Original Article

Roles of pyroptosis in myocardial ischemia/reperfusion injury diseases

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Ischemia-reperfusion injury (IRI) occurred when an organ lost its blood supply in a short . After that, the perfusion the perfusion was restored automatically or iatrogenically, leading to a burst of reactive oxygen species (ROS) from mitochondria. It is common in the clinic, resulting in lead to deterioration, even death, so an exploratory examination of the mechanism of ischemia-reperfusion injury is of great significance. Among the most common and fatal types of IR in myocardial tissue, myocardial IRI is one of the most fatal diseases in the modern world. The cellular and molecular mechanisms of IRI mainly include calcium overload, oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, energy metabolic disorders, neutrophil infiltration, cardiomyocyte autophagy, and apoptosis, etc. The main pathogenesis of IRI is programmed cell death, of which apoptosis is the most deeply studied processes. pyroptosis is a highly inflammatory form of programmed cell death (PCD), which depends on the activation of the caspase cascade and inflammatory mediators, which have been thought to be involved in the processes of IRI. Ptois has been referred to as a pattern. PCD with apoptosis characteristics Necrosis. It's stimulated by molecular signaling pathways similar to apoptosis, mainly including Caspase. The research progress in recent years is presented in this review. Among them, myocardial tissue and so on provide a theoretical basis for the burning organ system in I/R injury and provide theoretical practice for the clinical research of reducing ischemia-reperfusion injury.

Key Words: Myocardial Ischemia/Reperfusion Injury; Pyroptosis; Inflammation; caspase-1

INTRODUCTION

Reperfusion of ischemic tissues or cells is the most common pathologic processes in clinical practice, leading to widespread microvascular dysfunction and tissue/cell injury which in turn may affect prognosis and may directly endanger the patient's life. Nutrients carried in the blood are released to tissues via the permeable endothelium of blood vessels, vascular injury is the basis of the pathological processes of I/R injury, and it is also an

important barrier between vascular and tissue of endothelial cells. Studies have shown that vascular endothelium is a crucial site affected by I/R injury, apoptotic endothelial cells are before tissue cells. Damage of vascular endothelial cells leads to the infiltration of intravascular in flammatory mediators and inflammatory cells directly into the tissue, as a consequence aggravates tissue damage. Because the mechanism of IRI has not been fully elucidated, the main idea now is the outbreak of oxygen-free radicals, calcium overload, energy metabolic disorders, endo-

thelial dysfunction, neutrophil infiltration, apoptosis, and mitochondrial damage, etc, particularly in myocardial tissue.^{1,2} Myocardial I/R Injury like CHD are some of the leading causes of death and disability worldwide. 7,254,000 deaths worldwide (12.8% of all deaths) resulted from CHD in 2008 according to the WHO.³ What's more, the therapeutic effects and intervention on them are not satisfactory in recent years.⁴ Therefore, it is of great clinical significance and imperative to study the molecular mechanism of myocardial I/R injury and find new targets for intervention.

Pyroptosis, as a pro-inflammatory programmed cell death, has been valued and studied in depth by researchers in recent years. It has intrinsic properties and various pathological processes are inflammation, such as stroke, heart attack or cancer. is a caspase-1 dependent For example, in neurodegenerative diseases, a caspase-1 pathway of the neuron is abnormally activated, inducing the occurrence of pyroptosis; Gram-negative bacilli infection could induce pyroptosis through caspase-1, by helping our bodies to activate and eliminate infections and slow down inflammation, the response is mediated by macrophages. Besides a non-canonical pathway associated with caspase-4/5/11 has been proved to be relevant to the pathological processes mentioned above. However, an excessive reaction can lead to normal tissue damage. The processes of biological pyroptosis have the following characteristics: cell swelling and plasma membrane disruption.⁵ In myocardial tissues, pyroptosis could be stimulated and enhanced by cardiac I/R injury and then exerted detrimental effects on injured myocardial tissues, including inflammatory cell death and cardiac remodeling, in the pathology of myocardial ischemia diseases.⁶

In this editorial, we will briefly review the features of pyroptosis and its role in myocardial I/R injury, to highlight the significance of pyroptosis during IRI, to inspire some new directions for the research of molecular and cellular mechanisms of pyroptosis, and to provide the emerging therapeutic strategies for protecting the myocardium from its detrimental effects.

1. Morphological and Molecular Features of Pyroptosis

Pyroptosis is a kind of regulated cell death which is mediated through the inflammatory cysteine-dependent aspartate-specific (caspase) family, in particular, homologous genes of human and mouse are caspases. The classic morphological features of pyroptosis include chromatin condensation, pore formation in the cell membrane, cell swelling, cellular rupture and further release of cytoplasm contents. Those cell contents released during pore formation processes of pyroptosis usually were inflammatory hallmarks represented by IL-1 β and IL-18, which are helpful to initiate, amplify, and perpetuate inflammation.

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Similar to apoptosis, DNA damage has been found in pyroptosis with terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) positivity, while the intensity is lower than it is in apoptosis.⁷ Moreover, not only in apoptosis cells, Annexin-V positive has been detected in anthrax lethal toxin-induced pyroptosis cells.⁵ Further research shows that the translocation of phospholipid phosphatidylserine (PS) from the inner leaflet to the outer leaflet the plasma membrane creates opportunities for Annexin-V to bind to PS. It is not hard to understand that propidium iodide (PI) positive reaction was no more to be considered as a criterion for apoptosis. It is reported that PI can enter pyrophosphate cells and insert into nuclear DNA, releasing red fluorescence.⁸ This feature helps to differentiate pyroptosis cells from early apoptotic cells with an intact membrane, which may block translocation of PI from extracellular to intracellular.

Cellular pyroptosis has been subdivided into molecules, the different mechanisms of caspase-1 dependence are cysteine aspartate protease-1 independent pyroptosis. Despite a high morphological similarity, a caspase-1-dependent and a caspase-1-independent pyroptosis have particular features as following described. One of the most important steps is to bind caspase-1 nucleotides to the oligomeric receptor (NLR) family during pyrophosphate-dependent activation, absent in melanoma 2 (AIM2) or pyrin, NLRP1 and NLRP3 inflammatory bodies were activated by human periodontal ligament cells and subjected to cyclic stretch.^{9,10} NLRC4 responds to bacterial flagellin or components of bacterial type III secretion systems.¹¹ Linear double-stranded DNA activated by human adenovirus is AIM2.¹² Pyrin inflammasome responds to Rho-GTPase-modifying bacterial toxins.^{13,14} Some caspase-1 activated by the aforementioned inflammatory bodies were cut from 53 kDa substrate to 31 kDa by gas steirin and combined with terminal fragments 22 kDa and C-terminal fragments. While others engage in promoting the maturation of interleukin-1 β (IL-1 β) and interleukin-18 (IL-18). The activity of the N-terminal domain is inhibited by the C-terminal domain before they separated. A number of studies have shown that oligomers of GasDermin D fragments of each 16 N- terminals form cell pore membranes.¹⁵ Once these saboteurs destroy the integrity of the cell membrane, about 10-21 nm in diameter,¹⁶ low molecular weight unit length, such as water, sodium, potassium, IL-1 β (4.5nm), IL-18 (5.0nm) will cross the cell membrane immediately through the pore. As a result, cell volume increases rapidly until the membrane rupture a caspase-1-independent pyroptosis occurred through the non-canonical inflammasome pathway.¹⁷ Lipopolysaccharide (LPS) located at outer membrane of Gram-negative bacteria could directly bind to caspase-4, caspase-5 or a caspase-11 and activate them. Those activated cysteine aspartate proteases and their N terminal domains released, which takes part in the pore-forming activities. Unlike a caspase-1-dependent pyroptosis, caspase-4/5/11 do not process interleukins directly but keep the connections with a caspase-1 signaling pathway via NLRP3, which can be activated by N-terminal domain of GasDermin D (Figure1).¹⁸

2. Pyroptosis in vascular endothelial cells

Some studies have discussed the role of ptosis. Especially, endothelial cells (ECS) and potential unknown organisms damage the targeted therapy of endothelial pyroptosis. Chen H et al¹⁹ found

Figure 1 The caspase-1-dependent and caspase-1-independent pathways of activating cell pyroptosis. LPS of gram-negative bacterium can bind to caspase-4/5/11 and activates them directly. The activated caspase-4/5/11 cleaves GSDMD into a C-terminal domain and an N-terminal domain. Every 16 N-terminal domains join together to form a GSDMD pore with its diameter of about 10-21nm. Molecules with smaller diameters, like water, sodium, potassium, IL-1 β and IL-18 may leak out through the pore. NLRP1, NLRP3, NLRP4 and AIM2, respectively, activates caspase-1, whose activated forms, partly, promote the maturation of IL-1 β and IL-18, and partly, cleavage GSDMD, inducing cell pyroptosis. caspase-4/5/11 α =activated caspase-4/5/11; caspase-1 α =activated caspase-1

the presence of a caspase-1-dependent pyroptosis and inflammatory response in Cd-induced endothelial toxicity and demonstrated that activation of the NLRP3 inflammasome contributes to the pyroptosis in human umbilical vein endothelial cells (HUVECs) for the first time. In turn, Jahaira et al²⁰ detected the improvement of VEGFR-2 signaling, tube formation, and blood perfusion in ischemic tissues with the inhibition of a caspase-1 which means the suppression of a caspase-1 improves angiogenesis and ischemia prognosis. The studies above can provide an important relationship between pyroptosis and endothelial cell damage, which is a Caspase-1 dependent pathway. It can provide an important relationship between pyroptosis and endothelial cell damage, and it is a Caspase-1 dependent pathway. Moreover, the important role of group 2 intrinsic lymphocyte (ILC2) is to protect pulmonary edema caused by sepsis and further explore acute pulmonary inflammation. It showed that the increased ILC2 secreting IL-9 in the lungs prevents lung ECs from undergoing pyroptosis by attenuating a caspase-1 activation.²¹

Systemic exposure pulmonary tissue to the bacterial endotoxin LPS contributes to the development of fulminant pulmonary edema and acute lung injury (ALI) owing to severe endothelial pyroptosis that is dependent on the inflammatory caspases, the murine homolog a caspase-11 in mice in vivo, or caspases 4/5 in human ECs.²² In other words, the non-canonical pathway also plays a part in endothelial pyroptosis.

The results from other present studies indicate that DHM improves pyroptosis and concomitantly alleviates IL-1 β secretion by activating the Nrf2 signaling pathway to inhibit ROS-dependent NLRP3 inflammasome activation in palmitic acid (PA) induced HUVECs.²³ lncRNA revealed in several recent studies can function as a molecular sponge or a competing endogenous RNA (ceRNA) in modulating expression and biological functions of miRNAs.²⁴ MEG3, an endothelium-enriched lncRNA, as miRNA -223, cRNA has been proved to be negative regulation, and it is an

NLRP3 inflammatory small body.²⁵ In addition, the expression of NLRP3, can alleviate atherosclerosis by melatonin. As a result, the MEG3/miR223 / NLRP3 axis of melatonin preventing endothelial cell apoptosis can be believed.²⁶

These findings have expanded the current understanding of the roles of pyroptosis in endothelial injury, which may be a novel link in some vascular diseases including cardiovascular, pulmonary vascular diseases and so on.

3. The roles pyroptosis plays in myocardial I/R injury

Acute myocardial infarction (AMI) - The most likely consequence of atherosclerotic rupture and thrombosis, the leading cause of death and disability worldwide, is the coronary artery of ischemic heart disease. Restoration of the coronary blood flow as soon as possible is the most effective clinical strategy to narrow the extent of myocardial infarction and to improve the prognosis for AMI sufferers. However, this kind of treatment method may give rise to myocardial I/R injury through a variety of mechanisms, myocardial ischemia/reperfusion injury plays an important role in ptosis.

3.1 The phenomena of pyroptosis the myocardium during I/R injury

The current studies have indicated that the phenomenon of pyroptosis occurred in myocardial cells during acute myocardial infarction (AMI), myocardial ischemia (MI) and I/R injury. Unlike those immunogenically silent cell death like apoptosis, pyroptosis was more likely to promote myocardial I/R injury with a characteristic of inflammatory processes. It has been proved that the activated NLRP3 inflammatory corpuscle will cause further expression of the A inflammatory factor reverse gene. It has been proved that the activated NLRP3 inflammatory corpuscle will cause further expression of the A inflammatory factor reverse gene, especially IL-1 β and IL-18.^{5,27} Regarding myocardial I/R injury, many studies indicate that inflammasome activation occurred during the early stages of ischemia.²⁸ Nazir et al.²⁹ isolated hearts of mice at different time points after reperfusion and detected the infarcted area, a caspase-1, IL-1 β , IL-18, caspase-3, caspase-7, and BAX. Numerous data showed that these pyroptosis related factors (caspase-1, IL-1 β , and IL-18) increased at early time points than markers of apoptosis (caspase-3, caspase-7, and BAX), suggesting that pyroptosis precedes apoptosis in myocardial I/R injury.^{29,30}

Pyroptosis has been considered to plays a key role in I/R. IL-1 can release the effect on cardiomyocytes of adult male CD1 mice and further activate caspase 1. Wang, Q et al³¹ found that the protein expression of TLR4, NLRP3, and a caspase-1 was increased in I/R heart tissues of Sprague-Dawley rats at 24 hours following the treatment of 30 minutes ligating the left anterior descending coronary artery. Similarly, Toledo et al³² explored the timing of NLRP3 inflammasome formation and caspase-1 activity in the heart of CD1 mice during ischemia-reperfusion, the results showed that ischemia-reperfusion 3 h and 24 h I/R were used for injury control. Mastrocola et al³³ reported that increased protein expression of the Nlrp3 inflammasome and a caspase-1 were detected in heart tissues from C57Bl/6 male mice after being exposed to cardiac ex vivo I/R injury.

3.2 The effect of pyroptosis on I/R injury myocardium

Pyroptosis might participate in the pathogenesis of myocardial I/R

injury, the specific phenomenon of apoptosis will contribute to the prevention of myocardial ischemia /-reperfusion injury. Hearts from NLRP3-deficient mice showed a marked improvement of cardiac function after I/R injury compared with wild-type hearts.³⁴ A few studies have demonstrated that the depletion of NLRP3 and a caspase-1 can protect the heart from suffering the I/R injury and adverse cardiac remodeling.³⁵ Toledo et al³⁶ showed that inhibition of activated a caspase-1 could result in increased left ventricular (LV) end-diastolic diameter (EDD) and end-systolic diameter (ESD) after in vivo I/R injury of mice. As stated by the same authors,³⁶ significant preservation of cell viability and the promotion of favorable infarct healing due to caspase inhibitors were helpful to prevent adverse cardiac remodeling.

3.3 Possible mechanism of cell pyroptosis during myocardial I/R injury

Pyroptosis is a key process to mediate myocardial damage after I/R. Previous investigations have demonstrated that interventions targeted for canonical inflammasome pathway of pyroptosis could considerably prevent cardiomyocytes from cell death and attenuate myocardial I/R injury for myocardial ischemia/reperfusion injury, this means that caspase-1-dependent pyroptosis is an important mechanism. Wang, Q et al³¹ found that the myocardial I/R injury of Sprague-Dawley rats was associated with an exacerbated overexpression of TLR4 and NLRP3 inflammasome, resulting in remarkable activation of a caspase-1 pathway, following experiments showed ghrelin protected the heart via TLR4/NLRP3 signaling pathway. In recent work, pretreatment with synthesized NLRP3 inflammasome inhibitor INF4E could suppress the expression of a caspase-1 and cleaved IL-1 β by pro-survival risk pathway and improvement in mitochondrial function.³⁵ Of note, it has been demonstrated that NLRP3 plays a role in the myocardial microvasculature, i.e. endothelial cells (CMECs) rather than myocardial cells. During the last decade, microRNAs (miRNAs) have been considered to be a key regulator of cellular processes such as differentiation, survival, and death. And the development of pyroptosis is also regulated by miRNAs in away. Although it was not sufficient to draw any significant insights on the role of miRNAs in pyroptosis because of the limited studies, it might still make sense to offer some feasible researches and provide strategies for future studies. miRNAs can directly target key mediators of pyroptosis, such as adaptor proteins, a caspase-1, and pattern recognition receptors (NOD-like receptor (NLR) family of proteins). Besides miRNAs can indirectly affect pyroptosis via secondary mediators. In this case, they can be classified into two categories: miRNAs targeting negative regulators and miRNA targeted positive regulators and the former increased in CVDs facilitate enhanced pyroptosis. It is based on autophagy-mediated inhibition of inflammatory activation, transcription factors negatively regulating inflammasome and nitric oxide-mediated suppression of inflammasome formation.³⁷

CONCLUSIONS

One of the main objectives is to protect heart from I/R injury in patients with myocardial ischemia/reperfusion injury. The recent advances outlined above have indicated that a caspase-1-dependent pyroptosis plays an important role in the development and progression of I/R injury. Therefore, finding new targets for drug treatment of I/R injury may be a key factor in the

caspase-1-dependent pyrophosphate signaling pathway. However, it is a potential way to identify other possibilities and maximize the protective effect of this treatment. The suppression of a caspase-1 pathway may mitigate the effects of myocardial I/R injury. Experiments have proved that it can prevent I/R injury, and then achieve the treatment of kinase (risk) in reperfusion injury. The caspase one inhibitor, VX-765, has been shown to protect the heart from acute IR injury in vivo rat model.³⁸ have proved that it can reduce myocardial ischemia and alleviate the risk of perfusion injury in the model. Similarly, INF4E, synthesized NLRP3 inflammasome inhibitor, can suppress the expression of a caspase-1 and cleaved IL-1 β by pro-survival RISK pathway and improve the mitochondrial function.³⁵ Besides, a few of other researches also provide clues to confirm that depletion of NLRP3 and a caspase-1 protects the heart from suffering the I/R injury and adverse cardiac remodeling.³⁶

The caspases cleave to restrain the activity of its domain that executes pyroptosis through the pore-forming activity. It has been found to be a chemotherapeutic drug for most cancer cells and has shown GSDME-dependent activation of Caspase-3 in pyroptosis. On this account, we can assume that intervening the non-canonical inflammasome pathway at different stages may alleviate pyroptosis that plays a role in I/R injury. In other words, it is worthy of further studies on mitigating myocardial I/R Injury via avoiding LPS binding to caspase-4/5/11, cysteine aspartate proteases 时态 broken down into GSDMD and GSDMD. Besides, miRNAs can directly or indirectly affect pyroptosis as mentioned above, which may be considered as a potential therapeutic target for the treatment of myocardial I/R injury. (Table1.)

However, there are few studies on functions. Moreover, the molecular mechanism of myocardial ischemia-reperfusion injury is not perfect. The current research has just started, further and more detailed studies are needed to elucidate the pathogenic mechanism of pyroptosis and its role in the development of myocardial I/R injuries. The challenge in the future is to understand the specific mechanisms and burning factors associated with myocardial ischemia/reperfusion injury and to further explore appropriate therapeutic targets.

As an emerging researching field that combines inflammation reaction and PCD, pyroptosis provides a new perspective and has a broad prospect in realizing the occurrence, development, and outcome of diseases, including.^{40,41} Taking the high morbidity and mortality into account, more detailed studies on pyroptosis in ischemia diseases may provide new ideas and new drug targets for the intervention and treatment of related diseases.

Conflict of interest

The authors declared that they have no conflicts of interest in this work.

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