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Mechanism and Intervention of Pyroptosis Involved in Reperfusion Injury in Acute Coronary Syndrome

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Abstract: ***Opening occluded blood vessels to achieve myocardial reperfusion is the most critical treatment for acute coronary syndromes, but with the return of blood, the heart tissue produces a strong stress response, and the infarction may be expanded or the heart function may be reimpaired, which is called myocardial ischemia reperfusion injury (MIRI). As a programmed cell death mode, pyroptosis has been widely confirmed to be involved in the MIRI process, and the main morphological characteristics are the formation of cell membrane pores of about 10~20nm, cell swelling accompanied by plasma membrane rupture, and the release of cell contents. In this article, we will review the mechanism of pyroptosis in MIRI and the intervention of various drugs in the pyroptosis pathway, so as to provide new ideas for exploring and improving MIRI.***

Keywords: Acute coronary syndrome, Myocardial ischemia-reperfusion injury, Pyroptosis, Pathway mechanisms, Review**.**

1. Introduction

Multiple cellular insults are involved in the development of MIRI, with neutrophils attracted by chemokines infiltrating into the infarcted area, clearing infarcted cellular debris, and producing reactive oxygen clusters, cytokines, and proteases. Macrophages are also recruited to the infarcted and junctional areas to remove cellular debris and release pro-inflammatory cytokines or inhibitors. After about 5 days the macrophage phenotype enters a proliferative phase, producing anti-inflammatory factors, chemokines and growth factors, which accelerate activation of cardiac fibroblasts (CFs), accelerate angiogenesis and promote fibrosis in the infarct zone, ultimately leading to cardiac remodelling [1-3]. Cellular pyroptosis is a mode of inflammatory programmed cell death, mainly mediated by inflammasome-activated caspase-1, which is widely involved in the development of MIRI, and the main mechanisms of action are currently classified into classical and non-classical pathways [4]. The classical pyroptosis pathway relies on the activation of the cystatinase caspase-1. The process begins with the recruitment of apoptosis-associated speck-like protein (ASC), nucleotide-binding oligomerised structural domain-like receptor protein 3 (NOD-like receptor Pyrin3, NLRP3) activated by pathogen signals, which binds to Pro-caspase1, forming an inflammasome with concomitant activation of caspase-1. caspase-1, on the one hand, generates N-terminal aminopeptides (GSDMD N-terminal) and C-terminal carboxypeptides through shearing (Gastermin-D, GSDMD); on the other hand, it promotes the shearing of IL-1βand IL-18 precursors into mature inflammatoryfactors.The lipophilic GSDMD-N serves as the active region, promoting the formation of pyroptotic pores, increasing membrane permeability, and facilitating rapid cell lysis and release of contents, in which IL-1β and IL-18 released into the extracellular space further amplify the inflammatory response; the hydrophilic C-terminal serves as the inhibitory region. The non-classical pathway is activated by Caspase-11 (mouse)/4/5, which directly shears the GSDMD to form a focal death pore, which allows the passage of potassium ions, activates the NLRP3 inflammasome, releases inflammatory factors, etc. In contrast, cellular juxtaposition induced by Caspase-3/8, also known as apoptosis-like juxtaposition, is a third new pathway discovered in recent years, which causes perforation of the cell membrane by shearing GSDME, another protein of the Gasdermin family [5-7].

2. Cellular Pyroptosis is Involved in Reperfusion Injury in Acute Coronary Syndromes

In MIRI, cellular pyroptosis is involved in the pathologic process through different mechanisms in cardiomyocytes and non-cardiomyocytes, forming a complex injury network. Pyroptosis in non-cardiomyocytes (e.g., vascular endothelial cells, cardiac fibroblasts, and macrophages) is mainly mediated by the NLRP3 inflammasome/caspase-1/GSDMD pathway. During the early phase of reperfusion, Reactive Oxygen Species (ROS) release and potassium ion efflux activate the NLRP3 inflammasome, leading to caspase-1-dependent GSDMD shearing, release of its N-terminal fragment (GSDMD-N), formation of membrane pores, and triggering of cellular pyroptosis [8,9]. Scorched non-cardiomyocytes release large amounts of pro-inflammatory factors (e.g., IL-1β, IL-18), which further amplify the inflammatory response and promote myocardial tissue fibrosis. Pyroptosis of Vascular Endothelial Cells (VECs) disrupts the microvascular barrier, leading to neutrophil infiltration, microthrombosis, and tissue edema, and exacerbating microcirculatory disorders [10]. In addition, cardiac fibroblasts (CFs) pyroptosis accelerates myocardial fibrosis and expands infarct size through IL-18-mediated release of secondary cytokines. Macrophages, on the other hand, influence the process of myocardial repair by regulating the initiation and regression of inflammatory responses [3,11,12].

The mechanism of cardiomyocyte focal death differs from

that of non-cardiomyocytes and is mainly characterized by activation of the caspase-11/GSDMD pathway. After hypoxia-reperfusion, the level of GSDMD-N in cardiomyocytes increases in a time-dependent manner, which activates caspase-11, shears GSDMD and releases IL-18, which not only directly damages cardiomyocytes, but also activates CFs and promotes their proliferation and fibrosis, forming a positive feedback loop and exacerbating the cardiac dysfunction. Membrane rupture of scorched cardiomyocytes leads to the release of intracellular inclusions, which further triggers the inflammatory cascade and expands the scope of myocardial injury.

Scorched death of cardiomyocytes and noncardiomyocytes synergize with each other in MIRI to drive the pathological process. Non-cardiomyocytes directly or indirectly damage cardiomyocytes through inflammatory factors (e.g., IL-1β, IL-18), and IL-18 released from cardiomyocyte pyroptosis further activates non-cardiomyocytes, creating a vicious cycle.

3. NLRP3 Inflammasome Activation and MIRI

As a key endogenous danger signal perception and effector complex, NLRP3 inflammasome plays a central role in the pathological process of myocardial ischemia-reperfusion injury by integrating multiple intra- and extracellular stimulus signals, triggering inflammatory responses and exacerbating cellular pyroptosis [13]. The activation mechanisms are complex and diverse.

3.1 Reactive Oxygen Species (ROS)-mediated Activation of NLRP3

ROS is one of the core triggers of NLRP3 inflammasome activation. During ischemia/reperfusion, mitochondrial electron transport chain dysfunction and NADPH oxidase activation lead to the overproduction of ROS, which directly promotes oligomerisation by oxidatively modifying the thiol groups of the NLRP3 protein, and activates thioredoxin-interacting protein (TXNIP), which interacts with NLRP3. At the same time, ROS can activate the thioredoxin-interacting protein (TXNIP), which binds to NLRP3 and promotes the assembly of inflammatory vesicles, and ROS can further amplify oxidative stress signals by inhibiting autophagic cleavage, leading to the accumulation of damaged mitochondria and the formation of a ROS/NLRP3/IL-1β inflammatory waterfall, which can aggravate cardiomyocyte death and microvascular damage [14,15].

3.2 Endoplasmic Reticulum Stress (ER Stress)-Related Signals

Endoplasmic Reticulum Stress (ER Stress) activates the NLRP3 inflammasome through the Unfolded Protein Response (UPR).Protein misfolding induces the IRE1α/XBP1 pathway (Inositol-Requiring Enzyme 1α/X-box Binding Protein 1), which up-regulates the expression of NLRP3 and precursor IL-1β (pro-IL-1β); at the same time, an imbalance in sterol synthesis (e.g., cholesterol crystal deposition) and endoplasmic reticulum calcium stores ($ERCa^{2+}$) release triggers cytoplasmic Ca^{2+} overload via the STIM1/Orai1 channel (Stromal Interaction Molecule 1/Orai Calcium Release-Activated Calcium Modulator 1), which activates calcium-dependent cytoplasmic Ca^{2+} overload. overload, which activates the calcium-dependent phosphatase Calcineurin (CaN) and promotes NLRP3 inflammatory vesicle assembly. In addition, endoplasmic reticulum stress-induced C/EBP Homologous Protein (CHOP) inhibits Bcl-2 (B-cell lymphoma 2) expression, exacerbates the opening of the mitochondrial membrane permeability transition pore (MPTP), and amplifies ROS.MPTP) opening, amplifying ROS signalling and forming an ER-mitochondrial interactive activation loop [3].

3.3 Mitochondrial Dysfunction and Endothelial Damage

Mitochondrial DNA (mtDNA) leakage, cardiolipin ectopia, and ATP depletion directly activate the NLRP3 inflammasome [16]. mtDNA, as a Damage-Associated Molecular Pattern (DAMP), is activated by the Toll-Like Receptor 9 (Toll-Like Receptor 9, DAMP).Like Receptor 9 (TLR9), which triggers Myeloid Differentiation Primary Response 88/Nuclear Factor-kappa B-dependent NF-κB pathway to up-regulate NLRP3 expression; and cardiolipin up-regulates NLRP3 expression by binding to NLRP3-rich leucine repeat sequence (Leucine-rich).Leucine-Rich Repeat (LRR) structural domain, which directly promotes its oligomerisation. Mitochondrial dysfunction also leads to uncoupling of endothelial Nitric Oxide Synthase (eNOS), which reduces Nitric Oxide (NO) production, exacerbates microvascular spasm and leukocyte adhesion, and further amplifies the inflammatory response [17].

3.4 Direct Activation of Periostin (Periostin)

Periostin, as an extracellular matrix protein, is highly expressed in myocardial fibrosis. It activates the PI3K/Akt pathway (Phosphatidylinositol 3-Kinase/Protein Kinase B) via Integrin αvβ3 receptor (Integrin αvβ3), induces the assembly of NLRP3 inflammasome, and promotes Apoptosis-Associated Speck-like Protein (Apoptosis-Associated Speck-like Protein containing a CARD (ASC) spot formation. It was shown that knockdown of periosteal proteins significantly reduced NLRP3 activation and interleukin-1β (IL-1β) release in ischemic areas and improved myocardial function.

3.5 GSK3β-ASC Interaction Promotes NLRP3 Activation

Glycogen Synthase Kinase-3β (GSK3β) is inhibited by Akt phosphorylation in the resting state, whereas inactivation of Akt during ischemia-reperfusion leads to activation of GSK3β dephosphorylation. Activated GSK3β directly binds to the Caspase Recruitment Domain (CARD) of ASC and promotes ASC oligomerisation and caspase-1 recruitment, especially in Cardiac Fibroblasts (CFs), where the GSK3β-NLRP3 axisdrives interleukin-18 secretion and accelerates myocardial fibrosis. Inhibition of GSK3β attenuates focal death-associated injury by blocking ASC speckle formation.

4. Progress of Drugs Involved in Inhibiting Cellular Pyroptosis

MCC950, INF4E, calpain, and small miRNA-495, as small

molecule inhibitors of NLRP3 inflammasome, can block ASC oligomerization, inhibit MIRI-induced cellular scotomies, and reduce the release of IL-1β and LDH, which can significantly reduce the extent of myocardial infarction, and colchicine, as a clinically used inhalational anesthetic, can reduce the extent of heart fibrosis by inhibiting cellular scotomies through blocking the assembly and activation of NLRP3 in a dose-dependent manner. Sevoflurane, a commonly used inhalational anesthetic in clinical practice, dose-dependently reduces the expression of focal death-related proteins by inhibiting the P2X7-NLRP3 signaling pathway. Rosuvastatin and trimetazidine can inhibit the expression of Caspase-1, ACS, and NLRP3, reduce the extent of infarction, and attenuate MIRI [18]. Some other small molecule inhibitors play a role in inhibiting cellular focal death by blocking the formation of GSDMD such as necrolysinamide (NSA), bisulfite, Bay11-7082, etc. IL-1β-related drugs Canakinumab and Canna protect the myocardium by inhibiting the expression of IL-1β proteins and reducing the release of pro-inflammatory factors [19].

Some drugs affect cellular pyroptosis by regulating upstream and downstream factors of NLRP3 activation and formation, thus involving multiple molecular pathways. Exacerbating the inflammatory response by activating some of the pathways, for example, thioredoxin interacting protein (TXNIP) activates NLRP3 assembly to accelerate the onset of pyroptosis, and sodium 4-phenylbutyrate (4-PBA) acts as an endoplasmic reticulum stress inhibitor to inhibit cardiomyocyte pyroptosis through this pathway; nuclear factor κB ((NF-κB)) acts as an essential molecule for GSDMD protein transcription, and mitochondrial mitochondria in the late phase of MI DNA DAMP exacerbates cellular pyroptosis by activating the TLRs/NF-κB/NLRP3 pathway, and M2 macrophage-derived microRNA-148aCLEC5A, nicorandil affects cellular pyroptosis mainly by inhibiting this pathway [20]. p38 mitogen-activated protein kinase (MAPK) promotes expression of the pyroptosis pathway, and the small RNA molecule miR-19a- 3p inhibits this pathway [21]. c-Jun N-terminal kinase (JNK), which activates NF-κB and MAPK to exacerbate inflammatory expression, and the neuroendocrine inhibitor LCZ696 inhibits cellular juxtaposition mainly through this pathway. The JAK/STAT signaling pathway is involved in MIRI during the reperfusion phase, and STAT1 activation exacerbates myocardial injury. Extracellular vesicles carry miRNA-155-5P to promote the polarization of M1-like macrophages, and increase the expression of pro-inflammatory cytokines through the activation of the JAK2/SATA1 pathway [22,23].

JAK/STAT signaling pathway activation is dominated by STAT3 activation during ischemia, which inhibits cellular injury, and resuvastatin and HDL attenuate MIRI by activating the JAK2/STAT3 signaling pathway [22,24]. Activation of nuclea factor erythroid-2-related factor 2 (Nrf2), a key antioxidant transcription factor, inhibits ROS/NLRP3 and ameliorates cell death. Adenosine monophosphate-activated protein kinase (AMPK) is an important factor regulating energy metabolism and intracellular stress in eukaryotic cells, and activation of this factor inhibits NLRP3 activation. Condensin and metformin significantly improved hemodynamic disturbances, reduced infarct size, and provided cardioprotection in MIRI rats by decreasing the release of pro-inflammatory factors and inflammasome activation through the activation of AMPK [3,25]. Increasing reperfusion protective enzyme (PI3K) as a protective factor against cellular focal death and activating the PI3K/Akt pathway [26]. It can reduce the expression of related injury factors, increase the expression of related protective factors, and ultimately ameliorate MIRI injury, inhibitor LY294002 can aggravate myocardial injury by inhibiting this pathway, and cagliflozin, dagliflozin [27], calpain, and Caspase-1 inhibitor VX-765 can also play a role in protecting the myocardium by activating this pathway. Another information regulatory factor 2-related enzyme 1 (SIRT1) also inhibits oxidative stress and cellular focal death.

5. Discussion

MIRI injury after acute coronary syndrome has a complex physiopathological mechanism involving multiple cellular involvement, which regulates cellular focal death, apoptosis, etc. through multiple pathway mechanisms. According to the progression of the disease, a complex signal transduction process involving multifactors and multilink cross-linking is involved, and the mechanism of injury presents different trends due to the occurrence of its events, and there is great difficulty in accurately determining its targets and pathways. Cellular pyroptosis as a mode of programmed cell death with multiple mechanisms in which some of the targets overlap with other forms of cell death. There are real difficulties in accurately determining its targets and influencing factors, the relevant mechanisms have not been fully elucidated, and there are limitations in the study of the relevant targets of action.

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