



Research and Exploration of Programmed Cell Death in Infectious Diseases

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Abstract. Cell death is a fundamental biological phenomenon that is essential for the survival and development of living organisms. Cell death can be either a spontaneous programmed host process or an accidentally triggered process. Programmed cell death presents a lysed/non-lytic cell morphology depending on the signaling pathway activated by the stimulus. For example, apoptosis is characterized by cell shrinkage and the formation of apoptotic bodies, which is a typical non-lytic death mode. However, cysteine aspartate-specific protease 1/11-mediated pyroptosis and necroptotic processes can cause inflammatory responses, and promote cell lysis and release inflammatory cytokines by triggering the membrane pore-forming mechanism, which is a typical lytic death mode. In addition, the mitochondrial damage process triggered during the pathogen infection phase can further induce the release of intracellular reactive oxygen species, which in turn triggers ferroptosis. Studies have shown that these programmed cell death modalities can exert immune defense by eliminating infected cells and pathogens within cells, and stimulating an innate immune response with the help of the resulting cell cadavers. In this review, we summarize the molecular mechanisms of multiple cell death pathways and their roles in the defense of innate immunity against microbial infection, and briefly elaborate the interactions between different programmed cell death pathways, in order to provide new ideas for further study of the pathogenic mechanism of infectious diseases.

Keywords: Infectious diseases, programmed cell death, apoptosis, pyroptosis, ferroptosis, necroptosis.

1 Introduction

The programmed cell death (PCD) equation mainly shows coordinated disintegration, which is a typical "immunosilencing" and can be divided into lytic and non-lytic methods. Among them, apoptosis is the non-lytic death mode of the cell. However, recent studies have shown that apoptosis can be classified as immune cell death in some cases. Apoptotic cells not only induce tissue regeneration, but also limit the degree of pathogen infection, which in turn causes acute/chronic inflammatory responses. On the other hand, pyroptosis and necroptosis are relatively "violent" lytic cell death modes, which are mainly manifested in the formation of pores in the cell membrane, which causes

abnormal exchange of water and/or ions, resulting in cell swelling and eventually cell rupture.

Infectious diseases are caused by microorganisms. Pathogenic agents such as microbial autotoxins or enzymes can enter the host through the mouth, nose, skin, and blood, thereby interfering with the normal function of the host organs and/or systems. The host immune system acts as the first line of defense and can eliminate pathogens in a variety of ways, including PCD. When a pathogen invades the body, phagocytic cells first gather at the site where the pathogen is located to engulf the pathogen, and then kill themselves through apoptosis to limit the spread of the pathogen. The resulting dead cells and the damage-associated molecular patterns they release further regulate innate immune responses by activating a variety of PPRs, such as membrane-bound pattern recognition receptors and cytosolic PPRs, nucleotide oligomeric domain-like receptors. Among them, the activation of inflammation-related pathways such as receptor-interacting protein kinase 1/nuclear transcription factor κ B (Nuclearfactor- κ B, NF- κ B) can induce the death of immune cells that phagocytoses pathogens, and then promote the clearance of pathogens, but the process of PCD elimination of host immune cells can also facilitate the spread of pathogens^[1].

In addition, the immune system recognizes other red flags. Non-protein factors such as RNA, DNA, adenosine triphosphate, lipopolysaccharides, exotoxins, and protein factors such as histones are released into the cell as pathogen-related molecular patterns and are recognized by macrophage surface pattern recognition receptors, promote the release of inflammatory cytokines such as tumor necrosis factor, and activate the NF- κ B pathway, thereby triggering PCD pathways such as apoptosis and pyroptosis^[2].

There are cross-polluting mechanisms between different PCD pathways, such as cysteine, a key protein in apoptosis, aspartate-specific protease 3, which can cleave pyroptosis protein E, thereby promoting mitochondrial membrane pore-forming and the release of cytochrome c, thereby enhancing apoptosis signaling. Here, this review mainly expounds the molecular mechanisms of different PCD pathways, and discusses the research progress of these pathways in infectious diseases, in order to provide a new direction for the clinical treatment of infectious diseases.

2 Apoptosis

Apoptosis comes from the Greek word meaning "to stay away". In 1842, the German scientist Karl Vogt first observed apoptosis in the study of toad cell development, and proposed the concept of apoptosis for the first time. Apoptosis is mainly controlled by the caspase family, IAP family, bcl-2 family, tumor suppressor gene p53, etc., and is a process of self-consciously ending cell life taken by cells themselves to regulate the growth and development of the body and maintain the stability of the internal environment, and is the most typical form of cell death in programmed death. It has been found that the apoptosis pathways mainly include the mitochondria-mediated endogenous apoptosis pathway, the death receptor-mediated exogenous apoptosis pathway, the perforin-granzyme-mediated apoptosis pathway, and the endoplasmic reticulum stress-mediated apoptosis pathway. Although the signaling pathways of apoptosis are

different, they are all accomplished by the same apoptosis execution signaling pathway. Morphologically, apoptosis is mainly manifested as cell shrinkage and rounding, volume reduction, loss of microvilli, intact cell membrane, valgus of phosphatidylserine on the inner side of the cell membrane, disintegration of the cytoskeleton, loss of contact with the cells around the cell, cytosolic condensation, condensation of organelles in the cytoplasm, condensation of nucleoli, condensation of chromatin around the nuclear membrane, marginalization and condensation into crescent-shaped or cap-like protrusions, and formation of apoptotic bodies. Apoptotic bodies contain cytoplasmic components, nuclear debris, and organelles, and finally apoptotic bodies are engulfed and eliminated by macrophages, epithelial cells, etc. Because the cell contents are still in the cell membrane during the whole process of apoptosis, the cell membrane is intact, so it does not cause an inflammatory response in the surrounding tissues^[3].

Apoptosis plays a very important role in the stability of normal cells in tissues, the body's immune and defense responses, the growth and development of embryos, the occurrence and development of tumors, and cell damage caused by disease or poisoning. Therefore, the careful study of apoptosis has a wide range of physiological significance for the development and aging of the body, the formation and degeneration of organs, the proliferation and differentiation of cells, the immune system, and the living environment of the nervous system.

3 Autophagy

The word autophagy is derived from the Greek word auto-, which means "self", which is phagein, i.e. "devour". Christian de Duve et al. discovered that there are some organelles such as Peroxisome and Lysosome in cells, and in 1963, the concept of Autophagy was first proposed. In 1993, Japanese scientists Yoshinori Ohsumi and Tsukada found a batch of yeast mutants related to autophagy in yeast, screened autophagy-related genes (ATG) for the first time, and successfully identified 15 genes related to autophagy, deepening the research method of autophagy to the era of molecular genes. In 2016, Professor Yoshinori was awarded the Nobel Prize in Physiology or Medicine for his discovery of autophagy, a fundamental process of degradation and recycling of cellular components^[4].

Autophagy is a kind of cell self-phagocytosis phenomenon widely existing in nematodes, yeast and other lower eukaryotic cell organisms, which is a kind of cell self-phagocytosis phenomenon that is encapsulated by a double membrane derived from rough endoplasmic reticulum or Golgi apparatus under the action of physiological or pathological factors, and forms autophagosomes by combining with lysosomes to form autophagic lysosomes and carries out digestion and degradation of a variety of enzymes, and the decomposed nucleotides, amino acids, free fatty acids, monosaccharides, etc. can be reused by cells to meet the metabolic needs of the cell itself and realize the renewal of some organelles. Autophagy maintains cellular homeostasis and provides energy to the body by degrading intracellular proteins and damaged organelles, but in some cases, excessive autophagy can lead to cell death, so autophagy is considered to be a form of cell death that distinguishes it from apoptosis. Autophagy is

genetically regulated, so this form of cell death is also known as type II programmed cell death. Autophagy is generally divided into 3 types: macroautophagy, microautophagy, and chaperone-mediated autophagy. Among them, macroautophagy is one of the most studied forms of autophagy, and unless otherwise specified, autophagy generally refers to macroautophagy. The phenomenon of autophagy generally goes through three processes: phagocytosis, autophagosomes and autophagolysosomes, and the morphological characteristics of cell autophagy can be observed by transmission electron microscopy, and the phagophagocytosis has a structure with a bilayer or multilayer membrane that does not completely surround the cytoplasmic components, and the shape is mostly crescent-shaped or cup-shaped; autophagosomes contain ribosomes, mitochondria, endoplasmic reticulum and other organelles, which are most easily observed under transmission electron microscopy, and their diameter is generally 300–900 nm, the average is about 500 nm, it is a vacuole-like structure of a bilayer or multilayer membrane, and the cytoplasmic components in autophagic lysosomes have been degraded and have a monolayer membrane structure^[5].

In addition, the combination of allicin and rapamycin has been shown to induce more cell death compared to allicin or rapamycin alone. These results suggest that allicin regulates autophagy through the Akt/mTOR signaling pathway, which can improve the multidrug resistance of thyroid cancer cells as an adjuvant therapy for thyroid cancer patients. Wang L et al. found that β 1-adrenergic receptor autoantibodies can induce cardiomyocyte apoptosis, and inhibiting cardiomyocyte apoptosis can partially reverse cardiomyocyte death, and can also induce cardiomyocyte autophagy to increase first and then decrease, and the upregulation of Rapa or down-regulation of cardiomyocyte autophagy level by 3-MA can significantly change β 1-AA-induced cardiomyocyte apoptosis, which generally indicates that the decrease in autophagy level is involved in β 1-AA-induced cardiomyocyte apoptosis. It has been found that in addition to the mTOR signaling pathway, the Beclin-1 signaling pathway and the p53 signaling pathway can also inhibit or promote the occurrence of autophagy, which is closely related to the incidence of a variety of chronic inflammatory diseases, cardiovascular and cerebrovascular diseases, and ophthalmic diseases, especially tumors, viral infections, neurodegenerative diseases, aging, and tissue fibrosis^[6].

4 Ferroptosis

4.1 Molecular mechanisms of ferroptosis

Until now, the process of programmed cell death that cannot be prevented by inhibition of cas-pase is called non-apoptotic cell death. Recently, ferroptosis has received widespread attention because it can play an important role in regulating the body's metabolism and immune response, and has attracted widespread attention because ferroptosis is an oxidative cell death mode driven by lipid peroxidation. It was initially thought that ferroptosis could be regulated by small molecule compounds that selectively killed cancer cells with mutations in proto-oncogenes. However, subsequent studies have shown that ferroptosis can occur in an RAS independent manner when the body's circadian clock is disturbed or when the body is in an environment such as ischemia-reperfusion

injury^[7]. At present, the two key initial signals of ferroptosis are recognized as excessive iron accumulation and inhibition of glutathione peroxidase, which can induce ferroptosis by affecting iron ion absorption, storage, utilization and other metabolic pathways, thereby increasing the concentration of free iron in cells, for example, the degradation of iron storage protein by ferroptin can promote ferroptosis, and the reactive oxygen species production process mediated by the Fenton reaction or iron-binding enzyme oxidation promotes lipid peroxidation and causes ferroptosis. In addition, there are multiple pathways for the generation of ROS. Studies have shown that impaired mitochondrial and dipeptidyl peptidase-mediated activation of nicotinamide adenine dinucleotide oxidase may synergistically induce lipid peroxidation and promote ferroptosis through synergistic cytotoxicity with iron ion accumulation.

Glutathione is a cysteine-containing 3-peptide that is an important intracellular antioxidant. The production of GSH is mainly dependent on cysteine uptake by cystine/glutamate transporters and cysteine production regulated by cysteine-transferring RNA synthetase 1. Among them, the activity of the key subunit solute carrier family members of systemxc- is regulated by various mechanisms such as protein-protein interaction, nuclear factor erythrocyte line 2-related factor 2 and deubiquitination, and its down-regulation will directly inhibit the cysteine metabolic pathway, reduce GSH synthesis and induce the decrease of GPX4 activity.

4.2 Ferroptosis and infectious diseases

Studies have shown that ferroptosis-related injuries are involved in the development and development of a variety of diseases, such as nonalcoholic steatohepatitis, diabetes, asthma, rheumatoid arthritis, and some infectious diseases. Among them, ferroptosis can further exacerbate tissue damage caused by bacterial infection. Cystic fibrosis is a chronic inflammatory disease characterized by elevated levels of oxidative AA-phospholipids in the respiratory tract, and *Pseudomonas aeruginosa* is one of the main pathogens causing cystic fibrosis in the host. Studies have shown lipoxygenase expressed by *Pseudomonas aeruginosa*. Mammalian homologs are cologs that induce lipid peroxidation processes in bronchial epithelial cells and trigger ferroptosis. This process can be blocked by xanthophin and ferritin-1, suggesting that this substance may be a new treatment for cystic fibrosis.

Mycobacterium tuberculosis is another pathogen [57] that can cause host cell death through the ferroptosis mechanism, which contradicts the previous claim that cell necrosis plays a major role in its infection process, and [58]Jo's pharmacological study of ferrostatin-1 has demonstrated ferroptosis to mediate lung tissue damage induced by *Mycobacterium tuberculosis* infection, however, the above studies cannot rule out the off-target effects of other cell death modalities. In addition, KANG et al. [60] found that a mouse model of cecal ligation and puncture-induced sepsis was accompanied by systemic inflammation and symptoms of multi-organ failure induced by pyroptosis, and that conditional depletion of GPX4 in bone marrow cells could further accelerate this process. Ferroptosis inhibitory drugs can prevent cecal ligation and puncture or tissue damage caused by LPS, suggesting that GPX4 is involved in different types of

programmed cell death. Further exploration of the role of GPX4 in PCD such as pyroptosis will help to understand the mechanism of ferroptosis in infectious diseases.

5 Necroptosis

Necroptosis is also a form of programmed inflammatory cell death characterized by cell swelling and plasma membrane rupture. This pathway can be activated by TNF receptors, Fas, TLR3, TLR4, and Z-DNA-binding proteins to defend against pathogen infection and cell damage.

Activation of mixed lineage protein kinase-like domains and RIPK3 is a critical step in necroptosis. Cell membrane surface tnf receptors can recruit adapters TRADD, RIPK, cIAPs, and TRAF2/5 upon stimulation. When caspase8 is missing, RIPK1 phosphorylates RIPK3 through the RIP homology interaction domain and forms an amyloid filamentous signaling complex called the necrotic complex. In addition, RIPK3 can also be activated by interacting with other RHIM-containing proteins such as TLR4 and ZBP1. Phosphorylated RIPK3 activates the autophosphorylation process of MLKL and promotes its oligomerization to the plasma membrane, thereby binding to phosphoinositide and cardiolipin, disrupting the integrity of the membrane and triggering cell necrosis. Current research data on necroptosis and infectious diseases suggest that necroptosis alone cannot play a role in protecting against bacterial infections, such as wild-type and *Ripk3*^{-/-} mice showing similar susceptibility to *Salmonella typhimurium*, *Yersinia pseudotuberculosis*, and Citrate. However, RIPK3 can further activate necroptosis by interacting with the NLRP3 inflammasome.

RIPK3 induces NLRP3 inflammasome activation by activating caspase8 in response to LPS stimulation, and this process is independent of the RIPK3/MLKL signaling pathway. Therefore, when IAPs and caspase8 are not functional, activation of this mechanism will play an important role in the process of defending against pathogen infection. Interestingly, another study showed that necrotic complexes induce mitochondrial ROS production, followed by NLRP3 inflammasome activation via the serine threonine kinase pathway to defend against *Streptococcus pneumoniae* infection. However, RIPK3 is not involved in the activation of NLRP3 inflammasome induced by blistering stomatitis virus infection with bone marrow-derived dendritic cells. In conclusion, there are some contradictions in the mechanism of RIPK3 in NLRP3 inflammasome activation, which may be related to the different mechanisms triggered by different stimuli in different cells.

ZBP1, as a newly discovered DNA-dependent activator of necroptotic chemokines, is involved in the regulation of the activation process of NLRP3 inflammasome. ZBP1 activates NLRP3 inflammasome and promotes inflammatory cytokine production through the RIPK1-RIPK3 axis during influenza virus infection. In addition, ZBP1 and its Za2 domain are also necessary for inflammasome activation during diphtheria and *Aspergillus fumigatus* infection.

These results suggest that RIPK3 and ZBP1 are key regulators of NLRP3 inflammasome activation when the host defends against pathogen infection. This may be related to the membrane pore-forming mediated by the key protein MLKL in the

necroptotic pathway, which causes potassium efflux, but the detailed mechanism of the differences needs to be further explored. Further research is needed on how necroptosis interacts with the mechanism of programmed cellular death associated with NLRP3 inflammasomes.

6 Summary

Proper cell death can maintain normal homeostasis, metabolism and physiological functions, which in turn will induce and participate in various diseases. With the in-depth study and attention to the mechanism of cell death, a variety of types of cell death have been discovered in the academic community, forming a specialized research field. Cell death is a state in which cells cease to perform their functions, either as a result of the natural death process of old cells and their replacement by new cells, or biological death that may be caused by things like disease, local damage, or detachment of cells from tissues. In addition to the above, incorporation of death, excitability poisoning, apoptosis, cytokeratosis, and Wallerian degeneration. There is an interrelationship between different forms of death, and in some cases, a specific stimulus can cause one form of cell death, but at other times, a stimulus acts as a "master key" and often causes multiple forms of death. This article focuses on the sources, concepts, and mechanisms of several forms of programmed cell death, including apoptosis, autophagy, programmed necrosis, pyroptosis, and ferroptosis, as well as some recent studies, hoping to lay a foundation for in-depth research on the role of cell death in the pathogenesis of various diseases in the future, and promote the development of new therapeutic targets, so as to provide valuable clues and means for the research and development of related molecular target drugs.

Cell death eliminates the replication space available to pathogens. The release of infectious material from dying cells will provide a potent signal to initiate the inflammatory cascade. Among them, the activation of NLRP3 inflammasome can aggravate the inflammatory response of infectious diseases. By exploring various agonists and inhibitors of NLRP3 inflammasome, it may provide a direction for the clinical treatment of excessive inflammatory damage caused by infectious diseases, and then improve the prognosis of patients. In addition, ROS is not only involved in the activation of multiple PCD pathways, but also exacerbates the damage caused by the host oxidative stress mechanism triggered by pathogen infection. Therefore, exploring the application of natural antioxidants such as resveratrol in infectious diseases may also become the focus of future research.

At this stage, there is still a lot of work to be done to fully understand the mutual regulatory mechanisms of programmed cell death such as apoptosis and pyroptosis in vivo to resist pathogen infection. For example, loss of caspase 8 prevents exogenous apoptosis, but it may also trigger necroptosis, leading to lymphoproliferative disorders and suppressing innate immune receptor signaling. In addition, when PCD is sensitive to pathogen-specific virulence factors, the pathogen may initiate other mechanisms to evade the response. Therefore, the search for new infectious substances that cannot escape specific cell death modes and key common substances in the PCD crosstalk

mechanism may further help to understand the true function of PCD during pathogen infection, thus providing a direction for the synergistic use of multiple cellular programmed death modalities to jointly exert immune defense.

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