Noncanonical pyroptosis pathway: a promising target of traditional Chinese medicine in the treatment of sepsis-related injury

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Abstract
Sepsis is a life-threatening disease of organ failure caused by dysregulated host responses to infection and other infectious factors. Multi-organ injury is the leading cause of high mortality and septic shock during sepsis. Recent studies suggest that noncanonical pyroptosis, characterized mainly by the direct activation of caspase-11-gasdermin D-mediated pyroptosis by cytoplasmic lipopolysaccharide, is closely related to sepsis-related organ injury. Here, this review summarizes recent advances in the regulatory mechanisms and targeted natural products from traditional Chinese medicine of the noncanonical pyroptosis pathway in sepsis-related injury.

Keywords: sepsis-related injury; noncanonical pyroptosis; caspase-11; gasdermin D

Citation
**Background**

Sepsis is a life-threatening disease of organ failure caused by dysregulated host responses to infection and other infectious factors [1]. As the leading cause of in-hospital death globally, sepsis accounts for about 13.1% in-hospital deaths and results in a large financial burden in China [2]. The pathogenesis of sepsis is quite complicated and has not yet been fully understood, although numerous literatures provide information on its pathophysiology. There have been some advances in the clinical treatment of sepsis over the past two decades, particularly during the past COVID-19 pandemic, while no proposed targeted drug or “druggable” target for sepsis and septic shock can improve survival in phase 2 and 3 clinical trials [3]. Therefore, the clinical treatment of sepsis is in urgent need of reliable therapeutic targets and targeted drugs with good accessibility.

**Pyroptosis pathway in sepsis**

In 2016, the third international consensus definitions for sepsis and septic shock (Sepsis 3.0) were published [1]. According to Sepsis 3.0, the new definition of sepsis suggests two major concerns: immune dysfunction responsible for local and systemic inflammation, and the resulting organ injury and failure associated with the high mortality of sepsis. Innate immune cells such as macrophages, natural killer cells, and neutrophils play critical roles in immune defense, including pathogen engulfment, antigen presentation, and inflammation in sepsis. However, the release of toxins and other stressors in sepsis leads to functional dysfunction and exhaustion of these cells, leading to programmed cell death, including necroptosis, PANoptosis, pyroptosis and ferroptosis, which are closely linked to sepsis-related organ injury [4].

Among the various forms of cell death, pyroptosis is a special form that occurs in monocytes and macrophages, and is characterized by short-term inflammatory activation of the caspase family, the formation of pores in the cell membrane and DNA damage but preservation of the cell nucleus intact [5]. According to the trigger mechanism, pyroptosis consists of canonical pyroptosis and noncanonical pyroptosis. Canonical pyroptosis is a well-studied form known as pattern-recognition receptors (PRRs) and caspase-1-dependent. In detail, pathogens and their pathogen-associated molecular patterns are recognized by PRRs, then sequentially induce the priming and activation of inflammasomes and then activate caspase-1, ultimately leading to the maturation and secretion of IL-1β and IL-18 and to activate gasdermin D (GSDMD) into N-terminal GSDMD (NT-GSDMD), which triggers pyroptosis by forming GSDMD pores on the plasma membrane. Canonical pyroptosis has been well-studied in various models and diseases. However, studies on noncanonical pyroptosis mainly focus on immune cells. In 2011, Kayagaki N and colleagues reported a novel “noncanonical pyroptosis” pathway in mice in which cytosolic lipopolysaccharide (LPS) circumvents PRRs and caspase-1, which directly activates caspase-11 (known as caspase-4/5 in human), can also trigger NT-GSDMD-mediated pyroptosis of monocytes and macrophages [6].

In the last decade, numerous groups, notably the laboratory of Shao F [7], have elucidated the noncanonical pyroptosis pathway, which was not only related to but also distinct from the canonical pathway (As shown in Figure 1).

**Regulatory mechanisms of noncanonical pyroptosis in sepsis**

Numerous regulators have been reported to be involved in the regulation of the noncanonical pyroptosis pathway. Previous studies showed that some endogenous antimicrobial peptides, such as LL-37 and ECPep D, could bind directly to LPS, thereby attenuating LPS-induced pyroptosis in mice with cecal ligation puncature or bacteremia [8, 9]. In addition to the LPS-antagonizing mechanism, cytosolic LPS delivery is also involved in the regulation of noncanonical pyroptosis in sepsis. In 2018, Deng MH and colleagues reported that high mobility group box 1 (HMGB1) released from hepatocytes bound LPS and resulted in LPS leakage into the cytosol and caspase-11 activation. Depletion of HMGB1 prevented caspase-11-dependent noncanonical pyroptosis and protected mice from lethal sepsis [10]. Subsequently, caspase-11 activation is another crucial regulatory mechanism in noncanonical pyroptosis. Except for interferon β and toll-like receptor 4 which were well-studied

Figure 1 Canonical pyroptosis requires inflammasomes primed by the TLR4-NF-κB signaling activated by Gram-negative bacteria. The activated inflammasome cleaves pro-caspase-1 into mature caspase-1, which further cleaves full-length GSDMD into NT-GSDMD, which eventually forms GSDMD pores indicative of pyroptosis on the plasma membrane, and also pro-IL-1β/18 into IL-1β/18 which are involved in the cytokine cascade. In noncanonical pyroptosis, bacteria-containing vesicles induce IFN-β production which primes pro-caspase-11 via the IFN-STAT1 pathway, and then cytolytic LPS escaped from the vesicle activates caspase-11-dependent maturation of NT-GSDMD, which results in the formation of GSDMD pore. TLR4, toll-like receptor 4; GSDMD, gasdermin D; NT-GSDMD, N-terminal gasdermin D; IFN-β, interferon β; LPS, lipopolysaccharide.
activators that primed caspase-11 transcription, OspC3, an effector of type III secretion system, was reported to induce ADP-ribosylation of caspase-11 thereby inhibit LPS-caspase-11 binding and paralyze pyroptosis-mediated defense in Shigella-infected mice [11]. The formation of GSDMD pores is also a key factor in the regulation of pyroptosis, regardless of the canonical or noncanonical form. A previous report indicated that IpaH7.8, a Shigella ubiquitin ligase, targeted gasdermin D degradation to prevent pyroptosis [12]. Recently, another group further reported the crystal structure of the IpaH7.8-GSDMB complex, which showed how IpaH7.8 acted on GSDMB pore formation [13].

Traditional Chinese medicines target noncanonical pyroptosis in the treatment of sepsis

Sepsis-related organ damage is the leading cause of high mortality and septic shock. The Sepsis 3.0 consensus recommends a Sequential Organ Failure Assessment scoring system to assess multi-organ failure [1]. In the clinic, the Sequential Organ Failure Assessment score is applied to assess whether the physiological function of six systems, including the cardiovascular, respiratory, renal, neurological, hematological and hepatic systems, is normal. Although both canonical and noncanonical pyroptosis are involved in the inflammatory immune response, pathogen clearance, and cell damage in sepsis, recent literature suggests that the noncanonical form is more closely related to organ injury in sepsis [14]. Furthermore, genetic modification of caspase-11 and GSDMD, the key regulators of noncanonical pyroptosis, has shown benefits in the treatment of sepsis organ injury [5]. Therefore, noncanonical pyroptosis is considered a promising target for sepsis organ injury studies.

Several antimicrobial peptides such as LL-37 and ECPep D showed protective effects on mice with cecal ligation puncture or bacteremia by directly binding to LPS [8, 9]. This intervention strategy is achieved by directly antagonizing pathogenic molecules, but is also limited by the transmembrane transport efficiency of peptides. Therefore, the medicinal chemicals that easily cross the cell membrane are considered more ideal modulators of noncanonical pyroptosis. Recently, glycyrhrizin was reported to be a competitive inhibitor of HMGB1, blocking HMGB1-dependent cytokotic delivery of LPS and showing a protective effect on organ injury in endotoxia and experimental sepsis [15]. A Radix iatrodis-derived alkaloid, goitrin, was reported to possess protective effect on LPS-induced septic mice by inactivating the noncanonical inflammasome pathway, thereby decreasing the levels of cleaved gasdermin-11 and NT-GSDMD [16]. Similarly, another report indicated that caffeic acid protected macrophages from both nigericin-induced canonical and cytokotic LPS-induced noncanonical pyroptosis and alleviated LPS-induced sepsis in mice by directly acting on GSDMD and thus preventing the formation of NT-GSDMD [17]. In addition, a recent report suggested that maclurin markedly suppressed the proteolytic activation of caspase-11 and GSDMD and could significantly alleviate acute lethal sepsis in mice [18]. In contrast, gossypol was reported to increase the mortality of mice in a bacterial sepsis model by inducing noncanonical pyroptosis in macrophages [19]. In addition to these natural products, some traditional Chinese medicine extracts have also been shown to inhibit noncanonical pyroptosis in sepsis model. A recent literature suggested that Artemisia argyi methanol extract alleviated inflammatory responses by inactivating caspase-11-dependent noncanonical pyroptosis in macrophages, thereby increasing the survival rate of LPS-induced lethal sepsis in mice without significant toxicity [20]. Another study suggested that Danhong injection could protect mice with endotoxia by suppressing the formation of GSDMD pore, and the main active ingredient was salvianolic acid E [21]. Overall, these natural product-based strategies show good inhibition of noncanonical pyroptosis activation and there is no problem in drug delivery into cells. Further research is needed to elucidate the intricate interplay between noncanonical pyroptosis pathways and to develop effective drug candidates against sepsis-related injury.

Conclusions and future directions

In summary, cytosolic LPS induces a caspase-11-dependent noncanonical pyroptosis pathway that plays a crucial role in sepsis-related organ injury. Although great progress has been made in this area, there are still many questions. Future studies on noncanonical pyroptosis should focus on two questions: first, the detailed mechanism of LPS escape from phagocytic vesicles into the cytoplasm and second, the details of the molecular interaction of caspase-11 activated by LPS. This will contribute to the development of promising drugs. In addition, it is also challenging for researchers to find new inhibitors of noncanonical pyroptosis from a variety of traditional Chinese medicines and their active ingredients such as flavonoids and terpenoids.

References


