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Mechanistic Interactions Driving Nucleus Pulposus Cell Senescence in Intervertebral Disc Degeneration: A Multi-Axial Perspective of Mechanical, Immune, and Metabolic Pathways

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Received: 16 March 2025 | **Revised:** 2 June 2025 | **Accepted:** 6 June 2025

Funding: This study was supported by the Affiliated Hospital of Southwest Medical University Research Fund (Project No. 04-3636), titled "VEGF-CS-NGF Microspheres Regulating Angiogenesis and Neurogenesis for Spinal Cord Injury Repair."

Keywords: intervertebral disc degeneration | macrophage polarization | mechanical stimulation | mitophagy | nucleus pulposus cell

ABSTRACT

Background: The senescence of nucleus pulposus cells (NPCs) at the heart of the pathogenesis of intervertebral disc degeneration (IVDD), which causes low back pain. Abnormal mechanical stress causes intracellular Ca^{2+} overload by activating the Piezo-type mechanosensitive ion channel component 1 (PIEZO1) channel.

Aims: This creates a positive feedback loop of oxidative-inflammatory damage by inducing endoplasmic reticulum stress and mitochondrial reactive oxygen species (ROS) bursts, as well as directly activating the NLRP3 inflammasome/NF-κB axis to promote the release of pro-inflammatory factors like IL-1β.

Results: Energy metabolism collapsed as a result of mechanistic cause that caused excessive activation of mitophagy via the ROS-PINK1/Parkin pathway, and SIRT1 functional suppression further compromised mitochondrial quality control. The inflammatory nucleus pulposus (NP) brought on by mechanical stimulation caused macrophages to polarize toward the M1 type, and the p38MAPK pathway was activated by the TNF-α/IL-1β released, which in turn increased senescence markers like p16/p21. Notably, ROS both triggers mitophagy and activates the p53 pathway. On the one hand, oxidative damage-induced ATM/ATR kinase activation leads to p53 phosphorylation, which triggers p21-mediated cell-cycle block. On the other hand, p53 exacerbates mitochondrial dysfunction by inhibiting SIRT1 expression, creating a triangular amplification loop of p53-ROS-mitophagy. Furthermore, p53 stimulates apoptosis by altering the Bax/Bcl-2 balance and works in concert with inflammatory substances secreted by M1-type macrophages to cause the development of senescence-associated secretory phenotype (SASP).

Conclusion: This interaction network reveals the dynamic coupling of mechano-immune-metabolic pathways in the course of IVDD, providing a theoretical basis for the development of multi-targeted intervention strategies, such as PIEZO1 inhibitors combined with M2-type macrophage polarization modulation, which are expected to delay disease progression by blocking key nodes.

The first two authors have the same contribution and are considered as the first author.

1 | Introduction

The primary pathogenic cause of neurological dysfunction and chronic low back pain, intervertebral disc degeneration (IVDD), is directly linked to around 40% of the worldwide population. IVDD is a serious public health problem that is becoming more common as a result of population aging and the preference for sedentary lifestyles. The aging process is significantly positively correlated with the onset and development of IVDD, as nucleus pulposus cells (NPCs) are the primary functional unit responsible for maintaining the mechanical stability and metabolic balance of the intervertebral disc [1]. Recent studies have shown that aberrant mechanical stress, immunological microenvironment imbalance, mitochondrial malfunction, and anomalies in critical signaling pathways all contribute to the onset of NPC aging. For the creation of focused treatment plans, a thorough examination of their interaction mechanism is quite beneficial [2, 3]. The intervertebral disc, the biggest avascular tissue in the human body, can withstand dynamic mechanical pressure for an extended period of time. While aberrant mechanical stimulation (such as prolonged high pressure or shear stress) initiates a harmful cascade reaction by activating the Piezo-type mechanosensitive ion channel component 1 (PIEZO1) mechanically sensitive ion channel, physiological stress can sustain the creation of NPCs' extracellular matrix. PIEZO1 opening accelerates the release of pro-inflammatory proteins (IL-1β, TNF-α) by inducing intracellular Ca^{2+} excess, activating the NLRP3 inflammasome, and improving NF-κB signal transduction. Simultaneously, mechanical stress causes endoplasmic reticulum stress and excessive reactive oxygen species (ROS) production in the mitochondria, which results in oxidative damage buildup and the development of senescence-related secretory phenotype (SASP), which in turn causes NPCs to cycle arrest [1, 4]. This mechanism provides a theoretical basis for mechanical intervention strategies, such as dynamic load regulation.

During the degeneration process, the intervertebral disc's immunological immunity was damaged. The two main features of the inflammatory aging microenvironment were M1 macrophage polarization and infiltration. By secreting IL-6 and TNF-α, M1 macrophage directly activate the NPCs p38MAPK pathway. They also upregulate the expression of p16INK4a and other aging markers. The secretory Secreted Extracellular Vesicles (SEVs) have the ability to activate the TLR4/NF-κB signal axis and supply pro-aging factors like Lcn2. It is important to remember that SASP components (including MMP-13 and CCL5) generated by aging NPCs can reverse stimulate M1 macrophage polarization, creating a vicious cycle. The main tactic to prevent inflammatory aging may be target the balance of macrophage polarization (e.g., by encouraging M2 transition) [5]. The aberrant autophagy flux of mitochondria, which regulates oxidative stress and cellular energy metabolism, is a key factor in nucleus pulposus aging. Excessive PINK1/Parkin pathway activation brought on by mechanical damage or inflammatory stimulation can lead to excessive mitochondrial clearance and disorders of ATP production. This process is exacerbated, the antioxidant defense system (SOD2, GPX) is weakened, and the ROS level is encouraged to surpass the steady-state threshold when SIRT1 deacetylase function is down-regulated. Notably, a triangular amplification loop of mitochondrial damage, inflammation, and aging can be formed when mitochondrial DNA leakage triggers the innate immune response via the CGAs sting route. Restoring the equilibrium of energy metabolism may be possible by controlling the mitophagy threshold (e.g., by employing UBDCA1 inhibitors) [6].

Phosphorylation of p53, the molecular center of the cellular stress response, is triggered by ROS and DNA damage, and transcriptional control of p21 results in G1/S phase arrest. It has a dual effect on aging: prolonged activation results in an irreversible aging phenotype, while short-term activation protects against growth retardation. According to recent research, p53 and HIF-1α can work together to control the glycolytic reprogramming of NPCs, and the degree of intervertebral disc hypoxic microenvironment degradation is positively connected with p53 expression. Furthermore, by controlling the Bax/Bcl-2 balance, p53 stimulates mitochondrial apoptosis, indicating a key function in various cell fate decisions [7]. Even while recent research has identified a number of distinct regulatory modules, it is still unknown how mechanical stress, immunological response, and metabolic reprogramming interact dynamically. It is anticipated that the use of spatial transcriptome technology and single-cell sequencing would uncover the mechanism of microenvironmental cellular dialog as well as the heterogeneity of NPCs senescence. Translational research avenues that have demonstrated promise for clinical use include mechanoregulation-based biomaterials, small chemical modulators that target mitophagy, and inhibitors specific to the p53 pathway. A thorough examination of the multifaceted regulatory network of NPCs aging will open up new avenues for the development of targeted intervention techniques, which are crucial from a therapeutic standpoint for delaying the intervertebral disc degeneration process.

Recent studies suggest a complex interplay between mechanical stress, immune imbalance, and mitochondrial dysfunction in the regulation of NPCs senescence during IVDD. However, a unified model that integrates these multifactorial mechanisms remains lacking. In this review, we systematically synthesize current evidence on the mechano-immune-metabolic regulation of NPCs aging. We propose an integrative framework where mechanical stimulation acts as the initial insult, priming immune activation and oxidative stress, which further drive mitochondrial dysfunction and p53-mediated senescence signaling. This interconnected network offers novel insights into therapeutic opportunities for halting or reversing IVDD progression (Figure 1).

1.1 | Aging of NPCs Induced by Mechanical Stimulation via PIEZO1 Activation Related Signaling Pathway

Mechanical overload is an important causative factor in the pathogenesis of IVDD. It has been shown that excessive compressive forces disrupt disc homeostasis by inducing extracellular matrix (ECM) degradation through the upregulation of matrix metalloproteinases (MMPs) and ADAMTS family enzyme expression [8]. In contrast, moderate dynamic mechanical

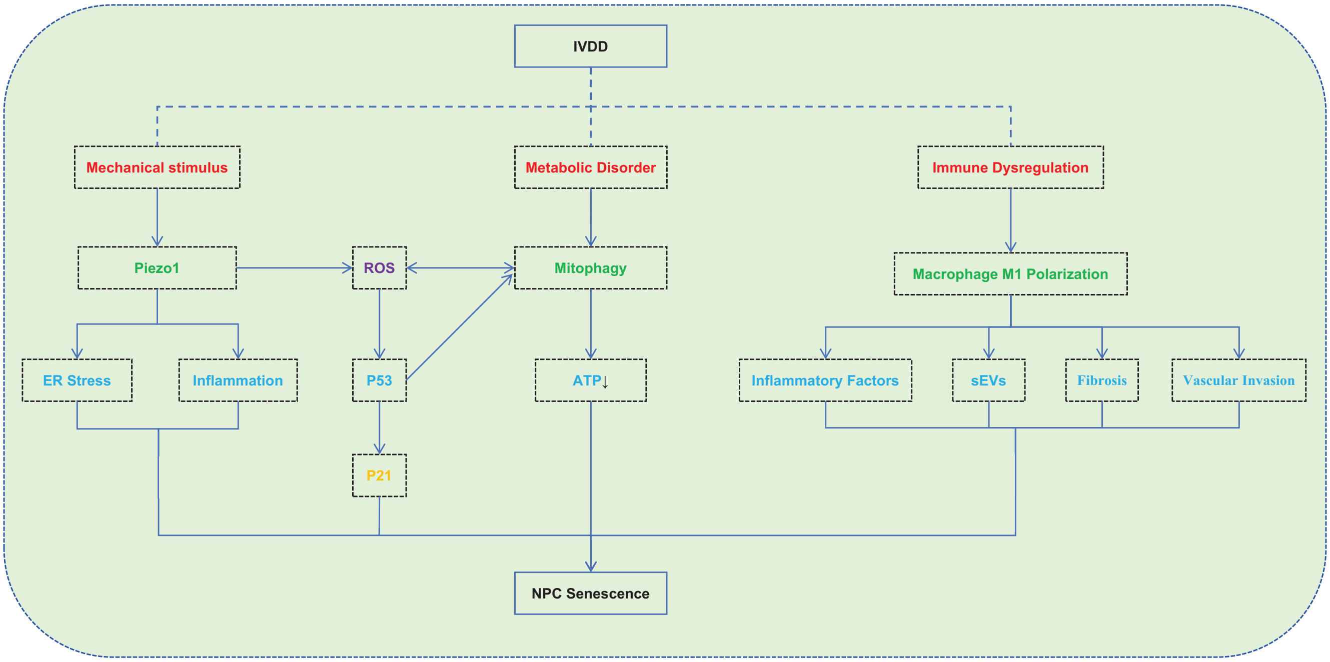

FIGURE 1 | Conceptual framework of NPCs senescence in IVDD: Mechanical, immune, and metabolic axes interact dynamically to accelerate cell aging.

stimulation promotes the anabolic activity of NPCs in in vitro experiments. Clinical studies further confirmed that prolonged mechanical stress due to occupational heavy physical labor or abnormal posture is significantly associated with disc herniation and chronic low back pain [9]. These findings reveal the dual role of mechanical stimulation in the progression of IVDD. Mechanical stimulation is one of the many pathogenic aspects of IVDD, and the PIEZO1 ion channel is a crucial "sensor" of the aging of NPCs brought on by mechanical stimulation. The mechanism of NPCs aging brought on by mechanical stimulation through the PIEZO1 activation related signaling pathway has advanced significantly in recent years, offering a crucial foundation for a deeper comprehension of the pathophysiology of IVDD [10].

In daily living, intervertebral discs are frequently subjected to mechanical stimulation. The normal physiological balance of NPCs will be disrupted and they will age as a result of prolonged aberrant mechanical stimulation, such as high pressure or stretching. PIEZO1 is a common ion channel found in many cells, including NPCs, that is extremely sensitive to mechanical stimulation [1]. When mechanically stimulated, the conformation of PIEZO1 will change, rapidly opening the ion channel, causing a large amount of extracellular Ca^{2+} influx, causing a sharp increase in intracellular Ca^{2+} concentration [8]. This alteration serves as the "fuse" that triggers the activation of several other signaling pathways. The NLRP3 inflammasome was initially triggered by the rise in Ca^{2+}. One significant inflammatory regulatory complex in cells is the NLRP3 inflammasome. It will encourage the development and release of pro-IL-1β and other inflammatory factors when it is active. IL-1β is a major pro-inflammatory cytokine that can trigger the NF-κB signaling pathway [11]. NF-κB is an important transcription factor. In the inactive state, it binds to inhibitor protein IκB and exists in the cytoplasm. When IL-1β binds to its receptor, it will trigger a series of cascade reactions, which will phosphorylate and degrade IκB, thereby releasing NF-κB. NF-κB can enter the nucleus and combine with the promoter region of target genes to promote the transcription of inflammation-related genes, such as IL-6, TNF-α, and so on [12]. In addition to aggravating the local inflammatory response, the high production of these inflammatory factors will also disrupt the regular metabolism and function of NPCs and hasten the aging process of cells. The study discovered that mechanical stimulation significantly increased the expression of aging-related markers p16 and p21, up-regulated PIEZO1 in NPCs, activated the NLRP3 inflammasome, and raised levels of inflammatory factors like IL-1β, IL-6, and TNF-α [1].

Endoplasmic reticulum stress (ERs) and impairment to the endoplasmic reticulum's normal function might result from an ongoing rise in intracellular Ca^{2+} concentration. The endoplasmic reticulum, which is crucial for protein synthesis, folding, and transport in cells, is highly responsive to variations in Ca^{2+} concentration. The unfolded protein response (UPR), which is initially a cell self-defense mechanism to restore the endoplasmic reticulum's normal function, can be triggered by errors. UPR will, however, trigger the signaling pathways linked to aging and apoptosis if ERS persist. In this mechanism, the perk-eIF2α-ATF4 route is crucial. Upon activation, perk will phosphorylate eIF2α, prevent total protein synthesis, and lessen endoplasmic reticulum burden [13]. ATF4 has the ability to control the expression of several genes linked to aging, apoptosis, and cell stress. For instance, ATF4 can increase the expression of the pro-apoptotic protein chop. Its overexpression can cause aging and cell death [13, 14]. Research has demonstrated that the expression of endoplasmic reticulum stress-related proteins, including perk, eIF2α, ATF4, and chop, is markedly up-regulated in the aging model of NPCs caused by mechanical stimulation.

This suggests that ERS and its UPR are active during this process [14].

Additionally, oxidative stress and PIEZO1 activation are closely related. NADPH oxidase can be activated by Ca^{2+} influx, which significantly increases the production of ROS. Because of their potent oxidative activity, ROS can oxidize biological macromolecules including proteins, lipids, and DNA within cells, resulting in oxidative damage. Overexposure to ROS will damage the cell membrane's structure and function, impairing the movement of materials and signals within cells; oxidative protein modification will cause proteins to lose their typical biological activity and disrupt cell metabolism; DNA damage can result in gene mutation and cell cycle disorders. Further activation of intracellular stress signaling pathways, including the p38MAPK pathway, can result from these oxidative damages. In order to promote the expression of genes linked to cell senescence and prevent cell proliferation, p38MAPK phosphorylates a number of downstream target proteins, such as transcription factors and cell cycle regulatory proteins. According to the study, mechanical stimulation greatly raised the ROS levels in NPCs, activated the p38MAPK pathway, and exacerbated the degree of cell aging. Cell aging may be somewhat mitigated by the use of antioxidants or p38MAPK inhibitors, suggesting that PIEZO1 under mechanical stimulation triggered the oxidative stress and p38MAPK pathway [15].

Together, these findings position PIEZO1 not merely as a sensor of mechanical overload but as a central hub linking biomechanical perturbation to downstream inflammatory, oxidative, and aging responses. The amplification of Ca^{2+}-mediated NLRP3 and NF-κB activation illustrates how mechanical cues initiate cellular senescence cascades, providing a mechanistic entry point into the broader pathological network (Figure 2).

1.2 | Macrophage Polarization Leads to Inflammatory Microenvironment and Aging of NPCs

Recent studies have emphasized that immune-mediated inflammation is a central driver of IVDD. Macrophages and T cells infiltrate the degenerated disc and secrete pro-inflammatory cytokines (e.g., IL-1β, TNF-α) that exacerbate ECM catabolism and neural sensitization [3]. Notably, the microenvironment of the degenerated disc is dominated by pro-inflammatory M1-type macrophages, while the proportion of anti-inflammatory M2-type macrophages is reduced [5, 16]. This imbalance creates a positive feedback loop of “tissue damage-pain signaling,” a mechanism supported by elevated cytokine levels in patients with discogenic low back pain. The inflammatory NP brought on by macrophage polarization during IVDD has a significant effect on NPCs aging, which is mediated by a number of intricate processes and intimately linked to several cytokines and signaling pathways [17]. A thorough investigation of this mechanism is necessary to comprehend the pathophysiology of IVDD and create novel therapeutic approaches. As the main immune system cells, macrophages are highly malleable and can polarize into various phenotypes in response to cues from the NP. The two primary polarization states of macrophages are M1 and

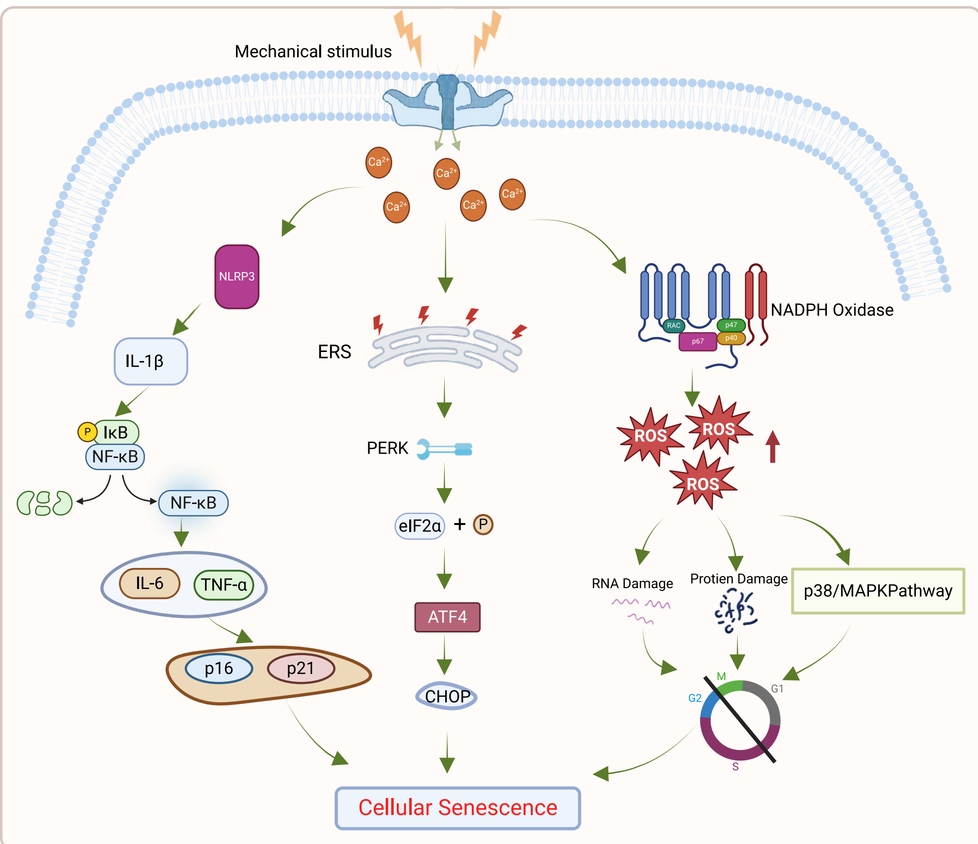

FIGURE 2 | Mechanism of senescence in NPCs induced by mechanical stimulation of the related signaling pathways via PIEZO1.

M2. To maintain tissue homeostasis, macrophages under normal physiological conditions maintain a specific polarization balance. Numerous causes, however, will upset this equilibrium during intervertebral disc degeneration, because macrophages to change to M1 polarization, cause a lot of pro-inflammatory substances to be released, and then produce an inflammatory microenvironment that will encourage NPCs to age.

After being activated, M1 macrophages secrete a series of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and so on [18]. These cytokines have a range of effects on NPCs' physiological states. The NF-κB signaling pathway can be activated by TNF-α binding to the receptors on the surface of NPCs. When at rest, NF-κB remains in the cytoplasm in an inactive state after binding to its inhibitor protein IκB. Following its binding to the receptor, TNF-α sets off a sequence of signal transduction events that phosphorylate and break down IκB, releasing NF-κB [19]. Following its entry into the nucleus, NF-κB joins with the promoter region of particular genes to increase the transcription of genes linked to inflammation and cell aging. According to the findings, NPCs treated with TNF-α exhibited significantly higher level of senescence-related β-galactosidase (SA-β-gal) activity, significantly higher levels of p16, p21, and other senescence markers, and reduced cell proliferation. Additionally, IL-1β has a potent pro-inflammatory effect. It can increase the inflammatory response by causing NPCs to create more prostaglandin E2 (PGE2), IL-6, and IL-8. By triggering mitogen-activated protein kinase (MAPK) pathways such p38MAPK and JNK, IL-1β can also accelerate the aging process of NPCs and increase the expression of proteins linked to aging.

Small extracellular vesicles (SEVs) produced by macrophages are essential for both intercellular communication and the aging process of non-proliferative cells. SEVs generated from type M1 macrophages are abundant in bioactive substances such lipids, proteins, and nucleic acids. The ability of M1 SEVs to transmit Lcn2 to NPCs was discovered. Lcn2 enters NPCs, activates the NF-κB signaling pathway, phosphorylates the p65 subunit, enters the nucleus, and subsequently increases the production of genes linked to cell aging and inflammatory factors. After NPCs were treated with M1 SEVs, the results showed that the aging process of NPCs was accelerated and the extracellular matrix metabolism was unbalanced. The expression of Lcn2 increased, the phosphorylation level of p65 increased, the proportion of SA-β-gal positive cells increased significantly, the expression of extracellular matrix related proteins like col-2 decreased, and the expression of matrix metalloproteinase 13 (MMP13) increased [20]. When siRNA was used to interfere with the expression of Lcn2, the aging phenomenon of NPCs induced by M1 SEVs was significantly alleviated, which further confirmed the key role of Lcn2 in the aging of NPCs mediated by M1 SEVs.

Macrophage infiltration during intervertebral disc degradation can result in abnormal vascular development and nucleus pulposus fibrosis, both of which are directly linked to NPCs aging. Macrophages release transforming growth factor-β (TGF-β), a crucial cytokine that promotes nucleus pulposus fibrosis. TGF-β can cause fibroblasts to proliferate and become activated, as well as produce and secrete a variety of extracellular matrix components, including collagen and fibronectin. The natural tissue structure and function were damaged as a result of the buildup of these extracellular matrix components, which caused the nucleus pulposus tissue to eventually become fibrotic. NPCs age more quickly in the fibrotic NP because of altered interactions between NPCs and extracellular matrix, reduced cell living space, and impeded nutrition and signal transmission. Studies have shown that in the fibrotic nucleus pulposus, the expression level of TGF-β is significantly increased, and the expression of aging markers of NPCs is correspondingly increased. The use of TGF-β inhibitors can partially reduce the degree of nucleus pulposus fibrosis and cell aging [21].

The chronic pro-inflammatory milieu fostered by M1 macrophage polarization is not only a consequence but also a driver of NPCs aging. This bidirectional communication, mediated in part by SEVs carrying LCN2, underscores the immune-metabolic feedback loop that perpetuates disc degeneration. As shown in Figure 3, this axis connects inflammation to metabolic dysfunction and fibrosis, highlighting macrophage modulation as a promising therapeutic target.

1.3 | Activation of Mitophagy Induces Aging of NPCs

IVDD is closely related to mitochondrial metabolic dysfunction. The hypoxic microenvironment of the avascular NP prompts cellular metabolism to be skewed toward glycolysis, leading to lactic acid buildup and decreased pH. The acidic environment impairs NP cell survival and accelerates ECM breakdown. In addition, mitochondrial dysfunction in aging discs exacerbates oxidative stress, further impairing cellular repair. Clinical imaging studies have shown that the aforementioned metabolic abnormalities are significantly associated with advanced stages of IVDD. Mitophagy, a crucial quality control system in cells, is essential for preserving cell homeostasis. However, in NPCs, cell senescence—which involves a number of intricate signaling cascades and molecular mechanisms—is tightly linked to the aberrant activation of mitophagy. To comprehend the degenerative process of intervertebral disc degeneration and create efficient treatment plans, more research into the molecular mechanism of mitophagy activation-induced aging of NPCs is crucial [2].

An important factor in the aging process of NPCs is the traditional PINK1/Parkin pathway, which controls mitophagy. PTEN-induced kinase 1 (PINK1) cannot be routinely delivered to the inside of mitochondria for destruction when mitochondria are injured because to a drop in membrane potential. Instead, it accumulates on the outer membrane of damaged mitochondria. E3 ubiquitin ligase Parkin will be recruited by accumulated PINK1 to move from the cytoplasm to the mitochondrial surface [22]. Numerous proteins on the mitochondrial membrane will be ubiquitinated by Parkin once it has been activated. Autophagy receptors will recognize these ubiquitinated proteins, which will then attract autophagy-related proteins to encourage the creation of autophagosomes and wrap damaged mitochondria before fusing with lysosomes to finish the destruction of damaged mitochondria [23].

Numerous stressors, including mechanical and oxidative stress, can damage mitochondria in NPCs, which in turn triggers the PINK1/Parkin pathway. Overactivation of the PINK1/Parkin

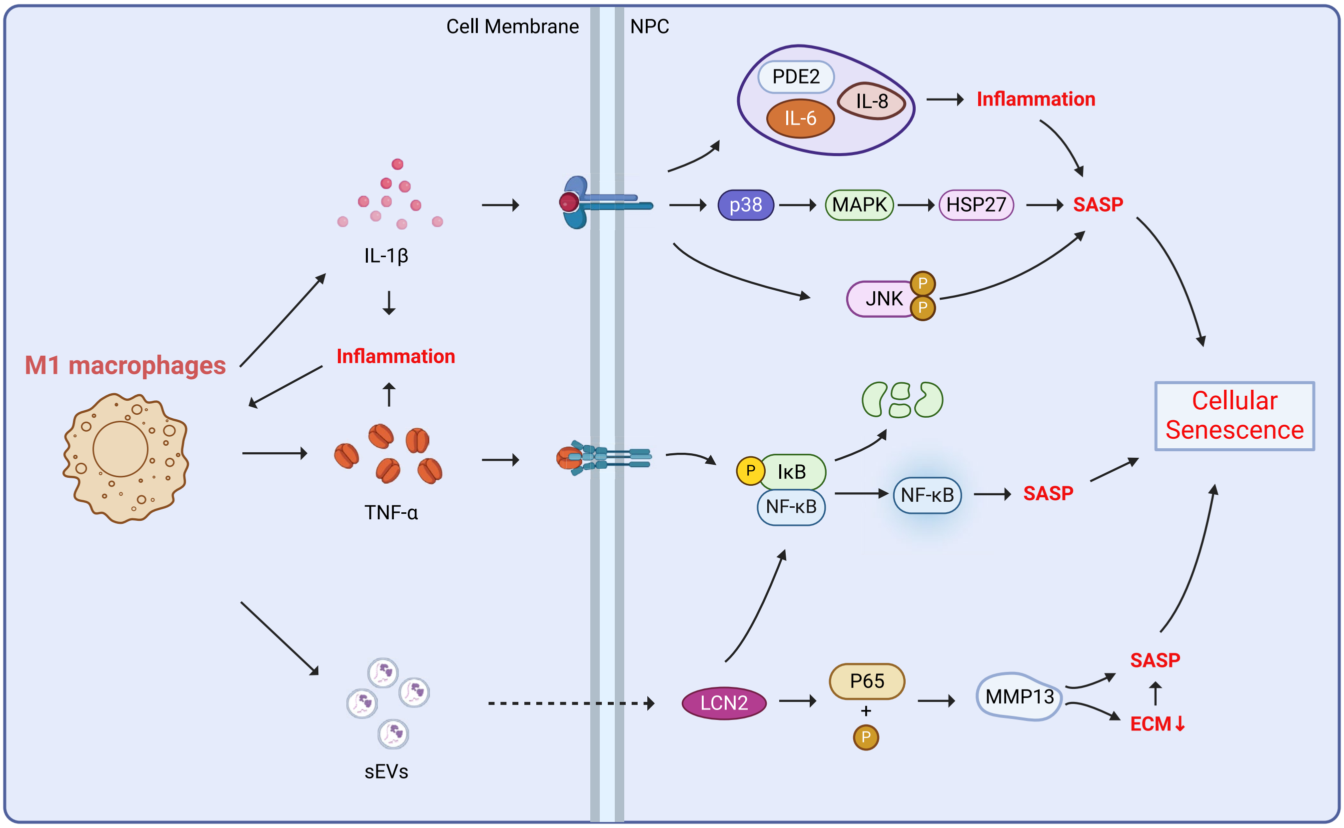

FIGURE 3 | Mechanism of macrophage polarization leading to an inflammatory microenvironment leading to senescence of NPCs.

pathway can remove damaged mitochondria, but it can also cause an abnormal decrease in the number of mitochondria and alter how cells use energy [24]. Cells' energy generators are called mitochondria. Their decrease will result in an imbalance in the energy metabolism of cells and inadequate ATP synthesis. According to studies, the PINK1/Parkin pathway is considerably more active, mitophagy is higher, intracellular ATP content is lower, cell viability is lower, and the expression of aging-related markers like p16 and p21 is up-regulated in NPCs as they age [25]. When using drugs to inhibit the activity of PINK1/Parkin pathway, it can reduce mitophagy, partially restore the energy metabolism of cells, and delay the aging process of NPCs [26]. This indicates that the overexpression of PINK1/Parkin pathway plays a promoting role in the aging of NPCs.

The SIRT family is crucial for stress response, metabolism, and cell aging [27]. SIRT1 is intimately associated with NPC aging and mitophagy. SIRT1 is a NAD-dependent deacetylase that uses deacetylation to control the activity of several different proteins. SIRT1 can interact with PINK1 and deacetylate it during PINK1-dependent mitophagy. Following deacetylation, PINK1's stability and activity rose, which encouraged the development of mitophagy. Under typical circumstances, SIRT1's modest expression aids in preserving cell homeostasis and mitochondrial function [28, 29]. However, with intervertebral disc degeneration, SIRT1 expression tends to decline. Numerous things, including oxidative stress and inflammatory reactions, could be the cause of this. The regular regulation of mitophagy will be impacted by the reduction of SIRT1 expression, which will raise the acetylation level of PINK1 and impede its function. According to studies, SIRT1 overexpression in NPCs can prevent PINK1-dependent mitophagy from being overactivated, lessen the breakdown of damaged mitochondria, preserve the quantity and functionality of mitochondria in cells, and postpone the aging of NPCs [29, 30]. On the contrary, inhibition of SIRT1 expression can promote PINK1 dependent mitophagy and accelerate the aging process of NPCs [30]. This implies that via controlling PINK1-dependent mitophagy, SIRT1 contributes significantly to the aging process of NPCs.

Mitochondrial quality control, particularly via the PINK1/Parkin pathway, plays a dual role in maintaining cellular homeostasis and promoting senescence when dysregulated. SIRT1 emerges as a key regulatory node, and its decline under pathological stress tilts the balance toward excessive mitophagy, impaired energy metabolism, and ROS accumulation. This metabolic dysfunction feeds back into both immune activation and cell cycle arrest mechanisms (Figure 4).

One of the byproducts of cell metabolism is ROS; it has a physiological role in the process of cell signal transmission, and its amount in cells is in dynamic equilibrium under physiological conditions. However, ROS production will sharply rise in pathological circumstances such as intervertebral disc degeneration, upsetting this equilibrium and resulting in oxidative stress. ROS buildup in NPCs can trigger the autophagy signaling pathway. On the one hand, ROS can directly affect proteins linked to autophagy, like ATG5 and atg4, and encourage the development of autophagosomes. On the other hand, ROS can indirectly regulate the expression of autophagy-related genes by activating MAPK pathways, such as p38MAPK and JNK [31]. Cells can preserve the stability of the intracellular environment and

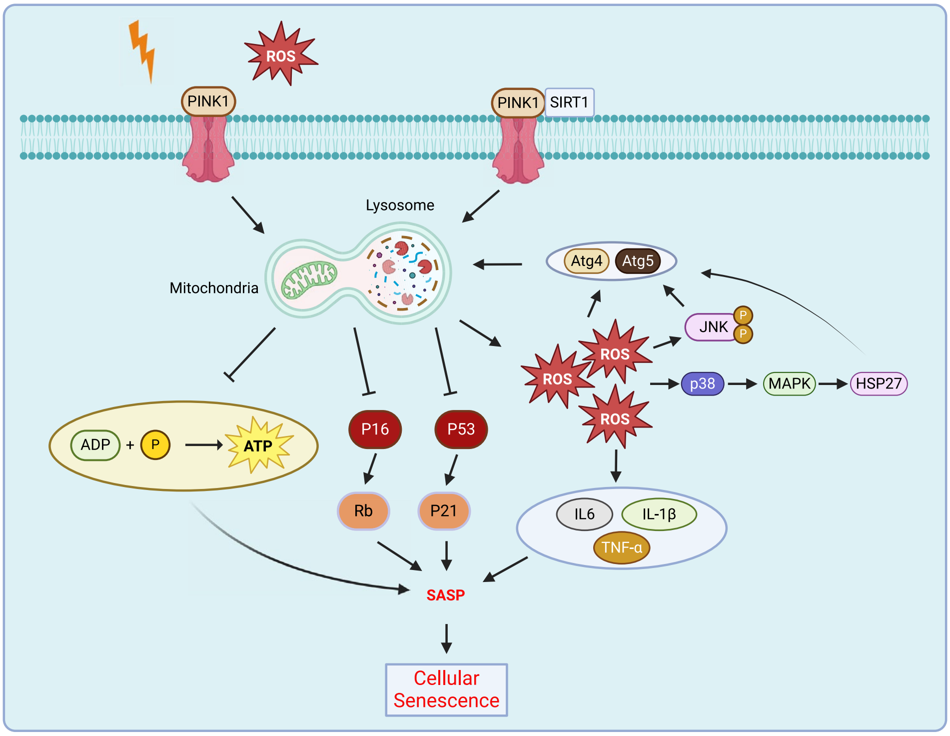

FIGURE 4 | Plot of the senescence mechanism induced by mitophagy activation in NPCs.

remove damaged proteins and organelles with the aid of moderate autophagy. Overactivated autophagy, on the other hand, can cause excessive intracellular material degradation, interfere with normal cell metabolism and function, and ultimately accelerate the aging of NPCs.

1.4 | ROS Mediated p53 Pathway Regulates Cell Cycle and Aging of NPCs

The p53 pathway and ROS are intimately related to the aging process of NPCs. They work together to control the cell cycle and play a significant role in IVDD formation. It is crucial to investigate this process further to comprehend the pathophysiology of IVDD and create focused treatment plans. ROS participates in physiological activities including cell signal transduction and is in a dynamic equilibrium state in cells under normal physiological settings. However, a number of variables, including mechanical stress and inflammatory response, can cause excessive ROS production in NPCs throughout the intervertebral disc degeneration process. Because of their high oxidative activity, excessive ROS can harm biological macromolecules like proteins, lipids, and DNA in cells. According to research on degenerative intervertebral disc tissue, the amount of ROS in this tissue was considerably higher than in normal tissue, and it was positively connected with the degree of NPC aging. This suggests that the aging process of NPCs is significantly influenced by ROS buildup [32, 33].

P53, a significant tumor suppressor protein, is essential for the way cells react to different types of stress. The p53 pathway was triggered when ROS-induced oxidative stress harmed NPCs. ROS can activate p53 in a number of ways, including by oxidatively altering the p53 protein, which makes it more stable and causes it to build up in cells. As a transcription factor, activated p53 enters the nucleus, combines with the promoter region of particular genes, controls the production of a number of downstream genes, and subsequently influences the cell cycle and aging process [34, 35]. According to studies, the p53 pathway is activated in ROS-mediated NPC senescence because the expression and activity of the p53 protein are markedly increased in ROS-treated NPCs, along with the upregulation of markers linked to cell senescence. The foundation for cell proliferation and preserving tissue homeostasis is the regular functioning of the cell cycle [36]. The cell cycle is significantly regulated by the activation of the p53 pathway, and in the aging process of NPCs, this regulation encourages cell cycle arrest. Cyclin dependent kinase inhibitors (CKIs) are expressed when p53 is activated, and p21 is a crucial downstream target gene. P21 has the ability to attach itself to cyclin-dependent kinases (CDKs) and stop them from working. CDKs are essential for controlling the cell cycle. Together with cyclins, they create complexes that propel the cell cycle forward [37]. The CDK cyclin complex's activity is suppressed when p21 attaches to CDKs, stopping the cell cycle and causing cells to stop at a particular stage, typically G1 or G2 [38, 39]. According to studies, ROS-treated NPCs exhibited significantly higher p21 expression, a much higher proportion of cell cycle arrest in the G1 phase, and a significantly lower capacity for cell proliferation. After p53 or p21 was knocked down, this effect was much reduced, confirming that p53 may inhibit the cell cycle and accelerate the aging of NPCs via controlling p21 [34, 40].

By controlling other cell cycle-related proteins, p53 can influence cell cycle progression in addition to controlling p21. For instance, the proto-oncogene c-myc's expression can be suppressed by p53. One significant transcription factor that is essential for cell development, proliferation, and metabolism is C-myc [41]. C-myc can accelerate the cell cycle and increase cyclin expression. The p53-induced suppression of c-myc further impairs the capacity for cell division and accelerates the aging of NPCs [42]. Furthermore, p53 has the ability to control the expression of proteins belonging to the Bax/Bcl-2 family. Bcl-2 is an anti-apoptotic protein, while Bax is a pro-apoptotic one. By upregulating Bax expression, downregulating Bcl-2 expression, and altering the Bax/Bcl-2 ratio—one of the key elements contributing to NPC aging—P53 stimulates apoptosis [43, 44].

The inflammatory response, ROS, and p53 pathways interact intricately in the NP of intervertebral disc degeneration. To further increase the generation of ROS and worsen the condition of oxidative stress, inflammatory substances including IL-1β and TNF-α would be released. In addition, the p53 pathway may interact with the inflammatory signaling system. For instance, NF-κB has a complex regulatory interaction with p53 and is a major regulator of the inflammatory response. In certain situations, NF-κB activation can increase p53 expression and activity and improve cells' ability to respond to oxidative stress. Inflammation and ROS interact with p53 pathways to generate a complex regulatory network that contributes to the development of intervertebral disc degeneration and the aging of NPCs.

Beyond its canonical role in DNA damage response, p53 integrates signals from oxidative stress and inflammatory pathways to regulate cell cycle arrest, apoptosis, and senescence. The p53–p21 axis, activated downstream of ROS accumulation, bridges metabolic stress to irreversible NPC aging. Moreover, its crosstalk with NF-κB signaling exemplifies the convergence of immune and metabolic pathways in the degenerative niche.

2 | Discussion

We evaluated the literature on mechanical stimulation, macrophage polarization, mitophagy, and ROS-mediated regulation of the p53 pathway with an emphasis on the aging process of NPCs in IVDD. By activating the NLRP3 inflammasome and NF-κB signaling pathway, mechanical stimulation raises intracellular Ca^{2+} concentration through the piezo1 ion channel, causes an inflammatory response, disrupts endoplasmic reticulum function, activates oxidative stress and the p38MAPK pathway, and speeds up the aging of NPCs [8, 45]. During intervertebral disc degeneration, macrophages polarize to the M1 type and release cytokines that promote inflammation. Their resultant SEVs produce pathological vascular expansion and nucleus pulposus fibrosis, which together accelerate the aging of NPCs, and they also carry Lcn2 to activate the NF-κB signaling pathway [6, 20]. Excessive PINK1/Parkin pathway activation will decrease the amount of mitochondria and impact energy metabolism in terms of mitophagy. While ROS buildup will inappropriately activate autophagy, which is linked to the aging of NPCs, SIRT1 can regulate this pathway [3, 35]. Furthermore, the rise in ROS in NPCs during intervertebral disc degeneration triggers the p53 pathway, causes p21 production, stops the cell cycle, and controls other proteins linked to the cell cycle. Together, the inflammatory response and ROS and p53 pathways accelerate the aging of NPCs and the degeneration of the intervertebral disc [46]. In conclusion, in IVDD, the core mechanism of NPCs aging stems from the synergistic effects of mechanical stimuli, immune dysregulation, and metabolic disorders, which are intertwined with each other: mechanical injury drives inflammation and oxidative stress, immune dysregulation exacerbates metabolic disorders and fibrosis, and metabolic abnormalities reinforce inflammation and mechanical signaling through ROS and autophagy imbalance feedback to form a vicious circle, which ultimately accelerates the aging of NPCs and intervertebral disc degeneration process. This multiaxial network provides a new intervention strategy for targeting the mechanical-immune-metabolic axis.

Even though current research on the aging process of NPCs has advanced significantly, many issues and obstacles need to be resolved. Although a number of signal pathway alterations brought on by piezo1 activation are known to occur in the aging process of NPCs triggered by mechanical stimulation through piezo1, it is unclear if piezo1 and other mechanically sensitive ion channels have an antagonistic or synergistic effect. The perception and processing of mechanical signals may involve a range of ion channels. More research is required to determine how their interaction impacts the aging process of NPCs [47–49]. Furthermore, it is important to investigate if other important bioactive molecules, besides Lcn2, play a role in controlling the aging of NPCs in SEVs produced from macrophages [50, 51]. Even though ROS is crucial for autophagy activation, a major challenge in the development of related treatment approaches is precisely controlling ROS levels to prevent the negative consequences of excessive autophagy suppression or activation on cells. Although the crucial role of the ROS-mediated p53 route in controlling the cell cycle and the aging of NPCs has been established, the intricate relationship between the p53 system and other signaling pathways linked to cell aging remains unclear. For instance, more research is required to determine how the aging of NPCs is impacted by the interaction between p53 and notch, Wnt, and other signaling pathways. Further clarification of the dynamic shifts in the interplay between the inflammatory response and ROS and p53 pathways at various phases is also necessary in order to create more specialized treatment plans [52].

Proteomics, metabonomics, and single cell sequencing are examples of multi-omics technologies that will be used extensively in the future to thoroughly examine the intricate mechanism of NPC aging. Multi-omics technology allows us to uncover new possible treatment targets as well as the overall regulatory networks and relationships between various signaling pathways. Simultaneously, the key development direction of research in this field is to strengthen the transformation of basic research and clinical application, apply basic research results to clinical practice more quickly, develop more effective methods for treating intervertebral disc degeneration, and improve therapeutic effects for patients.

In this review, we delineated a multi-axial framework encompassing mechanical, immune, and metabolic contributions to NPC senescence in IVDD. Rather than treating each axis in isolation, our model emphasizes their interdependence: mechanical overload activates PIEZO1, which triggers inflammatory and oxidative cascades, macrophage polarization sustains a chronic inflammatory environment that disrupts mitochondrial dynamics, and metabolic stress, particularly excessive ROS and mitophagy, drives p53-dependent senescence.

3 | Conclusion

In IVDD, the core mechanism of cellular senescence in NPCs involves multifaceted regulation: mechanical stimuli activate NLRP3 inflammatory vesicles and the NF-κB/p38MAPK pathway via PIEZO1 channel-mediated Ca^{2+} in-flow, triggering inflammatory cascade responses and oxidative stress that disrupts endoplasmic reticulum homeostasis. After polarization of M1-type macrophages, their secreted sEVs carrying LCN2 protein activate NF-κB signaling, which promotes myelofibrosis and abnormal vascular nerve invasion, forming a pro-senescence microenvironment. Mitochondrial dysfunction (e.g., abnormal mitophagy due to over-activation of PINK1/Parkin, dysregulation of SIRT1) synergistically interacts with ROS accumulation, inducing cell cycle arrest through activation of the p53–p21 pathway, while aberrant autophagy exacerbates the metabolic imbalance, which ultimately drives senescence in NPCs. In addition, ROS and inflammatory factors further amplify the p53-mediated senescence effect through a positive feedback loop, accelerating disc degeneration. In the future, we need to integrate single-cell transcriptomic, spatial proteomic, and metabolomic technologies to systematically analyze the aging-related signaling network (e.g., mechano-inflammatory-metabolic axis) of NPCs, and to explore the key targets, such as PIEZO1, LCN2, or SIRT1. Meanwhile, we will validate the targeted intervention strategies by combining organoid models and gene editing technologies, and promote the clinical translation of regenerative therapies based on aging regulation (e.g., small-molecule inhibitors, engineered modification of sEVs, or mitochondrial transplantation), so as to provide a new direction of precision therapy for reversing IVDD.

Author Contributions

Yunbo Yang: data analysis, writing – original draft. **Haoming Li:** images analysis. **Fei Lei:** funding acquisition, conceptualization, methodology. All authors participated in this article.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting the findings of this study are available from the respective authors upon reasonable request.

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