The Nrf2 antioxidant defense system in intervertebral disc degeneration: Molecular insights

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Intervertebral disc degeneration (IDD) is a common degenerative musculoskeletal disorder and is recognized as a major contributor to discogenic lower back pain. However, the molecular mechanisms underlying IDD remain unclear, and therapeutic strategies for IDD are currently limited. Oxidative stress plays pivotal roles in the pathogenesis and progression of many age-related diseases in humans, including IDD. Nuclear factor E2-related factor 2 (Nrf2) is a master antioxidant transcription factor that protects cells against oxidative stress damage. Nrf2 is negatively modulated by Kelch-like ECH-associated protein 1 (Keap1) and exerts important effects on IDD progression. Accumulating evidence has revealed that Nrf2 can facilitate the transcription of downstream antioxidant genes in disc cells by binding to antioxidant response elements (AREs) in promoter regions, including heme oxygenase-1 (HO-1), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and NADPH quinone dehydrogenase 1 (NQO1). The Nrf2 antioxidant defense system regulates cell apoptosis, senescence, extracellular matrix (ECM) metabolism, the inflammatory response of the nucleus pulposus (NP), and calcification of the cartilaginous endplates (EP) in IDD. In this review, we aim to discuss the current knowledge on the roles of Nrf2 in IDD systematically.

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INTRODUCTION

Lower back pain (LBP) has become a profoundly debilitating and increasingly prevalent disorder, causing a heavy socioeconomic burden worldwide. The leading cause of LBP is intervertebral disc degeneration (IDD). However, the pathogenesis of IDD is associated with multiple complex factors, including genetic, epigenetic, and environmental factors, and the knowledge about the molecular mechanisms underlying IDD remains elusive. The clinical treatments for IDD are limited to surgery, pharmacological or other nonpharmacological interventions to relieve the symptoms, and more effective therapeutic strategies to address the underlying pathology are needed for this degenerative spine disorder. Therefore, a better understanding of the molecular signaling involved in IDD has been a research focus, which may help to develop novel therapeutic targets for the successful treatment of IDD.

Redox homeostasis is crucial for the physiological maintenance of many cellular processes, and dysregulation of redox homeostasis is closely associated with various pathological conditions affecting human health. Oxidative stress is described as the disruption of redox homeostasis, which occurs when the balance between reactive oxygen species (ROS) production and the scavenging activity of the antioxidant system becomes disrupted. Excessive accumulation of ROS induces oxidative stress, which can cause damage to biological macromolecules such as carbohydrates, lipids, nucleic acids, and proteins, impairing the regular functional integrity of cells in the body. Accumulating evidence has revealed the roles played by oxidative stress in the pathogenesis of various human diseases, especially age-related disorders such as degenerative musculoskeletal diseases.

Degenerated disks exhibit oxidative stress as well as increased oxidation product levels, contributing to the development of IDD. Importantly, mounting evidence has revealed that therapies targeting oxidative stress might effectively alleviate or prevent IDD progression.

Nuclear factor E2-related factor 2 (Nrf2) is a master endogenous antioxidant transcription factor that has been increasingly reported to play crucial roles in protecting cells against oxidative stress. Physiologically, Nrf2 is critical for the expression of antioxidative genes, cytoprotective enzymes, and export transporters, which constitute an antioxidant defense system that maintains intracellular redox homeostasis. The activation of Nrf2 signaling is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1), which functions as a redox sensor for ROS and electrophiles. Under resting conditions, the activity of Nrf2 is tightly controlled by Keap1, which mediates ubiquitination-dependent proteasomal degradation of Nrf2 in the cytoplasm. In the presence of oxidative stress, Keap1 undergoes a conformational change and releases Nrf2, which moves to the nucleus, resulting in the initiation of the transcription of multiple antioxidant genes. Nrf2 signaling is considered a central hub that modulates redox homeostasis in cells, and aberrant Keap1-Nrf2 signaling is functionally involved in the pathology of many diseases. Interestingly, increasing evidence has revealed the crucial roles played by the Nrf2 signaling pathway in protecting against IDD progression. To our knowledge, no systematic
review has yet summarized the involvement of Nrf2 in disc degeneration diseases. Therefore, in this review, we synthesize and evaluate the results from the available literature and comprehensively discuss the roles of the Nrf2 antioxidant defense system in IDD.

PATHOPHYSIOLOGY OF IDD AND OXIDATIVE STRESS

Situated between the vertebral bones, each intervertebral disc (IVD) is made of fibro-cartilaginous tissues and is one of the most important structures of the spine. The IVD can distribute the axial compressive load transmitted from the vertebral bodies and enables physiological lateral and rotational flexibility of the spine.30 Anatomically, the disc consists of three major parts: the hydrated gel-like nucleus pulposus (NP) in the center, elastic annulus fibrosus (AF) surrounding the NP, and cartilaginous endplates (EP) on the inferior and superior sides.31 Oxidative stress, compressive overload, nutrient stress, enhanced inﬂammation, and other factors can act on these parts and stimulate aberrant cellular responses and progressive structural deﬁciency, leading to disc degeneration.32 IDD is characterized by a loss of centrally situated NP cells, which are replaced with cells with a ﬁbroblast-like phenotype.33 Another typical pathological change in disc degeneration is accelerated extracellular matrix (ECM) degradation, such as decreased deposition of type II collagen (Col II) and aggrecan, which is caused by imbalanced anabolism and catabolism.34 Additionally, cellular senescence and programmed cell death induced by inﬂammatory responses or other factors in the disc signiﬁcantly contribute to the pathological changes during the complicated process of IDD.9

Oxidative stress is a critical mediator in the initiation and progression of IDD. Oxidative stress occurs when the balance between ROS production and scavenging activity of the antioxidant defense system is disrupted.11 Excessive ROS accumulation can induce oxidative stress, which causes damage to the integrity and regular function of cells.35 Accumulating evidence has suggested that oxidative stress exerts signiﬁcant effects on cell fate and function and is closely related to cell viability, senescence, programmed cell death, matrix metabolism, and signaling network transduction of disc cells within the IVD.27.36–38

Previous studies have reported that aged and degenerated disks exhibit decreased antioxidant activity and elevated concentrations of oxidation products during IDD development.16,17 Excessive ROS accumulation and dysfunction of the antioxidant defense system induce cell apoptosis and senescence and trigger inﬂammatory responses in disc NP cells, accelerating IDD progression (Fig. 1). Redox homeostasis in the disc also plays a crucial role in the ECM anabolism and catabolism balance, and oxidative stress has been found to promote ECM degradation by interacting with various important signaling pathways in NP cells, including NF-κB signaling, p38/MAPK signaling, and the Nrf2/ARE signaling pathway.7,40,41 Moreover, the annulus ﬁbrosus is a crucial part of the IVD, and oxidative stress is involved in the cell senescence, apoptosis, and ferroptosis of disc AF cells in the pathogenesis of IDD.37,42–44 Disc EP degeneration is another critical contributor to IDD initiation because it hinders the nutrient supply to the NP and leads to disrupted disc homeostasis. It has been demonstrated that oxidative stress can induce autophagy, apoptosis, and calcification of endplate chondrocytes to modulate the EP degeneration process.38,45–47 Therefore, elucidating the key molecular mechanisms of oxidative stress in the disc might lead to effective therapeutic strategies for IDD.

NRF2 MEDIATED ANTIOXIDANT DEFENSE IN IDD

Nuclear factor E2-related factor 2 (Nrf2), also known as nuclear factor erythroid 2-like 2 (NFE2L2), is a master antioxidant transcription factor encoded by the NFE2L2 gene in humans.18 The Nrf2 protein is composed of approximately 605 amino acid residues and possesses seven highly conserved domains, namely, Neh1 to Neh7. Speciﬁcally, the Neh2 domain in Nrf2 participates in binding with the Keap1 homodimer and the degradation of Nrf2.19 The Neh2 domain contains two conserved motifs, DLG and ETGE, with an intervening sequence possessing seven lysine residues that can be ubiquitinated. DLG and ETGE are both associated with the interaction between Nrf2 and Keap1 homodimer. Physiologically, the activation of Nrf2 is regulated by Keap1, which functions as a cysteine-rich oxidative stress sensor. Keap1 is a substrate adaptor protein for the Cullin3 (Cul3)-containing E3 ubiquitin (Ub) ligase complex and is a cytosolic protein that

![Fig. 1](image-url) The effects of oxidative stress on disc cells during IDD pathogenesis. Excessive ROS accumulation exerts important effects on the three major types of IVD cells. Oxidative stress induces autophagy, apoptosis, and calcification of EP chondrocytes, while autophagy can act as a protective response to oxidative damage. Oxidative stress promotes cell apoptosis, senescence, ECM degradation, and inflammation response of disc NP cells. Oxidative stress induces cellular senescence, apoptosis, and ferroptosis in disc AF cells.
negatively modulates Nrf2 activity. Structurally, the Keap1 peptide is composed of 624 amino acid residues and possesses five functional regions, namely, the N-terminal region (NTR), intervening region (IVR), Broad complex Tramtrack and Bric-a-Brac (BTB) domain, double glycine repeat (DGR) domain and C-terminal region (CTR). The BTB domain is associated with the formation of the Keap1 homodimer, and the DGR and CTR domains (collectively known as the DC region) are involved in the interaction of Keap1 with Nrf2. The ubiquitin-proteasome system (UPS) is responsible for protein quality control and degradation and plays key roles in the maintenance of intracellular protein homeostasis. Under unstressed conditions, Keap1 binds to the Cul3-containing E3 ubiquitin ligase complex, and two molecules of Keap1 form a homomeric dimer. The Keap1 complex binds to Nrf2 for ubiquitination and subsequent degradation of Nrf2 by the proteasome. Under conditions of oxidative stress, Keap1 undergoes a conformational change, which leads to blocked ubiquitination of Nrf2 and accumulation of newly synthesized Nrf2. Subsequently, free Nrf2 is translocated to the nucleus, where it forms a heterodimer with small Maf proteins. Then, Nrf2-Maf interacts with the ARE in the promoter regions of DNA to promote the transcription of multiple targeted antioxidant genes, including HO-1, GSH, SOD, CAT, and NQO1. Activating Nrf2 signaling protects against oxidative stress in disc cells to alleviate IDD.

**Fig. 2  Nrf2 mediates antioxidant defense in IDD.** In unstressed conditions, Keap1 binds to the Cul3-containing E3 ubiquitin ligase complex, and two molecules of Keap1 form a homomeric dimer. The Keap1 complex binds to Nrf2 for the ubiquitination and subsequent degradation of Nrf2 by the proteasome. Under conditions of oxidative stress, Keap1 undergoes a conformational change, which leads to blocked ubiquitination of Nrf2 and accumulation of newly synthesized Nrf2. Subsequently, free Nrf2 is translocated to the nucleus, where it forms a heterodimer with small Maf proteins. Then, Nrf2-Maf interacts with the ARE in the promoter regions of DNA to promote the transcription of multiple targeted antioxidant genes, including HO-1, GSH, SOD, CAT, and NQO1. Activating Nrf2 signaling protects against oxidative stress in disc cells to alleviate IDD.

Multiple antioxidants, including ulinastatin, dimethyl fumarate, and cyanidin-3-glucoside, have been reported to alleviate oxidative stress in disc NP cells by promoting the activity of the Nrf2-mediated HO-1 signaling pathway. Dimethyl fumarate has also been demonstrated to activate Nrf2 to promote the production of GSH in NP cells, which is one of the most important ROS scavengers. Another study reported that Nrf2/HO-1 signaling activated by moracin dramatically promoted the expression levels of SOD and CAT in NP cells induced by LPS challenge. Acacetin and wogonin were also reported to activate the Nrf2 pathway to upregulate the expression of important antioxidant proteins, including HO-1, SOD, and NQO1, to ameliorate IDD progression. Interestingly, it has been reported that activating autophagy promoted Nrf2 signaling to upregulate the expression of antioxidant proteins, including SOD1 and SOD2, and thus protect cartilage endplate stem cells against calcification and ECM degradation during IDD. In summary, activating Nrf2 signaling facilitated the transcription of downstream antioxidant genes, including HO-1, GSH, SOD, CAT, and NQO1, to defend against oxidative stress in disc cells. The molecular mechanism of the Nrf2 antioxidant pathway is indicated in Fig. 2.

**THERAPEUTIC POTENTIAL OF TARGETING NRF2 SIGNALING IN IDD TREATMENT**

**Targeting Nrf2 to alleviate apoptosis of NP cells**

The highly hydrated NP is the structural and functional center of a disc. Dysregulated NP cell apoptosis causes damage to the normal metabolism in the NP, which disrupts the normal structure and physiological function of the disc and is considered a key contributor to IDD pathogenesis. It has been
demonstrated that the apoptosis ratio of NP cells is more than 50% in human degenerative disks, and preventing or alleviating apoptosis of NP cells is a potential effective therapy to treat disc degeneration,12,62. Accumulating evidence has reported anti-apoptotic roles played by Nrf2 activation in various types of human cells.33–65. Unexpectedly, targeting Nrf2 signaling regulated the apoptosis of NP cells during IDD progression. Long noncoding RNAs (lncRNAs) constitute a common and diverse class of noncoding RNAs that do not encode proteins.66 In 2019, Cherif et al.82 reported that lncRNA ANPODRT activated Nrf2 signaling to inhibit oxidative stress and apoptosis in human NP cells. Mechanistically, the lncRNA ANPODRT facilitated Nrf2 accumulation and nuclear translocation to activate downstream target genes by disrupting the Keap1-Nrf2 interaction. Moreover, Nrf2 knockdown obliterated the antioxidative and antiapoptotic effects of the lncRNA ANPODRT, indicating that Nrf2 activation is required for the lncRNA ANPODRT to exert protective effects on NP cells. MicroRNAs (miRNAs) comprise another important and large class of short-chain noncoding RNAs that regulate downstream genes by targeting the 3’ untranslated region (3’UTR) post-transcriptionally. A study by Xu et al.67 revealed that a miRNA termed miR-141-3p, which was enriched in platelet-rich plasma (PRP)-derived exosomes, activated Keap1-Nrf2 signaling to reverse the cell apoptosis, pyroptosis, and inflammatory response of NP cells stimulated by H2O2. Mechanistically, miR-141-3p interacted with the 3’UTR of Keap1 mRNA to induce its degradation, thus leading to Nrf2 translocation to the nucleus. More recently, Hu et al.28 reported the critical roles played by the Nrf2 agonist tert-butylhydroquinone (TBHQ) in retarding NP cell apoptosis. The results showed that TBHQ rescued TBHQ-induced apoptosis and oxidative stress by promoting Nrf2 expression and translocation to the nucleus. Mechanistically, TBHQ resisted oxidative stress by inducing Nrf2 activity and increasing the Sirt3 expression level to maintain mitochondrial homeostasis and enhance mitochondrial autophagy. Furthermore, the authors validated the therapeutic function and mechanism of TBHQ in a rat tail disc degeneration model in vivo. Mitoquinone (MitoQ) is a known mitochondria-targeted antioxidant that has shown protective effects in various oxidative damage-related diseases.86 It has been suggested that MitoQ alleviates sustained mitochondrial dysfunction, oxidative stress, and apoptosis of NP cells by stimulating the AKT-ERK1/2-Nrf2 signaling pathway cascades by directly binding to the Keap1-Nrf2 complex. A previous work reported that kinsenoside activated the AKT-ERK1/2-Nrf2 signaling pathway in NP cells to attenuate IDD both in vitro and in vivo.87 It has been reported that polydatin rescued mitochondrial dysfunction, suppressed senescence, and preserved ECM homeostasis in nucleus pulposus cells to attenuate IDD progression by promoting Nrf2 activity.87 In summary, triggering Nrf2 activation to inhibit NP cell senescence is a potential therapeutic strategy for IDD.

Targeting Nrf2 to regulate ECM metabolism in NP cells

Physiologically, the ECM endows the IVD with elastic and weight-bearing properties, allowing it to absorb compression loads while maintaining flexibility in the spine.88 The ECM is mainly composed of proteoglycans (mainly aggrecan) and Col II in disc NP tissues.89 ECM metabolism is generally modulated by degradative enzymes, including matrix metalloproteinases (MMPs) and aggrecanases, and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs).90,91. Degenerative disks are biochemically characterized by an imbalanced ECM metabolism of NP cells, implicating attenuated anabolic activities and enhanced catabolic activities in the disc. In this process, excessive degradation of aggrecan and Col II leads to NP dehydration and resorption and a decline in the ability of the cells to resist mechanical loading, thus contributing to IDD progression.92,93.

A recent study reported that IncRNA NEAT1 overexpression accelerated the ECM degradation of NP cells, while the Nrf2 activator TBHQ partially reversed the effects of the IncRNA NEAT1 on ECM metabolism.11 These results suggested that the IncRNA NEAT1 ameliorated ECM degradation of NP cells by regulating Nrf2 signaling pathway activation. Dimethyl fumarate is a known agonist of Nrf2-responsive genes and has been applied in certain clinically degenerative diseases.94. It has been revealed that dimethyl fumarate helped maintain the ECM balance of human NP cells, mainly by regulating the Nrf2/HO-1 signaling pathway.35. As mentioned above, the anti-inflammatory acidic protein extracted from human urine, ulinastatin, also protected human NP cells from ECM degradation by activating the Nrf2/HO-1 signaling pathway and...
suppressing the NF-κB signaling pathway^{24}. Treatment with either curcumin or o-vanillin increased the proteoglycan and type II collagen content and inhibited MMP3 and MMP13 expression in human disc cells. Further experiments suggested that curcumin and o-vanillin promoted ECM synthesis in IVD, which was mediated by the Nrf2 and NF-κB pathways^{72}. Furthermore, it has been reported that some other important biologically active components also regulated ECM metabolism in NP cells by targeting Nrf2 signaling; these compounds included cardamonin^{95}, sinapic acid^{70}, cyanidin-3-glucoside^{72}, moracin^{58}, acacetin^{59}, wogonin^{60}, lycopenes^{78}, genistin^{89}, and polydatin^{87}. Altogether, these results revealed that targeting Nrf2 signaling to alleviate ECM degradation of NP cells is a potential therapeutic strategy for IDD.

Targeting Nrf2 to regulate the inflammatory response in NP cells

In the initiation and progression of IDD, inflammation is widely acknowledged as a major characteristic feature^{96,97}. Accumulating evidence has demonstrated that excessive inflammatory responses can significantly affect the normal function of NP cells and thus contribute to IDD development^{3,32}. Dysregulated expression of proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-17, and tumor necrosis factor (TNF)-α has been observed in degenerated disc NP tissues and has been involved in the inflammatory response during IDD^{79}. These proinflammatory cytokines also play critical roles in the pathophysiological processes of IDD, including NP cell apoptosis, senescence, ECM remodeling, neovascularization, and oxidative stress^{4,32,99}. Therefore, regulating the inflammatory microenvironment in NP cells is essential for IDD treatment.

Nrf2 is widely involved in the modulation of the inflammatory response in NP cells. As mentioned above, the study by Xu et al^{67} revealed that exosomal miR-141-3p activated Keap1-Nrf2 signaling to regulate the inflammatory response of NP cells stimulated by \( \text{H}_2\O_2 \). Mechanistically, miR-141-3p directly interacted with the 3'UTR of Keap1 mRNA, causing Keap1 degradation, resulting in Nrf2 translocation to the nucleus, and thus inhibiting proinflammatory cytokine (IL-1β, IL-18, TGF-β, and IL-6) production and secretion by NP cells. It has been reported that the anti-inflammatory acidic protein extracted from human urine, ulinatasin, also dramatically suppressed the expression levels of proinflammatory mediators in human NP cells, including IL-6, TNF-α, INOS, and COX-2, by activating the NF-κB signaling pathway and suppressing the NF-κB signaling pathway^{23}. Interestingly, studies have shown that cardamonin^{95}, sinapic acid^{70}, and plumbagin^{71} protected NP cells against inflammation by modulating Nrf2/NF-κB axis activation. The known agonist for the Nrf2-responsive gene dimethyl fumarate has been found to ameliorate NP cell inflammation by promoting the activity of the Nrf2/HO-1 signaling pathway in IDD^{55,56}. Moreover, evidence has suggested that some other biologically active components, including moracin^{58}, acacetin^{59}, and wogonin^{60}, regulated the inflammatory response in NP cells by regulating the Nrf2 signaling pathway. Collectively, these data revealed that targeting Nrf2 signaling to regulate the inflammatory response in NP cells may be a promising therapeutic strategy for IDD.

Targeting Nrf2 to alleviate degeneration and calcification of EP

The human IVD has large vascular channels passing through the cartilaginous endplates at birth. With increasing age, however, these vessels recede, leaving the disc with little direct vascular supply^{100}. The IVD becomes the largest avascular organ of the body in adulthood. The cartilaginous endplates that attach the disc to the adjacent vertebral bodies provide the major portal for the diffusion of nutrients into the disc inner tissues^{100,101}. Therefore, the integrity of the EP structure is of great significance to the maintenance of homeostasis in the IVD. Histology and pathology have revealed that cartilaginous endplate calcification is a major pathological characteristic of disc degeneration^{100,102}. The degeneration and calcification of EP hinder the transport of nutrients and metabolite clearance in IVD and thus impair the survival and functions of disc cells, which is considered a crucial initiating mechanism of IDD^{103}.

Recently, Kang et al^{29} revealed the critical roles of oxidative stress and Nrf2 signaling in the cartilaginous endplate homeostasis of IVD. The authors found that \( \text{H}_2\O_2 \) stimulated oxidative stress, mitochondrial dysfunction, and cell apoptosis of human endplate chondrocytes, which were enhanced by Nrf2 knockdown. Moreover, upregulation of Nrf2 expression by polydatin treatment significantly protected endplate chondrocytes against these detrimental \( \text{H}_2\O_2 \)-induced effects. The study also applied the puncture-induced rat IDD model to validate the beneficial effects of Nrf2 activation on EP and disc degeneration. Interestingly, another study reported that rapamycin, a lipophilic macrolide antibiotic isolated from the actinomycete Streptomyces hygroscopicus, activated autophagy-Nrf2 signaling to protect cartilage endplate stem cells against calcification and ECM degradation^{71}. Tumor necrosis factor-α (TNF-α) treatment induced oxidative stress, cell senescence and the osteogenic differentiation of cartilage endplate stem cells. Mechanistically, rapamycin-induced autophagy to upregulate antioxidant protein expression, scavenge ROS, alleviate cell senescence and promote the chondrogenic differentiation potential of cartilage endplate stem cells. Moreover, the function of rapamycin-activated autophagy in inhibiting TNF-α-induced EP degeneration was realized through the regulated expression and nuclear translocation of Nrf2. Hence, targeting Nrf2 signaling to alleviate degeneration and calcification of EP might be an effective therapeutic means of IDD intervention. A list of the functional mechanisms of Nrf2 activation and related signaling pathways in IDD treatment is presented in Table 1.

CONCLUSIONS AND PERSPECTIVES

Oxidative stress has been demonstrated to play pivotal roles in the initiation and progression of a plethora of age-related diseases in humans. IDD is one of the most prevalent degenerative musculoskeletal disorders, and its pathogenesis is closely associated with oxidative stress. Nrf2 is a master antioxidant transcription factor and protects cells against oxidative stress damage, similar to its role in disc cells. As mentioned above, certain noncoding RNAs, including IncRNAs and miRNAs, and important antioxidants, such as bioactive compounds and small molecules from natural products, can activate Nrf2 signaling to alleviate IDD progression. Activating Nrf2 helps maintain the structural and functional integrity of IVD by inhibiting cell apoptosis, senescence, inflammation response, and ECM degradation of NP cells and alleviating degeneration and calcification of EP (Fig. 3). Therefore, targeting the Nrf2 antioxidant defense system is an effective therapeutic strategy for IDD. Although pharmacological Nrf2 activators have proven the benefits of defending against oxidative stress to prevent IDD progression in vitro and in vivo models, further investigations are needed to discover the details of the underlying molecular mechanism. In addition, mitochondria are intimately related to oxidative stress, as they are the main sources of intracellular ROS. Whether and how Nrf2 signaling regulates mitochondrial quality control in IDD might be a difficult but interesting area to address in the future. In addition, crosstalk between Nrf2 and important signaling pathways or cellular protective mechanisms, such as autophagy, is evident. Therefore, there remains a need for further systematic studies to clarify the multiple connected and intertwined mechanisms involved in IDD.
Table 1. Functional mechanisms of Nrf2 activation and related signaling pathways in IDD treatment.

| Experimental models                  | Molecular agents | Signaling pathways | Functional mechanisms                                                                 | References |
|--------------------------------------|------------------|--------------------|---------------------------------------------------------------------------------------|------------|
| Human NP cells (in vitro)            | LncRNA ANPODRT   | Keap1/Nrf2         | Inhibit oxidative stress and cell apoptosis                                           | Ref. 27    |
| Mouse NP cells (in vitro)            | Exosomal miR-141-3p | Keap1/Nrf2         | Promote cell proliferation and viability, inhibit cell apoptosis, pyroptosis, and inflammation | Ref. 67    |
| Human NP cells (in vitro), rat caudal disc (ex vivo) | Mitoquinone | Keap1/Nrf2         | Inhibit oxidative stress, mitochondrial impairment, and cell apoptosis               | Ref. 69    |
| Human NP cells (in vitro)            | LncRNA NEAT1, tert-butylhydroquinone (TBHQ) | Nrf2/ARE | Inhibit ECM degradation                                                               | Ref. 41    |
| Human NP cells (in vitro), rat tail disc (in vivo) | Quercetin | Nrf2/NF-κB         | Inhibit cell senescence                                                               | Ref. 85    |
| Human NP cells (in vitro)            | Ulinastatin      | Nrf-2/HO-1/NF-κB   | Inhibit oxidative stress, inflammation, apoptosis, and ECM degradation               | Ref. 54    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Cardamomin | Nrf2/NF-κB         | Inhibit inflammation and ECM degradation                                              | Ref. 95    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Sinapic acid | Nrf2/NF-κB         | Inhibit apoptosis, inflammation, and ECM degradation                                  | Ref. 70    |
| Rat NP cells (in vitro)              | Plumbagin        | Nrf2/NF-κB         | Inhibit oxidative stress, inflammation, and apoptosis                                 | Ref. 71    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | TBHQ | Nrf2/Sirt3         | Inhibit oxidative stress and cell apoptosis, promote mitophagy                        | Ref. 28    |
| Human NP cells (in vitro)            | Dimethyl fumarate | Nrf2/HO-1          | Inhibit oxidative stress, inflammation, ER stress-associated apoptosis, and ECM degradation | Ref. 55    |
| Human NP cells (in vitro), mouse tail disc (in vivo) | Dimethyl fumarate | Nrf2/HO-1          | Inhibit oxidative stress and inflammation                                              | Ref. 56    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Luteoloside | Nrf2/HO-1          | Inhibit apoptosis and ECM degradation                                                  | Ref. 72    |
| Rabbit NP cells (in vitro)           | CDDO-ethyl amide | Nrf2/HO-1          | Inhibit oxidative stress and cell apoptosis                                           | Ref. 73    |
| Human NP cells (in vitro)            | Cyanidin-3-glucoside | Nrf2/HO-1          | Inhibit oxidative stress, apoptosis, and ECM degradation                               | Ref. 57    |
| Rat NP cells (in vitro)              | Moracin          | Nrf2/HO-1/NF-κB/ TGF-β | Inhibit oxidative stress, inflammation, and ECM degradation                          | Ref. 58    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Kinsenoside | AKT-ERK1/2-Nrf2    | Inhibit apoptosis, senescence, and mitochondrial impairment                            | Ref. 74    |
| Mouse NP cells (in vitro), mouse tail disc (in vivo) | Keap1 siRNA | Keap1/Nrf2/p62 | Promote autophagy, inhibit oxidative stress                                            | Ref. 104   |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Wogonin | Nrf2/ARE           | Inhibit inflammation and ECM degradation                                               | Ref. 60    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Acacetin | Nrf2              | Inhibit inflammation and ECM degradation                                               | Ref. 59    |
| Human NP cells (in vitro)            | Sulforaphane     | Nrf2              | Inhibit cell apoptosis and oxidative stress                                            | Ref. 105   |
| Human NP cells (in vitro)            | Lycopene         | Nrf2              | Inhibit cell apoptosis and ECM degradation                                              | Ref. 75    |
| Human NP cells (in vitro)            | Curcumin and o-Vanillin | Nrf2          | Inhibit cell senescence and ECM degradation                                             | Ref. 82    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Genistein | Nrf2              | Inhibit cell apoptosis and ECM degradation                                              | Ref. 76    |
| Rat NP cells (in vitro), rat tail disc (in vivo) | Polydatin | Nrf2              | Inhibit mitochondrial impairment, cell senescence, and ECM degradation                  | Ref. 87    |
| Human endplate chondrocytes (in vitro), rat tail disc (in vivo) | Polydatin | Nrf2              | Inhibit oxidative stress, mitochondrial impairment, and cell apoptosis                 | Ref. 45    |
| Mouse cartilage endplate stem cells (in vitro), mouse tail disc (in vivo) | Rapamycin | Keap1/Nrf2        | Inhibit calcification and ECM degradation                                              | Ref. 61    |
Fig. 3 Schematic diagram showing the major mechanisms by which Nrf2 is activated to prevent IDD. Noncoding RNAs, such as lncRNAs and miRNAs, and other antioxidants can activate Nrf2 to alleviate IDD progression by inhibiting cell apoptosis, senescence, inflammation response, and ECM degradation in NP cells and alleviating the degeneration and calcification of EP.

DATA AVAILABILITY
The data used to support this study were included in the article.

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AUTHOR CONTRIBUTIONS
Q.X. conceived and drafted the manuscript. Q.X., Y.Z., J.L., and S.J. collected the references, and Y.Z. and W.L. proofread and revised the manuscript. All authors approved the final manuscript. Q.X. and Y.Z. contributed equally.

COMPETING INTERESTS
The authors declare no competing interests.

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