Pyroptosis in Spinal Cord Injury: Implications for Pathogenesis and Therapeutic Approaches

Guangjin Gu¹,²,³,±, Huaqi Yu¹,†, Huishuang Zou¹,‡, Wenjuan Kou¹, Pingping Zhang¹, Guangjie Gu⁵, Jie Lu¹, Weihan Shi¹, Pengcheng Chu¹, Yaning Zhang¹,*; Guangwei Sun¹,*; Jun Shang¹,6,*

¹Department of Orthopedics, Shanxi Medical College Seventh Affiliated Hospital: Linfen People’s Hospital, 041000 Linfen, Shanxi, China
²Department of Orthopedics, Qilu Hospital of Shandong University, Shandong University, 250033 Jinan, Shandong, China
³Shandong University Centre for Orthopedics, Advanced Medical Research Institute, Shandong University, 250012 Jinan, Shandong, China
⁴Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmaceutical Sciences and Research Center of Basic Medical Sciences, Tianjin Medical University, 300070 Tianjin, China
⁵Department of Medicine, Nantong University Xinglin College, 226008 Nantong, Jiangsu, China
⁶Department of Orthopaedics, The Second Hospital of Shandong University, 250033 Jinan, Shandong, China
*Correspondence: 2452462520@qq.com (Yaning Zhang); lfsunguangwei@163.com (Guangwei Sun); shang2108@126.com (Jun Shang)
†These authors contributed equally.

Academic Editors: Igor Lavrov and Nadia Lampiasi
Submitted: 7 February 2024 Revised: 21 April 2024 Accepted: 24 April 2024 Published: 11 June 2024

Abstract

Traumatic spinal cord injury (SCI) is a serious disease of the central nervous system. Aside from the limited intrinsic regenerative capacity of neurons, complex microenvironmental disturbances can also lead to further cellular damage and growth inhibition. Programmed cell death regulated by pyroptosis has an important role in the pathogenesis of SCI. While there has been a wealth of new knowledge regarding cellular pyroptosis, a detailed understanding of its role in SCI and possible therapeutic strategies is still lacking. This review summarizes current advances in the regulatory role of pyroptosis-regulated cell death and inflammasome components in the inhibitory microenvironment following SCI, as well as recent therapeutic advances.

Keywords: spinal cord injury; pyroptosis; gasdermin D; inflammasome; NLRP3

1. Background

Spinal cord injury (SCI) causes permanent and devastating loss of sensory-motor and autonomic function and greatly reduces patient quality of life. SCI increases public health costs and presents a challenge to clinics and public health professionals globally. There are 759,302 patients with traumatic SCI in China, with 66,374 new cases reported each year [1]. Moreover, about 17,000 people are diagnosed with SCI each year in the United States, with the financial cost of a high-grade quadriplegic patient being approximately $1 million in the first year [2]. Following a strike to the spinal cord, deleterious pathophysiological changes are induced by a variety of traumatic injuries such as shear stress, contusion, torsion, and compression. This results in transient or permanent impairment of normal sensory, motor, and autonomic functions of the spinal cord. In addition, the death of neurons in damaged tissue and the activation of inflammatory cells in damaged areas may cause secondary damage that induces axonal degeneration, accumulation of pro-inflammatory cytokines and chemokines, demyelination, and cellular damage. Due to the irreversible and unavoidable nature of primary SCI, therapeutic modalities have focused on ameliorating disturbances of the spinal cord microenvironment caused by secondary injuries, as well as promoting the intrinsic regeneration of host nerves in order to improve the clinical outcome and prognosis of patients with SCI. Several pathophysiological changes occur after SCI, including oxidative stress, inflammation, pyroptosis, and apoptosis. Moreover, there is growing evidence that modulation of cellular pyroptosis following secondary injury may prove beneficial in the treatment of SCI.

Previous studies have identified cellular pyroptosis as an important event leading to increased cell death and neuroinflammatory responses after SCI. Pyroptosis is a novel, pro-inflammatory type of cell death regulated mainly by Caspase-1 and Gasdermin D (GSDMD). It is also accompanied by the production of different inflammatory mediators, such as interleukin (IL)-1β and IL-18. Caspases cleave GSDMD by releasing its N-terminal domain (GSDMD-NT), thereby forming a pore in the cell membrane after cleavage. This leads to the release of IL-1β and IL-18, and the entry of H₂O into the cell, demonstrating that GSDMD plays a central role in cellular pyroptosis. Excessive production of pro-inflammatory factors (e.g., IL-1β and IL-18) and oxidative stress exacerbate the inflammatory response and aggravate secondary injury. Mechanistically, inflammasomes can be further activated to produce biologically active caspase-1 that triggers downstream cascade reactions leading to cell
death and inflammation. Due to the participation of various caspases, pyroptosis has also recently been characterized as “Gasdermin-mediated programmed necrotic cell death”. In addition to differences in the triggering signals and cystein-aspartic enzymes compared to necrotic cells, pyroptotic cells undergo membrane blistering, swelling, and flattening prior to cell lysis. This contrasts with the explosive rupture observed in necrotic cells, or the shrinking and wilting of apoptotic cells. Thus, the formation of inflammasomes, GSDM-dependent cell membrane rupture, and the release of IL-1β and IL-18 are considered to be typical features of cellular pyroptosis.

Despite the wealth of newly discovered information on pyroptosis, its regulatory role in SCI is still not fully understood. Moreover, it remains unclear how the inhibition of pyroptosis can improve clinical outcomes. Therefore, this review will systematically summarize new findings on the role of pyroptosis in the pathogenesis of SCI. Furthermore, we discuss potential biomarkers and drugs for therapeutic strategies involving pyroptosis in SCI (Fig. 1).

2. Cellular Pyroptosis in Spinal Cord Injury

Immediate contusion and compression of the spinal cord tissue as a result of the impact of a direct external force is accompanied by vascular injury and disruption of the blood-brain barrier [3]. The “primary injury” to spinal cord tissue results in the local and systemic release of damage-associated molecular patterns (DAMP), and binding to leucine-rich repeat sequence (LRR) domains. This directly disrupts the spinal cord microenvironment and induces local cellular pyroptosis [4]. The mechanism underlying NOD-like receptor protein 3 (NLRP3) inflammasome formation remains unclear. Previous reports suggest that ionic imbalance, oxidative stress, and cathepsin B (CTSB) leakage are the three main activators of NLRP3 inflammasomes. Moreover, the pathways linking ionic imbalance, oxidative stress and CTSB leakage with cellular death are not mutually exclusive. Each of these signals may interact independently with NLRP3, leading to different conformational changes in the molecules involved in inflammasome assembly [5]. Here, we discuss in detail how ion imbalance, ROS, and CTSB activate NLRP3 inflammasomes after SCI (Fig. 2).

2.1 Ionic Imbalance
2.1.1 Potassium Efflux

Potassium (K+) efflux is a necessary, but not sufficient upstream signaling event for the activation of NLRP3 inflammasomes [6]. A decrease in intracellular K+ concentration and cell swelling induced by a hypo-osmotic solution was first observed in macrophages treated with a variety of NLRP3 agonists, including ATP, cytoplasmic double-stranded RNA, Staphylococcus aureus hemolysin, crystalline and granular molecules, and bacterial lipoproteins [7]. Second, the activation of NLRP3 inflammasomes in response to a variety of stimuli was shown to be inhibited by elevated concentrations of extracellular K+ (30–45 mM) [8,9]. Additionally, macrophages that were activated and then cultured in K+ -free medium were capable of inducing the activation of NLRP3 inflammasomes [9].
Fig. 2. Schematic illustration of different pyroptosis pathways. (A) Damage associated molecular patterns (DAMPs) such as ATP or Reactive Oxygen Species (ROS) are released from parenchymal cells undergoing death after primary spinal cord injury (SCI). These promote the transcription of pro-IL-1β, pro-IL-18 and NLRP3 through activation of the TLR4/NF-κB pathway. P2X7R promotes K+ efflux and Ca2+ influx following ATP recognition, thereby further promoting the activation of inflammasomes. (B) Following SCI, ROS upregulation occurs through several pathways: (1) Ca2+ promotes NADPH activation via NMDAR and other means of non-excitotoxicity endocytosis; (2) intracellular ROS upregulation is further promoted by mitochondrial Ca2+ overload and Endoplasmic reticulum (ER) stress; (3) the blood-spinal cord barrier is disrupted after SCI, and ROS is released from infiltrating neutrophils, macrophages, etc.; (4) ferric ions are released from ferritin and transferrin, and ROS production is promoted by ferrous ions via the Fenton reaction. (C) Following SCI, the upregulation of CTSB expression promotes inflammasome production. IL-1β, Interleukin-1β; NLRP3, Nucleotide oligomerization domain (NOD)-like receptor thermal protein domain associated protein 3; CTSB, Cathepsin B; P2X7R, P2X7 Receptor. This figure was created with Biorender (https://www.biorender.com/).

Indeed, the impact of changes in ion channels on cellular pyroptosis following SCI remains unclear. The P2X7 receptor (P2X7R) is a member of the P2X purinergic receptor family. P2X7R is preferentially expressed in microglia and specifically detects ATP signaling in DAMP after SCI. This in turn promotes K+ efflux-induced cellular pyroptosis and inflammatory responses in a two-step biological process [10–12]. Initially, activation of Toll-like receptor 4 (TLR4) results in the buildup of cytoplasmic pro-IL-1β and pro-IL-18. In the second step, ATP-dependent activation of P2X7R leads to the activation of nucleotide-conjugated leucine-rich repeat (LRR) sequences, which in turn initiates the formation of NLRP3 inflammasomes. When activated, NLRP3 induces the cleavage of pro-caspase-1 to caspase-1. This leads to the enzymatic degradation of pro-IL-1β and pro-IL-18 to IL-1β and IL-18, respectively, via caspase-1-induced protein hydrolysis [13].

2.1.2 Calcium Influx

Excessive accumulation of intracellular Ca2+ is a common phenomenon after SCI and is the most critical step in ionic dysregulation. Winkler et al. [14] showed that intra-axonal Ca2+ accumulates 30 min after SCI, whereas extracellular Ca2+ decreases rapidly after SCI. These two opposing changes result in the intracellular Ca2+ level increasing within 45 min after trauma, peaking by 8 h post-
injury, and remaining high for one week after SCI [14]. The increase in intracellular Ca\textsuperscript{2+} after SCI is largely dependent on rapid Ca\textsuperscript{2+} influx via NMDAR and other means of non-excitotoxicity. The dysfunction of these homeostatic regulators leads to progressive overloading with Ca\textsuperscript{2+} [15]. Although the Ca\textsuperscript{2+} pathway does not affect activation of the AIM2 and NLRC4 inflammasomes, it is critical in promoting the formation of NLRP3 inflammasomes [16,17]. AIM2 is a member of the ALR family of proteins and is characterized by a C-terminal HIN (nuclear localization, hematopoietic, and interferon-inducible) domain and an N-terminal PYD (pyrin) domain [18]. The HIN domain is directly responsible for binding to dsDNA in the cytoplasm, while the PYD domain is responsible for recruiting the adaptor protein ASC during inflammasome assembly. This important adaptor molecule recruits the effector protein caspase-1 to the inflammasome complex [19,20]. NLRC4 is critical for cytoplasmic recognition of Gram-negative bacteria. It was originally named IPAF (ICE protease-activating factor) due to its ability to activate caspase-1 [21]. NLRC4 features a three-domain structure comprising a carboxy-terminal LRR, a central nucleotide-binding domain (NACHT), and an amino-terminal caspase recruitment domain (CARD). NLRC4 can directly bind to pro-caspase-1 through CARD-CARD interactions, thereby triggering the processing and activation of caspase-1 [22]. Lee et al. [16] reported that activation of inositol 1,4,5-trisphosphate receptor (IP3R) on the endoplasmic reticulum (ER) triggers Ca\textsuperscript{2+} release and NLRP3 activation. IP3 is a product of phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2). These workers also reported that NLRP3 activation could be induced by direct activation of PLC in the absence of other exogenous stimuli. In contrast, the inhibition of PLC blocked IL-1\beta secretion induced by a variety of stimuli. Moreover, Ca\textsuperscript{2+} mobilization and NLRP3 activation could be reduced by the pharmacological inhibition or downregulation of IP3R [16,17].

Two mechanisms have been proposed for the Ca\textsuperscript{2+}-mediated regulation of NLRP3 inflammasome activation. First, it was observed that Ca\textsuperscript{2+} facilitated the spontaneous binding of NLRP3-ASC in cell-free lysates obtained from LPS-stimulated macrophages, indicating that Ca\textsuperscript{2+} directly regulates the activation of NLRP3 inflammasomes. However, the direct targets for Ca\textsuperscript{2+} remain to be identified, and the underlying mechanisms by which Ca\textsuperscript{2+} promotes complex formation are still unknown [16]. A second possible mechanism is that excess release of Ca\textsuperscript{2+} from the ER leads to mitochondrial damage and mitochondrial Ca\textsuperscript{2+} overload, including cardiolipin externalization, mtDNA release, and mtROS production. This would further promote the activation of NLRP3 inflammasomes. The second mechanism is supported by considerable evidence [23–26].

2.2 Reactive Oxygen Species (ROS)

2.2.1 Sources of ROS after SCI

ROS are highly reactive, oxygen-containing substances produced mainly by redox reactions during normal physiological activities. Oxidative damage to proteins, lipids and nucleic acids is mainly driven by ROS and reactive nitrogen species (RNS), which can in turn induce pyroptosis, inflammation, autophagy, and apoptosis [27]. In the minutes or hours after SCI, the reduced activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase results in oxidative stress because superoxide anion (i.e., O\textsubscript{2}\textsuperscript{−}) produced by various pathways is not scavenged promptly [28].

2.2.1.1 Enzymatic Response of Inflammatory Cells. Neutrophils are the first inflammatory cells of the immune system to be activated following SCI and to reach the lesion, with their number peaking 24 h after injury [27,29]. Neutrophils and monocytes migrate to the injury site due to the release of chemokines and cytokines, and to the upregulation of several adhesion molecules on endothelial cells. The three main functions of these immune cells include phagocytosis and scavenging of cellular debris, secretion of myeloperoxidase proteases and elastase, and the release of ROS [30]. Activation of microglia also occurs immediately after SCI, with the cells exhibiting altered protein expression profiles and morphology, while also migrating towards the lesion where they localize and proliferate. In addition, circulating monocytes infiltrate the injury site and differentiate into macrophages. These cells are very similar to activated microglia in terms of their protein expression profile, morphology, and function [31,32]. The rapid increase in the number of macrophages generates substantial quantities of O\textsubscript{2}\textsuperscript{−} via nicotinamide adenine dinucleotide phosphate oxidase (NOX) activity [33]. Moreover, infiltrating macrophages deliver ROS, protein hydroxyls, and inflammatory cytokines to the lesion microenvironment [34]. Central nervous system (CNS) cells have significant levels of phosphorylated adenine dinucleotide [NAD(P)H], with oxidation of this molecule being the primary source of O\textsubscript{2}\textsuperscript{−} production in these cells [35].

2.2.1.2 Ferritin and Transferrin. Iron homeostasis plays a crucial role in the metabolic activities of the CNS, including oxidative phosphorylation, neurotransmitter production, and myelin synthesis [36]. Under physiological conditions, iron exists as a complex within ferritin and transferrin. However, due to the significant decrease in tissue pH in the injured area after SCI, ferric ions tend to be released from ferritin and transferrin. In the presence of iron, the Fenton reaction generates O\textsubscript{2}\textsuperscript{−}, resulting in the formation of ·OH. In addition, the reduction of O\textsubscript{2}\textsuperscript{−} allows the release of iron bound to the transferrin and ferritin proteins [37,38]. Furthermore, during hemorrhage after SCI, the iron released from hemoglobin generates ·OH by catalyzing hydrogen peroxide [39].
2.2.1.3 Mitochondria and the Endoplasmic Reticulum.

The mitochondrial respiratory chain is a prominent intracellular generator of ROS. As a significant site of ROS synthesis, mitochondria can trigger and regulate both pyroptosis and autophagy following SCI [40,41]. Under normal physiological conditions, the energy-dependent intramitochondrial Ca$^{2+}$ buffering system maintains homeostasis by removing excess Ca$^{2+}$ [42]. However, mitochondria in calcium-dependent neurons initiate cell death by regulating cytoplasmic Ca$^{2+}$ levels under pathological conditions [42]. The excessive demethylation that occurs after SCI leads to depletion of adenosine triphosphate (ATP), imbalance of the standard Ca$^{2+}$ buffer system, and an increase in the intracellular Ca$^{2+}$ level [43]. This results in activation of Complex-I (NADH), followed by increased ROS production and ATP [44]. Elevated ROS levels lead to increased permeability of the mitochondrial membrane, resulting in the formation of pores on the mitochondrial surface. The formation and opening of these pores lead to mitochondrial Ca$^{2+}$ overload, resulting in reduced mitochondrial membrane potential and disruption of the mitochondrial respiratory chain. These events lead to the continued release of excess ROS [45,46]. Chronic mitochondrial dysfunction results in the initiation of a mitochondrial self-destruct signal, the generation of significant ROS, and the induction of mitochondrial autophagy. In addition, changes in REDOX homeostasis are highly sensitive to the disulfide bonds of proteins translated in the ER. Inflammation and glutamate excitotoxicity during SCI can cause ER stress, resulting in atypical protein aggregation, protein misfolding, and impaired Ca$^{2+}$ homeostasis [47]. During oxidative protein folding, the protein disulfide isomerase receives electrons from the protein folding substrate and oxidizes the sulfhydryl group on the cysteine residue to form disulfide bonds [48]. ROS is then generated by endoplasmic reticulum oxidase 1α (ERO1α), which receives electrons from protein disulfide isomerase and transmits them to molecular oxygen. As a signaling intermediate, ROS alleviates ER stress through periodic responses. Under conditions of ER stress, ROS levels increase due to the delayed expression of proteins, including C/EBP homologues [49,50]. At the ER-mitochondrial junction, Ca$^{2+}$ transfer from the ER leads to mitochondrial dysfunction and a subsequent increase in mitochondrial ROS production. This results in cellular oxidative damage, or neuronal cell death.

2.2.2 ROS-Induced Inflammasomes

Previous studies have demonstrated that ROS, particularly those produced by mitochondria, contribute to the activation of NLRP3 inflammasomes [51–54]. Many NLRP3 inflammasome activators have been reported to trigger mitochondrial ROS production in a variety of cell types, and NADPH oxidase was originally thought to be the source of ROS production. ROS is therefore thought to be a common signal for NLRP3 inflammasome activation [55]. Palmi-

tate, a saturated fatty acid, has been shown to activate NLRP3 inflammasomes, leading to the release of active IL-1β from mitochondria in a ROS-dependent manner [56]. In response to several NLRP3 triggers, the level of oxidized mitochondrial DNA (mtDNA) in the cytoplasm increases and binds to NLRP3 to activate the NLRP3-inflammasome complex, leading to mitochondrial dysfunction and cell death [24]. According to Nakahira et al. [54], the production of mtROS by malfunctioning mitochondria is required for the activation of NLRP3 in response to LPS and ATP, and the release of mtDNA into the cytosol depends on NLRP3 and mtROS. Subsequently, Nakahira et al. [54] found that mtDNA interacts with NLRP3 and AIM2, and that oxidized mtDNA is specific for the activation of NLRP3 inflammasomes. Another study reported that both ROS-dependent and ROS-independent NLRP3 activators play a role in mitochondrial instability and dysfunction, leading to the activation of NLRP3 inflammasomes [23].

2.3 Release of CTSB

Several studies have shown that macrophages induce the activation of NLRP3 inflammasomes following the phagocytosis of particulate matter such as amyloid beta, silica, MSU, calcium crystals, and cholesterol crystals [55,57–59]. The phagocytosis of particulate matter damages lysosomes and causes leakage of lysosomal contents into the cytoplasm. Release of CTSB through lysosomal disruption appears to be a crucial step in the particulate-induced activation of NLRP3 inflammasomes, as this is inhibited in macrophages treated with CA-074-Me, a chemical inhibitor of CTSB [58,60]. Ellis et al. [61] have shown that CTSB expression is upregulated following SCI, suggesting that it may be involved in the secondary injury cascade that lasts for up to a week. However, the source of CTSB after SCI has not yet been clearly identified. Further elucidation of the source of CTSB after SCI and its relationship with cellular pyroptosis should therefore help to understand its mechanism of action.

3. Overview of the Cellular Pyroptosis Pathway

Pyroptosis plays a crucial role in the pathophysiological mechanisms of multicellular organisms by maintaining a dynamic balance between cell proliferation and cell death. Currently, four distinct cellular pathways of pyroptosis have been identified: canonical inflammasomal pathways, noncanonical inflammasomal pathways, apoptotic caspase-mediated pathways, and granzyme-based pathways (Fig. 3). In all four pathways, GSDM protein acts as the final target and is cut by upstream caspase or granzyme. In general, inflammasome-dependent pyroptosis includes caspase-1-dependent pathways (canonical) and caspase-4/5/11-dependent pathways (noncanonical) [62,63]. Recent studies have identified novel path-
ways that are not reliant on inflammasomes, including the caspase-3/8-mediated pathway, as well as other GSDM-mediated pathways [64,65].

3.1 Canonical Pathway

The classical inflammasome pathway was the first to be discovered. Inflammasomes are multiprotein complexes that assemble in response to either pathogen-associated molecular patterns (PAMPs), or non-pathogen-related damage-associated molecular patterns (DAMPs). In general, inflammasomes are composed of intracellular pattern recognition receptor (PRR), apoptosis-associated speck-like protein containing CARD (ASC), and inflammatory caspase [66]. In the canonical pyroptosis pathway, PRR forms functional inflammasomes by sensing DAMPs after SCI. The PRR, which recognizes the pathogenic stimulus, then binds to pro-caspase 1 with the help of the adaptor protein ASC, thus eventually forming the inflammasome. After formation of the inflammasome, caspase-1 is activated and cleaves inactive pro-IL-1β and pro-IL-18 through proteolysis. It also cleaves GSDMD to release GSDMD-N and free the pore-forming domain from GSDMD-C inhibition. The released GSDMD-N is recruited to the inner part of the cell membrane and oligomerizes to form transmembrane pores [67]. These pores further exacerbate the inflammatory response in the localized spinal cord by allowing the efflux of K+ and influx of water. This causes cell swelling and rupture, and the release of cellular contents including bioactive IL-1β and IL-18 [68].

3.2 Noncanonical Pathway

The majority of Gram-negative bacteria activate atypical inflammasomes via the noncanonical inflammasome pathway, which is separate from the classical inflammasome complex. Caspase-4/5/11 in host cells is activated upon recognition of Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, which subsequently initiates GSDMD cleavage and pyroptosis [68,69]. Although caspase-11 is able to lyse GSDMD, it is unable to convert the precursors of IL-1β and IL-18 into their biologically active forms. Thus, the noncanonical pathway requires activation of NLRP3 and caspase-1 through K+ efflux induced by the GSDMD-NT pore, which then plays a role in the production of mature IL-1β and IL-18 [70]. In addition, activated caspase-11 can cleave the Pannexin-1 channel, allowing ATP to flow into the extracellular space and bind to P2X7R. This ultimately leads to K+ efflux and subsequently to NLRP3-associated pyroptosis [71]. Since aseptic inflammation is the main manifestation after SCI, the noncanonical approach is not applicable to secondary SCI.

3.3 Inflammasome-Dependent Pathways

3.3.1 Caspase-3/8-Mediated Pathway

Caspase-3 has long been regarded as a key marker of cell apoptosis and is generally considered to be a pivotal effector of apoptosis-mediated cell death. However, recent studies have shown that it can also stimulate GSDME to perform pyroptosis. Members of the gasdermin protein family are structurally highly conserved. All gasdermins except DFNB59 contain C-terminal and N-terminal domains, with the latter being an activator of pyroptosis [72]. Notably, activation of caspase-3 results in the cleavage of GSDME and subsequent formation of GSDME-N termini. This leads to the creation of pores in the cell membrane region, ultimately resulting in pyroptosis in a process akin to GSDMD-N [64,73].

Furthermore, pro-caspase-8 serves as an initiating caspase that can undergo self-processing upon binding to the Fas tumour necrosis factor family of death receptors. Upon binding the Fas ligand (FasL or CD95L), the Fas receptor (a 45 kDa membrane receptor) associates with the adaptor protein FADD (a member of the death domain superfamily) and pro-caspase-8 to form a death-inducing signaling complex (DISC). Subsequently, either directly or through a mitochondria-dependent mechanism, the activation of caspase-8 can trigger cleavage of downstream targets such as caspase-3 [74]. Thus, the regulation of cell pyroptosis by GSDME and GSDMD may provide new insights into the inflammatory cell death process. Further studies are needed to investigate the underlying mechanisms and significance of this process in various diseases, including CNS disorders such as SCI.

3.3.2 Granzyme-Mediated Pathway

Recent work indicates that granzymes derived from cytotoxic T lymphocytes and natural killer cells can be delivered to target cells via perforin to induce cancer cell pyroptosis through the cleavage of specific gasdermin family members [75]. Granzyme A (GZMA) is the most prevalent serine protease in the granyme family and can promote the release of inflammatory factors and induce pyroptosis by cleaving GSDMB. Furthermore, granzyme B (GZMB) from natural killer cells can trigger GSDME-dependent pyroptosis in tumor cell targets through direct cleavage of GSDME and indirect activation of caspase-3 [76]. Recent studies by Wu et al. [77] have shown that ferric ions can induce the pyroptosis of B cells after SCI by activating the Tom20-Bax-caspase-GSDME pathway. However, these pathways have yet to be explored in other immune cell types following SCI.

4. Therapeutic Strategies Targeting Pyroptosis in SCI

Therapeutic strategies that target cellular pyroptosis after SCI are still relatively limited and focus on the following areas: (1) inhibition of priming and activation of
Fig. 3. Schematic illustration of the molecular mechanisms of NLRP3 activation after SCI. (A) In the canonical inflammasome pathway of SCI, DAMPs such as ATP or ROS stimulate inflammasomes, which then activate caspase-1 to cleave Gasdermin D (GSDMD) for pore formation. (B) In vitro simulation of post-SCI inflammation. Lipopolysaccharide (LPS) from Gram-negative bacteria is used to directly activate caspase-4/5/11, followed by GSDMD-mediated lysis leading to atypical inflammasome pyroptosis pathways. However, this pathway is rarely reported in mouse SCI models. (C) The caspase-mediated apoptosis pathway following SCI involves caspase-3/Gasdermin E (GSDME), caspase-8/Gasdermin C (GSDMC), GSDMD, and other mechanisms. (D) In the granzyme-mediated pathway, Granzyme A (GZMA) or GZMB derived from cytotoxic lymphocytes, Chimeric Antigen Receptor (CAR) T cells and natural killer cells can cleave GSDMB or GSDME to cause pore formation or pyroptosis, respectively. ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; TNFR, tumor necrosis factor receptor. This figure was created with Biorender (https://www.biorender.com/).

the NLRP3 inflammasome; (2) the promotion of cellular autophagy to inhibit pyroptosis based on autophagy-pyroptosis crosstalk; and (3) targeted inhibition of the pyroptosis of different cell types in the spinal cord (Fig. 4).

4.1 Targeting of NLRP3 Inflammasomes

NLRP3 is the most well-studied inflammasome sensor and is comprised of the nucleotide-binding domain (NACHT), the amino terminal PYRIN domain (PYD), and the carboxy-terminal LRR domain [78]. NLRP3 is a crucial integrator of cellular stress due to its capacity to react to multiple stimuli in cases of SCI. Stimuli such as extracellular ATP, lysosomal damage, and K⁺ membrane penetration can cause loss of NLRP3 inhibition [78,79]. NLRP3 is expressed in the majority of spinal cord cells and tissues, and is up-regulated in various cell types following SCI.

It is also worth noting that Nek7 plays a crucial role in the formation of mouse NLRP3 inflammasomes by binding to the NAHT of both NLRP3 and LRR [79]. Nek7 is essential for activation of NLRP3 inflammasomes, but not for activation of NLRC4 and AIM2 inflammasomes. Nek7 also regulates the formation of ASC specks, the oligomerization of NLRP3, and the activation of caspase-1 [80]. However, NLRP3 activation cannot be induced by the mere presence of Nek7. Both priming and activation are obligatory steps to achieve complete activation of NLRP3 [79]. The priming step enables cells to increase NLRP3 expression by stim-
Targeting Pyroptosis in SCI

Fig. 4. Therapeutic strategies for pyroptosis after SCI. (A) Inhibition of NLRP3 inflammasomes through: (1) Inhibition of NLRP3 transcription and post-transcriptional modification; and (2) Inhibition of the expression of caspase-1, and activation of NLRP3. (B) The promotion of autophagy after SCI inhibits pyroptosis through the caspase-3/8 pathway. (C) The targeting of neurons, microglia, astrocytes, pericytes and other cell types to attenuate parenchymal cell pyroptosis after SCI. This figure was created with Biorender (https://www.biorender.com/).

4.1.1 Inhibition of NLRP3 Inflammasomes: Priming after SCI

Two main signals are involved in the priming stage of NLRP3 [5]. Firstly, NLRP3 is upregulated by promoting transcription [78,79]. Once PAMP or DAMP molecules are recognized by PRR, the nuclear factor-κB (NF-κB) transcription factor is activated, thereby inducing transcription of NLRP3, pro-IL-1β and pro-IL-18 [78]. Several mediators, including Fas-associated proteins with death domains (FADD) and Myeloid Differentiation Primary Response 88 (MyD88), assist in promoting PRR signal transduction, which then readies the cells for the next step [79]. Priming not only upregulates NLRP3, but also induces its phosphorylation, ubiquitination and SUMOylation. Modified NLRP3 is kept in an inactive, self-inhibited, but signaling-potent state [5,78,79]. Wang et al. [81] demonstrated that TLR4 upregulates the dead-box helicase 3 X-linked (DDX3X)/NLRP3 axis through activation of the JAK2/STAT1 signaling pathway. Moreover, STAT1 was identified as a transcription factor that promotes the expression of DDX3X and is involved in the NLRP3 vesicle initiation program. Similarly, Shao et al. [82] found that TLR4 phosphorylated NF-κB p65 via PI3K/AKT signaling in BV-2 cells, thereby activating downstream pathways and releasing pro-inflammatory factors. Xu et al. [83] reported that TLR4 promotes the expression of lncRNA-F630028O10Rik following SCI. This results in activation of the PI3K/AKT pathway through the miR-1231-5p/Col1a1 axis, which ultimately regulates the pyroptosis of microglia.

4.1.2 Inhibition of NLRP3 Inflammasomes: Activation after SCI

Several mechanisms of NLRP3 activation are now widely recognized. These can act together or independently, and include pore formation and metabolic disorders, mitochondrial dysfunction, ion redistribution, lysosomal disorders and noncanonical models [78,84]. NLRP3 begins to activate and assemble when primed cells are subjected to activation stimuli under appropriate conditions [85]. Multiple factors can act as second signals for the activation and assembly of NLRP3 after SCI, including chole-
terol and ATP levels, cytoarchitectural instability such as lysosomal rupture and mitochondrial dysfunction, and ionic or molecular perturbations such as K+ efflux, Ca2+ signaling, and ROS [86]. Liu et al. [87] showed that treatment of BV2 cells with an advanced oxidative product (AOPP) induced NLRP3 inflammasome activation and GSDMD cleavage, leading to cleavage of caspase-1, downstream production of mature IL-1β and IL-18, and apoptosis. In addition, Zhao et al. [88] showed that lithium inhibits cell pyroptosis and reduces inflammation after SCI by suppressing caspase-1 expression, reducing oxidative stress, and inhibiting the activation of NLRP3 inflammasomes. Similarly, Xu et al. [89] showed that CD73 has an anti-focal death effect in SCI, partly by inhibiting activation of the NLRP3 inflammasome complex via adenosine-A2B AR-PI3K-AKT-Foxo1 signaling, thereby reducing the maturation of GSDMD.

4.2 Inhibition of Autophagy

Autophagy is a well-recognized lysosomal degradation process that breaks down protein aggregates and damages organelles and cytoplasmic proteins [90]. Autophagy-induced neuroprotection is initiated in both neurons and microglia during SCI and other CNS disorders [91,92]. This is a crucial process that helps to refresh cells and maintain homeostasis, which is especially vital for the health of terminally differentiated cells like neurons [93]. Many studies have demonstrated the vital role of autophagy in neurons [94,95]. Increased autophagy has been observed after SCI, and there is accumulating evidence that autophagy has pro-survival, neuroprotective effects by regulating the death of nerve cells [96]. In support of this, the stimulation of autophagy hinders both pyroptosis and necrotic apoptosis [97].

Necrotic apoptosis is similar to cellular pyroptosis in that both are inflammatory cell death pathways [98]. Mechanistically, RIPK1 and RIPK3 assemble to form a multi-protein complex known as the RIPK1/RIPK3 necrosome following stress-induced activation of the TNF receptor 1 (TNFR1) [99]. Subsequently, phosphorylation of the downstream molecule MLKL is facilitated by the necrosomes. p-MLKL then oligomerizes and moves to the plasma membrane, where it increases permeability to induce cell death [100]. Caspase-8 is also thought to be an important inhibitor of necrotic apoptosis [101].

A recent study based on Gene Expression Omnibus (GEO) analysis and RNA sequencing revealed the molecular mechanism by which cyclic helix B peptide improves functional recovery after SCI. It does this by inhibiting pyroptosis and reducing necroptosis through the enhancement of autophagy. Support for this mechanism was obtained using the autophagy inhibitor 3-methyladenine (3-MA), with regulation of autophagy shown to involve the transcription factor EB (TFEB) [102]. Two other studies also reported that promotion of autophagy by facilitating dephosphorylation and nuclear translocation of TFEB was effective in ameliorating pyroptosis and necrotic apoptosis in cells after SCI [103,104]. In addition, Wang et al. [105] showed that Bexarotene enhances the nuclear translocation of transcription factor E3, activates autophagy and mitochondrial autophagy, inhibits ROS production, and suppresses pyroptosis through stimulation of the AMPK-mTOR pathway in the cytoplasm and the AMPK-SKP2-CARM1 pathway in the nucleus. In conclusion, an effective therapeutic strategy after SCI may be to improve downstream pyroptosis by inhibiting autphagic flow through multiple pathways (Fig. 3).

4.3 Targeted Cell Types

4.3.1 Neurons

One of the main current therapeutic strategies is to maximize the preservation of residual neurons in secondary spinal cord injuries with microenvironmental imbalances. Pyroptosis is closely related to neuronal loss and is a key pathway for neuronal death following SCI [106,107]. Therefore, it is important to find a feasible treatment for neuronal pyroptosis after SCI. Jiang et al. [108] demonstrated that inhibiting the activation of NLRP3 inflammasomes and reducing GSDMD expression in neurons after SCI could inhibit the onset of pyroptosis and decrease the levels of pro-inflammatory cytokines. Similarly, Zheng et al. [107] showed that carbon monoxide inhibits signaling from inflammasomes and cell pyroptosis by reducing the activation of inositol-requiring enzyme 1 (IRE1), thereby reducing neuronal death and improving motor function recovery after SCI. Furthermore, substantial evidence indicates that inhibition of neuronal pyroptosis at the lesion site can improve the prognosis of rats with SCI [104,109,110].

4.3.2 Microglia

Pyroptosis is a stimulus-associated, programmed cell death pathway that can significantly influence neuroinflammation. It is a key factor driving selective injury after SCI and is activated by inflammasomes. Microglia are intrinsic immune cells in the CNS. They are significantly activated after SCI and are major participants in the subsequent neuroinflammatory process. There is substantial evidence showing increased microglial pyroptosis in CNS disorders such as SCI. An important step in the neuroinflammation observed following CNS injury is the activation of cytoplasmic inflammasome complexes leading to cell pyroptosis [111–113]. Xiong et al. [114] showed that application of Treg cell-derived exosomes after SCI significantly improved local neuroinflammation and facilitated functional recovery by inhibiting microglial pyroptosis in vivo.

4.3.3 Other Cell Types

Pyroptosis occurs widely after SCI, even in cell types that are less numerous than neurons and microglia. Therefore, the targeting of pyroptosis in other cell types may im-
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Examples</th>
<th>Animals</th>
<th>Experimental Models</th>
<th>Methods of administration</th>
<th>Mechanisms of action</th>
<th>Therapeutic effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exosomes</strong></td>
<td><strong>Treg cell-derived exosomes</strong></td>
<td><strong>Foxp3DTR mice</strong></td>
<td>Contusion SCI model</td>
<td>tail vein injection</td>
<td>miR-709/NKAP/NF-xB</td>
<td>Inhibition of microglia pyroptosis</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td><strong>BMSC-EXO</strong></td>
<td><strong>Sprague-Dawley rats</strong></td>
<td>Contusion SCI model</td>
<td>tail vein injection</td>
<td>miR-21/PTEN/PDCD4</td>
<td>Improvement of neuronal survival; inhibition of pericyte pyroptosis</td>
<td>[115]</td>
</tr>
<tr>
<td></td>
<td><strong>MSCs-EV</strong></td>
<td><strong>Sprague-Dawley rats</strong></td>
<td>Crush SCI model</td>
<td>tail vein injection</td>
<td>Inhibition of GSDMD</td>
<td>Inhibition of GSDMD; inhibition of microglia activation and pyroptosis</td>
<td>[117]</td>
</tr>
<tr>
<td><strong>TCM</strong></td>
<td><strong>Hesperetin</strong></td>
<td><strong>Sprague-Dawley rats</strong></td>
<td>C5</td>
<td>gastric gavage</td>
<td>increased Nrf2 signaling</td>
<td>Reduction of oxidative stress, inhibition of NLRP3 inflammasomes activation and pyroptosis</td>
<td>[119]</td>
</tr>
<tr>
<td></td>
<td><strong>Taxifolin</strong></td>
<td><strong>Sprague-Dawley rats</strong></td>
<td>Crush SCI model</td>
<td>gastric gavage</td>
<td>PI3K/AKT</td>
<td>Inhibition of pyroptosis-related gene and inflammatory factor expression; promotion of axonal regeneration</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td><strong>Betulinic acid</strong></td>
<td><strong>C57/BL6 mice</strong></td>
<td>Contusion SCI model</td>
<td>intraperitoneal injection</td>
<td>AMPK-mTOR-TFEB signaling pathway</td>
<td>Enhancement of autophagy and mitochondrial autophagy; reduction of ROS accumulation; inhibition of pyroptosis</td>
<td>[104]</td>
</tr>
<tr>
<td><strong>Tissue engineering</strong></td>
<td><strong>RM-LIP/MC</strong></td>
<td><strong>Mice</strong></td>
<td>/</td>
<td>injected into tail veins</td>
<td>/</td>
<td>Inhibition of activation of inflammasomes; inhibition of pyroptosis</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td><strong>H2S@SF hydrogel</strong></td>
<td><strong>Male ICR mice</strong></td>
<td>Control cortical impact</td>
<td>local coverage</td>
<td>H2S antioxidant effect</td>
<td>Reduction of endothelial cell pyroptosis; amelioration of oxidative stress</td>
<td>[122]</td>
</tr>
<tr>
<td></td>
<td><strong>EX-netrin1</strong></td>
<td><strong>Sprague-Dawley rats</strong></td>
<td>Contusion SCI model</td>
<td>tail vein injection</td>
<td>Uncl5b/PI3K/AKT/mTOR pathway</td>
<td>Reduction of inflammatory response and pyroptosis; promotion of axonal growth</td>
<td>[118]</td>
</tr>
</tbody>
</table>

Abbreviations: BMSC, Bone marrow mesenchymal stem cell; EXO, exosomes; TCM, Traditional Chinese Medicine; RM-LIP/MC, fabricated macrophage membrane-camouflaged liposomes; H2S@SF, H2S-releasing silk fibroin; EX-netrin1, engineered EX enriched in netrin-1; SCI, Spinal cord injury; TBI, Traumatic brain injury.
prove histological and functional outcomes after SCI. Zhou et al. [115] reported that exosomes derived from bone marrow mesenchymal stem cells effectively reduced pericellular pyroptosis, improved the integrity of the blood-spinal barrier, and promoted functional recovery after SCI. It was also reported that astrocytes play a role in neuroinflammation. Neuroinflammation induced by pyroptosis of astrocytes has been shown in several CNS diseases. A bioinformatics analysis by Shan et al. [116] demonstrated that erythropoietin reduces astrocyte pyroptosis both in vivo and in vitro by targeting the miR-325-3p/Gsdmd axis, thereby promoting functional recovery in a rat model of SCI.

5. Modes of Treatment

5.1 Exosomes

Extensive neuroprotection studies have demonstrated the involvement of exosomes sourced from cells of the CNS, PNS, mesenchymal, and other tissues involved in neural regeneration (Table 1, Ref. [104,114,115,117–122]). Compared to cell transplantation, exosomes are easier to obtain and store, and are subject to fewer ethical restrictions [123]. In addition, the outer body size is much smaller than the cell secretion, and therefore will not be captured in lung and liver tissue. Moreover, exosomes can penetrate the blood-spinal cord barrier [124]. Recent attention has therefore focused on the use of exosomes to treat pyroptosis in SCI. Sheng et al. [117] showed that loading miRNA-22 on MSCs-EV could inhibit the occurrence of microglial pyroptosis, reduce the expression of GSDMD, and reduce the opening of cell membrane pores and subsequent release of inflammatory factors. Similarly, another study found that netrin-1 enrichment with engineered exosomes alleviated LPS-induced inflammation and pyroptosis, while promoting recovery in SCI rats [118].

5.2 Traditional Chinese Medicine

Traditional Chinese medicine (TCM) is a promising supplementary treatment method that has attracted considerable attention in recent years. Studies have demonstrated that herbal active extracts, metabolites, and traditional botanical formulas are effective and can play an important role in the prevention and treatment of SCI, especially in reducing pyroptosis [125]. Hu et al. [120] showed that Taxifolin significantly reduced oxidative stress mediated by microglial activation, and inhibited the post-SCI expression of pyroptosis-associated proteins (NLRP3, Caspase-1, GSDMD, and ASC) and inflammatory cytokines (IL-1β and IL-18). Betulinic acid is a natural pentacyclic triterpenoid commonly used in TCM. It can penetrate the blood-brain barrier and play a neuroprotective role by promoting autophagy, reducing ROS, and inhibiting inflammation and pyroptosis [104]. Hesperetin (Hst) is a subclass of flavonoids that is widely found in citrus fruits such as oranges and pomelos. Hst has a variety of bioactive properties, including anti-inflammatory, antioxidant, and neuroprotective effects [126]. Recent studies have shown that Hst can attenuate pyroptosis in traumatic brain injury [127] and SCI [119]. In addition, Zhang et al. [128] showed that Piperine was able to promote functional recovery after SCI by inhibiting oxidative stress, inflammation and cellular death mediated by autophagy activation.

5.3 Tissue Engineering Material

With the rapid advances in tissue engineering, several biological substances have been extensively studied with regard to the treatment of SCI. These include natural elements such as alginate, hyaluronic acid, chitosan, etc., as well as synthetic materials such as polymers, nitrocellulose membranes, and biodegradable synthetic materials. The purpose of tissue engineering therapy is to reconstruct damaged tissues by embedding living cells or loading drugs, improving the injured microenvironment, and promoting nerve regeneration. Biomaterials employed for SCI typically require that they be biocompatible [129]. Moreover, they should possess adequate softness to avoid compressing the adjacent spinal cord tissue, while being sufficiently robust structurally to maintain local fixation [130]. In addition, they should have an appropriate rate of degradation [131]. Xiao et al. [132] implanted miR-138-modified umbilical cord mesenchymal stem cell-derived exosomes in a thermo-responsive hydrogel system into rats with SCI. This material was found to be biocompatible and able to decrease inflammation in BV-2 cells via the NLRP3-caspase-1 signaling pathway, while reducing neuronal apoptosis through down-regulation of intracellular ROS levels by Nrf2 [132]. Tang et al. [121] employed macrophage membrane-modified, liposome-loaded minocycline to effectively attenuate the pyroptosis process after SCI with high biocompatibility and advanced targeting efficacy. Although extensive data are still lacking, tissue engineering has shown great therapeutic potential for the inhibition of pyroptosis after SCI.

6. Conclusions and Perspectives

The important role of programmed cell death in SCI, and in particular pyroptosis, is gradually being uncovered. This article has reviewed the mechanisms and pathways of pyroptosis following SCI, including key players such as inflammasomes and gasdermin. In addition, we reviewed current therapeutic strategies for targeting pyroptosis after SCI. The inflammatory response and parenchymal cell death are important components of secondary SCI. Pyroptosis is a pro-inflammatory mode of cell death characterized by rapid rupture of the plasma membrane and subsequent leakage of cellular contents and cytokines (IL-1β and IL-18). It plays an important role in secondary SCI. The mechanism of pyroptosis in different cell types following SCI requires further investigation. In addition, the study of upstream and downstream proteins associated with the signaling pathway may provide new ideas for the treatment of CNS trauma.
Although newly developed pyroptosis therapeutic strategies have shown great promise for SCI, the research and potential applications of pyroptosis in SCI are still quite limited. For experimental purposes, the use of TCM, exosomes, miRNAs, etc., in combination with tissue-engineered materials, may further improve therapeutic efficacy. Further research should focus on the flexibility of pyroptosis in regulating cell death, and explore potential mechanisms of pyroptosis in other cell death types in SCI, rather than just a single function of pyroptosis such as lethality or mediation of one mode of death. In addition, there are few studies showing cell specific effects in vitro. Specially in neuronal cultures, thus cell specific targets of the drugs in an in vitro experiment with neuronal cultures will be essential. Moreover, the discovery of marker molecules and associated metabolic mechanisms of pyroptosis-related death, the exploration of clinically applicable targeted agents, and the clinical application of these markers in combination with pyroptosis regulation may transform disease treatment.

In summary, there is growing evidence that pyroptosis plays a dual role in promoting inflammation and cell death after SCI. Elucidation of the pathogenic mechanism of pyroptosis, and the potential benefit of inhibiting pyroptosis, could provide a rational, mechanism-guided model for treating SCI.

**Author Contributions**

JS, GWS and YNZ were responsible for the study conception and design. GJinG, HQY, and HSZ performed the literature review. GJinG, HQY, HSZ, WJK, and PPZ wrote the original manuscript. PPZ, GJieG, JL, WHS and PCC contributed to preparation of the table and figures. JS, WHS and PCC revised the manuscript. All authors have contributed to the acquisition and analysis of literatures. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

**Ethics Approval and Consent to Participate**

Not applicable.

**Acknowledgment**

Some of the figures were created with biorender.com ([https://www.biorender.com](https://www.biorender.com)). We apologize to authors whose excellent studies were within the scope of this review, but were not mentioned owing to space limitations.

**Funding**

The authors are grateful to the following organizations for partial funding of this research: the Scientific Research Project of Traditional Chinese Medicine in Shanxi Province (No.2023ZYJB2048), key medical research projects of Shanxi Province (2020XM51), and Basic Research Project of Shanxi Province (20203021221295).

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


[55] Dostert C, Péttrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflamma-


[63] Wang YY, Liu XL. Zhao R. Induction of Pyroptosis and Its Im-


[66] Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: toward a better understanding of complex mecha-


[70] Rühl S, Broz P. Caspase-11 activates a canonical NLRP3 inflam-
masome by promoting K(+) efflux. European Journal of Im-
munology. 2015; 45: 2927–2936.


