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Pyroptosis and inflammation-mediated endothelial dysfunction may act as key factors in the development of erectile dysfunction (Review)

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Abstract. Erectile dysfunction (ED) is a prevalent disease that causes sexual dysfunction in males. Inflammation-induced endothelial dysfunction is a fundamental pathophysiological symptom of ED, which is impacted by cell death. Pyroptosis is a type of programmed cell death mediated by the inflammasome that was discovered in inflammatory disorders. The activation of nucleotide-binding oligomerization domain-like receptors, particularly downstream inflammatory factors, such as IL-1β and IL-18, is indicative of caspase-dependent pyroptosis. Although the underlying mechanisms of pyroptosis have been investigated in several disorders, the role of pyroptosis in ED remains to be fully elucidated. At present, studies on pyroptosis have focused on improving the understanding of ED pathogenesis and promoting the development of novel therapeutic options. The present review article aimed to discuss the literature surrounding the mechanisms underlying pyroptosis, and summarize the role of pyroptosis in the development and progression of inflammation-mediated ED.

Contents

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1. Introduction

Erectile dysfunction (ED) is a common disease that prevents males from achieving or maintaining a penile erection that is sufficient for satisfactory sexual intercourse (1-3). ED exerts notable effects on the quality of life of patients and their sexual partners. Several risk factors, including diabetes, metabolic syndrome and systemic disease may increase the prevalence of ED (4-6). Approximately 52% of males worldwide have been reported to experience ED, which affects ~150 million men worldwide (7). A previous study predicted that the number of patients with diabetes will reach 592 million by 2035, and reported that 75% of male patients with diabetes experience differing degrees of ED (8). ED may also arise as a discrete idiopathic condition, as a consequence of an infection or in conjunction with systemic conditions, such as autoimmune disorders. Notably, imbalances in regulatory inflammatory cytokines lead to epithelial cell dysfunction, which induces the release of endotoxins into the peripheral blood circulation, thereby causing a systemic inflammatory response (9). Numerous therapeutic options are currently available for the treatment of ED, including oral phosphodiesterase type 5 (PDE5) inhibitors, alprostadil intraurethral suppositories, intracavernosal injection therapy, vacuum devices and penile prostheses or implants. PDE5 inhibitors are the most commonly used treatment options, which exert anti-inflammatory effects; however, 30% of patients are not sensitive to them (10). Furthermore, a previous review reported that PDE5 inhibitors induce headache, inflammation, digestive issues and other adverse effects, which limits their widespread usage in clinical practice (11).

The present review analyzed the literature on ED and created a graph that included 36,447 articles obtained from the Web of Science, with publication dates ranging from 1945 to 2020. Key words were used to provide a high-level description of the topic in each article, and high-frequency key words were considered to reflect the research hotspots in ED. Following the screening and analysis of all ED-associated key words, 141 high-frequency key words were collected. In addition, a symbiotic map of core key words was created to summarize the research hotspots of ED. Notably, ED was found to be associated with injury, endothelial dysfunction,

smooth muscle, infection, and oxidative stress (Fig. 1) (12-20). Numerous previous studies have demonstrated the effects of pyroptosis in cardiovascular disease, including atherosclerosis, myocardial infarction, diabetic cardiomyopathy and cardiac hypertrophy, and in cancer (21-27). However, studies focused on the role of pyroptosis in ED, and the associated molecular mechanisms and pathogenic pathways are lacking.

The results of a previous study demonstrated that a poly ADP-ribose polymerase inhibitor decreased apoptosis in the corpus cavernosum of diabetic rats; however, this inhibitor improved erectile function, but not fully restore the erectile response, indicating that types of cell death other than apopotosis may be involved in ED (28). Types of cell death include pyroptosis, apoptosis and necrosis, and these three types have multiple similarities and differences. Notably, these types of cell death play key roles in tissue homeostasis, basic biological functions and disease occurrence (Table I) (14,24). The inflammasome-induced activation of the pyroptosis pathway accelerates cell swelling and causes a large number of pro-inflammatory cytokines to be released from the cell, leading to cell death (Fig. 2). Pyroptosis is inflammatory programmed necrosis, which was initially reported in *Salmonella*-infected macrophages (29). Notably, pyroptosis occurs in response to a variety of pathogens and non-infectious factors, and is dependent on caspase-1, -4, -5 and -11, which are associated with the activation of downstream inflammatory factors. Cell death induced by caspase-1 activation is known as canonical pyroptosis, while cell death induced by caspase-4, -5 and -11 activation is known as noncanonical pyroptosis (30). Inflammasome activation in the canonical signaling pathway may induce the release of functional caspase-1 *in vivo*, the activation of immune cells, and the secretion of chemokines, inflammatory factors and adhesion molecules. This exacerbates the inflammatory response, leading to severe inflammation (31). A previous study demonstrated that activation of the pyroptosis signaling pathway is associated with the development of ED (32). Increases in proinflammatory cytokines in ED indicate that inflammatory factors play a significant role in the progression of this disease (33,34). The present study aimed to review the mechanisms and the biological importance of pyroptosis in inflammation-mediated ED.

2. Canonical pyroptosis pathway

Luo *et al* (35) demonstrated that the pyroptosis of endothelial cells leads to endothelial dysfunction by reducing the expression of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO). As a lytic and inflammatory type of regulated cell death, pyroptosis involves an inflammasome-mediated signaling pathway of programmed cell death (36). When cells are stimulated, inflammasomes recognize various exogenous and endogenous signals, including pathogen- and endogenous damage-associated molecular patterns, leading to auto-oligomerization (37). Pattern recognition receptors, such as nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) are activated in the cytoplasm and interact with homologous domains to form a multi-protein complex known as an inflammasome (38). Inflammasomes hydrolyze caspase-1 precursors into active caspase-1 (39). Activated caspase-1 not only mediates the cleavage of pro-IL-1β and pro-IL-18 to form mature cytokines IL-1β and IL-18, but also cleaves gasdermin D (GSDMD) to form cell membrane pores and secrete inflammatory cytokines (40). Moreover, large numbers of water molecules enter the cell, thus promoting cell swelling and inducing pyroptosis (Fig. 3) (41). Present research is focused on the dynamic composition of these signaling pathway complexes under different pathological conditions; however, the association between inflammatory-mediated ED and pyroptosis in penile cavernous tissue has not been fully elucidated.

Inflammasomes. The activity of inflammasomes in the corpora cavernosa may modulate the function of this tissue at a physiological level. In addition, inflammasome activation may contribute to functional changes occurring in pathophysiological states (40). Notably, inflammasomes are important components of the pyroptosis pathway that participate in immune regulation. NLRP3 is a member of the NOD-like receptor family of pattern recognition receptors, which also includes NLRP1, NOD-like receptor family caspase recruitment domain containing 4, absent in melanoma 2 receptor and pyrin. Among this family, the most commonly studied inflammasome is NLRP3 (42). Following the specific stimulation of NLRP3, apoptosis-associated speck-like proteins containing a caspase-1 precursor recruitment domain are activated. These apoptosis-associated speck-like proteins are bridging adaptors in inflammatory complexes that interact with cell death activators and are essential for inflammasome integrity. They form a multi-protein complex known as the NLRP3 inflammasome, which is associated with various immune and inflammatory diseases, including chronic obstructive pulmonary disease, bronchial asthma and hepatic fibrosis. The NLRP3 inflammasome is crucial for innate immunity; however, its aberrant activation promotes various inflammatory disorders, including atherosclerosis and ED (43). The findings of a previous study suggested that NLRP3 inflammasomes mediate innate immune responses to induce ED. Moreover, the study indicated that NLRP3 increases the accumulation of inflammatory factors in the blood vessel wall, activates caspase-1, increases the thickness of the vessel wall and reduces blood circulation, thereby leading to ED (44). Another study demonstrated that the NLRP3 inflammasome participates in the mediation of lipopolysaccharide-induced pyroptosis, and is positively associated with vascular inflammation (45). Activation of the NLRP3 inflammasome decreases the sensitivity of the corpora cavernosa to NO and endothelium-dependent relaxation; these are processes that may be associated with NLRP3-mediated vascular functional and structural damage (46,47). The inhibition of NLRP3 has been demonstrated to prevent the endothelin-1-induced impairment of endothelial relaxation in the corpora cavernosa in mice, which exhibits a positive effect on erectile function (48). Thus, the NLPR3 inflammasome may play a role in the pathogenesis of ED. Notably, inflammasomes stimulate proinflammatory cytokines and promote the expression of inflammatory factors, which induces systemic inflammation (45). Therefore, NLRP3 activation may serve as a novel

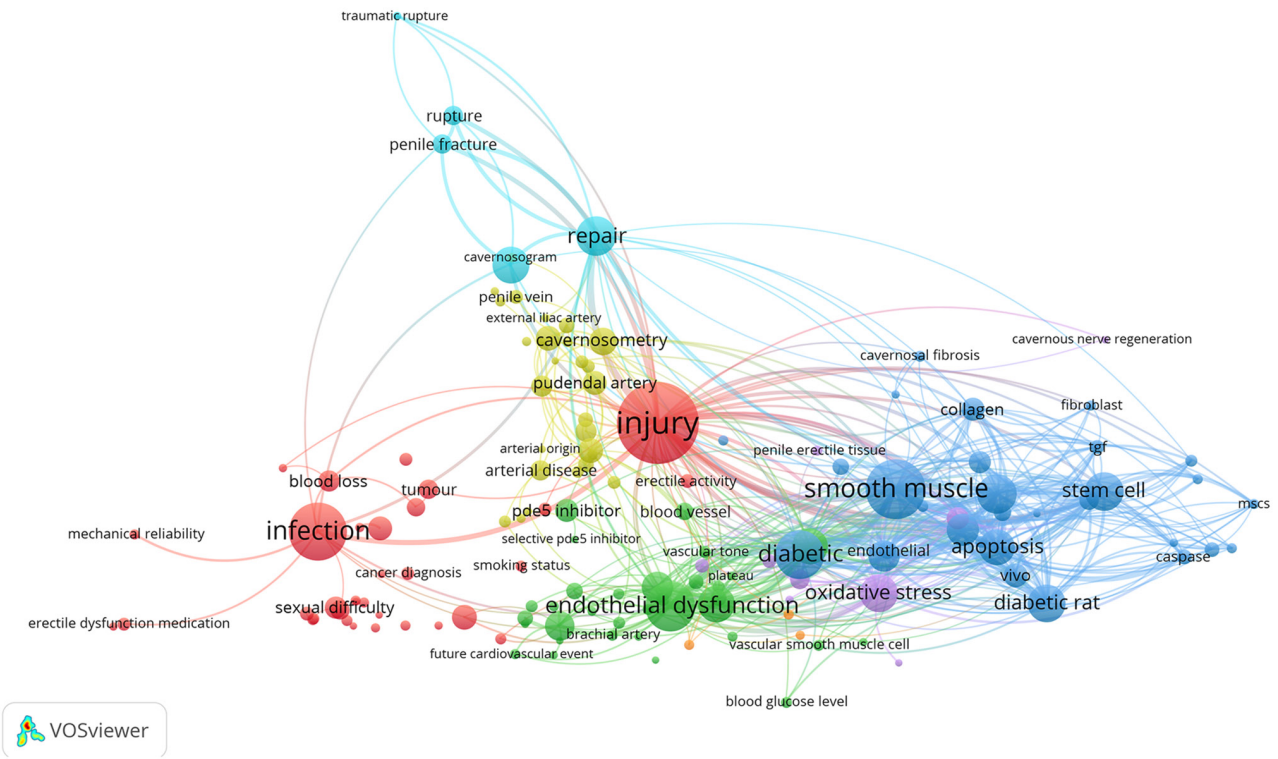

Figure 1. ED keyword co-occurrence analysis. Injury, infection, oxidative stress, smooth muscle and endothelial dysfunction have been extensively studied with high frequency in articles on ED. The different colors in the figure represent different clustering methods. The size of the circle and font represents the centrality of the keyword; the larger the circle and font, the higher the centrality of the keyword. This data was analyzed using VOSviewer 1.6.16 software (Centre for Science and Technology Studies, Leiden University). ED, erectile dysfunction; pde5, phosphodiesterase type 5; tgf, transforming growth factor; MSCs, mesenchymal stem cells.

target for the modulation of ED. Further investigations are required to determine whether inhibition of the inflammasome alleviates inflammation-mediated ED.

Caspase-1. A study by Matsui *et al* (49) described the elevation of caspase-1 expression in the corpora cavernosa of rats with ED, which indicated that pyroptosis may be involved in the development of ED. The caspase family comprises cysteine-containing proteolytic enzymes that exist in the form of inactive zymogens under normal conditions (50). When activated, caspases may trigger a reaction cascade that regulates inflammation and cell death. The precursor of caspase-1 undergoes oligomerization and hydrolysis into the p20 and p10 subunits that form caspase-1. Caspase-1 is activated via the NLRP3 inflammasome. When active caspase-1 cleaves the GSDMD protein, the active N-terminal of the latter is exposed and transferred to the cell membrane. The N-terminal domain combines with phosphatidylinositol phosphates, phosphatidic acid, phosphatidylserine and cardiolipin to create pores in the lipid structure of the cell membrane (51). These pores have a β-barrel structure with a diameter of 10-20 nm. The destruction of the cell membrane associated with the formation of these pores alters intracellular pressure and cell permeability, causing the cell to swell, rupture and lyse. Notably, caspase-1 also cleaves immature IL-1β and IL-18 precursors to form active IL-1β and IL-18, which are released from the cell through the membrane pores to induce cell pyroptosis (52). Caspase-1-mediated pyroptosis is important in the regulation of ED. Caspase-1 also inhibits eNOS phosphorylation, reduces NO synthesis and causes endothelial dysfunction, suggesting that pyroptosis may be associated with inflammation in the mediation of ED (53). Yuan *et al* (54) reported that the inhibition of caspase-1 expression regulates the release of downstream inflammatory factors, thereby reducing vascular endothelial damage and inflammation. Furthermore, another study demonstrated that caspase-1 knockdown significantly reduced the expression of IL-18, inhibited inflammation, promoted blood circulation and repaired injured vascular endothelial cells, which ameliorated diabetes rats with ED (49). In addition, a review by Chen and Xu (39) reported on the ability of inflammation to stimulate the expression of caspase-1 precursors, promote the activity of caspase-1 and increase pyroptosis, and highlighted the pathological effect of caspase-1-mediated pyroptosis in vascular disease. Furthermore, androgen deprivation may cause inflammation and endothelial dysfunction via the acceleration of caspase-1 activation and induction of the pyroptosis of endothelial cells (31). Collectively, these findings may provide a novel theoretical basis for elucidating the mechanisms underlying inflammation-induced ED.

GSDMD. Di *et al* (55) reported that GSDMD is mainly expressed in the cytoplasm of endothelial cells in rat blood vessels, suggesting that pyroptosis occurs in endothelial cells. The gasdermin protein family is a conserved family of proteins with pore-forming activity. GSDMD is a well-established member of the GSDM family, which is the substrate

Table I. Comparison of apoptosis, necroptosis and pyroptosis.

Items	Apoptosis	Necroptosis	Pyroptosis
Cell morphology	Cells shrink and become smaller in volume	Cells become round and swell	Cells gradually flatten
Cytoplasm	Cytoplasm becomes condensed, and is packaged into apoptotic bodies with organelles	Cytoplasmic swelling occurs and the intra-cellular contents are released into the immediate cellular milieu	Cytoplasmic swelling occurs and the intra-cellular contents are released into the immediate cellular milieu
Organelle	Organelle condensation	Swelling of organelles, mitochondrial dysfunction, loss of membrane potential	Mitochondria and lysosomes are damaged
Nucleus	Nuclear condensation and rupture, nucleolus disappears and chromatin is condensed	Retention of the integral nucleus; loss of nuclear chromatin	Condensation of nuclei, random fragmentation and degradation of chromatin DNA
Cell membrane	Membrane blebbing occurs, but a certain integrity is maintained	Membranous pore formation, membrane rupture and loss of integrity	Membranous pore formation, membrane rupture and loss of integrity
Micro structure	Apoptotic body formation	Necrotic body formation	Pyroptotic body formation
Process of cell death	Apoptotic bodies are phagocytosed and cleared by neighboring cells and macrophages	Explosion-like rupture of plasma membrane	Cells gradually swell until the plasma membrane ruptures
Caspase dependence	Depends on caspase-3	Depends on caspase-8	Depends on inflammatory caspase-1, -4, -5 and -11
Surrounding changes	No obvious changes	Amplified inflammation	Amplified inflammation

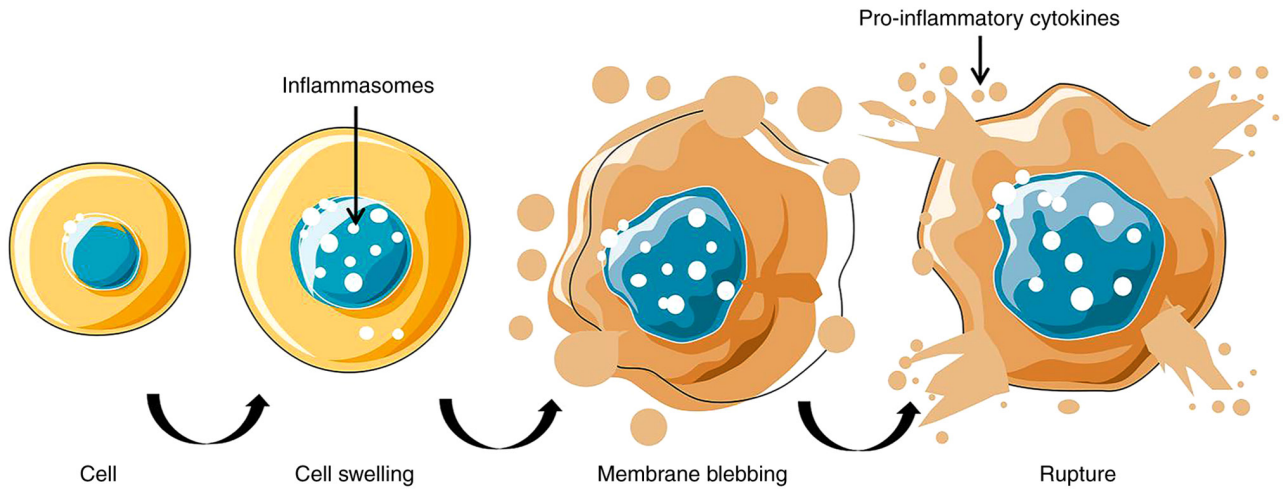

Figure 2. Morphological changes in cell death induced by pyroptosis. In the early stage of pyroptosis, cell swelling and membrane blebbing occur. During later stages, the plasma membrane ruptures.

of caspase-1 and is cleaved during pyroptosis. Barnett and Ting (56) reported that GSDMD is a critical protein in pyroptosis. It is composed of two fragments, namely the N- and C-terminals. The activation of caspase-1 releases the N-terminal, which has pore-forming activity. The activated N-terminal specifically recognizes the lipid bilayer on the cell membrane to form a circular structure, which releases the segment with toxicity through self-oligomerization and perforation on the cell membrane. The presence of the circular structure destroys the integrity of the cell membrane, resulting in changes in cell osmotic pressure and the release of inflammatory cytokines, which lead to pyroptosis (57,58).

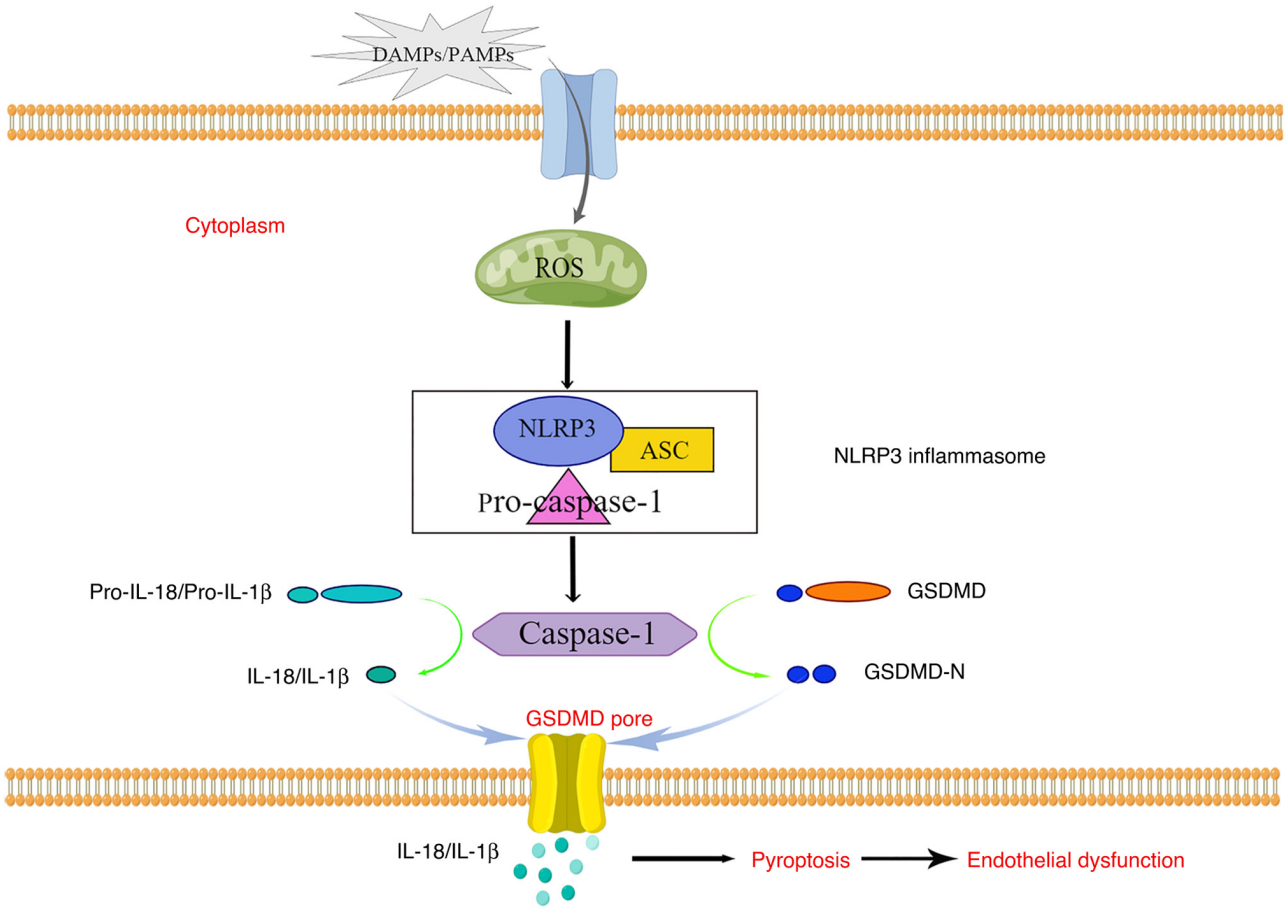

Figure 3. Schematic representation of the cell pyroptosis mechanism. When the cell is stimulated, NLRP3 inflammasomes comprising NLRP3, pro-caspase-1 and ASC are assembled. Subsequently, the assembled NLRP3 inflammasomes stimulate the activation of caspase-1 precursors and promote the maturation of intracellular IL-18 and IL-1β precursors. The activated caspase-1 triggers pyroptosis by cleaving GSDMD, and promotes the release of mature inflammatory molecules, including IL-18 and IL-1β. DAMPS, damage-associated molecular patterns; PAMPS, pathogen-associated molecular patterns; ROS, reactive oxygen species; NLRP3, nucleotide-binding oligomerization domain-like receptor protein-3; ASC, apoptosis-associated speck-like protein; pro-caspase-1, caspase-1 precursor; caspase-1, cysteine aspartic acid specific protease 1; GSDMD, gasdermin D; GSDMD-N, GSDMD N-terminal domain; IL, interleukin.

GSDMD is involved in the occurrence and development of various inflammatory diseases. For example, IL-6 prevents the death of pulmonary macrophages and alleviates pneumonia-mediated damage via the inhibition of GSDMD-mediated pyroptosis in pneumonia-associated sepsis (59). In addition, an increase in reactive oxygen species promotes a downstream inflammatory response and the expression of GSDMD, and induces endothelial dysfunction in pneumococcal pneumosepsis (60). Ye *et al* (61) revealed that GSDMD knockout significantly reduces the release of inflammatory factors related to pyroptosis. In addition, Lei *et al* (62) demonstrated that the inhibition of reactive oxygen species in endothelial cells reduces the activity of GSDMD, suggesting that reactive oxygen species mediate inflammatory responses through the NF-κB-GSDMD signaling pathway in vascular diseases. However, the association between GSDMD and inflammation-mediated ED remains to be fully elucidated.

IL-1β and IL-18. The results of a transcriptomic study demonstrated that IL-1β and IL-18 mediate multiple pathways in ED induced by a high-fat diet (63). The IL-1 family has numerous members, including IL-1α, IL-1β and IL-18, which play a key role in the regulation of innate immunity and the adaptive immune response. IL-1β and IL-18 are cellular inflammatory factors that exist on the surface of cell membranes, and are produced by a variety of cells, including monocytes, macrophages and neutrophils. These factors induce the inflammatory immune response and lead to inflammation. Mature IL-1β and IL-18 contribute to numerous biological processes, including the occurrence and development of ED. Song *et al* (64) demonstrated that IL-1β and IL-18 reduce the production of NO via downregulation of the expression and activity of eNOS, which suggests that IL-1β and IL-18 may be involved in the development of ED. In addition, another study demonstrated that the local levels of the inflammatory factors IL-1β and IL-18 were significantly increased in patients with ED, and the elevation of these levels was sustained for a prolonged period (65). Activation of the inflammasome induces the maturation of inactive IL-1β and IL-18, and increases the synthesis, expression and release of these cytokines. The release of IL-1β and IL-18 through GSDMD pores recruits immune cells to the site of inflammation, stimulates secondary cytokine production and triggers acute-phase immunity responses. Studies have revealed that the IL-18 receptor is associated with susceptibility to

vascular damage, and its expression increases the risk of disease (66,67). Moreover, the pyroptosis-mediated release of IL-1β and IL-18 initiates systemic inflammatory cascades and the development of ED (10). However, the association between pyroptosis and inflammation-mediated ED requires further investigation.

3. Effects of pyroptosis on ED

Numerous factors influence the development and progression of ED, including immunological and inflammatory microenvironments. Notably, inflammatory cytokines are closely associated with ED. Ferlin *et al* (68) observed that the volume of inflammatory components in the blood of patients with ED was increased compared with that in healthy individuals, and was inversely associated with sexual performance. The occurrence of oxidative stress in penile tissues in response to inflammatory factors promotes the accumulation of reactive oxygen species and triggers endothelial dysfunction, leading to ED. Matos *et al* (69) demonstrated that inflammatory factors can be used as predictors of ED, which further verifies the role of inflammatory factors in the occurrence of ED. Pyroptosis is associated with the pathogenesis of numerous chronic inflammatory diseases due to being a pro-inflammatory process. Oxidized low-density lipoproteins activate the NF-κB/NLRP3 signaling pathway and cause endothelial cell dysfunction, thereby increasing the occurrence of pyroptosis in vascular smooth muscle cells (48). A study in atherosclerotic mice demonstrated that the numbers of atherosclerotic plaques and pyroptotic factors were significantly reduced, and oxidative low-density lipoprotein-induced pyroptosis was inhibited following treatment with the caspase-1 inhibitor VX-765 (70). It has also been reported that hypercholesterolemia promotes activation of the NLRP3 inflammasome, downregulates the expression of eNOS and NO in coronary arteries, induces endothelial dysfunction and accelerates the pyroptosis of endothelial cells (71). Moreover, inflammation induces endothelial dysfunction in human umbilical vein endothelial cells through the increased expression of IL-1β, IL-18, caspase-1 and NLRP3 induced via activation of the PI3K/AKT pathway (72). A combination of lipopolysaccharides and adenosine triphosphate has been demonstrated to inhibit the erectile function of mice via activation of the NLRP3 inflammasome and upregulation of the expression of caspase-1 and IL-1β (73). It has also been demonstrated that upregulation of the expression of yes-associated protein, a component of the hippo signaling pathway, is associated with inhibition of the proliferation of corpus cavernosum smooth muscle cells. In addition, activated caspase-1 cleaves GSDMD into its N-terminal form, thereby inducing an inflammatory response in cells and pyroptosis, which leads to a decline in erectile function (63). Luo *et al* (74) revealed that adipose-derived stem cells improve erectile function in rats via the suppression of NLRP3 inflammasome-mediated pyroptosis and inflammation in the corpus cavernosum tissue. Notably, IL-1β and IL-18 exert inflammatory effects (53), with IL-1β playing a key role in the inflammatory response (75). The release of IL-1β from pyroptotic cells promotes collagen production and downregulates the expression of eNOS, leading to penile fibrosis, reduced NO synthesis (64,76) and inflammation-mediated ED. Furthermore, serum IL-1β levels have been demonstrated to be elevated in patients with ED (77). IL-18 plays a key role in inflammatory cells and is a chemotactic cytokine that attracts leukocytes to the site of inflammation (78). It has been demonstrated that NLRP3, caspase-1 and IL-1β expression levels are increased in rats with ED, indicating that pyroptosis may play a role in the development of ED. Therefore, the inflammatory microenvironment created by the release of a high number of inflammatory factors during pyroptosis may induce the initiation and progression of ED (34).

4. Conclusions

Present research is focused on pyroptosis as a form of pro-inflammatory programmed cell death. Notably, pyroptosis plays an important role in the inflammatory response and accelerates the progression of ED. The pathogenesis of ED is complex, and cell death is important in the occurrence and development of inflammatory-mediated ED. The present review demonstrates that data surrounding the mechanisms underlying ED and associated treatment options are lacking, despite the high incidence of ED. As pyroptosis plays a key role in inflammation, it may exhibit potential as a target in the treatment of inflammation-mediated ED. However, the specific cell death pathways and key upstream factors activated in inflammatory ED remain to be fully elucidated. Thus, further investigations into the pathogenesis of ED and cell pyroptosis are required. In addition, the development of novel pyroptosis inhibitors and strategies for the prevention and treatment of inflammatory-mediated ED is necessary.

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Availability of data and materials

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Authors' contributions

XJ collected the data. YN edited the manuscript. BZ and FL performed data management and wrote the manuscript. HG was responsible for protocol/project development and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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