury, ensuring wound closure [37]. Interestingly, senescent cells provoke plasticity of neighboring cells to enter transient reprogramming, which enhances differential potential and is conducive to tissue repair [38, 39]. The different role of senescent cells exists under embryonic development pressure. A p21-mediated senescence is found in the apical ectodermal ridge (AER). Growth factors like fibroblast growth factors (FGFs) are secreted to drive limb patterning and optimize tissue remodeling, which is recognized as a programmed senescence [40, 41]. Cells also encounter the stress from intracellular bioenergetic metabolism due to increasing dysfunctional mitochondria during aging [42]. As a metabolically active cell state, mitochondrial

dysfunction-associated senescence (MiDAS) was proved to be implicated in this cell state transformation via adenosine 5'-monophosphate-activated protein kinase- (AMPK-) mediated p53 activation [42]. Despite the different stressors and complex signal pathways, the passive cell fate eventually arrives at the senescence outcome.

3. Cell Senescence Influences IDD

The intervertebral disc is composed of an inner highly hydrated colloid named the nucleus pulposus (NP) encircled by a dense fibrous ring, the annulus fibrosus (AF). The above parts are interfaced with the adjacent vertebrae through the vertebral endplates (EPs) [43]. There are specific features of oligocells and abundant matrix in intervertebral discs compared with other tissues like nervous or myocardial tissues [44]. Resident cells ensure long-term matrix renewal to maintain the balance of extracellular matrix (ECM) anabolism and catabolism with the huge survival burden during aging. Disc cells with slow turnover are suggested to be chronic senescence prone before apoptosis under cell replication and other stressors (Figure 2). A detrimental role of persistent senescent cells is predominant in chronic senescence processes like IDD [16, 33, 38]. Contrary to effective self-clearance of senescent cells in other tissues, the avascular nature of discs partly limits the immune-mediated clearance to cause the abnormal accumulation [11, 45]. Therefore, cell senescence in IDD results from not only the general aging process but also the structure and tissue specificity of discs.

3.1. Senescence Influences Functional Cells in IDD. As the functional cells in discs during intervertebral disc growth, NP, AF, and EP cells are in charge of ECM anabolic balance in separate regions, which play a key role in maintaining normal extracellular microenvironment [43]. These functional cells derived from degenerative discs all undergo the decline of proliferating potential, with high expressions of SA- β -gal

and p16 (Ink4a) as well as decreased telomere length [4, 46, 47]. Interestingly, these senescence-associated changes in EPs seem to appear later than in AF and NP [46]. Relatively rich nutrition as well as milder local mechanical stress may explain this temporal sequence [48]. Although the cellular turnover in discs is slow, cell division during an expected lifespan of several decades is sufficient for inducing telomere length erosion and DNA damage to cause replicative stress. Subsequent activation of the p53/p21/RB pathway drives functional cells into the senescent cell state of cell cycle arrest [49]. The increasing stagnation during aging depletes the reservoir of cell renewal to further decrease remaining functional cells in IDD. Recent study found that deleting cell cycle inhibition gene p16 of NP cells could significantly decrease the proportions of senescent cells and SASP in degenerative discs [50]. Interestingly, Novais et al. observed the opposite result. They found that deficiency of p16 (Ink4a) did not alter the disc cell viability and the percentage of senescent cells [51]. Probably because p16 (Ink4a) acted as a main trigger for SIPS, knocking out this gene failed to avoid the replicative stress during degeneration [52, 53]. Despite the impotent inhibition of age-dependent degeneration, significant changes in the content and constituent of ECM occurred indeed. Changing senescent cells via targeting cell cycle relative genes or molecules has been focused on increasingly for ameliorating IDD.

In addition to replicative stress, as shock absorbers, the NP always suffers more mechanical stress than other tissues [54]. The accumulation of senescent NP cells was observed in scaffold and organ cultures under high-magnitude compression, confirming that mechanical-related SIPS existed in IDD [55]. Selective elimination of senescent NP cells protects tail suspension- (TS-) induced IDD mice from disc degeneration. Similar relationship between mechanical stress and cell senescence is proved in AF and EP cells [46, 47]. Further investigation indicated that the increased oxidative stress acted as a causative intermedator during mechanical factor-associated SIPS in degenerated discs [50]. In fact, intervertebral discs also have to face systemic stress derived from other diseases or risk factors. A critical review analysis indicated that vertebral EP capillaries got significantly narrower to block the nutrition supplement in diabetic animals, which might partly accelerate the diabetic-related IDD [56]. In diabetic patients, dysfunctional endothelial cells could exhibit senescence-like cell state via thrombospondin 1-cluster of differentiation 47- (TSP1-CD47-) dependent signaling to impair microvessels [57]. Although similar research in IDD is not reported, an endothelial senescence-related microcirculatory disturbance may partly promote the degeneration of EPs or even the whole discs under systemic risk factors like diabetes and hyperlipidemia. Therefore, cellular senescence in degenerative discs cannot always be ascribed to DNA replication and cell division as many cases of IDD occur in the stressful environment. In fact, RS and SIPS jointly accelerate the emergence of senescent cells in IDD compared with age-matched normal discs [4, 58].

3.2. Senescence Influences Extracellular Microenvironment in IDD. In addition to the stagnation of cell proliferation, the

senescent disc cells also overexpress a range of bioactive components as fundamental features of SASP [12, 59–62]. SASP during IDD plays a crucial role in altering tissue microenvironment [12, 63]. Among them, proinflammatory factors such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor- α (TNF- α) have been proved to be generally upregulated by senescent cells to trigger inflammatory cascades, which cause the chronic inflammation and accelerate IDD [51, 64]. Despite the low specificity, growing studies recognize them as indexes to cell senescence [18, 23, 24]. Apart from the inflammatory effect, some specific cytokines like IL-6 and IL-8 also provide intercellular dialogue to induce adjacent cells to be senescence or reprogramming prone [12, 21, 24, 39]. The persistent dedifferentiation of neighboring cells may inhibit the supplement for lost or damaged cells and disturb tissue rejuvenation [65]. Meanwhile, specific SASP like (C–C motif) ligand 2 (CCL-2), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), and chemokine (C–C motif) ligand 20 (CCL-20) are proved to induce adjacent normal cells to be “paracrine senescence” [63]. Although it has not been fully clarified in IDD, these cell-to-cell signals may partly promote the accumulation of dysfunctional disc cells.

As the fertile soil for NP cells, collagen matrix consisted of generous type II collagen and aggrecan provides nutrients for cell metabolism and maintains disc tensile stiffness [43]. Collagen falls into the stagnation period of renewal during the development of IDD. Che et al. observed that many matrix remodelling enzymes in SASP like matrix metalloproteinases (MMPs) family (MMP3, MMP9, MMP10, and MMP13) were secreted increasingly in senescent disc cells of TS mice [66]. MMPs degraded collagen II as well as ECM proteins like proteoglycan and aggrecan, decreasing hydration of NPs in IDD. In addition to MMPs, senescent disc cells also raise the level of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family (ADAMTS-4, ADAMTS-5). The increased proteolytic enzymes cleave the aggrecan interglobular domain and drive the matrix PG loss in vitro compared to nonsenescent disc cells, where certain pathways like β -catenin may play a key role [62, 67, 68]. As a crucial regulator in joint development, β -catenin is proved to mediate cell senescence in cartilages and discs, indicating that analogical cartilaginous tissue may be comparable in senescence mechanism [68, 69]. The continuous secretion of catabolic enzymes produced by senescent cells severely perturbs the ECM homeostasis since disc cells are unable to act as the major producer of ECM. Notably, SASP in senescent disc cells may be a unique response of tissue to different stress like physical activities, and the specific disc structure for low pH, low oxygen tension, and cellular environment may further amplify this effect of SASP on ECM [12, 70].

4. The Underlying Pathogenesis of Cell Senescence in IDD

As a multifactor-induced degenerative disease, IDD is associated with many potential risk factors such as abnormal

mechanical loading, inappropriate exercise, obesity, and even specific diet style [55, 71, 72]. Growing studies indicate that oxidative stress (OS) and epigenomic perturbations may act as major mediators between these risk factors and cell senescence in IDD [73].

4.1. Oxidative Stress. Reactive oxygen species (ROS) production is inevitable due to the oxygen-utilizing metabolism of disc cells despite the hypoxic microenvironment in IDD [74]. Subsequent OS increases cellular survival stress which activates the DDR to trigger cell senescence and force the dysfunction of resident cells [45, 75]. Apart from the harsher microenvironment like insufficient nutrient supply and metabolite clearance during aging, exogenous stimuli exacerbate excessive ROS generation in disc cells [76]. These excessive oxidizing products drive disc cells into the state of SIPS in IDD [14]. Underlying the cellular transformation, diverse signaling pathways have been proved to participate in the senescence process (Figure 3). Considering the nonmitochondrial-dependent ROS productions like nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase are scarcely reported in the discs, we focus on the role of mitochondrial-derived ROS in cell senescence during IDD [76, 77].

NP cells exhibit the stagnant state with senescence markers like high SA- β -gal activity by the accumulation of ROS in NP cells under various stimuli such as high-magnitude compression [4] and high glucose [58]. Indeed, the OS appears due to the imbalance between excessive ROS generation and impaired antioxidative molecules or enzymes disturbed by these stressors [78]. Recent studies significantly inhibited cell senescence through reversing this imbalance, emphasizing the role of OS in degenerative discs. The hydrogen peroxide (H_2O_2) was used to aggravate intracellular oxidative stress to provoke DNA damage and successfully induced cell cycle arrest as well as SASP of NP cells via the ataxia telangiectasia-mutated checkpoint kinase 2-p53-p21 (ATM-Chk2-p53-p21) pathway [58, 79]. This H_2O_2 -induced premature senescence could be partly reversed by the antioxidants like N-acetyl-L-cysteine [58]. Targeting the elimination of ROS components like H_2O_2 , hydroxyl ($\cdot OH$), and superoxide anion ($\cdot O_2^-$) becomes a potential therapeutic strategy. Recently, Larrañaga et al. constructed antioxidant polymer capsules, which can be engulfed by NP cells to scavenge intracellular ROS like H_2O_2 and $\cdot OH$. The decline of ROS level significantly attenuated the catabolic and proinflammatory cell phenotype [80]. In addition, the ROS-induced autophagy inhibition is also proved to cause oxidative stress in many cell types [81–83]. And by enhancing and restoring impaired autophagy during IDD, Kang et al. affirmed that excessive ROS production could be suppressed to protect the mitochondrial hemostasis and ameliorate NP cell senescence [84].

Mitochondria are not only the main source of excessive ROS production but also the major attacking target of ROS [37, 76, 85, 86]. Literature demonstrated that OS could be increased due to proinflammatory factors like TNF- α to induce mitochondrial dysfunction to aggravate the senescence in NP cells [87, 88]. Moreover, TNF- α -induced abnor-

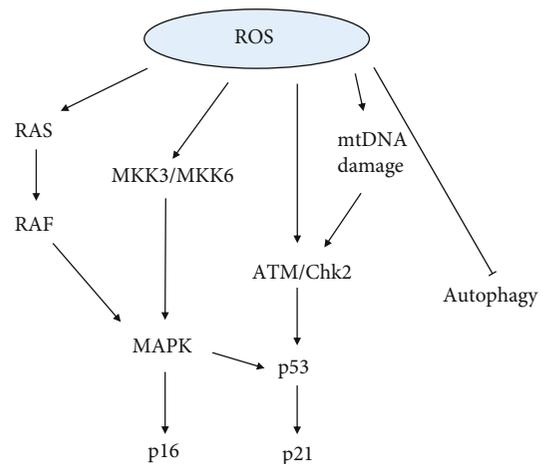


FIGURE 3: The signaling pathways between ROS and cell senescence.

mal OS can also disturb hypoxic conditions in senescent EPs, which transfer the cartilage endplate stem cell (CESC) differentiation from chondrogenesis to osteogenesis [89]. The ROS dysregulate respiratory enzyme activity and cause the respiratory chain defect in dysfunctional mitochondria, leaking extensive electrons to produce more ROS [76]. Lack of effective antioxidant mechanisms during senescence, a ROS-mediated positive feedback loop may act as a critical murderer to accelerate senescence. In turn, interventions targeting cellular senescence may attenuate the oxidative stress. For instance, Che et al. observed a reverse of senescent NP cells by p16 deletion in IDD, which was accompanied with a reduced oxidative stress [50]. A strong interaction between oxidative stress and disc cell senescence brings us more possibility for intervening IDD. Moreover, Wang et al. confirmed that ROS-mediated mitochondrial dysfunction could be inhibited by polydatin via activating the Nrf2/hemeoxygenase 1(HO-1) pathway to prevent senescence in rat NP cells [88], further testifying a significant effect of blocking this vicious circle. Although other pathways independent of ROS may also mediate the mitochondrial dysfunction to drive the senescent phenotype [85], maintaining a mitochondrial homeostasis should exert antioxidative benefits on preventing cell senescence.

4.2. Epigenomic Perturbations. Senescence phenotypes always appear in various forms no matter in different senescent cells or different senescence stages of the same cell [90]. Multiple stressors can be drivers of disc cell senescence through altering chromatin structural organization or gene expressions during aging, where epigenetic perturbations act as a critical link [91].

In recent years, increasing studies have focused on the role of epigenetics during IDD. As major protein components of chromatin, histone and related modifications participate in DNA damage-induced senescence. Under various stressors, the histone phosphorylation at the site of DNA damage initiates the first step of cascade reactions from DDR to the activation of p53/p21/Rb and eventually causes an irreversible cell cycle arrest [60, 92, 93]. The histone trimethylation also plays a critical role in the positive feedback

TABLE 1: Function characterization of epigenetic alterations in IDD in text.

Name	Type	Target	Expression	Functional role	Reference
/	Histone phosphorylation	Chk2	Up	Cell cycle arrest	[92, 93]
H3K27me3	Histone trimethylation	NOX4	Up	Oxidative stress	[94]
PARK2	DNA methylation	Parkin	Up	Mitophagy	[95, 96]
WNT5A	DNA methylation	Wnt- β	Up	Proinflammation	[98]
CARD14	DNA methylation	NF- κ B	Up	Proinflammation	[98]
EFHD2	DNA methylation	NF- κ B	Up	Proinflammation	[98]
RTKN2	DNA methylation	NF- κ B	Up	Proinflammation	[98]
MAPKAPK5	DNA methylation	MAPK	Up	Proinflammation	[98]
PRKCZ	DNA methylation	MAPK	Up	Proinflammation	[98]
miR-21	Noncoding RNA	PDCD4	Down	Proliferation	[100]
miR-146a	Noncoding RNA	TRAF6	Down	ECM degradation	[101]
miR-98	Noncoding RNA	IL-6/STAT3	Down	Inhibition of ECM degeneration and apoptosis	[102]
miR-494	Noncoding RNA	SOX9	Down	ECM degeneration and apoptosis	[103]
miR-132	Noncoding RNA	GDF5	Down	ECM degradation	[104]
SPARC	DNA methylation	SPARC	Down	ECM degradation	[106]

loop between enhancer of zeste homolog 2 (EZH2) and NADPH oxidase 4 (NOX4) to promote the NP cell senescence during IDD [94]. The latter of the loop has been proved as an important mediator of various stressors to induce OS-associated SIPS in degenerative discs [94]. Recently, Williams et al. observed a hypermethylation level at one cytosine guanine (CpG) site (cg15832436) of the parkinson protein 2 (PARK2) promoter in IDD [95]. The modification of PARK2 at transcriptional level may regulate mitochondrial-associated OS in degenerative NP and EP cells via parkin-mediated mitophagy [96, 97]. Therefore, epigenetic changes may affect cellular senescence via the regulation of OS at transcriptional level. In addition, the genome-wide analysis of DNA methylation profile indicated that DNA methylation profiles were significantly distinct between early and advanced stages of human IDD, especially in NP tissue [98]. The group further identified 220 differentially methylated loci comprising 187 individual genes. Among them, Wnt-5a, one of the representative ligands of the wntless/integration-1- (Wnt-) β -catenin pathway, was observed differentially methylated in advanced stage of IDD [98]. Notably, the Wnt- β -catenin pathway has been reported to be relative to promote disc cell senescence via a positive feedback loop of Wnt signaling and cytokines [60]. Moreover, they also found several hypermethylated genes that may play important roles in regulating catabolic molecules of IDD, such as caspase recruitment domain 14 (CARD14), EF-hand domain-containing protein D2 (EFHD2), rhotekin 2 (RTKN2), mitogen-activated protein kinase-activated protein kinase 5 (MAPKAPK5), and protein kinase C/ ζ (PRKCZ) associated with the MAPK pathway [98]. These mitogen-activated protein kinase (MAPK) and NF- κ B-associated genes may be involved in controlling SASP. The differential methylations dominantly exist in genes which are located upstream instead of directly regulating downstream target genes associated with proinflammatory cytokines and catabolic molecules [98]. However, considering a series of discoveries about DNA demethylation located in

promoter sites of catabolic enzyme MMPs in osteoarthritis (OA), further studies like ribonucleic acid (RNA) sequencing are needed to explore this possibility in IDD with more samples [99].

Based on previous literature, the overexpressions of many catabolic enzymes like MMPs and ADAMTS are recognized as characteristics of senescence-associated ECM remodelling in senescent discs during IDD [50, 62]. Many microRNAs have been proved to target these enzymes to modulate the ECM remodeling process through a specific pathway, respectively [100–102]. Notably, some of them such as microRNA-132 (miR-132) and microRNA-494 (miR-494) are reported to be upregulated by the methylation of CpG islands in the promoter region in degenerative human NP cells [103, 104]. Secreted protein acidic and rich in cysteine (SPARC) is widely accepted as another matricellular protein to regulate cell-matrix interactions and collagen fibrillogenesis in IDD [105]. And the hypermethylation of SPARC promoter is observed to decrease related gene expressions of degenerative discs in both humans and animal models, which may partly cause ECM remodeling in IDD [106].

Although the relationship between epigenetics and cell senescence in IDD has been primarily established, how various stressors trigger these epigenetic perturbations is still unclear. Further exploration for these epigenetic changes will provide us more potential biomarkers as well as therapeutic targets for the management of IDD (Table 1).

5. The Promising Therapy for IDD: Senotherapies

Optimizing disc health in an aging population requires maintaining normal renewal and proliferation as well as function of parenchymal cells [107]. Due to the key role that cell senescence plays in IDD, recent studies highlight the significance of developing senotherapeutic approaches that target senescent cells therapeutically to blunt or even reverse IDD.

There are several potential strategies for antisenescent cells including directly eliminating senescent cells, targeting SASP for reducing its adverse effects, and relieving senescence-associated growth arrest [108]. The first way seems to be the most direct and hitting home. Specifically, preventing accumulation of p16(Ink4a)-positive senescent cells in intervertebral discs has been proved to ameliorate matrix homeostasis and mitigate the progress of IDD [51, 109]. Furthermore, small molecules termed senolytics have emerged in recent years, and these selective eliminators of senescent cells are considered to exert great benefits on antiaging process [110]. Dasatinib and quercetin targeting ephrin (Eph) receptor tyrosine kinases and phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway modules, respectively, were the first molecules to be identified as senolytics in 2015 [5]. From then on, many drugs are found to be efficacious in animal models and human cells or tissues [73]. Some known antiaging compounds against other diseases exert new senotherapeutic activity on IDD. As an antidiabetic drug before, metformin confers antisenescence effect in NP cells and annulus fibrosus stem cells (AFSCs) which significantly ameliorate disc degeneration [111, 112]. Classic bisphosphonate antiosteoporosis (OP) drug has been demonstrated to benefit not only OA but also IDD [6]. The discs of rats treated with alendronate significantly retarded EP thickening and increased the aggrecan and type II collagen anabolism in discs [6]. Although the underlying mechanism has not been clarified, the analogical benefits in OP, OA, and IDD provide the possibility for preventing the whole musculoskeletal system aging and degeneration. Classic senotherapeutic drug repurposing emerges as an interesting aspect and may provide potential appealing therapeutic approaches for IDD [110]. Notably, whether the excellent result is a cause of antiaging effects or a consequence is worth considering because of the multifaceted effects of these compounds [12]. Despite their senotherapeutic effects, these drugs possess various disadvantages such as off-target effect and low potency. For example, the usage of dasatinib is reported to inhibit hypoxic pulmonary vasoconstriction to induce pulmonary hypertension independent of regular Src kinase and tyrosine kinase-induced mechanism [113]. As a broad-spectrum kinase inhibitor, it may target potential irrelevant kinases to cause nonessential damage [12]. Moreover, the avascular structures in intervertebral discs are not conducive to drug accumulation after systemic administration, which may further amplify the off-target effect and low potency. Therefore, delivering drugs directly to degenerative sites may partly avoid these deficiencies. In recent years, some drug delivery systems connected with antibodies against senescence-specific surface antigens are synthesized to solve the problem of targeted drug delivery. Qu et al. used the beta-2-microglobulin antibody (anti-B2MG) to successfully construct a nanoparticle tetrahedron which could recognize and selectively clear senescent cells [114]. Amor et al. screened a cell surface protein called the urokinase-type plasminogen activator receptor (uPAR) and constructed senolytic chimeric antigen receptor (CAR) T cells

to eliminate target cells in multiple senescence models, which emerged as a striking senolytic strategy [29]. Therefore, further screening and identifying senescence-specific surface antigens will provide more potential targets, which may facilitate the diagnosis and interventions of senescence [29, 115].

Another promising strategy is targeting the SASP for blocking the adverse effects of specific components like pro-inflammatory factors and matrix catabolic enzymes, without changing the inner antioncogenic pathways and cell cycle in senescent cells [108, 116]. This could be achieved by targeting signaling cascade upstream of the SASP of senescent disc cells, such as NF- κ B or p38 MAPK as well as related regulators [12, 87, 117]. For instance, depletion of ATM downregulates the expression of SASP factors like ADAMTS and MMPs and significantly attenuates age-associated IDD [118, 119]. However, the SASP expression on different cells, tissues, and organs is not always constant considering the diversity of senescence types [65]. Finding relative conserved molecular phenotypes existing in different senescence types will be meaningful for clarifying the mechanism underlying SASP and find potential targets [65]. Moreover, considering the self-clearing mechanism of senescent cells via SASP [120–122], it is essential to weigh the benefit between SASP-mediated immune clearance and inflammation damage in IDD and the conclusion is still not be casually established [12]. To avoid this dilemma, targeting specific proinflammation factors or proteins like TNF- α , MMP instead of the whole SASP is considered to be more promising for anti-IDD [43, 123]. Therefore, accurately identifying “the black sheep” of SASP and relative signal pathways in disc senescence may develop SASP-related interventions further.

The third approach, relieving senescence-associated growth arrest, could be realized through blocking the effect of cell cycle inhibitor like p16 and forkhead box O4 (FOXO4) or deleting relative genes [50]. Baar et al. synthesized a competitive inhibitor of FOXO4 to perturb the interaction between p53 and FOXO4 and successfully transferred the cell cycle from arrest to apoptosis [124]. However, this strategy needs to be treated with caution, considering that a potential excessive cell replication and proliferation may trigger tumorigenesis [108]. In contrast, reserving the senescent state instead of directly eliminating them seems to be more appealing because of the rare cell number in degenerative discs. There is no relative report in IDD, but Zhang et al. realized the reprogramming of senescent muscle stem cells via supplying cellular nicotinamide adenine dinucleotide (NAD⁺) and increased mouse lifespan [125]. Regardless of the difference of cell characteristics, restoring the function of senescent disc cells will greatly ameliorate the condition of oligocytes and cell dysfunction in degenerative discs.

6. Prospects of Cell Senescence in IDD

In this review, we demonstrated the pathogenesis of disc degenerative diseases from a perspective of cell senescence and highlighted the pivotal role of oxidative stress and epigenomic perturbations played. And we analyzed the potential of interventions targeting senescent cells and the SASP,

which seemed to be prospective strategies. Notably, there are some confusing questions to be answered: one unsolved is that most applications of current senescent animal models inevitably ignore the accumulation of time effect. And it is unconvincing to attribute the degeneration entirely to cell senescence, considering the real chronic degeneration process in our body. In addition, the heterogeneity of cell senescence in different cells or tissues has been proved, but little is known about that which may exist in the same cell types in intervertebral discs. Investigating every single senescent cell could be an interesting topic, which may help us further understand the detail of cell senescence in IDD. Finally, days are early to say whether changing the outcome of senescent cells can really ameliorate or even reverse the progression of disc degeneration, as many emerging strategies are only preliminarily proved in vitro. Cell senescence itself is a complex biological process that has not been fully clarified, and more problems could be readily solved when further cognition is established.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Supplementary Materials

The supplementary material is a summary list of abbreviations and their corresponding full names mentioned in this review. (*Supplementary Materials*)

References

- [1] GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, "Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017," *The Lancet*, vol. 392, no. 10159, pp. 1789–1858, 2018.
- [2] C. Fitzmaurice and Global Burden of Disease Cancer Collaboration, "Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 2006 to 2016: a systematic analysis for the Global Burden of Disease Study," *Journal of Clinical Oncology*, vol. 36, 15 Supplement, pp. 1568–1568, 2018.
- [3] M. Millicamps and L. S. Stone, "Delayed onset of persistent discogenic axial and radiating pain after a single-level lumbar intervertebral disc injury in mice," *Pain*, vol. 159, no. 9, pp. 1843–1855, 2018.
- [4] C. Le Maitre, A. Freemont, and J. Hoyland, "Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration," *Arthritis Research & Therapy*, vol. 9, no. 3, article R45, 2007.
- [5] Y. Zhu, T. Tchkonina, T. Pirtskhalava et al., "The Achilles' heel of senescent cells: from transcriptome to senolytic drugs," *Aging Cell*, vol. 14, no. 4, pp. 644–658, 2015.
- [6] Y. Gologorsky and J. Chi, "Bisphosphonate therapy for degenerative disc disease?," *Neurosurgery*, vol. 73, no. 4, pp. N12–N13, 2013.
- [7] J. Wang, A. K. Uryga, J. Reinhold et al., "Vascular smooth muscle cell senescence promotes atherosclerosis and features of plaque vulnerability," *Circulation*, vol. 132, no. 20, pp. 1909–1919, 2015.
- [8] P. Zhang, Y. Kishimoto, I. Grammatikakis et al., "Senolytic therapy alleviates A β -associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model," *Nature Neuroscience*, vol. 22, no. 5, pp. 719–728, 2019.
- [9] S. Lee and C. A. Schmitt, "The dynamic nature of senescence in cancer," *Nature Cell Biology*, vol. 21, no. 1, pp. 94–101, 2019.
- [10] M. P. Baar, E. Perdiguero, P. Muñoz-Cánoves, and P. L. J. de Keizer, "Musculoskeletal senescence: a moving target ready to be eliminated," *Current Opinion in Pharmacology*, vol. 40, pp. 147–155, 2018.
- [11] F. Wang, F. Cai, R. Shi, X. H. Wang, and X. T. Wu, "Aging and age related stresses: a senescence mechanism of intervertebral disc degeneration," *Osteoarthritis and Cartilage*, vol. 24, no. 3, pp. 398–408, 2016.
- [12] B. G. Childs, M. Durik, D. J. Baker, and J. M. van Deursen, "Cellular senescence in aging and age-related disease: from mechanisms to therapy," *Nature Medicine*, vol. 21, no. 12, pp. 1424–1435, 2015.
- [13] C. Michaloglou, L. C. W. Vredeveld, M. S. Soengas et al., "BRAFE600-associated senescence-like cell cycle arrest of human naevi," *Nature*, vol. 436, no. 7051, pp. 720–724, 2005.
- [14] D. Muñoz-Espín and M. Serrano, "Cellular senescence: from physiology to pathology," *Nature Reviews Molecular Cell Biology*, vol. 15, no. 7, pp. 482–496, 2014.
- [15] A. Chicas, X. Wang, C. Zhang et al., "Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence," *Cancer Cell*, vol. 17, no. 4, pp. 376–387, 2010.
- [16] A. Calcinotto, J. Kohli, E. Zagato, L. Pellegrini, M. Demaria, and A. Alimonti, "Cellular senescence: aging, cancer, and injury," *Physiological Reviews*, vol. 99, no. 2, pp. 1047–1078, 2019.
- [17] J. L. Kirkland and T. Tchkonina, "Cellular senescence: a translational perspective," *eBioMedicine*, vol. 21, pp. 21–28, 2017.
- [18] A. Hernandez-Segura, J. Nehme, and M. Demaria, "Hallmarks of cellular senescence," *Trends in Cell Biology*, vol. 28, no. 6, pp. 436–453, 2018.
- [19] S. G. Rao and J. G. Jackson, "SASP: tumor suppressor or promoter? Yes!," *Trends Cancer*, vol. 2, no. 11, pp. 676–687, 2016.
- [20] T. S. Chikenji, Y. Saito, N. Konari et al., "p16INK4A-expressing mesenchymal stromal cells restore the senescence-clearance-regeneration sequence that is impaired in chronic

- muscle inflammation,” *eBioMedicine*, vol. 44, pp. 86–97, 2019.
- [21] L. Mosteiro, C. Pantoja, A. de Martino, and M. Serrano, “Senescence promotes in vivo reprogramming through p16INK4a and IL-6,” *Aging Cell*, vol. 17, no. 2, 2018.
- [22] X. Zhou, F. Perez, K. Han, and D. A. Jurivich, “Clonal senescence alters endothelial ICAM-1 function,” *Mechanisms of Ageing and Development*, vol. 127, no. 10, pp. 779–785, 2006.
- [23] D. A. McCarthy, R. R. Clark, T. R. Bartling, M. Trebak, and J. A. Melendez, “Redox control of the senescence regulator interleukin-1 α and the secretory phenotype,” *The Journal of Biological Chemistry*, vol. 288, no. 45, pp. 32149–32159, 2013.
- [24] P. Ortiz-Montero, A. Londoño-Vallejo, and J. P. Vernot, “Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line,” *Cell Communication and Signaling: CCS*, vol. 15, no. 1, p. 17, 2017.
- [25] S. Roberts, E. H. Evans, D. Kletsas, D. C. Jaffray, and S. M. Eisenstein, “Senescence in human intervertebral discs,” *European Spine Journal*, vol. 15, no. S3, pp. 312–316, 2006.
- [26] H. E. Gruber, J. A. Ingram, H. J. Norton, and E. N. Hanley Jr., “Senescence in cells of the aging and degenerating intervertebral disc: immunolocalization of senescence-associated β -galactosidase in human and sand rat discs,” *Spine*, vol. 32, no. 3, pp. 321–327, 2007.
- [27] W. Erwin, D. Islam, R. D. Inman, M. G. Fehlings, and F. W. L. Tsui, “Notochordal cells protect nucleus pulposus cells from degradation and apoptosis: implications for the mechanisms of intervertebral disc degeneration,” *Arthritis Research & Therapy*, vol. 13, no. 6, p. R215, 2011.
- [28] M. Ogrodnik, H. Salmonowicz, D. Jurk, and J. F. Passos, “Expansion and cell-cycle arrest: common denominators of cellular senescence,” *Trends in Biochemical Sciences*, vol. 44, no. 12, pp. 996–1008, 2019.
- [29] C. Amor, J. Feucht, J. Leibold et al., “Senolytic CAR T cells reverse senescence-associated pathologies,” *Nature*, vol. 583, no. 7814, pp. 127–132, 2020.
- [30] G. Casella, R. Munk, K. M. Kim et al., “Transcriptome signature of cellular senescence,” *Nucleic Acids Research*, vol. 47, no. 14, pp. 7294–7305, 2019.
- [31] T. Tchkonja and J. L. Kirkland, “Aging, cell senescence, and chronic disease: emerging therapeutic strategies,” *JAMA*, vol. 320, no. 13, pp. 1319–1320, 2018.
- [32] J. Birch, P. J. Barnes, and J. F. Passos, “Mitochondria, telomeres and cell senescence: implications for lung ageing and disease,” *Pharmacology & Therapeutics*, vol. 183, pp. 34–49, 2018.
- [33] M. Serrano, A. W. Lin, M. E. McCurrach, D. Beach, and S. W. Lowe, “Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a,” *Cell*, vol. 88, no. 5, pp. 593–602, 1997.
- [34] A. Chandra, A. B. Lagnado, J. N. Farr et al., “Targeted reduction of senescent cell burden alleviates focal radiotherapy-related bone loss,” *Journal of Bone and Mineral Research*, vol. 35, no. 6, pp. 1119–1131, 2020.
- [35] A. F. Phoa, A. Recasens, F. M. S. Gurgis et al., “MK2 inhibition induces p53-dependent senescence in glioblastoma cells,” *Cancers*, vol. 12, no. 3, p. 654, 2020.
- [36] P. P. Shah, G. Donahue, G. L. Otte et al., “Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape,” *Genes & Development*, vol. 27, no. 16, pp. 1787–1799, 2013.
- [37] M. Demaria, N. Ohtani, S. A. Youssef et al., “An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA,” *Developmental Cell*, vol. 31, no. 6, pp. 722–733, 2014.
- [38] K. R. Jessen, R. Mirsky, and P. Arthur-Farraj, “The role of cell plasticity in tissue repair: adaptive cellular reprogramming,” *Developmental Cell*, vol. 34, no. 6, pp. 613–620, 2015.
- [39] L. Mosteiro, C. Pantoja, N. Alcazar et al., “Tissue damage and senescence provide critical signals for cellular reprogramming in vivo,” *Science*, vol. 354, no. 6315, article aaf4445, 2016.
- [40] M. Storer, A. Mas, A. Robert-Moreno et al., “Senescence is a developmental mechanism that contributes to embryonic growth and patterning,” *Cell*, vol. 155, no. 5, pp. 1119–1130, 2013.
- [41] D. Muñoz-Espín, M. Cañamero, A. Maraver et al., “Programmed cell senescence during mammalian embryonic development,” *Cell*, vol. 155, no. 5, pp. 1104–1118, 2013.
- [42] C. D. Wiley, M. C. Velarde, P. Lecot et al., “Mitochondrial dysfunction induces senescence with a distinct secretory phenotype,” *Cell Metabolism*, vol. 23, no. 2, pp. 303–314, 2016.
- [43] N. Henry, J. Clouet, J. le Bideau, C. le Visage, and J. Guicheux, “Innovative strategies for intervertebral disc regenerative medicine: from cell therapies to multiscale delivery systems,” *Biotechnology Advances*, vol. 36, no. 1, pp. 281–294, 2018.
- [44] L. J. Smith, N. L. Nerurkar, K. S. Choi, B. D. Harfe, and D. M. Elliott, “Degeneration and regeneration of the intervertebral disc: lessons from development,” *Disease Models & Mechanisms*, vol. 4, no. 1, pp. 31–41, 2011.
- [45] J. P. de Magalhães and J. F. Passos, “Stress, cell senescence and organismal ageing,” *Mechanisms of Ageing and Development*, vol. 170, pp. 2–9, 2018.
- [46] Q. J. Xing, Q. Q. Liang, Q. Bian et al., “Leg amputation accelerates senescence of rat lumbar intervertebral discs,” *Spine (Phila Pa 1976)*, vol. 35, no. 23, pp. E1253–E1261, 2010.
- [47] L. Zhao, B. Tian, Q. Xu, C. Zhang, L. Zhang, and H. Fang, “Extensive mechanical tension promotes annulus fibrosus cell senescence through suppressing cellular autophagy,” *BioScience Reports*, vol. 39, no. 4, 2019.
- [48] G. Fontana, E. See, and A. Pandit, “Current trends in biologics delivery to restore intervertebral disc anabolism,” *Advanced Drug Delivery Reviews*, vol. 84, pp. 146–158, 2015.
- [49] K. W. Kim, H. N. Chung, K. Y. Ha, J. S. Lee, and Y. Y. Kim, “Senescence mechanisms of nucleus pulposus chondrocytes in human intervertebral discs,” *The Spine Journal*, vol. 9, no. 8, pp. 658–666, 2009.
- [50] H. Che, J. Li, Y. Li et al., “p16 deficiency attenuates intervertebral disc degeneration by adjusting oxidative stress and nucleus pulposus cell cycle,” *eLife*, vol. 9, 2020.
- [51] E. J. Novais, B. O. Diekman, I. M. Shapiro, and M. V. Risbud, “p16Ink4a deletion in cells of the intervertebral disc affects their matrix homeostasis and senescence associated secretory phenotype without altering onset of senescence,” *Matrix Biology*, vol. 82, pp. 54–70, 2019.
- [52] J. Campisi, “Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors,” *Cell*, vol. 120, no. 4, pp. 513–522, 2005.
- [53] U. Herbig and J. M. Sedivy, “Regulation of growth arrest in senescence: telomere damage is not the end of the story,”

- Mechanisms of Ageing and Development*, vol. 127, no. 1, pp. 16–24, 2006.
- [54] Z. Zhou, M. Gao, F. Wei et al., “Shock absorbing function study on denucleated intervertebral disc with or without hydrogel injection through static and dynamic biomechanical tests in vitro,” *BioMed Research International*, vol. 2014, Article ID 461724, 7 pages, 2014.
- [55] P. Li, G. Hou, R. Zhang et al., “High-magnitude compression accelerates the premature senescence of nucleus pulposus cells via the p38 MAPK-ROS pathway,” *Arthritis Research & Therapy*, vol. 19, no. 1, p. 209, 2017.
- [56] K. Alpantaki, A. Kampouroglou, C. Koutserimpas, G. Effraimidis, and A. Hadjipavlou, “Diabetes mellitus as a risk factor for intervertebral disc degeneration: a critical review,” *European Spine Journal*, vol. 28, no. 9, pp. 2129–2144, 2019.
- [57] M. Bitar, “Diabetes impairs angiogenesis and induces endothelial cell senescence by up-regulating thrombospondin-CD47-dependent signaling,” *International Journal of Molecular Sciences*, vol. 20, no. 3, p. 673, 2019.
- [58] A. Dimozi, E. Mavrogonatou, A. Sklirou, and D. Kleatsas, “Oxidative stress inhibits the proliferation, induces premature senescence and promotes a catabolic phenotype in human nucleus pulposus intervertebral disc cells,” *European Cells & Materials*, vol. 30, pp. 89–103, 2015.
- [59] S. W. Jeong, J. S. Lee, and K. W. Kim, “In vitro lifespan and senescence mechanisms of human nucleus pulposus chondrocytes,” *The Spine Journal*, vol. 14, no. 3, pp. 499–504, 2014.
- [60] C. Feng, H. Liu, M. Yang, Y. Zhang, B. Huang, and Y. Zhou, “Disc cell senescence in intervertebral disc degeneration: causes and molecular pathways,” *Cell Cycle*, vol. 15, no. 13, pp. 1674–1684, 2016.
- [61] J. P. Coppé, C. K. Patil, F. Rodier et al., “Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor,” *PLoS Biology*, vol. 6, no. 12, pp. 2853–2868, 2008.
- [62] K. Ngo, P. Patil, S. J. McGowan et al., “Senescent intervertebral disc cells exhibit perturbed matrix homeostasis phenotype,” *Mechanisms of Ageing and Development*, vol. 166, pp. 16–23, 2017.
- [63] J. C. Acosta, A. Banito, T. Wuestefeld et al., “A complex secretory program orchestrated by the inflammasome controls paracrine senescence,” *Nature Cell Biology*, vol. 15, no. 8, pp. 978–990, 2013.
- [64] M. V. Risbud and I. M. Shapiro, “Role of cytokines in intervertebral disc degeneration: pain and disc content,” *Nature Reviews Rheumatology*, vol. 10, no. 1, pp. 44–56, 2014.
- [65] P. L. J. de Keizer, “The fountain of youth by targeting senescent cells?,” *Trends in Molecular Medicine*, vol. 23, no. 1, pp. 6–17, 2017.
- [66] J. M. van Deursen, “The role of senescent cells in ageing,” *Nature*, vol. 509, no. 7501, pp. 439–446, 2014.
- [67] X. Wang, M. Zou, J. Li et al., “LncRNA H19 targets miR-22 to modulate H₂O₂-induced deregulation in nucleus pulposus cell senescence, proliferation, and ECM synthesis through Wnt signaling,” *Journal of Cellular Biochemistry*, vol. 119, no. 6, pp. 4990–5002, 2018.
- [68] L. Ding, Z. Jiang, J. Wu et al., “ β -catenin signalling inhibits cartilage endplate chondrocyte homeostasis in vitro,” *Molecular Medicine Reports*, vol. 20, no. 1, pp. 567–572, 2019.
- [69] A. Hiyama, D. Sakai, F. Arai, D. Nakajima, K. Yokoyama, and J. Mochida, “Effects of a glycogen synthase kinase-3 β inhibitor (LiCl) on c-myc protein in intervertebral disc cells,” *Journal of Cellular Biochemistry*, vol. 112, no. 10, pp. 2974–2986, 2011.
- [70] J. Antoniou, T. Steffen, F. Nelson et al., “The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration,” *The Journal of Clinical Investigation*, vol. 98, no. 4, pp. 996–1003, 1996.
- [71] C. Ruiz-Fernández, V. Francisco, J. Pino et al., “Molecular relationships among obesity, inflammation and intervertebral disc degeneration: are adipokines the common link?,” *International Journal of Molecular Sciences*, vol. 20, no. 8, p. 2030, 2019.
- [72] D. Krishnamoorthy, R. C. Hoy, D. M. Natelson et al., “Dietary advanced glycation end-product consumption leads to mechanical stiffening of murine intervertebral discs,” *Disease Models & Mechanisms*, vol. 11, no. 12, article dmm036012, 2018.
- [73] J. Campisi, P. Kapahi, G. J. Lithgow, S. Melov, J. C. Newman, and E. Verdin, “From discoveries in ageing research to therapeutics for healthy ageing,” *Nature*, vol. 571, no. 7764, pp. 183–192, 2019.
- [74] D. C. Lee, C. S. Adams, T. J. Albert, I. M. Shapiro, S. M. Evans, and C. J. Koch, “In situ oxygen utilization in the rat intervertebral disc,” *Journal of Anatomy*, vol. 210, no. 3, pp. 294–303, 2007.
- [75] J. F. Passos, G. Nelson, C. Wang et al., “Feedback between p21 and reactive oxygen production is necessary for cell senescence,” *Molecular Systems Biology*, vol. 6, no. 1, p. 347, 2010.
- [76] C. Feng, M. Yang, M. Lan et al., “ROS: crucial intermediators in the pathogenesis of intervertebral disc degeneration,” *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 5601593, 12 pages, 2017.
- [77] Y. Lin, G. Tang, Y. Jiao et al., “*Propionibacterium acnes* induces intervertebral disc degeneration by promoting iNOS/NO and COX-2/PGE₂ activation via the ROS-dependent NF- κ B pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 3692752, 12 pages, 2018.
- [78] D. P. Jones, “Radical-free biology of oxidative stress,” *American Journal of Physiology Cell Physiology*, vol. 295, no. 4, pp. C849–C868, 2008.
- [79] H. Liu, H. Kang, C. Song et al., “Urolithin A inhibits the catabolic effect of TNF α on nucleus pulposus cell and alleviates intervertebral disc degeneration in vivo,” *Frontiers in Pharmacology*, vol. 9, p. 1043, 2018.
- [80] A. Larrañaga, I. L. M. Isa, V. Patil et al., “Antioxidant functionalized polymer capsules to prevent oxidative stress,” *Acta Biomaterialia*, vol. 67, pp. 21–31, 2018.
- [81] J. J. Wu, C. Quijano, E. Chen et al., “Mitochondrial dysfunction and oxidative stress mediate the physiological impairment induced by the disruption of autophagy,” *Ageing*, vol. 1, no. 4, pp. 425–437, 2009.
- [82] T. Toshima, K. Shirabe, T. Fukuhara et al., “Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice,” *Hepatology*, vol. 60, no. 1, pp. 290–300, 2014.
- [83] P. Davalli, T. Mitic, A. Caporali, A. Lauriola, and D. D’Arca, “ROS, cell senescence, and novel molecular mechanisms in

- aging and age-related diseases," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 3565127, 18 pages, 2016.
- [84] L. Kang, Q. Xiang, S. Zhan et al., "Restoration of autophagic flux rescues oxidative damage and mitochondrial dysfunction to protect against intervertebral disc degeneration," *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 7810320, 27 pages, 2019.
- [85] P. Vasileiou, K. Evangelou, K. Vlasis et al., "Mitochondrial homeostasis and cellular senescence," *Cells*, vol. 8, no. 7, p. 686, 2019.
- [86] M. C. Marazita, A. Dugour, M. D. Marquioni-Ramella, J. M. Figueroa, and A. M. Suburo, "Oxidative stress-induced premature senescence dysregulates VEGF and CFH expression in retinal pigment epithelial cells: implications for age-related macular degeneration," *Redox Biology*, vol. 7, pp. 78–87, 2016.
- [87] P. Li, Y. Gan, Y. Xu et al., "17beta-estradiol attenuates TNF- α -induced premature senescence of nucleus pulposus cells through regulating the ROS/NF- κ B pathway," *International Journal of Biological Sciences*, vol. 13, no. 2, pp. 145–156, 2017.
- [88] J. Wang, C. Huang, Z. Lin et al., "Polydatin suppresses nucleus pulposus cell senescence, promotes matrix homeostasis and attenuates intervertebral disc degeneration in rats," *Journal of Cellular and Molecular Medicine*, vol. 22, no. 11, pp. 5720–5731, 2018.
- [89] R. Zuo, Y. Wang, J. Li et al., "Rapamycin induced autophagy inhibits inflammation-mediated endplate degeneration by enhancing Nrf 2/Keap 1 signaling of cartilage endplate stem cells," *Stem Cells*, vol. 37, no. 6, pp. 828–840, 2019.
- [90] L. Q. Cheng, Z. Q. Zhang, H. Z. Chen, and D. P. Liu, "Epigenetic regulation in cell senescence," *Journal of Molecular Medicine (Berlin, Germany)*, vol. 95, no. 12, pp. 1257–1268, 2017.
- [91] C. Li, Y. Chai, L. Wang et al., "Programmed cell senescence in skeleton during late puberty," *Nature Communications*, vol. 8, no. 1, p. 1312, 2017.
- [92] U. Herbig, W. A. Jobling, B. P. C. Chen, D. J. Chen, and J. M. Sedivy, "Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21 (CIP1), but not p16 (INK4a)," *Molecular Cell*, vol. 14, no. 4, pp. 501–513, 2004.
- [93] V. Gire, P. Roux, D. Wynford-Thomas, J. M. Brondello, and V. Dulic, "DNA damage checkpoint kinase Chk 2 triggers replicative senescence," *The EMBO Journal*, vol. 23, no. 13, pp. 2554–2563, 2004.
- [94] C. Liu, L. Liu, M. Yang et al., "A positive feedback loop between EZH2 and NOX4 regulates nucleus pulposus cell senescence in age-related intervertebral disc degeneration," *Cell Division*, vol. 15, no. 1, p. 2, 2020.
- [95] F. M. K. Williams, A. T. Bansal, J. B. van Meurs et al., "Novel genetic variants associated with lumbar disc degeneration in northern Europeans: a meta-analysis of 4600 subjects," *Annals of the Rheumatic Diseases*, vol. 72, no. 7, pp. 1141–1148, 2013.
- [96] Z. Zhang, T. Xu, J. Chen et al., "Parkin-mediated mitophagy as a potential therapeutic target for intervertebral disc degeneration," *Cell Death & Disease*, vol. 9, no. 10, p. 980, 2018.
- [97] L. Kang, S. Liu, J. Li, Y. Tian, Y. Xue, and X. Liu, "Parkin and Nrf 2 prevent oxidative stress-induced apoptosis in intervertebral endplate chondrocytes via inducing mitophagy and anti-oxidant defenses," *Life Sciences*, vol. 243, p. 117244, 2020.
- [98] A. Ikuno, K. Akeda, S. I. Takebayashi, M. Shimaoka, K. Okumura, and A. Sudo, "Genome-wide analysis of DNA methylation profile identifies differentially methylated loci associated with human intervertebral disc degeneration," *PLoS One*, vol. 14, no. 9, article e0222188, 2019.
- [99] K. McCulloch, G. J. Litherland, and T. S. Rai, "Cellular senescence in osteoarthritis pathology," *Aging Cell*, vol. 16, no. 2, pp. 210–218, 2017.
- [100] B. Chen, S. G. Huang, L. Ju et al., "Effect of microRNA-21 on the proliferation of human degenerated nucleus pulposus by targeting programmed cell death 4," *Brazilian Journal of Medical and Biological Research*, vol. 49, no. 6, 2016.
- [101] S. X. Gu, X. Li, J. L. Hamilton et al., "MicroRNA-146a reduces IL-1 dependent inflammatory responses in the intervertebral disc," *Gene*, vol. 555, no. 2, pp. 80–87, 2015.
- [102] M. L. Ji, J. Lu, P. L. Shi et al., "Dysregulated miR-98 contributes to extracellular matrix degradation by targeting IL-6/STAT3 signaling pathway in human intervertebral disc degeneration," *Journal of Bone and Mineral Research*, vol. 31, no. 4, pp. 900–909, 2016.
- [103] L. Kang, C. Yang, Y. Song et al., "MicroRNA-494 promotes apoptosis and extracellular matrix degradation in degenerative human nucleus pulposus cells," *Oncotarget*, vol. 8, no. 17, pp. 27868–27881, 2017.
- [104] W. Liu, P. Xia, J. Feng et al., "MicroRNA-132 upregulation promotes matrix degradation in intervertebral disc degeneration," *Experimental Cell Research*, vol. 359, no. 1, pp. 39–49, 2017.
- [105] J. Bedore, A. Leask, and C. A. Séguin, "Targeting the extracellular matrix: matricellular proteins regulate cell-extracellular matrix communication within distinct niches of the intervertebral disc," *Matrix Biology*, vol. 37, pp. 124–130, 2014.
- [106] M. Tajerian, S. Alvarado, M. Millemcamps et al., "DNA methylation of SPARC and chronic low back pain," *Molecular Pain*, vol. 7, pp. 1744–8069, 2011.
- [107] S. Roberts, P. Colombier, A. Sowman et al., "Ageing in the musculoskeletal system," *Acta Orthopaedica*, vol. 87, Supplement 363, pp. 15–25, 2017.
- [108] T. Tchkonja, Y. Zhu, J. van Deursen, J. Campisi, and J. L. Kirkland, "Cellular senescence and the senescent secretory phenotype: therapeutic opportunities," *The Journal of Clinical Investigation*, vol. 123, no. 3, pp. 966–972, 2013.
- [109] P. Patil, Q. Dong, D. Wang et al., "Systemic clearance of p16INK4a-positive senescent cells mitigates age-associated intervertebral disc degeneration," *Aging Cell*, vol. 18, no. 3, article e12927, 2019.
- [110] V. Myrianthopoulos, "The emerging field of senotherapeutic drugs," *Future Medicinal Chemistry*, vol. 10, no. 20, pp. 2369–2372, 2018.
- [111] D. Chen, D. Xia, Z. Pan et al., "Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo," *Cell Death & Disease*, vol. 7, no. 10, article e2441, 2016.
- [112] Y. Han, F. Yuan, C. Deng et al., "Metformin decreases LPS-induced inflammatory response in rabbit annulus fibrosus stem/progenitor cells by blocking HMGB1 release," *Aging (Albany NY)*, vol. 11, no. 22, pp. 10252–10265, 2019.

- [113] N. Ö. Yurttaş and A. E. Eşkazan, “Dasatinib-induced pulmonary arterial hypertension,” *British Journal of Clinical Pharmacology*, vol. 84, no. 5, pp. 835–845, 2018.
- [114] A. Qu, X. Wu, S. Li et al., “An NIR-responsive DNA-mediated nanotetrahedron enhances the clearance of senescent cells,” *Advanced Materials*, vol. 32, no. 14, article 2000184, 2020.
- [115] M. Althubiti, L. Lezina, S. Carrera et al., “Characterization of novel markers of senescence and their prognostic potential in cancer,” *Cell Death & Disease*, vol. 5, no. 11, article e1528, 2014.
- [116] M. Amaya-Montoya, A. Pérez-Londoño, V. Guatibonza-García, A. Vargas-Villanueva, and C. O. Mendivil, “Cellular senescence as a therapeutic target for age-related diseases: a review,” *Advances in Therapy*, vol. 37, no. 4, pp. 1407–1424, 2020.
- [117] N. Malaquin, A. Martinez, and F. Rodier, “Keeping the senescence secretome under control: molecular reins on the senescence-associated secretory phenotype,” *Experimental Gerontology*, vol. 82, pp. 39–49, 2016.
- [118] Y. Han, C. M. Zhou, H. Shen et al., “Attenuation of ataxia telangiectasia mutated signalling mitigates age-associated intervertebral disc degeneration,” *Aging Cell*, vol. 19, no. 7, 2020.
- [119] J. Zhao, L. Zhang, A. Lu et al., “ATM is a key driver of NF- κ B-dependent DNA-damage-induced senescence, stem cell dysfunction and aging,” *Aging*, vol. 12, no. 6, pp. 4688–4710, 2020.
- [120] L. Hoenicke and L. Zender, “Immune surveillance of senescent cells—biological significance in cancer- and non-cancer pathologies,” *Carcinogenesis*, vol. 33, no. 6, pp. 1123–1126, 2012.
- [121] V. Krizhanovsky, M. Yon, R. A. Dickins et al., “Senescence of activated stellate cells limits liver fibrosis,” *Cell*, vol. 134, no. 4, pp. 657–667, 2008.
- [122] W. Xue, L. Zender, C. Miething et al., “Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas,” *Nature*, vol. 445, no. 7128, pp. 656–660, 2007.
- [123] A. Freund, A. V. Orjalo, P. Y. Desprez, and J. Campisi, “Inflammatory networks during cellular senescence: causes and consequences,” *Trends in Molecular Medicine*, vol. 16, no. 5, pp. 238–246, 2010.
- [124] M. P. Baar, R. M. C. Brandt, D. A. Putavet et al., “Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging,” *Cell*, vol. 169, no. 1, pp. 132–147.e16, 2017.
- [125] H. Zhang, D. Ryu, Y. Wu et al., “NAD⁺ repletion improves mitochondrial and stem cell function and enhances life span in mice,” *Science*, vol. 352, no. 6292, pp. 1436–1443, 2016.