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REVIEW

Pyroptosis and Intervertebral Disc Degeneration: Mechanistic Insights and Therapeutic Implications

Yuying Ge[1,*], Yuying Chen[2,*], Chijiao Guo[1,*], Huan Luo[3], Fangda Fu[1,4], Weifeng Ji[1,4], Chengliang Wu[1,4], Hongfeng Ruan[1,4]

[1]The First Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, 310053, People's Republic of China; [2]The Fourth Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, People's Republic of China; [3]Department of Pharmacy, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, People's Republic of China; [4]Institute of Orthopaedics and Traumatology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Traditional Chinese Medicine), Hangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Chengliang Wu, Institute of Orthopaedics and Traumatology, The First Affiliated Hospital of Zhejiang Chinese Medical University, 548 Binwen Road, Hangzhou, 310053, People's Republic of China, Email wcl@zcmu.edu.cn; Hongfeng Ruan, Institute of Orthopaedics and Traumatology, The First Affiliated Hospital of Zhejiang Chinese Medical University, 548 Binwen Road, Hangzhou, 310053, People's Republic of China, Email rhf@zcmu.edu.cn

Abstract: Low back pain (LBP) is a common problem worldwide, resulting in great patient suffering and great challenges for the social health system. Intervertebral disc (IVD) degeneration (IVDD) is widely acknowledged as one of the key causes of LBP. Accumulating evidence suggests that aberrant pyroptosis of IVD cells is involved in the pathogenesis of IVDD progression, however, the comprehensive roles of pyroptosis in IVDD have not been fully established, leaving attempts to treat IVDD with anti-pyroptosis approaches questionable. In this review, we summarize the characteristics of pyroptosis and emphasize the effects of IVD cell pyroptosis on the pathological progression of IVDD, including secretion of cytokines, nucleus pulposus cell apoptosis and autophagy, accelerated extracellular matrix degradation, annulus fibrosus rupture, cartilage endplate calcification, vascularization, sensory and sympathetic fiber neoinnervation, and infiltrating lymphatic vessels. Finally, we discuss several interventions used to treat IVDD by targeting pyroptosis. This review provides novel insights into the crucial role of IVD cell pyroptosis in IVDD pathogenesis, and could be informative for developing novel therapeutic approaches for IVDD and LBP.

Keywords: low back pain, intervertebral disc degeneration, pyroptosis, inflammasome, anti-pyroptotic approach

Introduction

Low back pain (LBP) is a common symptom of the musculoskeletal system and a primary cause of disability.[1] Epidemiological studies have revealed that intervertebral disc (IVD) degeneration (IVDD) is clinically related to LBP and is one of the key causes of LBP.[2,3] Despite the enormous efforts of researchers made to investigate the etiology and pathogenesis of IVDD, the exact mechanisms underpinning IVDD remain obscure, which accounts for the limited number of effective approaches to prevent and treat this pathological condition. Therefore, it is essential to further elucidate the cellular and molecular mechanism of IVDD to facilitate the development of novel therapeutic strategies.

IVD is fibrocartilage tissue that connects the bodies of adjacent vertebrae, accounting for 25–30% of the total length (height) of the spine and maintaining its stability.[4] IVD consists of a peripheral annulus fibrosus (AF) surrounding a central gelatinous nucleus pulposus (NP) and a thin layer of cartilage endplates (CEP) at the upper and lower ends. AF consists mainly of tough and elastic annular fibrous cartilage arranged in concentric circles. In normal adults, NP is a highly hydrated gelatinous structure consisting of NP cells derived from the embryonic notochord as well as NP cell-produced components of extracellular matrix (ECM), such as collagen type II (Col2) and proteoglycan, which are acknowledged as key contributors for resistance to axial stress and stress in the spine.[5,6] Meanwhile, CEP is a layered

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composite consisting of semi-porous thickened cancellous bone and hyaline cartilage,[7] which can transmit the compressive loads containing and pressurizing NP, and transfer water, nutrients, and waste into and out of the IVD.[8]

The etiology of IVDD is multifactorial, including aging, mechanical loading, trauma, genetic susceptibility and other factors leading to vertebral instability, spinal canal stenosis, and spinal segment deformity, resulting in LBP and mobility disability.[9] IVDD is characterized by abnormalities in local physiological structure, such as progressive NP dehydration, ECM degradation or degeneration, AF rupture, CEP calcification, and inflammation.[10] Interestingly, inflammatory factors such as interleukin (IL)-1β and tumor necrosis factor -α (TNF-α) have been identified as key cytokine mediators that participate in the pathological changes of IVDD and LBP, which can amplify inflammatory reaction, stimulate ECM degradation, accelerate cell senescence, impair IVD cell proliferation, aggravate oxidative stress, promote angiogenesis and neoinnervation in the process of IVDD.[9] It has been shown that AF rupture in IVD provides a microenvironment for sensory innervation and angiogenesis,[11] allowing ingrowth of sensory neurons and blood vessels, further accelerating the development of discogenic pain.[12] In addition, emerging evidence suggests that once the IVD tissue is compressed into the surrounding soft tissue, reparative fibrous tissue containing lymphatic vessels is induced to grow inwards, although it is widely acknowledged that normal adult IVDs do not contain lymphatic vessels.[13]

Pyroptosis is a newly discovered inflammatory programmed cell death mediated by inflammasome, which differs from other types of cell death, such as apoptosis, necrosis, and autophagy. The remarkable features of pyroptosis include pore formation on the plasma membrane, cell swelling and rupture, and subsequent release of inflammatory cytokines (such as IL-1β and IL-18) and intracellular materials.[14] It has been established that aberrant activation of pyroptosis can result in a massive inflammatory response, leading to inappropriate repair of damaged tissue and organ, causing inflammatory diseases, including arthritis, sepsis, atherosclerosis, cancers, Parkinson and other diseases.[15–21] Growing evidence suggests that the inhibition of pyroptosis may be a potential therapeutic direction for these diseases.[22–25] Importantly, our latest findings also demonstrated a significant increase in the expression of pyroptosis-related proteins, such as nod-like receptor protein-3 (NLRP3), Caspase-1, and Gasdermin-D (GSDMD) in IVD cells of IVDD patients and murine IVDD model.[26–28] Meanwhile, other in vitro experiments corroborated that exogenous stimuli, including lipopolysaccharide (LPS) and hydrogen peroxide (H_2O_2), could induce inflammatory responses and degenerative phenotype of NP cells by activating NLRP3.[29,30] The above findings suggest that pyroptosis is a prominent regulatory process in IVDD. However, the comprehensive roles of pyroptosis in IVDD have not been fully established, which accounts for the lack of consensus on attempts to treat IVDD via an anti-pyroptosis approach.

In this review, we briefly introduce the characteristics of pyroptosis and provide a brief overview of the latest findings on aberrant pyroptosis activation of IVD cells during IVDD progression and emphasize the comprehensive roles of pyroptosis on various pathological phenotypes of IVDD, including NP cell apoptosis and autophagy, ECM degradation, AF rupture, CEP calcification, sensory innervation, lymphoid ingrowth, and cytokine secretion. Finally, currently available anti-pyroptotic strategies for IVDD are listed and analyzed.

Characterization of Pyroptosis

Pyroptosis is a newly discovered caspase-dependent cell death, the characteristics of which are different from the well-known apoptosis, autophagy, and ferroptosis (Table 1), mainly manifested as cell swelling until the cell membrane rupture, resulting in the inability to regulate the ingress and egress of cell contents and the activation of a strong inflammatory response.[14,19] Pyroptosis is mediated by the formation of inflammasome complexes, which are cytosolic heptameric oligomers composed of the nucleotide-binding domain and leucine-rich repeat (NLR) pattern recognition receptors. The best-characterized NLR, NLRP3, is a redox-sensitive cytosolic sensor that triggers the recruitment and activation of pro-Caspase-1, thus cleaving pro-IL-1β and pro-IL-18 into the mature active form of pro-inflammatory factors, IL-1β and IL-18. In addition, Caspase-1 activation results in the oligomerization of the N-terminal of GSDMD and pore formation in the plasma membrane, which allows mature IL-1β/IL-18 of a diameter of 4.5 nm and Caspase-1 of a diameter of 7.5 nm to pass through.[31] Meanwhile, water entering through the pores causes cell swelling and osmotic lysis, resulting in the rupture of the plasma membrane and release of IL-1β and IL-18 (Figure 1).[14,32–35] A more in-depth discussion of the mechanisms underlying pyroptosis is available in previous reviews.[36–38]

Table 1 Characterizations and Key Molecules Involving Four Forms of Cell Death

Type	Characterization	Molecules Mainly Involved	References
Pyroptosis	Rupture of cell membrane; pore formation in the plasma membrane; strong inflammatory response	NLRP3, Caspase-1, GSDMD, IL-1β, IL-18	[14,19]
Apoptosis	Breakdown of the nuclear membrane; membrane blebbing; breakdown of genomic DNA into nucleosomal structures; release of cytochrome c from mitochondria	Caspase-3, Caspase-6, Caspase-9, Bcl-2, Bax	[39]
Autophagy	Formation of autophagosome; fusion of autophagosomes and lysosomes; protein degradation	AMPK, ATG1, ULK1, PI3K, LC3, p62	[40–42]
Ferroptosis	Smaller mitochondria, diminished or vanished of mitochondria crista, and condensed mitochondrial membrane densities	TFR1, DMT1, FTL, FTH1, HSPB1, IREB2	[43]

Aberrant Pyroptosis of IVD Cells During IVDD Progression

Accumulating evidence suggests that pyroptosis of IVD cells is induced during IVDD progression.[44–46] A previous study based on IVD tissues from IVDD patients showed that the expression of inflammasome-related proteins, including NLRP3 and its downstream targets CASPASE-1 and IL-1β, were upregulated in degenerative IVDs, and their expressions were positively correlated with the degree of disc degeneration.[47] It has been shown that NP cells from grade

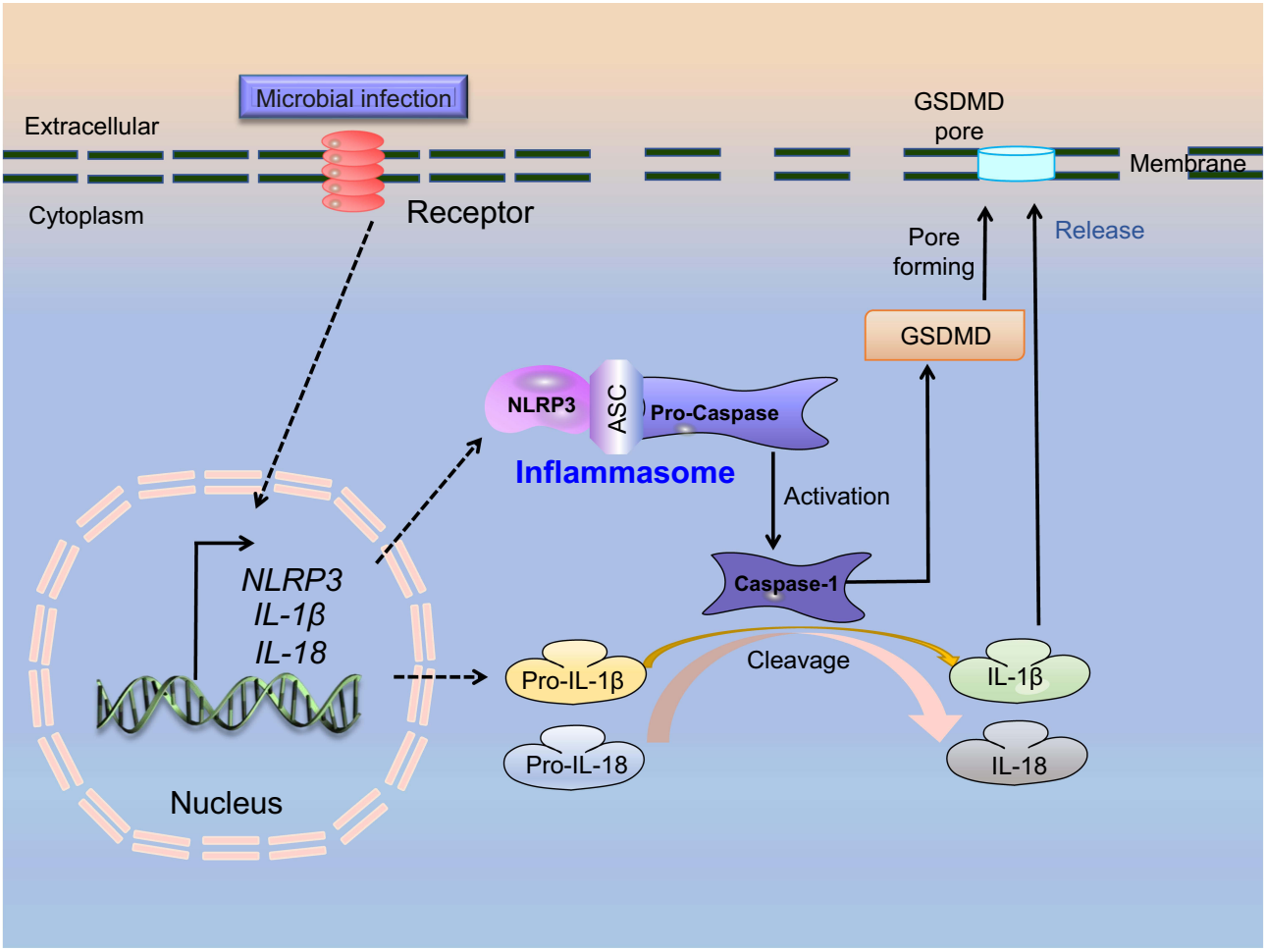

Figure 1 A schematic model depicting the activation process of cell pyroptosis.

V disc degeneration patients express a higher cleaved-Caspase-1, IL-1β, and IL-18 than grade IV patients, depending on the difference in the degree of disc degeneration defined by the Pfirrmann classification.[46] Current evidence suggests that the proportion of IL-1β-positive cells is particularly prominent in chondrocyte-like cells in inner AF tissue, and this immunopositivity increases with the severity of degeneration.[48] Beyond that, there is limited information that can be gleaned on cytological studies of the effects of pyroptosis on CEP. Increased expression of pyroptosis-related indicators, including NLRP3, Caspase-1 and IL-1β, has been documented in CEP tissues of IVDD patients and animal model.[27,49] Accumulating in vitro experiments show that treatment with H_2O_2 or LPS can increase ROS levels in human NP cells, which in turn augments NLRP3, cleaved IL-1, cleaved IL-18, and PYCARD expression, leading to activation of pyroptosis in NP cells.[26,46] These findings substantiate that IVD cells exhibit a pyroptosis phenotype during the pathogenesis of IVDD, suggesting pyroptosis may play an important role in disease progression.

Biological Roles of Pyroptosis of Different IVD Cells on the Pathological Phenotypes of IVDD

An increasing body of evidence suggests that pyroptosis of chondrocytes could promote apoptosis and autophagy in cartilages, production of inflammatory cytokines, and ECM metabolism homeostasis imbalance, resulting in osteoarthritis and other bone degenerative diseases.[20,50–54] Noteworthy, chondrocyte pyroptosis has been associated with the growth of blood vessels and nerves, triggering severe pain reactions.[48] Similar with chondrocyte cells, IVD cell pyroptosis has been demonstrated to play an important role in IVDD progression. Nonetheless, the relationship between pyroptosis of IVD cells and other pathological changes of IVDD progression is still unclear, which leads us to speculate that pyroptosis of different IVD cells can accelerate the IVDD process by aggravating other pathological changes in multiple IVD compartments.

Pyroptosis and Production of Cytokines in IVDD

Inflammation occurs in response to tissue injury and is a key factor in the degenerative process of IVD and LBP.[2] Overwhelming evidence substantiates that pyroptosis is involved in inflammatory responses during IVDD. It has been reported that Propionibacterium acnes induces NP cell pyroptosis activation via the NLRP3-dependent pathway, which accounts for inflammation in IVDD.[44] In an ex vivo bovine IVDD model, IL-1β exposure increased the expression of IL-6, IL-8, monocyte chemoattractant protein-1, and prostaglandin E_2 in IVDs.[55] Conversely, the binding of IL-6 with its soluble receptor promotes IL-1β-induced proteoglycan catabolism, suggesting a positive feedback loop involving IL-1β and IL-6 that could further amplify the IVD inflammatory response.[56] Another study showed that LPS treatment could dramatically enhance the expression of TNF-α, IL-6, and other inflammatory factors in NP cells by down-regulating *microRNA-200C-3p*.[57] IL-10 is a cytokine with anti-inflammatory properties that plays a central role during infection by limiting the immune response to pathogens and thereby preventing damage to the host.[58] The above literature substantiates that activation of pyroptosis in IVDD tissue induces inflammatory responses and aggravates disc degeneration by stimulating various cytokines, including IL-1β, IL-6, IL-8, IL-18, *etc*.

NP Cell Pyroptosis and NP Cell Apoptosis

Apoptosis is a form of programmed cell death distinguished from pyroptosis, whereby cells self-destruct and are involved in many biological events, including tissue homeostasis, removal of unwanted cells, and developmental sculpting.[59,60] It has been shown that increased NP cell apoptosis contributes to IVDD progression. In some bioprocesses, the activation of pyroptosis can trigger apoptosis, although the relationship between pyroptosis and apoptosis in NP cells remains obscure.[26,61] Pyroptosis is characterized by the release of inflammatory cytokines, while inflammatory inhibition reduces NP cell apoptosis, indicating that pyroptosis may be one of the main reasons for apoptosis in NP cells. Notably, IL-1β, an important downstream protein of pyroptosis, has been documented to induce apoptosis of NP cells by elevating pro-apoptotic proteins, including Caspase-3 and Bax, and reducing anti-apoptotic proteins.[62] A recent study showed that CY-09, an NLRP3 inflammasome-specific inhibitor, could reduce LPS-induced NP cell apoptosis in *vitro*, indicating that inhibition of NLRP3 inflammasome activation can protect against LPS-induced apoptosis of NP cells.[61]

NP Cell Pyroptosis and NP Cell Autophagy

Autophagy is an evolutionarily conserved intracellular recycling system that delivers cytoplasmic content to lysosomes for degradation, thus maintaining metabolism and homeostasis.[3] Growing evidence suggests that autophagy could limit the activation of inflammasomes and alleviate the secretion of inflammatory cytokines.[63,64] It is reasonable to assume that autophagy may regulate inflammasome activation and affect the outcome of cell pyroptosis. However, the relationship between NP cell pyroptosis and autophagy is complicated. Current evidence suggests that IVD cell autophagy can exert a protective effect when IVDs are subjected to oxidative stress, starvation, hypoxia, inflammation, and infection, triggering or exacerbating disc damage.[65] Meanwhile, it has been found that the expression of GSDMD and autophagy-related protein LC3 and ATG5 are increased in NP tissues of human IVDD patients and rat IVDD models.[66] Further analysis showed that treatment with autophagy inhibitor 3-MA resulted in increased expression of GSDMD, NLRP3, and Caspase-1 proteins, while autophagy inducer rapamycin significantly reversed these alterations.[46] The above studies substantiate a negative correlation between pyroptosis and autophagy in NP cells. Nonetheless, whether NP cell pyroptosis can influence autophagy warrants further investigation.

Pyroptosis and Matrix Degradation

The imbalance between ECM synthesis and degradation is an important pathological process in IVDD. Col2 and proteoglycan (predominantly aggrecan) content is crucial to proper disc function, particularly in NP, while MMP (MMP1, 3, 9, 10 and 13) and ADAMTS (ADAMTS-4 and −5) are the primary enzymes that degrade Col2 and Aggrecan, respectively. Interestingly, a study demonstrated that pyroptosis and its downstream protein could stimulate the production of MMP and ADAMTS during IVDD in vitro and in vivo. Moreover, it has been shown that IL-1β or LPS-stimulation regulates ECM anabolic and catabolic homeostasis by upregulating MMP1, 3, 9, 10 and decreasing Col2 and Aggrecan, thereby accelerating IVDD progression.[67–73] Conversely, hyperbaric oxygen reduces MMP3 amounts in degenerated human NP cells by suppressing of IL-1β.[74] Interestingly, lactoferricin, a cationic antimicrobial peptide, has been reported to exert a significant anti-inflammatory effect.[75] A study reported that lactoferricin could block IL-1β-induced expression of ADAMTS-4 and ADAMTS-5 in bovine IVD cells.[67]

Moreover, it has been shown that inhibiting NLRP3 inflammasome activity could suppress ECM degradation and slow the process of IVDD. Bromodomain-containing protein 4 is an important upstream regulator of NLRP3 inflammasome activity. It has been reported that JQ1, a bromodomain-containing protein 4 inhibitor, could significantly inhibit bromodomain-containing protein 4-mediated activation of the NLRP3 inflammasome, thereby inhibiting the expression of MMP3, 13, ADAMTS-4, −5 and upregulating Col2 and proteoglycan levels, ultimately slowing IVDD.[76] As a focal adhesion protein, Kindlin-2 inhibits inflammatory signals to maintain IVD homeostasis. Recent evidence from clinical samples and abnormal mechanical stress-induced mouse coccygeal IVDD model showed that Kindlin-2 is significantly decreased in NP cells in severe IVDD patients and aged mice. In contrast, Kindlin-2 loss activates the NLRP3 inflammasome and stimulates IL-1β expression in NP cells, further downregulating Kindlin-2 expression. Importantly, this vicious cycle promotes ECM catabolism by increasing MMP13 and decreasing Col2 expression.[77,78]

These findings lead us to speculate that NP pyroptosis contributes to the synthesis of IVD MMPs and ADAMTS, thereby aggravating the loss of ECM and the development of IVDD. Importantly, targeting pyroptosis may be a promising direction for inhibiting ECM degradation of IVDD tissues.

Pyroptosis and CEP Degeneration and Tearing

As one of the initiating factors of IVDD, CEP degeneration and tearing participate in or accelerate IVDD by disrupting the nutrient supply of IVD. Current evidence suggests that in IVDD patients, the avulsed CEP is characterized by multiple defects, apparent inflammation, and nucleus invasion, as well as activated NLRP3 inflammasome with upregulation of NLRP3, Caspase-1, and IL-1β, suggesting that pyroptosis is activated in CEP tissues of IVDD patients with Modic alterations on MRI, causing the progression of LBP.[49,79] Meanwhile, CEP cells induced by LPS can activate the cGAS/STING molecular pathway, resulting in cartilage tear damage with irregular structure and loss of hyaline cartilage and its cells.[70] In line with these findings, our recent study using a lumbar instability surgery-induced IVDD

mouse model indicated that the expression level of NLRP3 in the CEP ectopic bone formation area was significantly increased in IVDD mice.[27] These findings substantiate that pyroptosis of CEP tissues is closely related to CEP degeneration and tearing. Nonetheless, the specific mechanism remains to be further studied, especially ectopic bone formation in CEP in IVDD disease.

Pyroptosis, Vascularization and Nerve Ingrowth

It is well known that IVD is the largest avascular tissue in the organism with branches of the sinuvertebral nerve in the posterior aspect of the outer AF.[80,81] Paradoxically, IVD tissues gradually become vascularized and innervated during IVDD progression and positively correlate with the severity of IVDD.[80,81] It is widely believed that innervation follows vascular ingrowth.[82] In vivo and in vitro evidence from IVDD patient NP tissues showed a positive correlation between IL-1β and expression of VEGF as well as neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in human IVD tissues. At the same time, IL-1β generated during the degeneration of IVDs could further stimulate the expression of VEGF, NGF, and BDNF, suggesting pyroptosis may be associated with vascularization and nerve ingrowth in IVDD disease. Blocking pyroptosis may be an alternative way to impede vascularization and nerve ingrowth.[29]

Vascular endothelial growth factor (VEGF) is the most important pro-angiogenesis factor that stimulates the proliferation and migration of endothelial cells, thus contributing to the formation of neovessels. By using a rat IVD degeneration model with anterior limbs and a tail-removing method, Liu et al reported that positive expression of VEGF in degenerative IVD tissue is responsible for neovascularization and infiltration in IVD, which eventually accelerates the IVDD process.[83] Consistently, a study reported that IL-1β could stimulate NP cells to express more VEGF under hypoxic conditions, whereas treatment with IL-1β antibody reduced the production of VEGF in NP cells.[84,85] In conclusion, the above findings suggest that pyroptosis-mediated inflammation may stimulate neovascular ingrowth within the IVD by promoting the production of VEGF.

Neurotrophic factors such as NGF and BDNF secreted by blood vessels can promote the survival and differentiation of neurons, expressed in AF and NP cells of IVD, influencing and enhancing the innervation and pain in degenerative IVD.[86] A previous study documented that high molecular weight hyaluronic acid hydrogels could attenuate the expression of IL-1R, NGF, and BDNF in IL-1β induced inflammation model of NP cells, which may have a therapeutic effect on inflammation-associated pain in discs.[87] Beyond that, Netrin-1 also plays a positive role in promoting peripheral nerve regeneration.[88] Further evidence from clinical samples showed increased netrin-1 immunopositive IVD cells in degenerative IVD, which is closely related to the progression of neurovascular growth.[89] These pieces of evidence allow us to conjecture that pyroptosis may affect Netrin-1 and induce nerve ingrowth, although the specific mechanism warrants further study.

Pyroptosis and Lymphatic Involvement

The lymphatic system is part of the immune system that protects the body from infection and has long been considered to play an essential impact in immune-related diseases by modulating the inflammatory response to lesions or pathogens, and disrupting the lymphatic system by affecting its normal development or function can lead to severe consequences.[90] The quantification of specific lymphatic endothelial cell markers podoplanin and LYVE-1 in clinical vertebral samples has revealed no lymphatics in intact normal IVDs or spinal vertebrae of children or adults.[91] Unexpectedly, in infected and displaced degenerate disc tissues, scattered small vessels lined by LYVE-1^{+}/podoplanin^{+} endothelial cells were observed, demonstrating the presence of *de novo* lymphatic vessels in the outer periosteum, paraspinal ligaments, and surrounding connective tissue.[13,91] Additionally, monocytes/macrophages are cells of the innate immune system that regulate tissue homeostasis and host defense during pathogen infection by controlling the formation of lymphatic vessels.[92–95] Increasing evidence from human and rodent IVDD model samples indicates that monocytes/macrophages recruited from the peripheral blood infiltrate into IVDs and produce proinflammatory cytokines, contributing to the degenerative process.[96] In the LPS-induced rat IVDD model, abundant local production of pro-inflammatory cytokines, including IL-1β, is accompanied by the increased expression of M1 macrophages, as evidenced by elevated specific markers iNOS and CD68.[70] Meanwhile, IL-1β stimulation or IVD injury could increase chemoattractant protein-1

expression in macrophages, which may indirectly contribute to inflammation through monocyte recruitment from blood during IVD injury.[97] Therefore, we speculate that pyroptosis-induced inflammation is related to lymphatic ingrowth into IVD tissues.

Potential Therapeutic Strategies for IVDD via Targeting Pyroptosis

Given the importance of pyroptosis in many aspects of IVDD, various anti-pyroptosis strategies have been applied in animal models or clinical trials to treat IVDD. The agents targeting pyroptosis for the treatment of IVDD include natural small molecular compounds, such as icariin, morin, notoginsenoside R1, and protein inhibitors, such as NLRP3 inhibitors, Caspase-1 inhibitors, GSDMD inhibitors, and IL-1 inhibitors (Table 2). Therefore, cell pyroptosis might be an effective therapeutic target for IVDD.

Natural Small Molecular Compounds

Icariin

Icariin represents a class of prenylated flavonoids and a principal active component of the Traditional Chinese Medicine herb, *Epimedium grandiflorum*. It is well-established that the pharmacological effects of icariin include anti-osteoporosis, neuroprotective effects, protective effects of cardiovascular disease, anti-inflammation, improvement effects on the reproductive system, antioxidant stress effect, anti-depression, and anti-tumor effects,[105] which has been harnessed for the management of hypertension, coronary heart disease, osteoporosis, menopausal syndrome, rheumatism, neurasthenia, bronchitis, and hypogonadism.[106] A previous study reported that icariin treatment inhibits rat chondrocyte pyroptosis by inhibiting NLRP3-mediated inflammasome and production of IL-1β, thereby alleviating rat osteoarthritis progression.[98] In line with this finding, emerging evidence from an in vitro NP cell inflammation model suggested that icariin exerts a strong protective effect against IL-1β-induced inflammatory response in NP cells.[107]

Morin

Morin is a natural polyphenol originally isolated from members of the Mulberry family and can be extracted from the leaves, fruits, stems, and branches of many plants.[108] Growing evidence suggests that morin has antioxidant, anti-inflammatory, and

Table 2 Therapeutic Agents for Treating IVDD by Targeting Pyroptosis

Agents		Mechanism of Action	References
Natural small molecular compound	*Icariin*	Inhibits NLRP3-mediated NP cell pyroptosis	[98]
	Morin	Inhibits TXNIP/NLRP3/Caspase-1/IL-1β signaling to alleviate NP cell pyroptosis	[99]
	Notoginsenoside R1	Inhibits TNF-α-induced NP cell pyroptosis and ECM degradation	[100]
NLRP3 inhibitors	*Muscone*	Blocks IL-1β's effect on end-plate chondrocytes in *vitro*	[101]
	α-Mangostin	Inhibits LPS-induced increase of NLRP3 in NP cells	[102]
	Melatonin	Inhibites NLRP3 inflammasome priming and activation in NP cells to alleviate IVDD	[103]
Caspase-1 inhibitors	*VX-765*	Inhibits Caspase-1 and GSDMD-N expression to increase ECM protein expression	[66]
GSDMD inhibitors	*Disulfiram*	Directly inhibits the formation of GSDMD pore	[104]
IL-1 inhibitors	*Lactoferricin*	Inhibits IL-1β and LPS-induced ECM degradation around NP cells	[67]

Abbreviations: LBP, Low back pain; IVD, Intervertebral disc; IVDD, Intervertebral disc degeneration; AF, annulus fibrosus; NP, nucleus pulposus; CEP, cartilage endplates; ECM, extracellular matrix; Col2, collagen type II; IL, interleukin; TNF-α, tumor necrosis factor -α; NLRP3, nod-like receptor protein-3; LPS, lipopolysaccharide; NLR, nucleotide-binding domain and leucine-rich repeat; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; TRX, thioredoxin; TXNIP, thioredoxin-interacting protein; NR1, Notoginsenoside R1; TLR-2, toll-like receptor-2.

anti-pyroptosis properties.[109–111] A mechanistic study showed that thioredoxin (TRX)-interacting protein (TXNIP) could interact with NLRP3 and participate in NLRP3 inflammasome-dependent pyroptosis, while *TXNIP* deficiency impaired activation of the NLRP3 inflammasome and subsequent secretion of IL-1β.[99,112] Importantly, morin, an inhibitor of TXNIP, can attenuate NP cell pyroptosis and ameliorate IVDD via inhibition of the TXNIP/NLRP3/Caspase-1/IL-1β signaling pathway.[99,112]

Notoginsenoside R1

Notoginsenoside R1 (NR1) is one of the main bioactive compounds in the Chinese herb *Sanchi* root, which plays a certain role in bone metabolism regulation, cardiovascular protection, kidney protection, and anti-cancer effects.[79] Over the years, NR1 has been widely applied to treat IVDD.[113] Further mechanistic study suggested that NR1 significantly reduces the increased pyroptosis markers, such as NLRP3, cleaved-Caspase-1, IL-1β, and GSDMD-N and ECM degradation induced by TNF-α in AF puncture-induced rat IVDD model, indicating that NR1 may protect against IVDD via inhibiting NF-κB/NLRP3 pathway.[100]

Protein Inhibitors

NLRP3 Inhibitor

Muscone

Muscone (known as 3-methylcyclopentadecanone), one of the macrocyclic musk compounds, is primarily responsible for the characteristic odor of musk, and can relieve pain.[101] In China, muscone is widely used to treat neck pain.[101] A previous experiment indicated that muscone could significantly downregulate the levels of LPS-induced inflammatory cytokines and inhibit NF-κB-mediated activation of the NLRP3 inflammasome, thereby improving cardiac function in myocardial infarction mice.[114] Moreover, muscone has been shown to restore the structure of degenerative discs in cervical spine instability surgery-induced rat IVDD model (excision of the paraspinal muscles and supra- and inter-spinous ligaments from C2-C7). Further mechanistical analysis showed that muscone reversed IL-1β and reduced the viability of CEP chondrocytes and the corresponding upregulation of various proinflammatory cytokines, including prostaglandin E_2, 6-keto-prostaglandin F1α, IL-1β, TNF-α, cyclooxygenase 2, inducible nitric oxide synthase, *etc*.[101]

α-Mangostin

α-Mangostin is a natural xanthone product isolated from the pericarps of the mangosteen tree as a secondary metabolite, representing one of the most studied chemopreventive agents.[115] Substantial evidence suggests that α-mangostin can interfere with the major stages of carcinogenesis (initiation, promotion, and progression).[116,117] Moreover, α-Mangostin exhibits a novel biological function against inflammation in various inflammatory diseases.[116,117] A recent study has demonstrated that α-mangostin treatment inhibits LPS-induced increase of NLRP3, ASC, and pro-Caspase-1 expression, as well as IL-1β and IL-18 production, suggesting α-mangostin exerts a protective effect on LPS-induced NLRP3 inflammasome of NP cells.[102]

Melatonin

Melatonin is a methoxyindole principally synthesized and secreted by the pineal gland at night under normal light/dark conditions.[118] It is well established that melatonin can treat schizophrenia, primary headache disorders, and sleep disturbances.[119–121] Interestingly, melatonin was found to delay the progression of IVDD and relieve IVDD-related LBP in a needle puncture-induced IVDD rat model.[103] Subsequent in vitro and in vivo mechanistical analysis showed that melatonin impairs the IL-1β/NF-κB-NLRP3 inflammasome activation positive feedback loop by decreasing NLRP3, p20 and IL-1β levels.[103] These findings suggest that melatonin might be a potential therapeutic agent for IVDD.

Caspase-1 Inhibitor

VX-765

VX-765 is a newly developed selective small-molecule Caspase-1 inhibitor that can cross the blood–brain barrier and reduce inflammation in vitro and in vivo.[122] It can be orally taken for the treatment of epilepsy.[123] A recent study performed in a needle puncture-induced rat disc IVDD model has shown that Caspase-1 was significantly increased

during IVDD progression, and VX-765 efficiently reduced Caspase-1 and GSDMD-N expression, thus inhibiting cell pyroptosis.[66] Moreover, VX-765 could dramatically retard degenerative phenotypes of NP tissues in IVDD mice, such as loss of ECM proteins replaced by fibrous tissues.[66]

GSDMD Inhibitor

Disulfiram

Disulfiram is the most frequently used alcohol-aversive drug by American physicians in treating alcohol dependency disorders.[124] Moreover, many preclinical studies have shown the safety profile of disulfiram, which can target various cancers.[125] Interestingly, it has been found that disulfiram blocks pyroptosis and cytokine release in cells and LPS-induced septic death in mice by inhibiting the formation of GSDMD pore by targeting Cys191 on GSDMD, thereby preventing IL-1β release and pyroptosis.[104] However, disulfiram does not affect inflammatory Caspase cleavage or other upstream events in GSDMD.[104] Importantly, the inhibitory effect of disulfiram on GSDMD offers new therapeutic indications for repurposing this safe drug against inflammation associated with many diseases.

IL-1β Inhibitor

Lactoferricin

Lactoferricin was originally identified as an antimicrobial peptide derived from the digestion of lactoferrin (a multi-functional innate-defense protein in milk) by pepsin.[126] There is rich literature available substantiating that lactoferricin inhibits catabolic and inflammatory mediators in articular cartilage.[127] Currently available research suggests that lactoferricin significantly attenuates catabolic factor-induced stimulation of oxidative and inflammatory factors, such as iNOS, IL-6, and toll-like receptor-2 (TLR-2) and TLR-4, and disrupts IL-1β-mediated inflammation and suppression of proteoglycan production and synthesis, thus restoring proteoglycan accumulation and ECM formation in IVD.[67] Meanwhile, lactoferricin has been reported to antagonize IL-1 and LPS-mediated suppression of proteoglycan in an en bloc intradiscal microinjection model followed by ex vivo organ culture using both mouse and rabbit IVD tissue, suggesting a potential therapeutic benefit of lactoferricin against IVDD in the future.[67]

Conclusions and Prospects

IVDD is an intricate disease involving many types of pathological alterations. Accruing evidence from recently published studies demonstrate that IVD cell pyroptosis is activated in IVDD progression, suggesting its potential role in disease pathogenesis. Importantly, pyroptosis can affect the composition, structure, and functions of IVD tissues and facilitates IVDD progression by promoting inflammation, NP cell loss, disc matrix catabolism, AF rupture, and CEP calcification (Figure 2). Our previous study on the LSI surgery-induced mouse IVDD model found obvious pathological changes in the CEP 1 week after modeling, which were much earlier than those of NP and AF, indicating that the lesions of CEP may be more important. However, compared with NP and AF, the mechanism of pyroptosis in CEP remains largely unclear, which may hinder our understanding of pathogenesis. Further research on the relationship between CEP and pyroptosis may provide novel insights into the transport function of CEP, the compression of NP and the overall progress of IVDD. In addition, the mechanisms by which pyroptosis participates in sensory nerve and lymphoid ingrowth in the pathogenesis of IVDD remains obscure. The importance of sensory nerves and the lymphoid system in IVDD and LBP highlights the need for further research to understand the underlying mechanisms.

Ferroptosis is another newly discovered cell death that occurs via an iron-catalyzed process of lipid peroxidation initiated through non-enzymatic (Fenton reactions) and enzymatic mechanisms (lipoxygenases).[128] Like pyroptosis, it belongs to a genetically regulated type of non-apoptotic cell death, called regulatory necrosis,[129] characterized by the increased rupture of mitochondrial membrane increases.[130] An increasing body of evidence shows that oxidative stress could reduce phospholipid hydroperoxides on NP and AF cells by inhibiting glutathione peroxidase 4 (Gpx4), thus triggering ferroptosis and aggravating IVDD progression.[131–133] Another study using the kidney tissue of selenium-deficient broilers has reported that inhibiting Gpx4 could increase the release of ROS, activate the NLRP3 inflammasome, and release the inflammatory factors IL-1β and IL-18 to trigger pyroptosis,[134] whether a similar relationship exists in the context of IVDD progression remains obscure, which needs to be further clarified.

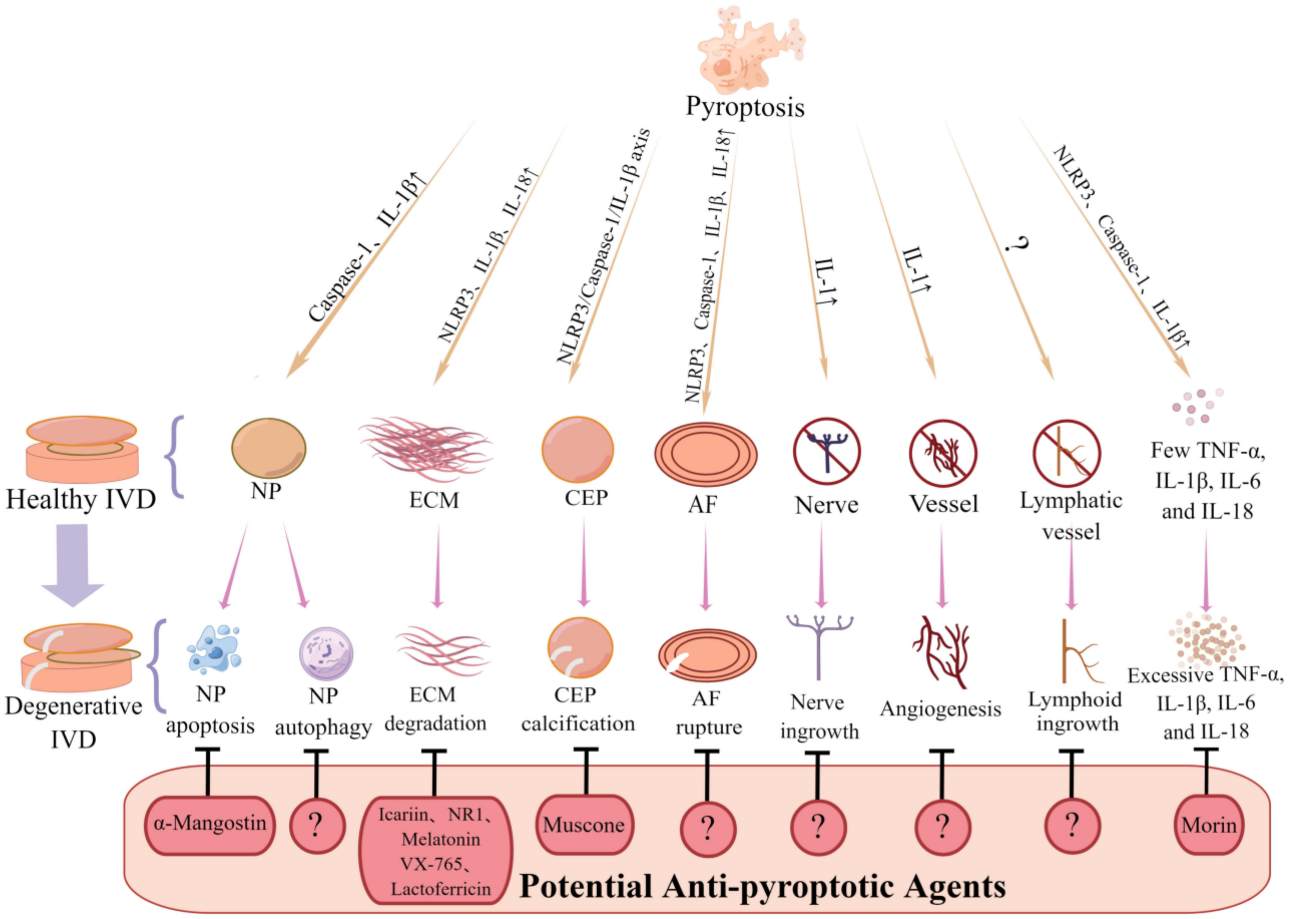

Figure 2 Potential roles of pyroptosis in the pathogenesis of IVDD disease.

Nevertheless, our understanding of the role of pyroptosis in IVDD remains to be improved. Considering that the pathogenesis in IVDD also includes the thoracolumbar fascia and vertebrae, zygapophyseal joints, ligaments, and muscles.[135] The IVDD processes may be usually the consequence of the biomechanical imbalance of the spinal axis caused by these tissue structural and functional disorders. But studies on pyroptosis or pyroptotic-related molecules involving the above tissue are limited, the involved mechanisms may be diverse and multifaceted, and a systematic investigation of the increased disorders of these tissues caused by pyroptosis and the IVDD progression is urgently needed.

It should be borne in mind that current studies on the treatment of IVDD are based on the mechanism of pyroptosis participating in IVDD. In vitro and in *vivo* studies substantiated that some of the developed anti-pyroptosis methods have good therapeutic effects on IVDD. Unfortunately, no IVDD-related preclinical studies have been conducted to further explore the efficacy and side effects of these drugs as well as other drugs that have been put into clinical use to treat other diseases by targeting pyroptosis. Indeed, these findings highlight the need to develop novel drugs that target the pyroptosis of IVD cells to improve IVDD treatment. Importantly, our future studies will assess treatment efficacy with Caspase-1, GSDMD, and IL-1 antibodies on IVDD. Moreover, we will also further explore the efficacy and side effects of other drugs that have been put into clinical use to treat other diseases by regulating pyroptosis.

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Disclosure

The authors declare no conflicts of interest in this work.

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