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REVIEW

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# Disc cell senescence in intervertebral disc degeneration: Causes and molecular pathways

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**ABSTRACT**

The accumulation of senescent disc cells in degenerative intervertebral disc (IVD) suggests the detrimental roles of cell senescence in the pathogenesis of intervertebral disc degeneration (IDD). Disc cell senescence decreased the number of functional cells in IVD. Moreover, the senescent disc cells were supposed to accelerate the process of IDD via their aberrant paracrine effects by which senescent cells cause the senescence of neighboring cells and enhance the matrix catabolism and inflammation in IVD. Thus, anti-senescence has been proposed as a novel therapeutic target for IDD. However, the development of anti-senescence therapy is based on our understanding of the molecular mechanism of disc cell senescence. In this review, we focused on the molecular mechanism of disc cell senescence, including the causes and various molecular pathways. We found that, during the process of IDD, age-related damages together with degenerative external stimuli activated both p53-p21-Rb and p16-Rb pathways to induce disc cell senescence. Meanwhile, disc cell senescence was regulated by multiple signaling pathways, suggesting the complex regulating network of disc cell senescence. To understand the mechanism of disc cell senescence better contributes to developing the anti-senescence-based therapies for IDD.

## Introduction

Nowadays, low back pain (LBP) is prevalent worldwide and causes huge socio-economic burdens.[1,2] Intervertebral disc degeneration (IDD) is a widely accepted cause of LBP. IDD is associated with the severity of LBP.[3,4] Thus, elucidating the pathogenesis of IDD in detail benefits the development of effective therapies for LBP. The pathogenesis of IDD is very complicated. It has been demonstrated to involve cell apoptosis, autophagy, pro-inflammatory cytokine storm and increased matrix catabolism.[5,6] Nevertheless, the mechanism of IDD is still not well established. More and more researchers devote themselves to elucidating the pathogenesis of IDD.

Since disc cell senescence was determined in degenerative discs, the number of studies discussing disc cell senescence increases every year. Cell senescence is classically defined as an irreversible cell-cycle arrest caused by telomere uncapping or various external stimuli. However, to date, the knowledge of characteristics of senescent cells has been expanded.[7-10] Senescent cells tend to aggregate in cell clusters, and show increased cell size and flattened and vacuolized morphology *in vitro*. *In vivo*, the morphology of senescent cells depends on tissue structure. They are unresponsive to mitogenic stimulation and fail to replicate. Consequently, their cell cycle is halted at the G1 phase. Although losing the replicative capability, senescent cells aberrantly secretes pro-inflammatory cytokines, matrix degradation proteases, growth factors and chemokines.[11,12] This pro-inflammatory phenotype of senescent cells is defined as senescence-associated secretory phenotype (SASP). The inflammatory microenvironment created by SASP via autocrine and paracrine has been identified as a key step in the occurrence and development of age-related diseases.[7-10]

With respect to the biomarkers of cell senescence, the first mentioned is senescence-associated $\beta$-galactosidase (SA-$\beta$-Gal). The activity of SA-$\beta$-Gal detected at pH 6.0 is related to the senescence-associated lysosomal activity. However, SA-$\beta$-Gal is not a specific biomarker of senescence because its activity is affected by the state of lysosome. Thus, other molecular biomarkers, such as p53 (tumor suppressor gene), cell-cycle kinase dependent (CDK) inhibitors (p16 and p21), cell cycle regulator (retinoblastoma protein, Rb), p38 and telomere length, are used to detect cell senescence. These molecular biomarkers are prominent cell-cycle regulators, and suggest the molecular signaling pathways of cell senescence. Based on the markers above mentioned, disc cell senescence in degenerative discs has been investigated in many researches. This phenomenon has been reviewed comprehensively.[13] However, cell senescence is regulated by various molecular signaling pathways. The molecular mechanism of cell senescence is cause-dependent and cell type-dependent.[7,14] Therefore, in the following sections, we will discuss the molecular mechanism of disc cell senescence in detail. The well-established molecular mechanism contributes to understanding the roles of disc cell senescence in the pathogenesis of IDD. More importantly, antagonizing disc cell senescence by regulating

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molecular signaling pathways helps to develop new potential therapies for IDD.

In this review, we briefly discussed the significance of disc cell senescence in the pathogenesis of IDD. Elucidating the effects of disc cell senescence on IDD helps to understand the pathogenesis of IDD better. Then, our review focused on the molecular mechanism of disc cell senescence. Because the mechanism is cause-dependent, we discussed the causes and signaling pathways of disc cell senescence respectively. In summary, regulating disc cell senescence on molecular level suggests a new potential therapeutic target of IDD.

## The significance of disc cell senescence in the pathogenesis of IDD

The SA-$\beta$-Gal positive disc cells existed in both nucleus pulposus (NP) and annulus fibrosus (AF) specimens harvested from patients with IDD. They preferred to aggregate in cluster.[15] The number of SA-$\beta$-Gal positive disc cells was positively correlated to the Thompson grade and Pfirrmann Grade of discs. It also was negatively related to the number of Ki67 positive cells (proliferated cells). Meanwhile, mean telomere length of disc cells shortened progressively with the degenerative grade of IDD increasing. NP cells isolated from degenerative discs proliferated slower and presented accelerated cell senescence than those from non-degenerative discs.[16-19] Moreover, various signaling pathways were activated in senescent disc cells, which we will discuss in a separate section.[18-22] All results above mentioned suggest that senescent disc cells accumulate in discs with IDD progressing. Enhanced disc cell senescence is a new hallmark of IDD.

The accumulation of senescent disc cells provides a novel insight into the pathogenesis of IDD. On the one hand, senescent disc cells are unable to generate new disc cells, thus the number of functional cells in discs decreases gradually due to cell death. On the other hand, the SASP of disc cells was characterized by a catabolic and pro-inflammatory phenotype.[19,23, 24] Senescent disc cells may alter the pattern of secretion to change the microenvironment of discs.[8,14] They decreased extracellular matrix (ECM) production and enhanced ECM degradation in IVD. Moreover, pro-inflammatory cytokines secreted by senescent disc cells, including TNF-$\alpha$, IL-1$\beta$, IL-17, IL-6, COX-2 and chemokines may promote the senescence of neighboring disc cells and the infiltration of immune cells, then reinforce inflammation in the microenvironment of degenerative discs.[6-10] In conclusion, senescent disc cells undergo a phenotypic shift and disrupt the balance between ECM anabolism and catabolism in discs. As a result, disc degeneration is accelerated (Fig. 1). However, we still need more evidence to elucidate the roles of disc cell senescence in the process of IDD. Further studies should investigate the precise phenotype of senescent disc cells to reveal
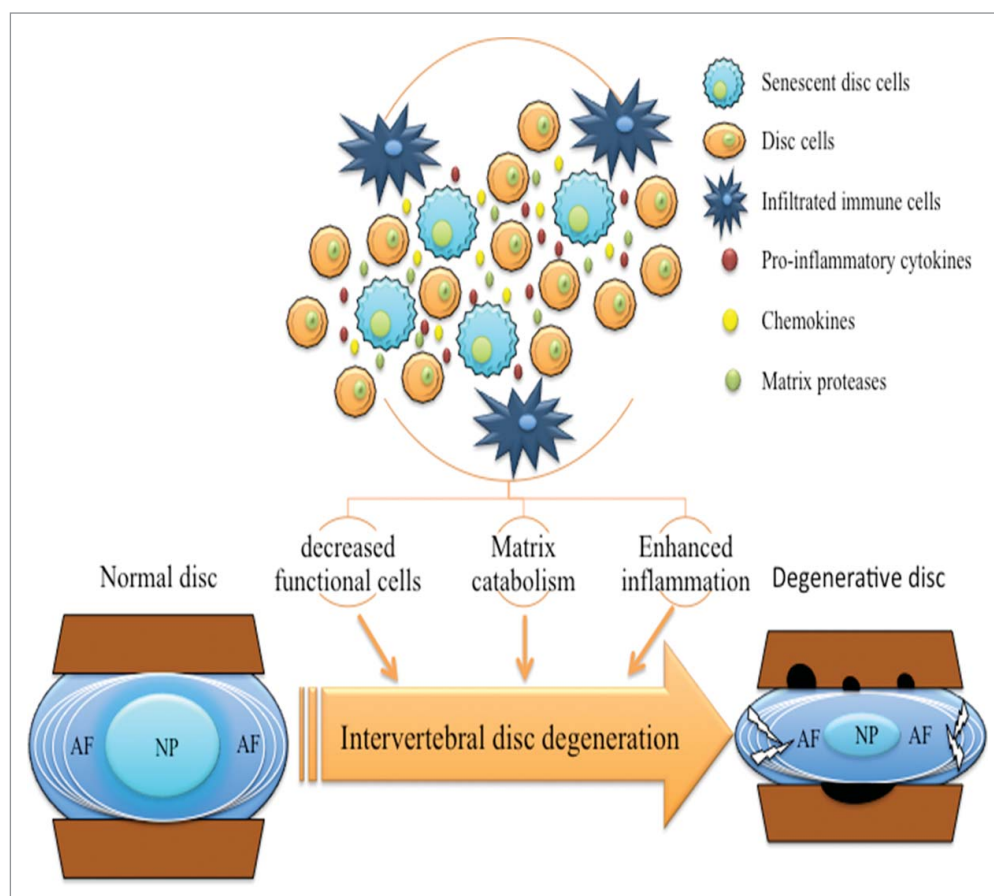


**Figure 1.** The roles of disc cell senescence in the pathogenesis of intervertebral disc degeneration. The senescent disc cells are unable to replicate, thus, the loss of functional cells occurs in discs. Furthermore, the senescence-associated secreted phenotype (SASP) of disc cells is characterized by a catabolic and pro-inflammatory phenotype. Senescent disc cells secrete matrix proteases to enhance extracellular matrix (ECM) catabolism in intervertebral disc. Meanwhile, pro-inflammatory cytokines secreted by senescent disc cells promote the senescence of surrounding disc cells and the infiltration of immune cells in discs, reinforcing the inflammation in the microenvironment of degenerative discs. As a result, disc degeneration is accelerated. NP, nucleus pulposus. AF, annulus fibrosus.

the significance of disc cell senescence in IDD. Preventing disc cell senescence indicates a new therapeutic strategy for IDD.

## The molecular mechanism of disc cell senescence

### Triggers of disc cell senescence

In this section, we focused on the molecular signaling pathways of disc cell senescence. As generally known, the activation of senescent molecular pathways is cause-dependent.[7,8,14] Hence, we reviewed different causes of disc cell senescence first. Various causes of cell senescence have been identified, including telomere shortening, DNA damage, oxidative stress, oncogene activation and development cues.[7-9,14,25] Herein, we just discussed the causes associated with disc cell senescence and IDD based on currently available evidence.

### Telomere erosion, DNA damage and aging

During the serial replication, the telomere length becomes shorter and shorter due to the incomplete replication of the ends of DNA, which is the first identified cause of cell senescence. It explains the limited replicative capacity of eukaryotic cells. It mainly activates the p53-p21-Rb signaling pathway (discussed in the next section) to induce replicative senescence.[26,27] In degenerative discs, decreased telomere length, declined telomerase activity and disc cell senescence were observed simultaneously. Telomere length decreased progressively with IDD advancing.[18,19] The senescent signaling pathways were activated by telomere shortening,[21] suggesting that telomere shortening triggers the replicative senescence of disc cells in the process of IDD (Fig. 2).

Cells activate DNA damage response (DDR) in respond to telomere shortening. Thus, in a larger sense, DNA damage is an intrinsic trigger of cell senescence.[28,29] DNA damage agents, both ionization radiation (IR) and mechlorethamine (MEC), have been found to increase the number of p16-positive cells in the NP of wild-type mouse. Deficiency of DNA repair gene, ERCC1, enhanced disc cell senescence in ERCC1-deficient mice. These deficient mice exposed to IR or MEC had more senescent disc cells.[30,31] In addition, the DDR pathway was activated by oxidative stress to induce disc cell senescence.[32] These
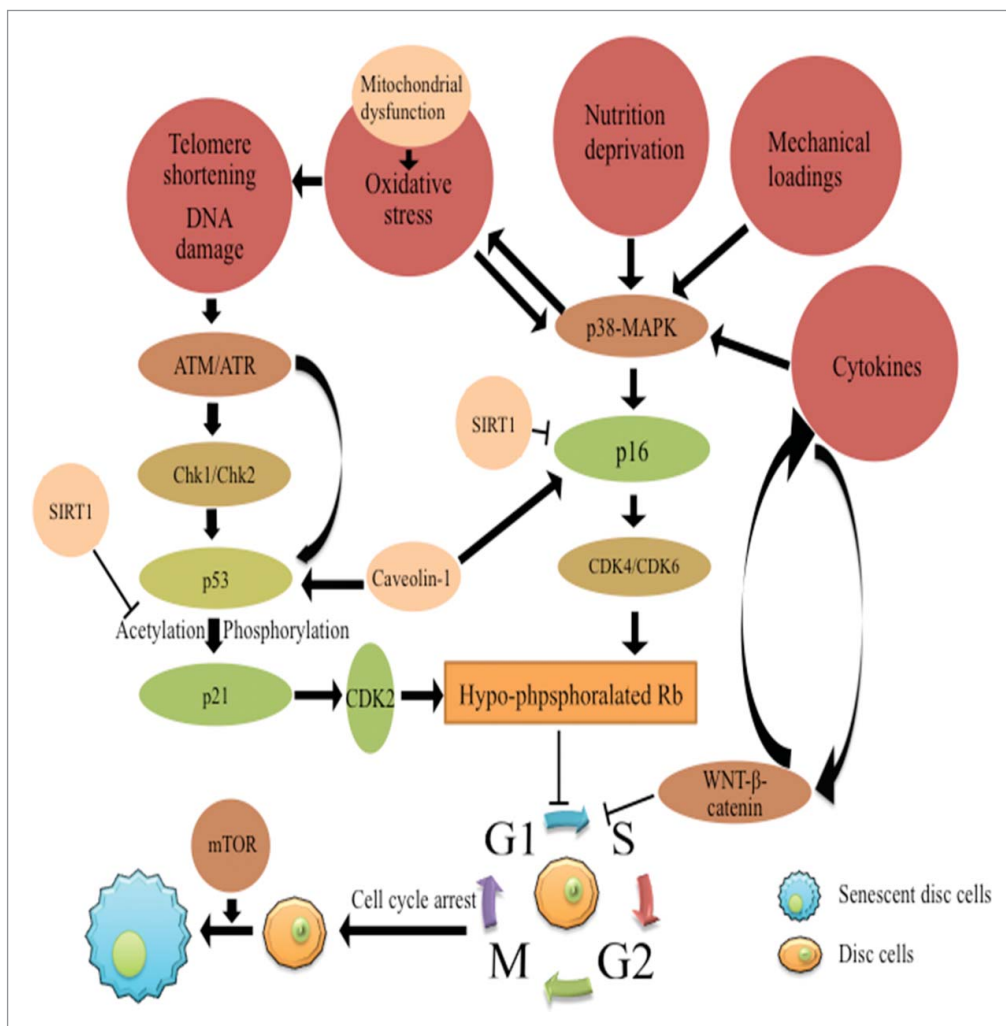


**Figure 2.** The molecular mechanism of disc cell senescence. The molecular mechanism underlying disc cell senescence includes 2 aspects, the arrest of cell cycle and the development of the senescent phenotype of disc cells. The p53-p21-Rb pathway and the p16-Rb pathway play major roles in the cell cycle arrest. Both pathways are activated by telomere shortening, DNA damage response or various stressful stimuli in the microenvironment of degenerative discs respectively. SIRT1 plays a protective role in disc cell senescence by suppressing p53 and p16. Caveoline-1 synergizes with p53 and p16 to accelerate disc cell senescence. The p38-MAPK pathway responds to various stimuli to activate the p53-p21-Rb and p16-Rb pathways. WNT-β-catenin pathway induces disc cell senescence. A positive-feedback loop of WNT signaling and cytokines enhances the pro-senescence effects of WNT-β-catenin pathway. Moreover, the mTOR pathway is required for cells to acquire the senescent phenotype.

findings were consistent with previous studies on other cell types. Inactivation of DNA repair genes, including Brca1, Xrcc4 and DNA ligase IV, also induced cell senescence.[33-36] DNA damage was able to activate both the p53-p21-Rb and p16-Rb pathways (discussed in the next section) to induce cell senescence.[37,38]

Interestingly, the wild-type mice exposed to IR or MEC showed IDD changes, including reduced disc height, decreased ECM synthesis and enhanced ECM degradation, along with the up-regulated expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5 in discs. Meanwhile, ERCC1-deficient mice also showed the same IDD changes reinforced by IR or MEC,[30,31] suggesting that DNA damage perturbs the disc homeostasis to cause IDD. Notably, disc cell senescence occurred in response to DNA damage. Senescent disc cells increased the production of matrix proteases.[19, 24] Thus, the loss of ECM homeostasis caused by DNA damage probably resulted from disc cell senescence. In other word, disc cell senescence mediated disc degeneration caused by DNA damage. These findings could explain why tobacco smoking is major risk factor for IDD.[39] It has been reported that rats exposed to tobacco smoke showed reduced collagen content, disorganized AF and upregulated expression of IL-1$\beta$ in IVD.[40] However, the underlying mechanism remains unknown. Some studies suggested that tobacco smoke induced vasoconstriction to reduce the nutrient supply of IVD, promoting disc degeneration.[41,42] A recent study reported that the mice chronically exposed to tobacco smoke exhibited enhanced disc cell senescence along with decreased proteoglycan (PG) content and reduced matrix synthesis in discs, suggesting that smoke-induced disc degeneration probably results from disc cell senescence. Many DNA damage agents in tobacco may induce disc cell senescence, leading to disc degeneration.[43] Future studies should investigate the incidence of DNA damage and cell senescence in smoker's discs to confirm this viewpoint.

Considering that telomere shortening and DNA damage are the most important aging-related molecular events, senescent disc cells accumulate in discs with aging can be speculated. Contrary to our expectations, current evidence are contradictory. On the one hand, the telomere length of disc cells decreased with patient's age.[18,19] The disc cells from older patients reached to senescence *in vitro* earlier than those from younger patients.[21] On the other hand, many studies did not find the relationship between disc cell senescence and patient's age,[15,17,19] except for the study done by Kim et al in 2009.[18] However, in this exceptional study, the age of specimen donors was positively correlated with the Pfirrmann Grade of disc specimens. Based on this bias of case selection, this fake positive correlation maybe just reflected the positive correlation between dis cell senescence and disc degeneration. Meanwhile, various external stimuli, including oxidative stress, high glucose, serum starvation and pro-inflammatory cytokines,[44-47] have been suggested as triggers of cell senescence. Therefore, except natural aging, there must be some environmental stimuli in degenerative discs causing disc cell senescence. Moreover, the diversity of the causes of disc cell senescence provides a support for the diversity of the risk factors of IDD. Aging-dependent disc cell senescence mediates age-related disc degeneration,[48] and premature IDD caused by acute fractures or abnormal mechanical loading is mediated by age-independent disc cell senescence.[49,50]

## Oxidative stress

The harsh microenvironment of degenerative discs is characterized by low nutrition,[51,52] high levels of cytokines[53,54] and oxidative stress.[55,56] These microenvironmental stimuli cause the stress-induced premature senescence (SIPS).[7,9,14] Oxidative stress is a major contributor to cellular senescence.[57,58] NP cells were a source of reactive oxygen species (ROS).[18] The levels of ROS in discs increased with IDD advancing.[55] Notably, hydrogen peroxide ($H_2O_2$) inhibited the proliferation of NP cells. It significantly enhanced the formation of H2A.X foci (a marker of DNA damage) in NP cells, and increased the number of SA-$\beta$-Gal positive disc cells.[32,59-61] Furthermore, $H_2O_2$ activated the senescent signal pathways to induce the cycle arrest of NP cells at the G0/G1 phase,[32] indicating that oxidative stress in the microenvironment of degenerative discs triggers disc cell senescence (Fig. 2).

## Nutrition deprivation

Reduced nutrient supply in degenerative discs affects the behavior of disc cells.[51] Serum starvation inhibited the proliferation of disc cells along with enhancing the senescence of disc cells. High concentration serum increased the proliferation rate of NP cells. Conversely, glucose deprivation with serum presence showed little effect on disc cell senescence.[62,63] High glucose enhanced oxidative stress through mitochondrial damage to induce disc cell senescence.[61,64] These results suggest that various nutrient components have different effects on disc cell senescence. The effects of glucose on disc cell senescence depend on the glucose concentration. Normal glucose level protects disc cells from cell senescence. On the contrary, uncontrolled high glucose promotes disc cell senescence. Moreover, serum components are crucial to the regulation of disc cell cycle. Numerous serum-derived growth factors have been demonstrated to enhance the proliferation of disc cells, including insulin-like growth factor (IGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF).[65,66] IGF-1 prevented disc cell senescence induced by oxidative stress.[59] However, the availability of these growth factors to disc cells depends on the diffusion rate of these proteins from blood vessels to the center of discs through cartilage endplate (CEP). The permeability of CEP decreased due to the endplate calcification or the microangiopathy induced by diabetes.[67,68] As a result, the major route of nutrient molecules to the center of discs was blocked, and the reduced availability of growth factors occurred in IVD, which accelerates the disc cell senescence (Fig. 2).

## Pro-inflammatory cytokines

The levels of pro-inflammatory cytokines in degenerative discs increased with IDD progressing.[6] These cytokines, including TNF-$\alpha$, IL-1$\alpha$, IL-1$\beta$, IL-6, IL-17, and various chemokines, are widely accepted mediators of IDD,[53,54, 69,70] which enhance the

ECM catabolism and inflammation in discs, leading to the structural and functional deterioration of discs. Recently, the effects of cytokines on disc cell senescence have been investigated. TNF-$\alpha$ increased the number of SA-$\beta$-Gal positive cells in organ cultured bovine discs, and induced the ECM metabolism shift from anabolism to catabolism.[71] Significantly increased SA-$\beta$-Gal positive cells were also observed in rat NP cells treated with TNF-$\alpha$ and IL-1$\beta$,[23] indicating that the cytokines in degenerative discs are powerful pro-senescence factors (Fig. 2).

## Abnormal mechanical loading

Abnormal mechanical loading is a risk factor of IDD.[72] Our spine resists multidirectional mechanical loadings, including compressive, tensile and shear loads from axial, radial and circumferential directions. Forelimb amputation was used to induce the upright posture of rats, which simulates the upright posture of human beings. Prolonged upright posture caused abnormal mechanical loadings in all directions. As a result, IDD changes were observed, including disorganized collagen structure, AF fissure and decreased disc height. The matrix anabolism decreased and the catabolism increased.[73] Interestingly, the prolonged upright posture also accelerated disc cell senescence in rats. The abnormal loadings increased the number of SA-$\beta$-Gal positive cells, and up-regulated the expression of senescence-associated genes, including p16, p27, RB, PTEN, p27KIP, p19ARF, and RAGE, in discs,[74] suggesting that abnormal mechanical loading promotes disc cell senescence (Fig. 2). Mechanical stress increased the production of ROS to accelerate the SIPS.[75] These results provide further evidence of the contribution of disc cell senescence to the pathogenesis of IDD. The disc cell senescence mediates the process of load-induced disc degeneration.

To sum up, there are many stressful stimuli in the harsh microenvironment of degenerative discs, which are the triggers of disc cell senescence. Establishing some measures to ameliorate the harsh microenvironment, such as antioxidants application,[32,60] growth factors supplementation,[76,77] keeping healthy posture and blood glucose control, could retard disc cell senescence. Interestingly, these measures also have been suggested as promising therapeutic measures for IDD. It is consistent with our viewpoint above mentioned. Disc cell senescence promotes IDD. These potential therapeutic measures could retard IDD through suppressing disc cell senescence. Furthermore, except these known pro-senescence stimuli, further studies should identify anti-senescence factors in the microenvironment of degenerative discs. The balance between the pro-senescence stimuli and anti-senescence factors is crucial to retarding disc cell senescence. To investigate this issue will help us to understand the pathogenesis of IDD better. Moreover, maintaining this balance suggests some new therapeutic targets for IDD.

## The signaling pathways of disc cell senescence

The explorations on the signaling network underlying disc cell senescence are still at the infant stage. Herein, we discussed several major and well-defined signaling pathways.

## Two central pathways mediating disc cell senescence (the p53-p21-Rb and p16-Rb pathways)

The p53-p21-Rb pathway is activated in response to telomere erosion or DDR, which induces the replicative senescence.[7,14] The tumor suppressor gene, p53, is involved in many aspects of cell biology, including cell proliferation, senescence and death. With regard to cell senescence, p53 responds to telomere shortening or DDR to initiate the first step to the irreversible cell-cycle arrest. The mechanism by which telomere erosion or DDR activate p53 is complicated and remains to be investigated further. So far, the widely accepted mechanism resulted from the studies of autosomal recessive disorder ataxia–telangiectasia (AT). At the sites of DNA damage, the histone H2AX is characteristically phosphorylated to $\gamma$-H2AX. $\gamma$-H2AX is involved in the changes of chromatin structure that assemble the cell-cycle checkpoint proteins, Chk1 and Chk2, and DNA repair proteins, ATM and ATR. ATM directly phosphorylates p53 to activate it. Furthermore, ATM and ATR phosphorylate Chk1 and Chk2 to activate p53 indirectly. On the downstream of p53, p21 inhibits CDK2, which suppresses the phosphorylation of Rb. Because the cell-cycle progression from G1 to S phase relies on the hyper-phosphorylation of Rb, this hypo-phosphorylated Rb is unable to activate the E2F factor to promote the expression of the genes necessary for G1 to S progression, eventually, it causes the cell-cycle arrest.[78,79] The senescent NP cells in degenerative NP specimens expressed p53, p21 and Rb, along with telomere shortening and decreased telomerase activity.[18] With culture passage increasing, the p53-p21-Rb pathway was activated in human NP cells to induce the replicative senescence of disc cells.[21] Meanwhile, the senescent AF cells in degenerative discs showed a significant up-regulation of p53.[20] A recent study demonstrated that the ATM-Chk2-p53-p21-Rb pathway was activated by oxidative stress in human NP cells. Hydrogen peroxide leaded to the formation of $\gamma$-H2AX foci in the nuclei of NP cells, then, activated the ATM-Chk2-p53-p21-Rb pathway to induce disc cell senescence (Fig. 2).

Other than the p53-dependent pathway, the p53-independent pathway, the p16-Rb pathway is activated by different stimuli, especially oxidative stress, to induce the SIPS (Fig. 2). The p16 is the inhibitor of CDK4 and CDK6. When p16 is activated by oxidative stress, the inhibition of CDK4 and CDK6 results in the hypo-phosphorylation of Rb and the halt of cell-cycle progression.[80,81] Previous studies have found the expression of p16 by the senescent disc cells in degenerative disc specimens.[18,19,82] The number of p16-positive cells in disc tissues positively correlated with the histological grade of disc degeneration.[19] In consistent with the p53-p21-Rb pathway, the p16-Rb pathway in disc cells was also activated with culture passage increasing.[21] Recently, several studies found that the p16-Rb pathway mediated the high glucose-induced disc cell senescence. High glucose concentration enhanced the generation of excessive ROS in disc cells by causing mitochondrial damage. Consequently, the p16-Rb pathway was activated to induce the SIPS of disc cells.[61,64,83] In summary, the disc cell senescence is mainly mediated by both p53-p21-Rb and p16-Rb pathways. These

pathways converge on RB to regulate the cell-cycle progression of disc cells. During the process of disc degeneration, both pathways are activated by DNA damage and various stimuli simultaneously to induce the cell-cycle arrest of senescent disc cells.

## Silent information regulator 2 ortholog 1 (SIRT1) suppresses disc cell senescence and protects discs from degeneration

SIRT1 is a highly conserved nicotinamide (NAD+)-dependent deacetylases and has been found to be associated with age-related diseases, cancer and degenerative disorders.[84] It plays key roles in lifespan extension.[85,86] Previous studies have investigated the roles of SIRT1 in cell senescence. In addition to be regulated at mRNA and protein levels, p53 is regulated by post-translational modifications, such as acetylation and phosphorylation. The promyelocytic leukemia protein (PML) has been reported to activate p53 by posttranslational acetylation. SIRT1 deacetylated p53 to retard the PML-induced cell senescence.[87] The overexpression of SIRT1 attenuated the $H_2O_2$-induced senescence of human endothelial cells.[88] It is noteworthy that SIRT1 was expressed in human NP cells. The mRNA levels of SIRT1 in NP cells increased with patients age increasing. SIRT1 also deacetylated p53 in NP cells.[89] Furthermore, the activation of SIRT1 down-regulated the expression of p16 by disc cells in mouse coccygeal discs, and relived the IDD changes caused by disc puncture. Compared to the wild-type mice, the genetic ablated mice (SIRT1+/− ) had more p16-positive cells in discs, and severer IDD changes.[90] In conclusion, SIRT1 plays a protective role in the process of disc degeneration, which probably depends on suppressing the p16-dependent senescence of disc cells (Fig. 2). In the future studies, the roles of SIRT1 in the p53-dependent senescent pathway should be investigated in detail.

## Caveolin-1 is associated with disc cell senescence

Caveolae are vesicular invaginations in cell membrane. Caveolin-1 is a 21-24 kDa integral membrane protein and crucial to the structure and function of caveolae.[91] Many signaling proteins are regulated by caveolins, including epidermal growth factors (EGF) receptors, lipid transport, integrin and protein kinase C. The expression caveolin-1 was reported in degenerative human NP specimens. The expression of caveolin-1 was positive correlated with the expression of p16.[82] Notably, caveolin-1 was suggested to be associated with the senescence of different cell types, because its key roles in maintaining the morphology and unresponsive character of senescent cells.[92,93] Thus, the positive correlation between p16 and caveolin-1 suggests the roles of caveolin-1 in the SIPS of NP cells (Fig. 2). Moreover, the expression of caveolin-1 was associated with the upregulation of p53 and p21, suggesting the roles of caveolin-1 in the p53-dependent pathway. Caveolin-1 and p53 probably synergistically induced the replicative senescence of disc cells (Fig. 2).[94] Future studies will be needed to investigate the mechanism by which caveolin-1 regulates the 2 central senescent pathways of disc cells.

## The p38-MAPK pathway mediates the premature senescence of disc cells

The SIPS is induced by p38-MAPK pathway.[95,96] The pathway is activated by various external stimuli, including cytokines, oxidative stress, growth factors and oncogenes. Particularly, p38 is activated by MAP kinase kinases (MKKs, MKK3 and MKK6) in respond to oxidative stress, followed by the activation of p16 and hypo-phosphorylation of Rb. As a result, the cell-cycle progression is halted.[96] On the other hand, the p38-MAPK-mediated oxidative stress activates the DDR to induce cell senescence. The p38 up-regulates the expression of NADPH oxidase to generate excessive ROS causing DNA damage. Subsequently, the p53-p21-Rb pathway is activated.[97] The general support for the roles of p38-MAPK pathway in disc cell senescence was provided by the *in vivo* observations that the expression of p38 was upregulated in the senescent AF cells selectively harvested from paraffin-embedded sections of human AF tissue using laser capture microdissection (LCM). Moreover, $H_2O_2$ was found to increase intracellular ROS levels and activate all 3 MAPK pathways (p38-MAPK, ERK and JNK) to induce the senescence of human NP cells. The results suggest that the p38-MAPK pathway mediates the SIPS of disc cells (Fig. 2).

## Mitochondrial dysfunction accelerates the senescence of disc cells

ROS result from oxygen metabolism or exogenous stimuli.[98,99] The generation of ROS involves various metabolic enzymes, such as lipoxygenase, cyclooxygenase and flavin oxidases. But in non-immune cells, the major generator of ROS is mitochondria.[100] With aging, the mitochondrial DNA damage leads to mitochondrial dysfunction and abnormal electron leakage, enhancing the reduction of oxygen and increasing the production of ROS. The mitochondria-derived ROS directly initiates cell senescence through DDR.[101] The enhanced mitochondrial function by pyruvate increased the production of ROS, and then activated both the p53-p21-Rb and p16-Rb pathways to induce cell senescence. On the contrary, mitochondrial respiration uncoupling decreased the production of $H_2O_2$ to delay the replicative senescence of cells.[101] Mitochondrial dysfunction has been determined in the senescent AF cells. The expression of genes associated with mitochondrial function, including substrate dehydrogenases, cytochromes and substrates carriers, was up-regulated significantly in the senescent AF cells, indicating mitochondrial dysfunction in the senescent AF cells.[20] More importantly, the mitochondrial-derived ROS not only accelerated the SIPS of disc cells, but also were involved in IDD (Fig. 2).[61,83,100] Elucidating the mechanism of mitochondrial dysfunction in disc cells contributes to revealing the underlying mechanism of disc cell senescence and the pathogenesis of IDD.

## The WNT-$\beta$-catenin signaling pathway accelerates disc cell senescence

The WNT-$\beta$-catenin signaling is crucial to the homeostasis of bone and cartilage tissues. Its roles in the development of

osteoarthritis have been widely investigated.[102,103] However, its roles in the development of IDD are not understood well. A previous study found that LiCl, an activator of WNT pathways, inhibited the disc cell proliferation and enhanced the senescence of disc cells, suggesting that the WNT/$\beta$-catenin signaling triggers disc cell senescence. Meanwhile, this signaling also upregulated the expression of matrix proteases in NP cells, including MMP-9 and MMP-10, indicating that this signaling pathway is involved in the matrix degradation of IDD. [24] WNTs bind to various receptors to activate different signaling pathways together with downstream transcriptional effectors. It regulates cellular proliferation, differentiation and apoptosis. [104,105] Thus, Following studies investigated the mechanism by which the WNT-$\beta$-catenin pathway regulates disc cell senescence. Both c-myc and cyclin-D1 are essential for the cell-cycle progression of NP cells. WNT signaling suppressed the expression of c-myc and cyclin-D1 in NP cells. [106,107] Klotho, an anti-aging gene on the downstream of $\beta$-catenin, was reported to induce the cell cycle arrest of NP cells. Furthermore, a positive-feedback loop of WNT signaling and TNF-$\alpha$ was observed in NP cells (Fig. 2). The activation of WNT pathway may stimulate the expression of TNF-$\alpha$ to induce disc cell senescence and disc degeneration (Fig. 2).[108] The inhibition of WNT signaling probably prevents the senescence of disc cells and retard disc degeneration.

## The mammalian target of rapamycin (mTOR) signaling pathway contributes to the development of the senescent phenotype of disc cells

Paradoxically, the senescent disc cells were unable to replicate but secreted more specific proteins and increased in cell size and mass, indicating that the arrest of cell cycle and the formation of senescent phenotype are uncoupled. [7] The mTOR pathway, a member of the phosphatidyl-inositol 3-kinase (PI3K) pathway family, is required for cells to acquire the senescent phenotype.[109] When cells are stuck in the cell cycle, this pathway determines whether these cells get the senescent phenotype or just become quiescent (reversible cell cycle arrest). Cell cycle arrest together with mTOR pathway activation cause cellular senescence, however, it just results in cell quiescence without mTOR pathway activation.[109] Noteworthy, the mTOR pathway was activated in the senescent NP cells. Glucosamine retarded the senescence of NP cells by inhibiting the mTOR pathway activation.[60] The roles of mTOR pathway in the development of the senescent phenotype of disc cells will be a new valuable issue to discuss. Uncoupling the cell-cycle arrest and the development of the senescent phenotype of disc cells is a potential approach to reduce the detrimental effects of disc cell senescence on IDD (Fig. 2).

## Conclusion

The accumulation of senescent disc cells in degenerative discs suggests crucial roles of cell senescence in the initiation and development of IDD. More studies will be needed to elucidate the cause-effect relationship between disc cell senescence and IDD. The molecular mechanism underlying disc cell senescence can be subdivided into 2 aspects, the halt of cell cycle and the development of the senescent phenotype of disc cells (Fig. 2). Regarding cell cycle arrest, the p53-p21-Rb pathway and the p16-Rb pathway play major roles. Both pathways are activated by DDR or various stressful stimuli in the microenvironment of degenerative discs respectively, and converge on Rb to retard the cell-cycle progression from the G1 to S phase. The two pathways are regulated by different signals. SIRT1 deacetylates p53 to suppress the p53-depenent senescent pathway. It also inhibits the activation of p16, then, plays a protective role in disc cell senescence. On the other hand, caveoline-1 synergizes with p53 and p16 to mediate disc cell senescence. In addition to these negative regulators, the p38-MAPK pathway responds to external stimuli to activate the p53-p21-Rb and p16-Rb pathways in disc cells indirectly or directly. Mitochondrial dysfunction increases the generation of ROS to enhance disc cell senescence. Furthermore, the enhanced disc cell senescence by WNT-$\beta$-catenin pathway has been reported, suggesting a complex cell-cycle regulating network in disc cells.

Referring to the development of the senescent phenotype in disc cells, the mechanism remains to be elucidated. The pro-inflammatory cytokines, matrix catabolism enzymes and chemokines secreted by senescent disc cells have detrimental effects on the process of IDD. However, the development of the senescent phenotype is suggested to be uncoupled with the cell-cycle arrest. There are several regulatory pathways for the formation of the senescent phenotype of disc cells.[7] Among them, the widely known is the mTOR pathway. The cell cycle arrested cells can't develop into the senescent phenotype without the activation of mTOR pathway. Understanding the mechanism of the development of the senescent phenotype of disc cells well helps us to elucidate the cause-effect relationship between disc cell senescence and disc degeneration.

Due to the relationship between disc cell senescence and disc degeneration, several anti-senescence biological therapies targeting at different molecular pathways have been proposed to retard disc degeneration. Telomerase transduction fixed shortening the telomere and blocked the activation of senescent pathways, thus, prevented disc cell senescence.[110,111] But the high risk of tumorigenesis should be considered. Regulating p53 activity via SIRT1 or caveoline-1 is possible approaches to attenuating the p53-dependent disc cell senescence. The therapeutic strategy aiming at the p38-MAPK pathway and mitochondrial dysfunction may alleviate the SIPS of disc cells. In light of that the development of the senescent phenotype and the cell-cycle arrest are uncoupled, manipulating the mTOR pathway probably ameliorates the aberrant paracrine signaling of senescent disc cells to relieve the harsh microenvironment of degenerative discs. Of course, more *in vivo* studies will be needed in the future to demonstrate the validity of these therapeutic strategies in preventing disc cell senescence and retarding IDD.

## Abbreviations

| | |
|---|---|
| ADAMTS | a disintegrin and metalloproteinase with thrombospondin motifs |
| AF | annulus fibrosus |
| CEP | cartilage endplate |
| DDR | DNA damage response |

| | |
|---|---|
| ECM | extracellular matrix |
| FGF | fibroblast growth factor |
| IDD | intervertebral disc degeneration |
| IGF | insulin-like growth factor |
| IR | ionization radiation |
| IVD | intervertebral disc |
| LBP | low back pain |
| LCM | laser capture microdissection |
| MEC | mechlorethamine |
| MMP | matrix metalloproteinase |
| mTOR | the mammalian target of rapamycin |
| NP | nucleus pulposus |
| OA | osteoarthritis |
| PDGF | platelet derived growth factor |
| PG | proteoglycan |
| PML | promyelocytic leukemia protein |
| Rb | retinoblastoma protein |
| ROS | reactive oxygen species |
| SA-$\beta$-Gal | senescence-associated $\beta$-galactosidase |
| SASP | senescence-associated secreted phenotype |
| SIPS | stress-induced premature senescence |
| SIRT1 | silent information regulator two ortholog 1 |

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No potential conflicts of interest were disclosed.

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