

Role of Autophagy and Pyroptosis in Intervertebral Disc Degeneration

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Abstract: Intervertebral disc degeneration is a chronic degenerative disease caused by the interaction of genetic and environmental factors, mainly manifested as lower back pain. At present, the diagnosis of intervertebral disc degeneration mainly relies on imaging. However, early intervertebral disc degeneration is usually insidious, and there is currently a lack of relevant clinical biomarkers that can reliably reflect early disease progression. Pyroptosis is a regulatory form of cell death triggered by the activation of inflammatory bodies and caspase, which can induce the formation of plasma membrane pores and cell swelling or lysis. Previous studies have shown that during the progression of intervertebral disc degeneration, sustained activation of inflammasomes leads to nuclear cell pyroptosis, which can occur in the early stages of intervertebral disc degeneration. Moreover, intervertebral disc nucleus pulposus cells adapt to the external environment through autophagy and maintain cellular homeostasis and studying the mechanism of autophagy in IDD and intervening in its pathological and physiological processes can provide new ideas for the clinical treatment of IDD. This review analyzes the effects of pyroptosis and autophagy on IDD by reviewing relevant literature in recent years, in order to explore the relationship between pyroptosis, autophagy and IDD.

Keywords: intervertebral disc degeneration, pyroptosis, autophagy

Introduction

Intervertebral disc degeneration (IVDD) is a main factor contributing to the chronic lower back pain (LBP). During IVDD, aberrant apoptosis, senescence and pyroptosis of disc cells, degradation of extracellular matrix (ECM), and infiltration of immune cells are the main molecular variants. Changes at the tissue level usually do not occur until the late stages of IVDD. Ectopic growth of nerves within the annulus fibrosus (AF) and nucleus pulposus (NP) tissues is considered as the main cause of IVDD.

Overview of IVDD

Disc degeneration is a major risk factor for low back pain. IVDD usually has no obvious symptoms.¹ However, as the disease progresses, there will be disc herniation, lumbar spondylolisthesis and even spinal stenosis, which makes IVDD and lead to chronic disability.² In addition, IVDD can present with neurological symptoms, including neuralgia, numbness, muscle weakness, and even paralysis.³ To date, most IVDDs have been treated with conservative treatments, such as bed rest, nonsteroidal anti-inflammatory drugs and analgesics, and surgical interventions such as lumbar discectomy and interbody fusion.^{4,5} These approaches focus on temporary relief of symptoms rather than targeting pathogenesis, so the progression of IVDD cannot be reversed. Hence, developing biotherapeutics is critical for the early recovery of IVDD.

Physiological Structure of IVDD

The intervertebral disc consists of the NP (nucleus pulposus) and the peripheral AF (annulus fibrosus) act as a buffer against pressure.^{6,7} NP is a highly hydrated gelatinous tissue composed of water, proteoglycans, and collagen, and it also contains large amounts of elastin, fibrin and laminin.⁸ Located around the nucleus pulposus, the annulus fibrosus is a circular structure composed of fibrous tissue rich in type I collagen.⁹ The CEP (cartilaginous endplate) consists of a layer of hyaline cartilage, which plays an important role in nutrient diffusion and metabolic waste expulsion of the intervertebral disc.⁹ (Figure 1).

Pathophysiology of IVDD

The nutrients required for NP (including oxygen, glucose, matrix generated substrates, amino acids and sulfate) are the roots in the permeation of vertebral capillaries through the CEP. The energy metabolism of NP is mainly performed through glycolysis, so glucose is necessary for the survival of the disc cells.¹⁰ However, in response to smoking, decreased blood supply, subchondral osteosclerosis, nutrients are reduced in NP, manifested by high lactate, low oxygen levels, and PH value indicated acidic. On account of the low natural cell density of NP and AF, it is believed that an enough nutrient supply is needful to maintain cellular activities. Cells in the intervertebral disc function gradually changes at high lactate levels, low oxygen and acidic pH, resulting in excessive apoptosis.¹¹ The oxygen pressure subsequently decreases below 5%, which will greatly inhibit matrix synthesis.¹² Meanwhile, under acidic pH environment, the matrix synthesis was suppressed and the matrix fracture rate was enhanced.

Intervertebral discs can undergo aging and degenerative degeneration over lifetime of a person.¹³ IVDD occurs as early as age 11, and develops with age.^{14,15} Slight microscopic degenerative changes, including aging and proliferation of NP cells, slight crack formation, changes in cell density, and degeneration of the CEP matrix were observed by 2 years. Latterly, the CEP undergoes cracking and thinning eventually leading to disc herniation and even spinal canal stenosis.¹⁶ Meanwhile, different genetic or environmental factor can damage NP, AF, or EP, leading to the development of IVDD.¹⁷ IVDD is characterized by abnormal extracellular matrix (ECM) metabolism, expedited cartilage and bone remodeling, shaped tissue fibrosis, resulting in the release of proinflammatory cytokines.¹⁸ Smoking, accidental trauma, tissue infection, genetic factors, and metabolism related diseases also further accelerate IVDD^{19–25} (Figure 2).

Autophagy Biology

In all cells, there are usually two ways to regulate energy supply. One approach is to obtain extracellular nutrients regulated by growth factor signaling pathways.²⁶ Extracellular hormones stimulate this system to fortify nutrient uptake, thereby offering the cell with oxidizable substrates to support adenosine triphosphate production and biosynthesis via

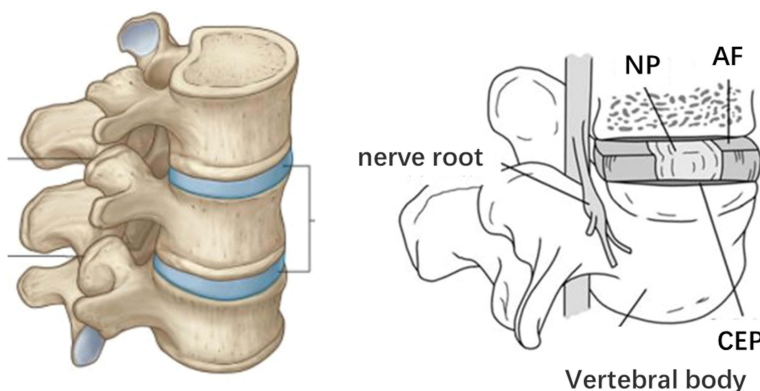


Figure 1 The Physiological structure of IVDD. The cell density of gel shaped nucleus pulposus tissue is low, and the extracellular matrix mainly includes type II collagen and proteoglycan. Protein polysaccharides (mainly aggregated proteoglycans and hyaluronic acid) can maintain intervertebral disc moisture, maintain disc height, and buffer axial loads on the spine. The fibrous ring is the fibrous tissue surrounding the nucleus pulposus, consisting of two layers: 1) the inner fibrous ring, and 2) the outer fibrous ring. The outer fibrous ring plays an important role in maintaining the integrity of intervertebral discs. The cartilage endplate contains abundant type II collagen and chondrocytes, which attach the intervertebral disc to the vertebral body and provide nutrients for the intervertebral disc. Blood vessels and nerves are only distributed in the outer fibrous ring of the intervertebral disc in healthy adults. Nutrients passively diffuse to the deep nucleus pulposus. The nerves in the scar in the degenerative intervertebral disc are the main cause of discogenic pain.

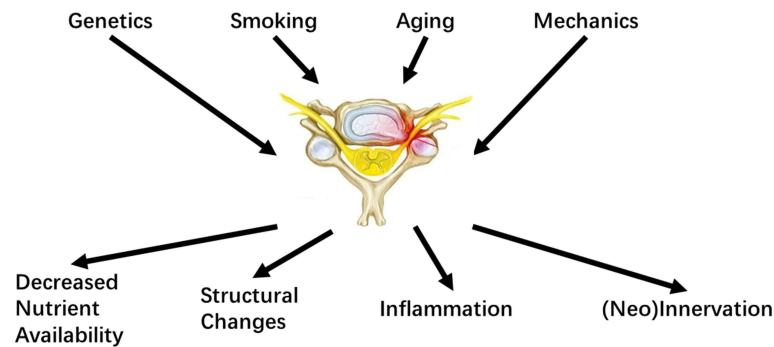


Figure 2 Risk factors for IVDD. Smoking, aging, genetics and mechanics related diseases also further accelerate IVDD.

anabolism. In the presence of attenuated growth factor signaling, autophagy at this stage can provide amino acids required for static cellular biosynthesis as well as cytoplasmic ATP.

Maintaining the integrity of organelles can help the cells adapt to various environmental changes.²⁷ Autophagy can remove dysfunctional and excess organelles including peroxisomes, mitochondria, nucleus, lysosomes, and ribosomes to maintain cell survival. Autophagosomes can provide nutrients to the cell during the removal of these organelles. Lipid degradation in autophagosomes/lysosomes supplies fatty acids for mitochondrion oxidation, which generates acetyl-CoA. Amino acids are substrates not only for the synthesis of new proteins, but also for mitochondrial oxidation in the tricarboxylic acid (TCA) cycle. Alanine and glutamate can generate ATP through the TCA to provide energy for the cell.

Normally, autophagy is related to apoptosis as well as the mechanism of cell death.^{28,29} There is a complex cross-regulation between autophagy and apoptosis, involving a variety of common regulatory factors and signal transduction pathways.³⁰ The initiation of autophagy protects cells from apoptosis in IVDD, highlighting its major cytoprotective role.³¹ With no other types of cell death, independent autophagy induced cell death, called autophagy cell death.³² Apoptosis was most commonly observed at some time after autophagy was upregulated, in this case, autophagy death may play a role in protecting and restoring tissue balance by clearing out cells with irreversible damage.

Expression and Mechanism of Autophagy in IVDD

Autophagy is regulated by many factors, including the serine/threonine protein kinase ULK1 complex, beclin1 complex, positive regulation of AMPK and PINK1/Parkin, and negative regulation of mTOR targets. Genes involved in autophagy (Beclin-1, Atg8, Atg12, Cathepsin B, Presenilin 1, and p62) were prominently upregulated in degenerative disc tissue compared with healthy disc tissue.³³ Meanwhile, more autophagic vacuoles and upregulation of LC3-II as well as lysosomal associated membrane glycoprotein 2 (LAMP-2A) were also found in disc tissues with the age of rats.³⁴ In addition, numerous studies have found increased expression and phosphorylation levels of mTOR, p70/S6K, and Akt.³⁵

mTOR Signaling

Recently, in a review of IVDD, Yurube et al described the mechanism by which the mTOR pathway regulates autophagy.³ mTOR is a member of the phosphatidylinositol 3-kinase family. In mammals, m can form two complexes with other proteins, mTORC1 or mTORC2. mTORC1 is mainly a complex sensitive to rapamycin, and mTORC2 is vice versa. mTORC2 is involved in the assembly of mTORC2 complexes that are resistant to rapamycin. Ito et al verified that the interference with targeted RNA against mTORC1 and mTORC2 could obviously enhance the autophagy process in NP cells.³⁶ PI3Ks are lipid kinases that can convert phosphatidylinositol to 3,4,5-trisphosphate (PIP3). Afterwards, The combined interaction of PIP3 and Akt can fully activate Akt and promote the activation of mTORC1, leading to the inhibition of autophagy.³⁷ Moreover, morpholino M could activate autophagy in NP cells, which further reversed LPS-induced IL-1 β , TNF- α and IL-6 Morpholino M can reduce the activity of PI3K/Akt/mTOR by down-regulating the ratio of pPI3K/PI3K, p-Akt/Akt and p-mTOR/mTOR, which leads to the reduction of PI3K/Akt/ mTOR activity and induces autophagy in NP cells.³⁸

PINK1/Parkin Signaling

PINK1 encodes a 581 amino acid residues PINK1 protein. The mature PINK1 protein has kinase activity and is cleaved from its precursor protein through the cytoplasm to the mitochondria. PINK1 is located in mitochondria and interacts with Parkin to maintain the integrity of mitochondrial structure and function, which is the main regulatory pathway of mitophagy.³⁹ Down-regulation of PINK1 in NP cells could obviously inhibit mitochondrial phagocytosis and accelerate oxidative stress-induced apoptosis of NP cells.⁴⁰ Zhang et al found that salicylic acid can promote Parkin expression in vivo and in vitro, and upregulation of Parkin can promote mitophagy and further inhibit TNF- α caused apoptosis of nucleus pulposus cells and ROS production.⁴¹ In parallel, Parkin-mediated mitophagy was verified to be essential in the elimination of mitochondrial dysfunction and apoptosis in NP cells.⁴² The current study identified a total of 12 immunoglobulins involved in the regulation of autophagy process in age-related diseases.⁴³

AMPK Signaling

AMP-activated protein kinases are sensors that stabilize the body's energy and can link cellular metabolic stress to energy homeostasis by controlling several homeostatic mechanisms such as autophagy and protein degradation.⁴⁴ AMPK/mTOR signaling pathway can regulate autophagy activation. AMPK directly activates ULK1 via phosphorylation of Ser317 to promote autophagy.⁴⁵ Active mTORC1 prevents ULK1 activation and inhibits autophagy by phosphorylating a specific ULK1 site (Ser757) and disrupting the interaction between ULK1 and AMPK.⁴⁶ Activation of the AMPK/mTOR pathway in vitro is associated with intercellular Ca²⁺ levels and inhibits apoptosis of human notochord cells by inducing autophagy. Using Ca²⁺ inhibitors, AMPK/mTOR pathway activation induced reduced autophagy and p62/SQSTM1 deposition, leading to accelerated apoptosis of human notochord cells.⁴⁷ In a separate experiment, researchers verified that curcumin activates the AMPK/mTOR/ULK1 pathway and leads to autophagy activation and autophagic flux increase., removing TBHP - induced apoptosis, ECM degradation, and senescence.⁴⁸ Nevertheless, other scholars confirmed that activation of the AMPK/mTOR pathway can facilitate autophagy and accelerate apoptosis, and ECM breakdown in human NP cells.⁴⁹

Autophagy Plays a Protective Role in IVDD

Protective Role of Autophagy in IVDD

Autophagy plays a twofold role in IVDD, activation of autophagy is a protective effect for IVDD.⁵⁰ Autophagy can be rapidly activated in response to oxidative stress, starvation, inflammation, and hypoxia. After intervention with sirolimus (an mTORC1 inhibitor), the levels of P70/S6K kinases were distinctly decreased. However, Akt phosphorylation and LC3-II expression were upregulated, which promoted autophagy.²³ Melatonin synthesis in the pineal gland is mainly regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus. A positive correlation between serum melatonin concentration and IVDD in patients was demonstrated in clinical studies.⁵¹ Experiments have suggested that removal of the pineal gland in chickens will induce the disease of IVDD.⁵² Melatonin could increase sSirt1 expression and activity, promote autophagy in inner plate chondrocytes. The protective effects of melatonin on apoptosis and calcification of lamellar chondrocytes were abolished by autophagy inhibitor 3-methyladenine (3-MA).⁵³ In addition, activation of the autophagy pathway can attenuate oxidative stress-induced mitochondrial dysfunction and inhibit apoptosis and senescence in NP cells. In addition, it regulates the expression of type II collagen, proteoglycans and matrix metalloproteinases in the intervertebral disc to maintain a stable level of extracellular matrix.⁵⁴

Autophagy Promotes IVDD

Excessive activation of autophagy can promote excessive degradation and self-digestion of important cellular components.⁵⁵ Researches verified that activation of autophagy facilitated apoptosis and senility in NP cells. Applying 1 MPa of the autophagy inhibitor 3-MA to mouse NP cells significantly increased the death of NP cells compared with NC group, and the promotion death effect of 3-MA was clearly reduced.⁵⁶ This research suggests that appropriate autophagy is beneficial for the survival of NP cells, but when the stimulus accumulates to a certain limit, the significantly increased autophagic flow may accelerate the apoptosis of NP cells.

Basal cell autophagy may avoid or delay the progression of intervertebral disc herniation by reducing or inhibiting cell apoptosis, ECM degradation, osteogenic differentiation and inflammatory response. The increase of autophagy flow can promote the apoptosis and senescence of intervertebral disc cells, thereby accelerating the development of IVDD.⁵⁷

Scorch Death and IVDD Pyrogenic Cell Biology

Pyroptosis is an inflammatory programmed cell death mode found in macrophages in recent years that depends on the activation of caspase-1. In addition to causing a large number of immune cells to decrease, pyroptosis can also trigger excessive inflammatory response of the body, cause tissue and organ damage and even lead to death.^{58,59}

Study of Focal Death in IVDD

Mechanism of Pyroptosis in the Progression of IVDD

The inflammatory response is mediated by the formation of the inflammatory body complex, which is a cell membrane heptamer consisted of nucleotide binding domains and leucine-rich repeat (NLR) pattern recognition receptors. NLR or NLR3 is a redox-sensitive cell membrane sensor that causes docking and activation of procaspase-1, which cleaves proIL-1 β into its mature form IL-1 β at 17kDa. Latest research have speculated that both chronic inflammation and innervation are key factors in disc degeneration.⁶⁰ In contrast to apoptosis, focal death during intervertebral disc degeneration has not been well studied.

In general, exogenous deleterious stimuli activate innate immune-dependent PRRS, which in turn activate various inflammatory bodies, including NLRP3, producing caspase-1 and gasdermin D (GSDMD), which ultimately mediate programmed death.⁶¹ Bai et al verified that hydrogen peroxide could increase the ROS level in human NP cells leading to increased pyroptosis. Compared with the control group, the expressions of NLRP3, cleaved IL-1, and PYCARD in the hydrogen peroxide treatment group reached the maximum at 3 h.⁶² Transfection of NLRP3-shrna and PYCARD-shRN reduced the thermal damage of NP cells. Zhang et al showed that pyroptosis in NP cells is mediated by LPS-induced upregulation of NLRP3, caspase-1, and GSDMD.⁶³ The expression of NLRP3, caspase-1 and GSDMD was down-regulated after the intervention of MSCs-derived exosomes, and miR-410 could significantly down-regulate their expression, thereby inhibiting pyroptosis. We also found that *P. acnes* activated NLRP3 inflammasome through TXNIP-NLRP3 pathway, which promoted pyroptosis of NP cells and ultimately led to IVDD.⁶⁴

IL-1 β secretion is associated with pyroptosis and the NLRP3 inflammatory factor triggers the coking process.⁶⁵ In addition, pyroptosis was found to be related to proinflammatory processes of intervertebral disc degeneration mediated by *Propionibacterium acnes*. The expression of NLRP3, IL-1 β and GSDMD in NPC was up-regulated after co-culture with *Propionibacterium acnes*.⁶⁴

The canonical inflammasome activation pathway needs to include the initiating event of the binding of proinflammatory cytokines to their receptors, such as by triggering pattern recognition receptors (PRRS).^{66,67} In response to PAMPs or DAMPs, the inflammasome complex assembles leading to caspase 1 activation, IL-1 β cleavage and pyroptosis. In addition, ROS also control pyroptosis of NPCs via the NLRP3/PYCARD pathway and are negatively regulated by promoting autophagy and the transcription factor erythrocytic 2-like 2 (NFE2L2).⁶²

Pyroptosis Promotes IVDD

Focal death of resident intervertebral disc cells promotes the progression of IVDD.⁶⁴ Inflammatory cytokines or ROS accumulate in senescent or degenerated intervertebral disc cells, thereby activating the NLRP3 inflammasome and caspase-1. Activated caspase-1 in the case of cleavage of GSDMD and release of the GSDMD-N fragment leads to membrane pore formation and cell death.⁶⁸ Knockout of GSDMD can inhibit the inflammatory response, thereby protecting the organ damage caused by the stimulation.⁶⁹ It was also shown that delivery of NLRP3 inflammasome inhibitor to degenerated rat intervertebral discs can effectively delay the progression of IDD.⁷⁰ In addition, caspase-1 inhibitors reduced GSDMD expression and ameliorated disc degeneration in vivo.⁷¹

Strategies Targeting Autophagy and Pyroptosis in IVDD

Autophagy and Pyroptosis in IVDD

Some studies have shown that autophagy inhibits inflammasome activation and reduces the secretion of inflammatory cytokines.^{72,73} Previous researches have confirmed that activation of autophagy protects NP cells from stress-induced cell death, whereas inhibition of autophagy has the opposite effect.⁷⁴ Autophagy has been reported to be activated during ROS-induced pyroptosis of NP cells, with negative regulatory and self-protective effects.⁶² The effect of increased ROS levels on pyroptosis may vary depending on the difference in autophagy levels in the inability cell lines. A study assessing the relationship between oxidative stress and pyroptosis in NP cells showed that ROS induced pyroptosis in NP cells via the NLRP3/PYCARD inflammasome and formed a negative regulatory relationship with NFE2L2 by activating autophagy.

Currently, some studies have shown that autophagy can inhibit the activation of inflammasome, and indicate the protective effect of autophagy on pyroptosis.⁷⁵ Bai et al showed that autophagy was activated in the process of pyroptosis induced by ROS, and inhibition of autophagy could aggravate pyroptosis.⁶² Since autophagy enzymatically hydrolyses its contents, its activation could remove some pyrogen inducers or remove damaged organelles to re-establish cellular balance.⁷⁵⁻⁷⁷ Similarly, increased autophagy markers led to pyroptosis, which was associated with terminal activation of autophagy and lysosomal instability, while autophagy inhibitors reduced pyroptosis. Since autophagy is a dynamic activity at different stages, the relationship between autophagy and pyroptosis is still controversial.

In fact, activation of inflammasomes, including NLRP3, plays an important role in pyroptosis.⁷⁸ It has been shown that activation of autophagy down-regulates NLRP3 expression and cleaved caspase-1 production.^{79,80} Houtman et al verified colocalization between NLRP3 and LC3-labeled structures, suggesting a selective degradation mechanism of NLRP3.⁸¹ In addition, mTOR signaling pathway is involved in autophagy activation. Autophagy intervention may regulate NLRP3 activation by regulating mTOR and NLRP3 binding.⁷⁹ Perforation of the plasma membrane is a sign of pyroptosis. Plasma membrane perforation leads to the leakage of cell contents and the release of inflammatory cytokines.⁸² Lysosomes can act as a membrane repair mechanism under autophagy activation.^{83,84} With the activation of small GTP-enzymes, lysosomes fuse with the plasma membrane and release degradation products via extravasation pathways that may repair thermalized perforations and promote cell survival.⁸⁵

Impaired autophagy accelerates PS-induced pyroptosis of NP cells.⁸⁶ VX-765, an inhibitor delivering caspase-1, inhibits inflammatory body activation and pyrogenesis, hence improving the progression of intervertebral disc degeneration in vivo. These studies indicate the deleterious role of pyroptosis in intervertebral disc degeneration and the protective role of autophagy in pyroptosis of NP cells.

Treatment of IVDD with Traditional Chinese Medicine

The treatment of LIDH, especially the treatment of traditional Chinese medicine is very important and necessary. At present, although a large number of conservative treatments are available, LIDH patients are still well treated. In addition, there are certain national characteristics in the conservative treatment of LIDH in different countries, but it is still difficult to widely apply these methods. How to select a conservative treatment method with less side effects and low cost has become a common concern for doctors and patients with LIDH. Among the non-surgical treatment methods, some TCM therapies such as acupuncture, massage, and Chinese herbal medicine are especially favored by patients with LIDH. Chinese medicine has developed its own unique theory, diagnosis and treatment system in Asian countries, especially China, after thousands of years of development. In the past few decades, these TCM therapies have been increasingly used worldwide and are known for their important role in the prevention and treatment of various diseases including LIDH.⁸⁷

Under various adverse stimuli, NP cells enhance their ability to clear damaged tissues through autophagy, suggesting that autophagy plays a key role in protecting the survival of NP cells and delaying the course of IVDD. Studies have shown that traditional Chinese medicine YQHXZ can promote the absorption of ruptured lumbar disc herniation to a certain extent.⁸⁸ Dai Feng et al studied the protective effect of Yiqi Huoxue recipe on IVDD by promoting autophagy, which provided a reference for the clinical treatment of IVDD with Yiqi Huoxue recipe. Fu et al found that lumbar spine

instability (LSI) in mice caused histological changes of intervertebral disc degeneration, interrupted stroma metabolism, activated Wnt signaling in VD tissues, promoted apoptosis of IVD cells, sensory nerve invasion into the annulus fibrosus, and induced focal death.⁸⁹ Maltol can improve IVDD by inhibiting the PI3K/AKT/NF- κ B pathway and regulating NLRP3 inflammasome-mediated focal disc death.⁹⁰

IVDD is a complex pathological process, which is affected by nutritional factors, mechanical factors and body metabolism. The specific mechanisms in the disease process of IVDD have not been fully elucidated. Autophagy and pyroptosis play a key role in the occurrence and development of IVDD. In recent years, the experimental research on the mechanism of delaying IDD through the signaling pathway of traditional Chinese medicine has become more and more in-depth. Moreover, the balance of autophagy in intervertebral disc cells and the effect of autophagy on pyrocytosis will not only expand the knowledge of molecular pathogenesis of IVDD, but also provide new ideas for the treatment and prevention of intervertebral disc degeneration and the exploration of the therapeutic value of traditional Chinese medicine.

Conclusions

The traditional treatment methods of IDD can no longer meet the patient's expectations for prognosis and their needs for quality of life. In recent years, autophagy has attracted widespread attention and in-depth exploration as a research hotspot. At present, most studies are still in the in vitro trial stage, and their exact clinical effects still need to be verified. How to regulate the levels of pyrocytosis and autophagy of intervertebral disc cells to the optimal level to minimize cell apoptosis is a major challenge in clinical application. However, it can be foreseen that therapies targeting pyrocytosis and autophagy related pathways will provide more options for the clinical treatment of IDD.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

References

1. Boden SD, Davis DO, Dina TS, Patronas NJ, Wiesel SW. Abnormal magnetic-resonance scans of the lumbar spine in asymptomatic subjects. A prospective investigation. *J Bone Joint Surg Am.* 1990;72(3):403–408. doi:10.2106/00004623-199072030-00013
2. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol.* 2014;10(1):44–56. doi:10.1038/nrrheum.2013.160
3. Yurube T, Ito M, Kakiuchi Y, Kuroda R, Kakutani K. Autophagy and mTOR signaling during intervertebral disc aging and degeneration. *JOR Spine.* 2020;3(1):e1082. doi:10.1002/jsp2.1082
4. Wang P, Zuo G, Du SQ, et al. Meta-analysis of the therapeutic effect of acupuncture and chiropractic on cervical spondylosis radiculopathy: a systematic review and meta-analysis protocol. *Medicine.* 2020;99(5):e18851. doi:10.1097/MD.00000000000018851
5. Raj PP. Intervertebral disc: anatomy-physiology-pathophysiology-treatment. *Pain Pract.* 2008;8(1):18–44. doi:10.1111/j.1533-2500.2007.00171.x
6. Kos N, Gradisnik L, Velnar T. A brief review of the degenerative intervertebral disc disease. *Med Arch.* 2019;73(6):421–424. doi:10.5455/medarh.2019.73.421-424
7. Tong W, Lu Z, Qin L, et al. Cell therapy for the degenerating intervertebral disc. *Transl Res.* 2017;181:49–58. doi:10.1016/j.trsl.2016.11.008
8. Guerrero J, Hackel S, Croft AS, Hoppe S, Albers CE, Gantenbein B. The nucleus pulposus microenvironment in the intervertebral disc: the fountain of youth?. *Eur Cell Mater.* 2021;41:707–738. doi:10.22203/eCM.v041a46
9. Fiordalisi MF, Silva AJ, Barbosa M, Goncalves RM, Caldeira J. Intervertebral disc decellularisation: progress and challenges. *Eur Cell Mater.* 2021;42:196–219. doi:10.22203/eCM.v042a15

10. Desmoulin GT, Pradhan V, Milner TE. Mechanical aspects of intervertebral disc injury and implications on biomechanics. *Spine*. 2020;45(8):E457–E64. doi:10.1097/BRS.0000000000003291
11. Bowles RD, Setton LA. Biomaterials for intervertebral disc regeneration and repair. *Biomaterials*. 2017;129:54–67. doi:10.1016/j.biomaterials.2017.03.013
12. Novais EJ, Choi H, Madhu V, et al. Hypoxia and hypoxia-inducible factor-1alpha regulate endoplasmic reticulum stress in nucleus pulposus cells: implications of endoplasmic reticulum stress for extracellular matrix secretion. *Am J Pathol*. 2021;191(3):487–502. doi:10.1016/j.ajpath.2020.11.012
13. Zhang Y, Yang B, Wang J, et al. Cell senescence: a nonnegligible cell state under survival stress in pathology of intervertebral disc degeneration. *Oxid Med Cell Longev*. 2020;2020:9503562. doi:10.1155/2020/9503562
14. Novais EJ, Tran VA, Johnston SN, et al. Long-term treatment with senolytic drugs dasatinib and quercetin ameliorates age-dependent intervertebral disc degeneration in mice. *Nat Commun*. 2021;12(1):5213. doi:10.1038/s41467-021-25453-2
15. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo award in basic science. *Spine*. 2002;27(23):2631–2644. doi:10.1097/00007632-200212010-00002
16. Hadjipavlou AG, Tzermiadianos MN, Bogduk N, Zindrick MR. The pathophysiology of disc degeneration: a critical review. *J Bone Joint Surg Br*. 2008;90(10):1261–1270. doi:10.1302/0301-620X.90B10.20910
17. Francisco V, Pino J, Gonzalez-Gay MA, et al. A new immunometabolic perspective of intervertebral disc degeneration. *Nat Rev Rheumatol*. 2022;18(1):47–60. doi:10.1038/s41584-021-00713-z
18. Chao-Yang G, Peng C, Hai-Hong Z. Roles of NLRP3 inflammasome in intervertebral disc degeneration. *Osteoarthritis Cartilage*. 2021;29(6):793–801. doi:10.1016/j.joca.2021.02.204
19. Rao PJ, Maharaj M, Chau C, et al. Degenerate-disc infection study with contaminant control (DISC): a multicenter prospective case-control trial. *Spine J*. 2020;20(10):1544–1553. doi:10.1016/j.spinee.2020.03.013
20. Cannata F, Vadala G, Ambrosio L, et al. Intervertebral disc degeneration: a focus on obesity and type 2 diabetes. *Diabetes Metab Res Rev*. 2020;36(1):e3224. doi:10.1002/dmrr.3224
21. To D, Rezaei M, Murnaghan K, Cancelliere C. Risk factors for low back pain in active military personnel: a systematic review. *Chiropractic & Manual Therapies*. 2021;29(1):52. doi:10.1186/s12998-021-00409-x
22. Avin-Wittenberg T. Autophagy and its role in plant abiotic stress management. *Plant Cell Environ*. 2019;42(3):1045–1053. doi:10.1111/pce.13404
23. Kakiuchi Y, Yurube T, Kakutani K, et al. Pharmacological inhibition of mTORC1 but not mTORC2 protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction. *Osteoarthritis Cartilage*. 2019;27(6):965–976. doi:10.1016/j.joca.2019.01.009
24. Yurube T, Buchser WJ, Moon HJ, et al. Serum and nutrient deprivation increase autophagic flux in intervertebral disc annulus fibrosus cells: an in vitro experimental study. *Eur Spine J*. 2019;28(5):993–1004. doi:10.1007/s00586-019-05910-9
25. Zhang TW, Li ZF, Dong J, Jiang LB. The circadian rhythm in intervertebral disc degeneration: an autophagy connection. *Exp Mol Med*. 2020;52(1):31–40. doi:10.1038/s12276-019-0372-6
26. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell*. 2019;176(1–2):11–42. doi:10.1016/j.cell.2018.09.048
27. Li W, He P, Huang Y, et al. Selective autophagy of intracellular organelles: recent research advances. *Theranostics*. 2021;11(1):222–256. doi:10.7150/thno.49860
28. Zhao H, Yang Y, Si X, Liu H, Wang H. The role of pyroptosis and autophagy in ischemia reperfusion injury. *Biomolecules*. 2022;12(7):1010. doi:10.3390/biom12071010
29. Cong L, Bai Y, Guo Z. The crosstalk among autophagy, apoptosis, and pyroptosis in cardiovascular disease. *Front Cardiovasc Med*. 2022;9:997469. doi:10.3389/fcvm.2022.997469
30. Zhao H, Liu H, Yang Y, Wang H. The role of autophagy and pyroptosis in liver disorders. *Int J Mol Sci*. 2022;23(11):6208. doi:10.3390/ijms23116208
31. Luo J, Yang Y, Wang X, Chang X, Fu S. Role of pyroptosis in intervertebral disc degeneration and its therapeutic implications. *Biomolecules*. 2022;12(12):1804. doi:10.3390/biom12121804
32. Kritschil R, Scott M, Sowa G, Vo N. Role of autophagy in intervertebral disc degeneration. *J Cell Physiol*. 2022;237(2):1266–1284. doi:10.1002/jcp.30631
33. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN Jr. Autophagy in the degenerating human intervertebral disc: in vivo molecular and morphological evidence, and induction of autophagy in cultured annulus cells exposed to proinflammatory cytokines-implications for disc degeneration. *Spine*. 2015;40(11):773–782. doi:10.1097/BRS.0000000000000865
34. Ye W, Xu K, Huang D, et al. Age-related increases of macroautophagy and chaperone-mediated autophagy in rat nucleus pulposus. *Connect Tissue Res*. 2011;52(6):472–478. doi:10.3109/03008207.2011.564336
35. Chen HW, Zhou JW, Zhang GZ, Luo ZB, Li L, Kang XW. Emerging role and therapeutic implication of mTOR signalling in intervertebral disc degeneration. *Cell Prolif*. 2022;56(1):e13338. doi:10.1111/cpr.13338
36. Ito M, Yurube T, Kakutani K, et al. Selective interference of mTORC1/RAPTOR protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism with akt and autophagy induction. *Osteoarthritis Cartilage*. 2017;25(12):2134–2146. doi:10.1016/j.joca.2017.08.019
37. Barnes PJ. Mechanisms of development of multimorbidity in the elderly. *Eur Respir J*. 2015;45(3):790–806. doi:10.1183/09031936.00229714
38. Guo F, Zou Y, Zheng Y, Moracin M inhibits lipopolysaccharide-induced inflammatory responses in nucleus pulposus cells via regulating PI3K/Akt/mTOR phosphorylation. *Int Immunopharmacol*. 2018;58:80–86. doi:10.1016/j.intimp.2018.03.015
39. Gan ZY, Callegari S, Cobbold SA, et al. Activation mechanism of PINK1. *Nature*. 2022;602(7896):328–335. doi:10.1038/s41586-021-04340-2
40. Wang Y, Shen J, Chen Y, et al. PINK1 protects against oxidative stress induced senescence of human nucleus pulposus cells via regulating mitophagy. *Biochem Biophys Res Commun*. 2018;504(2):406–414. doi:10.1016/j.bbrc.2018.06.031
41. Zhang Z, Xu T, Chen J, et al. Parkin-mediated mitophagy as a potential therapeutic target for intervertebral disc degeneration. *Cell Death Dis*. 2018;9(10):980. doi:10.1038/s41419-018-1024-9
42. Kang L, Liu S, Li J, Tian Y, Xue Y, Liu X. Parkin and nrf2 prevent oxidative stress-induced apoptosis in intervertebral endplate chondrocytes via inducing mitophagy and anti-oxidant defenses. *Life Sci*. 2020;243:117244. doi:10.1016/j.lfs.2019.117244

43. Hansen M, Rubinsztein DC, Walker DW. Autophagy as a promoter of longevity: insights from model organisms. *Nat Rev Mol Cell Biol.* 2018;19(9):579–593. doi:10.1038/s41580-018-0033-y
44. Gong Y, Tang N, Liu P, et al. Newcastle disease virus degrades SIRT3 via PINK1-PRKN-dependent mitophagy to reprogram energy metabolism in infected cells. *Autophagy.* 2022;18(7):1503–1521. doi:10.1080/15548627.2021.1990515
45. Zhang S, Xie Y, Yan F, et al. Negative pressure wound therapy improves bone regeneration by promoting osteogenic differentiation via the AMPK-ULK1-autophagy axis. *Autophagy.* 2022;18(9):2229–2245. doi:10.1080/15548627.2021.2016231
46. Saikia R, Joseph J. AMPK: a key regulator of energy stress and calcium-induced autophagy. *J Mol Med.* 2021;99(11):1539–1551. doi:10.1007/s00109-021-02125-8
47. Qiao L, Yan S, Dou X, et al. Biogenic selenium nanoparticles alleviate intestinal epithelial barrier damage through regulating endoplasmic reticulum stress-mediated mitophagy. *Oxid Med Cell Longev.* 2022;2022:3982613. doi:10.1155/2022/3982613
48. Kang L, Xiang Q, Zhan S, et al. Restoration of autophagic flux rescues oxidative damage and mitochondrial dysfunction to protect against intervertebral disc degeneration. *Oxid Med Cell Longev.* 2019;2019:7810320. doi:10.1155/2019/7810320
49. Zhan S, Wang K, Xiang Q, et al. lncRNA HOTAIR upregulates autophagy to promote apoptosis and senescence of nucleus pulposus cells. *J Cell Physiol.* 2020;235(3):2195–2208. doi:10.1002/jcp.29129
50. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science.* 2004;306(5698):990–995. doi:10.1126/science.1099993
51. Turgut M, Yenisey C, Akyuz O, Ozsunar Y, Erkus M, Bicakci T. Correlation of serum trace elements and melatonin levels to radiological, biochemical, and histological assessment of degeneration in patients with intervertebral disc herniation. *Biol Trace Elem Res.* 2006;109(2):123–134. doi:10.1385/BTER:109:2:123
52. Turgut M, Basaloglu HK, Yenisey C, Ozsunar Y. Surgical pinealectomy accelerates intervertebral disc degeneration process in chicken. *Eur Spine J.* 2006;15(5):605–612. doi:10.1007/s00586-005-0972-9
53. Zhang Z, Lin J, Tian N, et al. Melatonin protects vertebral endplate chondrocytes against apoptosis and calcification via the Sirt1-autophagy pathway. *J Cell Mol Med.* 2019;23(1):177–193. doi:10.1111/jcmm.13903
54. Liu W, Jin S, Huang M, et al. Duhuo jisheng decoction suppresses matrix degradation and apoptosis in human nucleus pulposus cells and ameliorates disc degeneration in a rat model. *J Ethnopharmacol.* 2020;250:112494. doi:10.1016/j.jep.2019.112494
55. Schwartz LM. Autophagic cell death during development - ancient and mysterious. *Front Cell Dev Biol.* 2021;9:656370.
56. Ma KG, Shao ZW, Yang SH, et al. Autophagy is activated in compression-induced cell degeneration and is mediated by reactive oxygen species in nucleus pulposus cells exposed to compression. *Osteoarthritis Cartilage.* 2013;21(12):2030–2038. doi:10.1016/j.joca.2013.10.002
57. Gong CY, Zhang HH. Autophagy as a potential therapeutic target in intervertebral disc degeneration. *Life Sci.* 2021;273:119266. doi:10.1016/j.lfs.2021.119266
58. Hou J, Hsu JM, Hung MC. Molecular mechanisms and functions of pyroptosis in inflammation and antitumor immunity. *Mol Cell.* 2021;81(22):4579–4590. doi:10.1016/j.molcel.2021.09.003
59. Shi J, Gao W, Shao F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci.* 2017;42(4):245–254. doi:10.1016/j.tibs.2016.10.004
60. Jia J, Nie L, Liu Y. Butyrate alleviates inflammatory response and NF-kappaB activation in human degenerated intervertebral disc tissues. *Int Immunopharmacol.* 2020;78:106004. doi:10.1016/j.intimp.2019.106004
61. Frank D, Vince JE. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. *Cell Death Differ.* 2019;26(1):99–114. doi:10.1038/s41418-018-0212-6
62. Bai Z, Liu W, He D, et al. Protective effects of autophagy and NFE2L2 on reactive oxygen species-induced pyroptosis of human nucleus pulposus cells. *Aging (Albany NY).* 2020;12(8):7534–7548. doi:10.18632/aging.103109
63. Zhang J, Zhang J, Zhang Y, et al. Mesenchymal stem cells-derived exosomes ameliorate intervertebral disc degeneration through inhibiting pyroptosis. *J Cell Mol Med.* 2020;24(20):11742–11754. doi:10.1111/jcmm.15784
64. He D, Zhou M, Bai Z, Wen Y, Shen J, Hu Z. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell pyroptosis via NLRP3-dependent pathway. *Biochem Biophys Res Commun.* 2020;526(3):772–779. doi:10.1016/j.bbrc.2020.03.161
65. Kovacs SB, Miao EA. Gasdermins: effectors of pyroptosis. *Trends Cell Biol.* 2017;27(9):673–684. doi:10.1016/j.tcb.2017.05.005
66. Abboud A, Namas RA, Ramadan M, et al. Computational analysis supports an early, type 17 cell-associated divergence of blunt trauma survival and mortality. *Crit Care Med.* 2016;44(11):e1074–e81. doi:10.1097/CCM.0000000000001951
67. Cunha LD, Silva ALN, Ribeiro JM, et al. AIM2 engages active but unprocessed caspase-1 to induce noncanonical activation of the NLRP3 inflammasome. *Cell Rep.* 2017;20(4):794–805. doi:10.1016/j.celrep.2017.06.086
68. Sborgi L, Ruhl S, Mulvihill E, et al. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* 2016;35(16):1766–1778. doi:10.15252/embj.201694696
69. Jia Y, Cui R, Wang C, et al. Metformin protects against intestinal ischemia-reperfusion injury and cell pyroptosis via TXNIP-NLRP3-GSDMD pathway. *Redox Biol.* 2020;32:101534. doi:10.1016/j.redox.2020.101534
70. Zhang W, Li G, Luo R, et al. Cytosolic escape of mitochondrial DNA triggers cGAS-STING-NLRP3 axis-dependent nucleus pulposus cell pyroptosis. *Exp Mol Med.* 2022;54(2):129–142. doi:10.1038/s12276-022-00729-9
71. Xing H, Zhang Z, Mao Q, et al. Injectable exosome-functionalized extracellular matrix hydrogel for metabolism balance and pyroptosis regulation in intervertebral disc degeneration. *J Nanobiotechnology.* 2021;19(1):264. doi:10.1186/s12951-021-00991-5
72. Claude-Taupin A, Bissa B, Jia J, Gu Y, Deretic V. Role of autophagy in IL-1beta export and release from cells. *Semin Cell Dev Biol.* 2018;83:36–41. doi:10.1016/j.semcdb.2018.03.012
73. Takahama M, Akira S, Saitoh T. Autophagy limits activation of the inflammasomes. *Immunol Rev.* 2018;281(1):62–73. doi:10.1111/immr.12613
74. Li S, Hua W, Wang K, et al. Autophagy attenuates compression-induced apoptosis of human nucleus pulposus cells via MEK/ERK/NRF1/Atg7 signaling pathways during intervertebral disc degeneration. *Exp Cell Res.* 2018;370(1):87–97. doi:10.1016/j.yexcr.2018.06.012
75. Wang X, Li H, Li W, et al. The role of Caspase-1/GSDMD-mediated pyroptosis in taxol-induced cell death and a taxol-resistant phenotype in nasopharyngeal carcinoma regulated by autophagy. *Cell Biol Toxicol.* 2020;36(5):437–457. doi:10.1007/s10565-020-09514-8
76. Wang Y, Song X, Li Z, et al. MicroRNA-103 protects coronary artery endothelial cells against H2O2-induced oxidative stress via bnip3-mediated end-stage autophagy and antiapoptosis pathways. *Oxid Med Cell Longev.* 2020;2020:8351342. doi:10.1155/2020/8351342

77. Qiu T, Pei P, Yao X, et al. Taurine attenuates arsenic-induced pyroptosis and nonalcoholic steatohepatitis by inhibiting the autophagic-inflammasomal pathway. *Cell Death Dis.* 2018;9(10):946. doi:10.1038/s41419-018-1004-0
78. Chauhan D, Vande Walle L, Lamkanfi M. Therapeutic modulation of inflammasome pathways. *Immunol Rev.* 2020;297(1):123–138. doi:10.1111/imr.12908
79. Cosin-Roger J, Simmen S, Melhem H, et al. Hypoxia ameliorates intestinal inflammation through NLRP3/mTOR downregulation and autophagy activation. *Nat Commun.* 2017;8(1):98. doi:10.1038/s41467-017-00213-3
80. Han X, Sun S, Sun Y, et al. Small molecule-driven NLRP3 inflammation inhibition via interplay between ubiquitination and autophagy: implications for Parkinson disease. *Autophagy.* 2019;15(11):1860–1881. doi:10.1080/15548627.2019.1596481
81. Houtman J, Freitag K, Gimber N, Schmoranzer J, Heppner FL, Jendrach M. Beclin1-driven autophagy modulates the inflammatory response of microglia via NLRP3. *EMBO J.* 2019;38(4). doi:10.15252/embj.201899430
82. Liu X, Zhang Z, Ruan J, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature.* 2016;535(7610):153–158. doi:10.1038/nature18629
83. Michelet X, Tuli A, Gan H, et al. Lysosome-mediated plasma membrane repair is dependent on the small GTPase arl8b and determines cell death type in mycobacterium tuberculosis infection. *J Immunol.* 2018;200(9):3160–3169. doi:10.4049/jimmunol.1700829
84. Radulovic M, Schink KO, Wenzel EM, et al. ESCRT-mediated lysosome repair precedes lysophagy and promotes cell survival. *EMBO J.* 2018;37(21):doi:10.15252/embj.201899753
85. Tan JMJ, Mellouk N, Osborne SE, et al. An ATG16L1-dependent pathway promotes plasma membrane repair and limits *Listeria monocytogenes* cell-to-cell spread. *Nat Microbiol.* 2018;3(12):1472–1485. doi:10.1038/s41564-018-0293-5
86. Liao Z, Li S, Liu R, et al. Autophagic degradation of gasdermin D protects against nucleus pulposus cell pyroptosis and retards intervertebral disc degeneration in vivo. *Oxid Med Cell Longev.* 2021;2021:5584447. doi:10.1155/2021/5584447
87. Zhang B, Xu H, Wang J, Liu B, Sun G. A narrative review of non-operative treatment, especially traditional Chinese medicine therapy, for lumbar intervertebral disc herniation. *Biosci Trends.* 2017;11(4):406–417. doi:10.5582/bst.2017.01199
88. Dai F, Yu P, Yu Z, Jiang H, Ma Z, Liu J. Yiqi huoxue recipe delayed intervertebral disc degeneration by activating autophagy. *Front Pharmacol.* 2021;12:705747. doi:10.3389/fphar.2021.705747
89. Fu F, Bao R, Yao S, et al. Aberrant spinal mechanical loading stress triggers intervertebral disc degeneration by inducing pyroptosis and nerve ingrowth. *Sci Rep.* 2021;11(1):772. doi:10.1038/s41598-020-80756-6
90. Gong Y, Qiu J, Jiang T, et al. Maltol ameliorates intervertebral disc degeneration through inhibiting PI3K/AKT/NF- κ B pathway and regulating NLRP3 inflammasome-mediated pyroptosis. *Inflammopharmacology.* 2022; 31:369–84.

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