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Progress of pyroptosis in acute pancreatitis

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Acute pancreatitis (AP) is a complex abdominal disease with high morbidity and mortality that involves a strong local and systemic inflammatory response. When AP develops into severe acute pancreatitis (SAP), it easily leads to multiple organ dysfunction syndrome, which is also one of the main reasons for death in SAP patients.[1] In the past decade, we have made substantial progress in our understanding of the pathophysiological mechanism of AP. The mechanism of calcium-mediated acinar cell damage and death has been elucidated. The cytoprotective effects of the unfolded protein response and autophagy in preventing continuous endoplasmic reticulum stress, apoptosis, and necrosis has been shown. The central role of unsaturated fatty acids in causing pancreatic organ failure has also been confirmed. In contrast, we know very little about the mechanism of parenchymal cell death.[2] Early and effective regulation of pancreatic acinar cell death may be an effective treatment for AP. In recent years, a new type of cell death called pyroptosis has become an important research topic in the field of inflammation and immune diseases. Recent studies have shown that pyroptosis is involved in the pathogenesis of pancreatic parenchymal cell death. Inhibiting pyroptosis can reduce pancreatic damage and has an important impact on the progression of AP.[3,4] This article reviews the research progress on pyroptosis in AP, with a focus on understanding the pathogenesis of AP and providing references for the identification of new therapeutic targets [Supplementary Figure 1, http://links.lww.com/CM9/A630].

In AP, a variety of cell death modes, including apoptosis, autophagy, necrosis, necroptosis, and pyroptosis are involved, and other types of programmed cell death (PCD) also affect the development and prognosis of AP.[4] Apoptosis and necrosis are the two main types of cell death. Apoptosis preserves the structural integrity of the plasma membrane, while necrotic cells release their components, damage neighboring cells, and promote the infiltration of inflammatory cells in the organ.[5] Necroptosis is also a type of PCD that stimulates the phosphorylation of receptor-dependent serine/threonine protein kinase 1 and receptor-interacting serine/threonine protein kinase 3 (RIPK3), eventually leading to the phosphorylation of the mixed-lineage kinase domain pseudokinase (MLKL). Phosphorylated MLKL multimerizes and forms pores in the plasma membrane, leading to cell lysis.[2] The various types of cell death are not independent, and they also have complicated regulatory mechanisms. For example, caspase-8 is an important initiator of apoptosis. Recent studies have shown that in the absence of apoptosis and necroptosis, the catalytic inactivation of caspase-8 may trigger pyroptosis.[3,5]

The concept of pyroptosis was first proposed by Cookson and Brennan in 2001 and was used to describe caspase-1-dependent rapid cell death in Salmonella-induced macrophages.[6] The current definition of pyroptosis is mainly as follows: pyroptosis is an inflammatory PCD process and a functional product of NLR family pyrin domain-containing protein 3 (NLRP3) and other inflammatory bodies regulated by pattern recognition receptors (PRRs). Caspase-1, caspase-4, caspase-5, and caspase-11 directly cleave the substrate gasdermin D (GSDMD) to produce the N-terminal fragment, GSDMD-mediated pore formation in the plasma membrane, followed by cell swelling and cell membrane rupture, which releases a large number of cytoplasmic contents. Later studies showed that caspase-3, caspase-7, and caspase-8, which play roles in apoptosis, are also involved in the regulation of pyroptosis.[7,8] For example, caspase-8, similar to caspase-1, may directly process interleukin-1β (IL-1β) and activate NLRP3 inflammatory mediators. Similarly, RIPK3 and MLKL-dependent necroptosis signals can activate the NLRP3 inflammasome and the production of IL-1β, an effector that indirectly induces pyrolysis.[8]

Studies have shown that damaged acinar cells have the characteristics of pyroptosis and participate in AP in a variety of ways.[1] When damage occurs, acinar cell

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damage or death induces damage associated molecular patterns (DAMPs) and activates Toll-like receptor 4 (TLR4) and TLR9 in immune cells and nucleotide-binding oligomerization domain contains protein 1. These pathways induce nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and the expression of the NLRP3 inflammasome and its effectors (IL-1β, IL-18, and high mobility group protein B1 [HMGB1]) to trigger sterile inflammation in the pancreas and play a role in expanding the inflammatory response. When AP further develops into SAP, intestinal endotoxemia-induced DAMPs and pathogen-related molecular patterns (PAMPs) and TLR4-mediated acinar cells release caspase-1 and IL-18, and the activation of IL-1β has a sensitizing effect on pancreatic damage.[4,5]

The innate immune system has many ways to fight pathogens, including antimicrobial peptides on the mucosal surface, activation of the complement system in the blood, the chemical attraction of immune cells to the infected site, and the coordinated activity of PRRs.[7,8] PRRs mainly detect PAMPs or DAMPs. Studies have shown that dead and injured pancreatic acinar cells can release DAMPs, such as histones, DNA, and heat shock proteins. The NLRP3 inflammasome is by far the most characteristic and largest multimeric protein complex, and it is also the main inflammasome that is involved in pyroptosis.

Current research mainly divides pyroptosis into classic and non-classic types according to the source of the signal and the method of activation. Caspase-1-dependent pyroptosis, commonly referred to as the classic pyroptosis pathway, is a vital part of the natural immune response to pathogens.[3] The classic approach involves the recognition of PAMPs or DAMPs through NLRP3. PRRs oligomerize with the linker apoptosis-associated speck-like protein containing caspase recruitment domain (ASC) to form the inflammasome, and the inflammasome recruits caspase-1 through the adaptor protein ASC, which leads to caspase-1 multimerization, promotes the maturation of pro-IL-1β and pro-IL-18, and cleaves GSDMD to produce N-terminal fragments, which induce pore formation, cytokine release, and pyroptosis.[8,9] The non-classic inflammasome pathway mainly involves the direct binding of murine caspase-11 or human caspase-4 or caspase-5 with toxins and lipopolysaccharides on gram-negative bacteria (including *Bacillus rotatum*, *Escherichia coli*, and *Vibrio cholerae*), leading to GSDMD cleavage and the release of its N-terminal domain, and also can indirectly activate the classic NLRP3-ASC-caspase-1 pathway, leading to the processing and release of IL-1β and IL-18.[9]

The gasdermin (GSDM) family consists of gasdermin A (GSDMA), gasdermin B (GSDMB), gasdermin C (GSDMC), GSDMD, gasdermin E (GSDME), and pejvakin.[10] GSDMD is necessary after the activation of classic and non-classic pyroptosis. Caspase cleaves GSDMD to release the N-terminal effector domain from the C-terminal inhibitory domain. The N-terminal domain oligomerizes on the cell membrane to form a pore with a diameter of 10 to 16 nm, leading to the loss of the cell ion gradient and water influx, after which the cell swells and secretes smaller diameter substrates, such as IL-1β and IL-18. Membrane pores will continue to increase the imbalance between the inside and outside of the cell, eventually leading to the occurrence of pyroptosis.[3,10] In addition to GSDMD, other GSDM family members, such as GSDMA, and GSDME (also known as DFNA5), all have N-terminal domains and show similar pore-forming activities.[11] The discovery of GSDMD has promoted explosive growth in research on the molecular mechanisms of inflammasome-mediated cell death and the crosstalk between pyroptosis and other cell death pathways (including apoptosis). However, the specific mechanism by which GSDMD triggers pyroptosis is still unclear.[11,12]

The membrane pores formed by the pyroptosis effector GSDMD can release cytoplasmic contents, with the result that inflammasome effectors (such as IL-1, IL-1β, and IL-18) and inflammatory mediators (such as eicosanoid compounds) are released to the extracellular environment, but the membrane pores are small enough to retain organelles and trap bacteria to prevent bacterial spread, thus forming a structure called pore-induced intracellular traps (PITs). PITs maintain the presence of bacteria, coordinate the innate immune response, and complement and scavenger receptors to drive the recruitment of neutrophils, the formation of neutrophil extracellular traps (NETs), or the recruitment of macrophages to engulf and kill the captured bacteria. This function of pyroptotic is considered to be a major defense mechanism against ubiquitous environmental pathogens.[9,10] Before GSDMD pores induce membrane rupture, cells can repair the membrane pores to a certain extent to negatively regulate the progression of pyroptosis and the release of cytokines. Studies have shown that in macrophages, this process is coordinated by the endosomal sorting complex (ESCRT), which is required for transport. ESCRT is a highly conserved transport system that is ubiquitous in yeast and other eukaryotic cells, and plays a role in repairing light-induced plasma membrane damage, multivesicular body formation, virus germination, and cell division.[13,14] The repair process is a signal for calcium influx through the GSDMD pore to initiate cell membrane repair. Ca^{2+} enters the cell through damaged sites and triggers local exocytosis, which is a necessary process for plasma membrane repair. Ultrastructural analysis shows that there are large intracellular vesicles near the wound, which suggests that Ca^{2+} influx induces the homotypic fusion of intracellular vesicles, forming large extracellular membrane plaques, which can promote resealing when fused with the wound through ESCRT-mediated plasma membrane budding and shedding.[13,15]

Caspase-1, IL-1β, IL-18, and HMGB1 are induced by the NLRP3 inflammasome in pancreatic acinar cells in AP patients and are key factors affecting the disease progression of AP.[1] HMGB1 is the main endogenous ligand of TLR4 in AP. HMGB1 is released by immune cells (mainly white blood cells), regulated by caspase-1 activation, and coordinates the inflammatory response in the early stages of sepsis or AP. When HMGB1 is released in large amounts by damaged somatic cells, it may cause pyroptosis and immunosuppression, thereby weakening the host's ability to eradicate microbial infections.[2,4]

During AP, immune cells infiltrate the pancreas, and the cellular contents released by necrotic and damaged cells activate monocytes and neutrophils to further promote inflammation.[2] Neutrophils can produce NETs, which are mainly composed of granule protein, DNA, and other proteins. NETs capture microorganisms, activate myeloid cells, and promote clotting, which can cause duct blockage; NETS also activate proinflammatory signals, and prematurely activate trypsinogen. NETs are very important for the occurrence and development of AP. In recent years, with the discovery of pyroptosis and its effector GSDMD, we have found that pyroptosis is also involved in the formation and release of NETs.[9] As an effector of NETs, pyroptosis may be an important cause of exacerbated AP secondary acute lung injury (ALI).[16] In endotoxemia, high concentrations of endotoxin may persist and be abnormally located in the cytoplasm. Then, activated TLR4 induces the type I interferon response and complement C3-C3aR axis together to upregulate the expression of caspase-11, triggering the overactivation of caspase-11, leading to high levels of pyroptosis and promoting NET release, thereby exacerbating AP-related lung damage. NETs are a double-edged sword, and the discovery of the relationship between NETs and pyroptosis provides new treatment strategies for AP-induced remote organ damage.[9,16] Although the specific mechanism is not clear, NETs provide another important target for SAP treatment.

The clinical treatment of AP has undergone different challenging developmental stages. The current treatment guidelines for AP include intravenous fluids, dietary changes, analgesics, pancreatic secretion inhibitors (somatostatin and its analog octreotide), L-arginine, calcium antagonists, and different inhibitors of inflammatory mediators. Unfortunately, the use of standard drugs in AP is still disappointing. In addition, the existing drugs (somatostatin and octreotide) have short half-lives and very limited clinical efficacy.[2,4] With the discovery of pyroptosis, a new perspective is provided for the treatment of AP. For example, studies have shown that CircHIPK3 can promote inflammation and pyroptosis by regulating the miR-193a-5p/GSDMD axis in AR42J cells. Therefore, CircHIPK3 may become a new biomarker and therapeutic target for AP.[17]

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Conflicts of interest

None.

References

1. Habtezion A, Gukovskaya AS, Pandol SJ. Acute pancreatitis: a multifaceted set of organelle and cellular interactions. Gastroenterology 2019;156:1941–1950. doi: 10.1053/j.gastro.2018.11.082.
2. Lee PJ, Papachristou GI. New insights into acute pancreatitis. Nat Rev Gastroenterol Hepatol 2019;16:479–496. doi: 10.1038/s41575-019-0158-2.
3. Ye A, Li W, Zhou L, Ao L, Fang W, Li Y. Targeting pyroptosis to regulate ischemic stroke injury: molecular mechanisms and preclinical evidences. Brain Res Bull 2020;165:146–160. doi: 10.1016/j.brainresbull.2020.10.009.
4. Sundar V, Senthil Kumar KA, Manickam V, Ramasamy T. Current trends in pharmacological approaches for treatment and management of acute pancreatitis – a review. J Pharm Pharmacol 2020;72:761–775. doi: 10.1111/jphp.13229.
5. Kist M, Vucic D. Cell death pathways: intricate connections and disease implications. EMBO J 2021;40:e106700. doi: 10.15252/embj.2020106700.
6. Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. Trends Microbiol 2001;9:113–114. doi: 10.1016/s0966-842x(00)01936-3.
7. Vince JE, Silke J. The intersection of cell death and inflammasome activation. Cell Mol Life Sci 2016;73:2349–2367. doi: 10.1007/s00018-016-2205-2.
8. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Res 2019;29:347–364. doi: 10.1038/s41422-019-0164-5.
9. Oh C, Verma A, Aachoui Y. Caspase-11 non-canonical inflammasomes in the lung. Front Immunol 2020;11:1895. doi: 10.3389/fimmu.2020.01895.
10. Feng S, Fox D, Man SM. Mechanisms of gasdermin family members in inflammasome signaling and cell death. J Mol Biol 2018;430:3068–3080. doi: 10.1016/j.jmb.2018.07.002.
11. Xue Y, Enosi Tuipulotu D, Tan WH, Kay C, Man SM. Emerging activators and regulators of inflammasomes and pyroptosis. Trends Immunol 2019;40:1035–1052. doi: 10.1016/j.it.2019.09.005.
12. Flores-Romero H, Ros U, Garcia-Saez AJ. Pore formation in regulated cell death. EMBO J 2020;39:e105753. doi: 10.15252/embj.2020105753.
13. Rosazza T, Warner J, Sollberger G. NET formation – mechanisms and how they relate to other cell death pathways. FEBS J 2020. doi: 10.1111/febs.15589. Online ahead of print.
14. Gong YN, Guy C, Olauson H, Becker JU, Yang M, Fitzgerald P, *et al*. ESCRT-III acts downstream of MLKL to regulate necroptotic cell death and its consequences. Cell 2017;169:286–300.e16. doi: 10.1016/j.cell.2017.03.020.
15. Andrews NW, Almeida PE, Corrotte M. Damage control: cellular mechanisms of plasma membrane repair. Trends Cell Biol 2014;24:734–742. doi: 10.1016/j.tcb.2014.07.008.
16. Wu XB, Sun HY, Luo ZL, Cheng L, Duan XM, Ren JD. Plasma-derived exosomes contribute to pancreatitis-associated lung injury by triggering NLRP3-dependent pyroptosis in alveolar macrophages. Biochim Biophys Acta Mol Basis Dis 2020;1866:165685. doi: 10.1016/j.bbadis.2020.165685.
17. Wang J, Li X, Liu Y, Peng C, Zhu H, Tu G, *et al*. CircHIPK3 promotes pyroptosis in acinar cells through regulation of the miR-193a-5p/GSDMD axis. Front Med (Lausanne) 2020;7:88. doi: 10.3389/fmed.2020.00088.

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