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Review Article

Pyroptosis in Myocardial Ischemia/Reperfusion Injury: Role of Endoplasmic Reticulum Stress and STING-IRF3 Pathway

Tuo Han[#], Ting Zhang[#], Yajie Fan, Congxia Wang[*] and Yan Zhang[*]

Department of Cardiology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

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Abstract

Myocardial ischemia/reperfusion (I/R) exacerbates ischemic cell death, in which endoplasmic reticulum (ER) stress and pyroptosis have major implications. Previous evidence has shown that the stimulator of interferon genes/interferon regulatory factor (STING/IRF3) pathway regulates numerous immune and inflammatory responses and is implicated in a range of autoimmune diseases, pathogenic infections, cancer, and cardiovascular diseases. Our most recent study linked STING activation to ER stress and the unfolded protein response (UPR). In addition, STING has a potential role in activating the NOD-like receptor protein 3 (NLRP3)/caspase-1 inflammasome cascade and inducing cell pyroptosis. Therefore, the STING/IRF3 pathway could be a bridge between the upstream ER and oxidative stress and downstream NLRP3/Caspase-1 pathway and pyroptosis and the expression and release of the inflammatory cytokines interleukin-1 (IL-1β) and interleukin-18 (IL-18) triggers, and therefore, myocardial I/R injury. The targeting of STING/IRF3 is of increasing interest in therapeutic agents to reduce I/R injury.

Introduction

Ischemic heart disease is one of the greatest health threats to human health, which causes numerous deaths annually worldwide.[1] Myocardial ischemia/reperfusion (I/R) injury induces apoptosis of cardiomyocytes and leads to adverse cardiovascular consequences, cardiac dysfunction, and ventricular remodeling.[2] The imbalance in oxygen supply and demand within the ischemic field leads to pervasive tissue hypoxia and microvascular dysfunction. Subsequent reperfusion further aggravates the damage by activating inflammatory responses and cell death.[3] With the application of revascularization therapy, myocardial I/R injury is becoming more common and gaining increasing attention. It has been well-established that the excessive accumulation of reactive oxygen species, inflammatory response, myocardial cell apoptosis, and intracellular calcium (Ca^{2+}) overload exert a synergistic impact on the pathogenesis of myocardial I/R damage.[4] However, no available drugs that target these mechanisms are applied in clinical treatment.

Pyroptosis is a newly discovered form of programmed cell death that is characterized by plasma membrane pore formation and mediated by gasdermin D (GSDMD) and the extracellular release of inflammatory cytokines, interleukin-1 (IL-1β) and interleukin-18 (IL-18).[5] There is evidence that inflammation induced during I/R injury by pyroptosis contributes to cardiac cell death, excessive fibrosis, and poor clinical outcomes.[6] Therefore, there is increasing interest in exploiting the components of pyroptosis as agents to reduce I/R injury.

The endoplasmic reticulum (ER) is an essential organelle that is involved in several cellular functions. In response to stressful insults, the ER environment is compromised, and protein maturation is impaired; this causes an accumulation of misfolded proteins in the ER, and therefore, activates the unfolded protein response (UPR).[7] The UPR protects cells from stress and helps to maintain

Keywords: Myocardial ischemia/reperfusion; Endoplasmic reticulum stress; Pyroptosis; NOD-like receptor protein 3; Inflammasome; Stimulator of interferon genes.

Abbreviations: I/R, ischemia/reperfusion; H/R, hypoxia/reoxygenation; ROS, reactive oxide species; ER, endoplasmic reticulum; UPR, unfolded protein response; ATF6, activating transcription factor 6; Grp78, 78-kDa glucose-regulated protein; SITNG, Stimulator of interferon genes; IRF3, interferon regulatory factor 3; NLRP3, NLR family pyrin domain-containing protein 3; ACS, apoptosis-related speckle-like protein; IL-1β, interleukin-1β; IL-18, interleukin-18; GSDMD, gasdermin D; PFD, pore-forming domain; LPS, lipopolysaccharide; cGAS, cyclic GMP-AMP synthase; ERGIC, ER-Golgi intermediate compartment.

[*]**Correspondence to**: Yan Zhang, Department of Cardiology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. ORCID: https://orcid.org/0000-0003-0895-5170. Tel: +86-029-86320423, Fax: +86-029-87678246, E-mail: zy1985525@126.com; Congxia Wang, Department of Cardiology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. ORCID: https://orcid.org/0000-0001-6262-2276. Tel: +86-029-87679770, Fax: +86-029-87678246, E-mail: wcxcirculation@163.com

[#]These authors contributed equally to this manuscript.

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cellular homeostasis. However, under sustained ER stress, UPR activation can result in cell death.[7] A variety of diseases have been implicated in ER stress, including metabolic disorders, neurodegenerative and cardiovascular diseases, cancer, and viral infections.[8] Targeting components of the UPR is gaining increased interest as a therapeutic strategy.

The stimulator of interferon genes (STING) was first discovered as an essential molecule that regulates inflammation and immune responses to viral infections.[9] It plays an important role in several diseases. STING deficiency in mice significantly improved their survival and cardiac function by downregulating inflammatory cytokines in the myocardium and serum, reducing cell apoptosis and pyroptosis in mice that suffered from sepsis-induced cardiomyopathy.[9] STING can be activated by a range of stressors, such as viral infection, ER stress, or cytosolic double-stranded DNA (dsDNA) that is released from the nucleus or mitochondria.[10] STING might act as a bridge that connects upstream ER stress and downstream pyroptosis, which could be a promising therapeutic target for the prevention of I/R injury. In this paper, the recent research will be summarized and a brief overview will be provided of ER stress, STING activation, and pyroptosis during myocardial I/R injury.

ER stress and myocardial I/R injury

Under myocardial I/R injury, the cardiomyocytes suffered from hypoxia and mitochondrial dysfunction in the ischemic state, which resulted in a lower level of ATP production and dysfunction of transmembrane ion exchange. If ischemia is prolonged, it can lead to energy depletion and necrotic cell death, which is accompanied by increased reactive oxide species (ROS) production and local inflammation during subsequent reperfusion due to reoxygenation; therefore, exacerbating secondary damage to myocardial cells.[4] Intracellular ROS accumulation induced ER stress and UPR, activated the inflammasome, and promoted the intracellular inflammatory response, and therefore, exacerbated cardiomyocyte necrosis and apoptosis.[11] Therefore, myocardial I/R damage is accompanied by ER stress, in which ROS overproduction and clearance dysfunction exert a major influence. The role of ER stress-related proteins, such as CCAAT/enhancer binding protein (C/EBP) homologous protein, activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK), has been extensively studied in myocardial I/R injury.[12] However, a recent study uncovered a previously unknown association between ER stress and oxidative stress. ATF6 deficiency in cardiomyocytes that were subjected to hypoxia/reoxygenation (H/R) injury revealed increased ROS and necrosis, both of which were alleviated by ATF6 overexpression. Knockdown of ATF6 caused increased infarct size and decreased cardiac function after I/R injury *in vivo*. ATF6 induced several oxidative stress response genes including catalase, which is responsible for clearing harmful ROS in the heart. ER stress response elements have been identified in the catalase gene and might bind directly to ATF6; therefore, increasing catalase promoter activity.[8] Therefore, the link between ER stress and I/R injury needs further investigation.

Pyroptosis and gasdermin family

Pyroptosis is a newly discovered form of programmed cell death that is mediated by the inflammasome, which includes canonical caspase-1-dependent signaling and non-canonical caspase-11/4/5-dependent signaling.[13] During canonical pyroptosis, the NOD-like receptor family with pyrine domain 3 (NLRP3) recruits the adapter apoptosis-associated (ACS) speckle-like protein that contains a caspase recruitment domain and the cysteine protease procaspase-1. They eventually assemble to form the NLRP3-inflammasome complex, which leads to self-cleavage of procaspase-1.[14] Non-canonical pyroptosis occurs via the binding of lipopolysaccharide (LPS) derived from Gram-negative bacteria to procaspase-4, -5, or -11 in the cytosol, which then triggers cleavage and activation of downstream targets.[15] The activated caspase-1 then cleaves GSDMD and releases its N-terminal fragment (GSDMD-NT), which oligomerizes and creates pores in the cell membrane. In addition, caspase-1 cleaves and activates pro-interleukin-1 β (IL-1β) and pro-interleukin-18 (IL-18) into mature IL-1β and IL-18, which could be released through the GSDMD-created pores into the intercellular space and increase inflammation.[14]

Gasdermins (GSDMs) act as the gatekeepers of pyroptosis. Upon activation, gasdermins are activated by proteolytic cleavage to release their NT fragment; therefore, forming large cell membrane pores that release pro-inflammatory cytokines and lead to cell death, for instance, pyroptosis.[16] Currently, gasdermins consist of six members: gasdermins A, B, C, D, E, and DFNB59.[17] Most GSDM members have conservative N- and C-terminal structures and there are differences in the linker region. Activated caspase-1 or caspase-11/4/5 are cleaved in the linker in GSDMD to release the N-terminus with pore-forming domain (PFD) activity. PFD oligomerizes to form 10–15 nm diameter holes on the cell membrane, which leads to the disruption of intracellular osmotic pressure and results in cell swelling and membrane rupture.[18] Most members of the gasdermin family have an intrinsic membrane pore-forming ability (except for DFNB59) *in vivo*, *in vitro,* or both. Among them, caspase-3 activation, previously thought to be a hallmark of apoptosis, can cleave gasdermin E to produce PFD with membrane pore-forming activity, which leads to cell pyroptosis.[19] Therefore, based on these findings, pyroptosis is currently classified as a programmed cell death that is mediated by proteins of the gasdermin family.[20]

Pyroptosis in myocardial I/R injury

Myocardial I/R injury is associated with a cascade of inflammatory responses that release damage-associated molecular patterns (DAMPs). Classical DAMPs consist of several nuclear or cytosolic proteins, such as histones, heat shock proteins and S100 proteins, the nuclear protein high mobility group box 1 (HMGB1), deoxyribonucleic acids (DNAs) (e.g., nuclear and mitochondrial), ribonucleic acids (RNAs), and adenosine triphosphate (ATP).[21] DAMPs could be recognized by intracellular pattern-recognized receptors (PRPs), such as NLR family pyrin domain-containing protein 3 (NLRP3). Normally, NLRP3 is automatically repressed by an internal interaction between its C-terminal leucine-rich repeats and a central NACHT domain. In the presence of danger signals, NLRP3 is opened and binds to an ASC to recruit procaspase-1; therefore, forming the NLRP3-inflammasome complex, which converts procaspase-1 into active caspase-1. Caspase-1 then cleaves pro-IL-1β and pro-IL-18 into active IL-1β and IL-18 to exacerbate the inflammatory response cascade.[22] The mechanism of how IL-1β and IL-18 enter the extracellular domain remains unclear, as both lack exocrine signaling peptides. In addition, activated caspase-1 could cleave GSDMD and release its intramolecular inhibition of the GSDMD N-domain, which has intrinsic pyroptosis-inducing and

pore-forming activity.[18] It was assumed that IL-1β and IL-18 escape through GSDMD-generated pores to exacerbate the inflammatory cascade, although the specific mechanism remains unclear.

Several studies have demonstrated the activation of NLRP3 inflammasome and pyroptosis in I/R-injured cardiomyocytes.[23] The pyroptosis-related proteins NLRP3, caspase-1, and IL-1β were significantly elevated by I/R induction.[24] In addition, the amount of GSDMD N-domains was upregulated in cardiomyocytes during I/R or H/R damage and was mediated by the activation of the TLR4/MyD88/NF-κB/NLRP3 inflammasome signaling pathway.[25] The subcutaneous injection of isoproterenol, which is a selective agonist of the β-adrenergic receptor, induced NLRP3 inflammasome activation and pyroptosis in mouse myocardial cells.[26] Further investigations revealed that the propagation of inflammatory injuries in the heart was facilitated via membrane nanotubes that mediated distant intercellular connections and communication.[26] In addition, our previous studies showed that the expression of the inflammatory cytokines IL-1β and IL-18 in the myocardium and serum of myocardial I/R rats was significantly upregulated, which indicated that the NLRP3/caspase-1 pathway was activated during I/R injury.[27] Therefore, several studies have focused on identifying components of the NLRP3-inflammasome for therapy in myocardial I/R injury.

Metformin exerts many health and disease benefits beyond its hypoglycemic effects. Post-conditioning with metformin can protect against myocardial I/R injury and cell pyroptosis via the AMPK/NLRP3 inflammasome pathway.[28] Sevoflurane, which is widely used as an anesthetic, exerted a protective effect on the myocardium by inhibiting the P2X7/NLRP3 pathway, and therefore, reduced IL-1β, IL-18, and GSDMD levels.[29] Dexmedetomidine alleviated myocardial I/R injury in rats and inhibited H/R-induced pyroptosis in myocardial cells by downregulating miR-29b, to activate the FoxO3a/ARC axis.[30] The traditional Chinese medicine *apigenin* might attenuate pyroptosis and apoptosis of H9c2 cells that are induced by H/R injury and reduce the elevated levels of IL-1β and IL-18.[31] Mesenchymal stem cells (MSCs) transplantation is considered a promising therapeutic strategy to improve cell viability after myocardial infarction and to regulate the inflammatory response. Exosomes, or extracellular vesicles that are derived from MSCs or anti-inflammatory M2 macrophages, protect against I/R injury by inhibiting NLRP3 inflammasome signaling and cell pyroptosis.[32] M2 macrophage-derived exosomes carried miR-148a could mitigate myocardial I/R damage by suppressing TXNIP and inactivating the TLR4/NF-κB/NLRP3 inflammasome cascade.[33] MSCs-derived exosomes could protect the myocardium from I/R injury through downregulation of pyroptosis likely mediated by miR-320b on NLRP3 inhibition.[34]

Micro RNA is a type of non-coding RNA, 20~22 nucleotides in length, which could bind the 3′-UTR of target messenger RNA (mRNA) and induce its degradation. Inhibition of miR-132 enhanced myocardial I/R injury via inhibiting oxidative stress and pyroptosis by activating PGC-1α/Nrf2 signaling through targeted Sirtuin 1.[24] miR-149 exacerbated pyroptosis by silencing FoxO3 in I/R stimulated H9c2 cells.[35] MiR-703 was repressed in mouse cardiomyocytes after H/R stimulation. Restoration of its expression in cardiomyocytes counteracted H/R-induced cytotoxicity and pyroptosis by inhibiting the NLRP3/caspase-1 pathway.[36]

IP3R1, an intracellular ion channel receptor that releases Ca^{2+} from the ER, was increased in I/R suffering rat myocardium. IP3R1 silencing alleviated myocardial I/R injury, decreased Ca^{2+} overload, inflammation, and pyroptosis in I/R rats and H/R-induced cells.[37] Uric acid (UA) is the end product of purine metabolism, and increasing evidence suggests that UA increases the sensitivity of cardiomyocytes to I/R injury.[38] Recent studies have shown that UA exacerbated the activation of the NLRP3 inflammatory cascade and pyroptosis by promoting ROS generation.[39] In addition, LPS could aggravate high glucose and H/R-induced H9c2 cell damage by promoting ROS production to induce NLRP3 inflammasome-mediated pyroptosis.[40] These studies confirmed the important role of pyroptosis mediated by NLRP3 inflammasome in myocardial I/R damage and indicate that it could be a therapeutic target to reduce I/R injury.

Targeting pyroptosis in IR injury

During myocardial I/R, the expression of canonical components of the NLRP3 inflammasome is highly upregulated, as is the activation of caspase-1 and the secretion of IL-1β and IL-18.[41] The NLRP3 inflammasome is an intracellular multiprotein complex whose activation then induces pyroptosis.[42] Therefore, several studies have focused on the intervention of the NLRP3 inflammasome to mitigate cell pyroptosis in myocardial I/R injury. To date, several reports have confirmed the contribution of pyroptosis to myocardial damage by targeting the NLRP3/caspase-1 inflammatory signaling.[14] Pharmacological inhibition of the NLRP3 inflammasome by INF4E, which is a newly synthesized NLRP3 inhibitor, weakened myocardial I/R injury by activating the prosurvival reperfusion injury salvage kinase (RISK) pathway and enhancing mitochondrial function.[43] VX765, which is a canonical caspase-1 inhibitor synthetically administered to rats at reperfusion with a P2Y12 receptor antagonist, resulted in the long-term reduction in myocardial infarction size and maintenance of ventricular function and decreased the release of lactate dehydrogenase, which is a marker for pyroptosis.[44] In addition, VX-765 could reduce myocardial infarction in Langendorff-perfused rat hearts with I/R injury by activating the RISK pathway.[45] Traditional Chinese medicines, such as *gastrodin* or *Cinnamomi Ramulus*, could inhibit pyroptosis of cardiac microvascular endothelial cells and reduce IL-1β production via an inhibitory NLRP3/caspase-1 inflammasome.[46] Polydopamine (PDA), which is a biodegradable class of nanomaterials with excellent antioxidant capacity, showed significant potential to prevent myocardial I/R damage. The PDA-based biomimetic nanoplatform (PDA@M), which is composed of a polydopamine core and a macrophage membrane to form an envelope–core structure, effectively alleviated myocardial I/R-induced oxidative stress and suppressed cell pyroptosis by inhibiting the NLRP3/caspase-1 pathway.[47] In addition, the inhibition of aquaporin 4 effectively ameliorated myocardial injury and reduced cardiomyocyte pyroptosis *in vitro* and could be proposed as a new strategy to treat I/R damage.[48]

The key roles of GSDMs in cell pyroptosis have prompted investigations into GSDMs-targeting therapeutics. Among them, GSDMD is a particularly promising drug target, because it is the critical point for all inflammasome activations and serves upstream of the IL-1β and cytokine secretion and downstream of the inflammasome sensors.[16] The targeting of GSDMD inhibition and possibly other GSDM members might therefore be an attractive strategy to treat cardiovascular diseases, such as myocardial I/R injury and other conditions that are aggravated by pyroptosis. To date, some chemicals or drugs have been reported as potential inhibitors of GSDMD. Disulfiram (DSF), an FDA-approved drug for alcoholism, is a potent inhibitor of GSDMD pore formation by modifying a reactive cysteine in GSDMD with a half-maximal inhibitory concentration (IC50) in the nanomolar range.[49] Dimethyl fumarate (DMF), a drug for the treatment of multiple sclero-

sis, reportedly reacted with GSDMD at critical cysteine residues to form S-(2-succinyl)-cysteine.[50] Succination prevents GSDMD from interacting with caspases; therefore, limiting its processing, oligomerization, and ability to induce cell pyroptosis.[50] In summary, targeting GSDMD and other GSDMs could be a promising strategy to reduce I/R damage.

STING-IRF3 pathway: bridge between ER stress and pyroptosis

STING is one of the pathogen recognition receptors and is located on the ER, which is known as *TMEM173, MPYS,* or *MITA*, and does not directly bind to DNA.[51] It is activated by cyclic dinucleotides (CDNs), which are second messengers that are derived from microorganisms or synthesized by cyclic GMP-AMP synthase (cGAS).[52] Cytosol-free DNA could bind to cGAS and then trigger ATP and GTP conversion into cyclic guanosine monophosphate adenosine monophosphate (cGAMP).[51] cGAMP is one of the canonical CDNs, which binds and activates STING, and triggers its conformational change, dimerization, and translocation to the ER-Golgi intermediate compartment (ERGIC). TANK-binding kinase 1 (TBK1) is recruited to STING dimers, which results in the phosphorylation of STING^{Ser366}, and creates itself as a docking site for interferon regulatory factor 3 (IRF3) binding and its eventual phosphorylation by TBK1. In addition, STING can activate the nuclear factor B (NF-κB) pathway.[53] IRF3 and NF-κB then translocate into the cell nucleus and bind to the promoter of downstream target genes (i.e., *IFN*) to induce the expression of inflammatory cytokines that are involved in host immune responses.[54]

STING is now recognized as an essential molecule that regulates inflammation and immune responses in numerous diseases.[55] LPS stimulation triggered the perinuclear translocation of STING, which binds to IRF3 and triggers its phosphorylation. Phosphorylated IRF3 subsequently migrated to the nucleus and increased the expression of NLRP3.[9] In addition, STING recruited IKK (an inhibitor of NF-κB), which resulted in the dissociation and release of NF-κB through self-phosphorylation. NF-κB then migrates into the cell nucleus and binds to the promoter of the inflammatory cytokines IL-1β and IL-18 to induce their expression. Pro-IL-1β and pro-IL-18 are then recruited by the NLRP3 inflammasome and cleaved into their activated forms by caspase-1 to exacerbate the inflammatory cascade.[53] Studies have shown that STING activates IRF3 and therefore exacerbates LPS-induced myocardial damage via the NLRP3 inflammasome pathway, which leads to inflammation, myocardial cell apoptosis, pyroptosis, and cardiac dysfunction.[9] In addition, phosphorylated IRF3 dimer enters the nucleus and binds to the promoter of intercellular adhesion factor 1 (ICAM1), which induces its expression in metabolic disorder-associated endothelial inflammation.[56] Our previous study found that STING expression was upregulated in human and mouse hypertrophic hearts, as the components of ER stress, such as *PERK, IRE-1,* and phospho-eukaryotic initiation factor 2 alpha (*eIF2*).[27] However, STING deficiency significantly reversed the previously mentioned abnormalities in mice with aortic banding-induced cardiac hypertrophy. Furthermore, angiotensin II (Ang II)-induced STING could be accelerated by the ER stress activator and significantly reduced by the ER stress inhibitor.[27] These results defined STING as an important signaling device in cardiac hypertrophy, which is closely related to ER stress and could be a bridge between ER stress and cell pyroptosis.

Prospectives

Canonical ER stress is modulated by ER-transmembrane stress sensors. Under normal conditions, these sensors are inhibited by the binding of their luminal domains to the chaperone 78-kDa glucose-regulated protein (BIP/Grp78).[55] Upon stress increases, the binding of misfolded proteins to BIP/Grp78 leads to the release of those stress sensors (e.g., PERK, IRE1, and ATF6) that allow their activation.[56] Since SINTG is located on the ER membrane, STING might interact with BIP/Grp78 to be inhibited. Previous evidence revealed that the STING/IRF3 pathway regulated immune and inflammatory responses, and participated in several autoimmune disorders, pathogen infections, cancer, and cardiovascular diseases, and was related to ER stress. In addition, STING/IRF3 has a potential role in activating the NLRP3/caspase-1 inflammasome cascade and inducing cell pyroptosis. According to our previous findings, the STING/IRF3 pathway could serve as a bridge between upstream ER stress and ROS, and the downstream NLRP3/caspase-1 pathway and pyroptosis, stimulating the expression and intercellular release of IL-1β and IL-18; therefore, modulating myocardial I/R injury. Targeting STING/IRF3 is of increasing interest as a therapeutic method to prevent I/R injury (Fig. 1).

Conclusions

Myocardial I/R aggravates ischemic cell injury, in which ER stress and pyroptosis might exert a significant impact. However, the specific pathogenesis is unclear, and therefore, limits the optimal treatments in clinical practice. The STING/IRF3 pathway might act as a bridge between upstream ER stress and ROS and the downstream NLRP3 inflammasome-pyroptosis cascade. Therefore, targeting the STING/IRF3 pathway could be a new and promising strategy with increasing interest to prevent myocardial I/R injury. Several related clinical trials and preclinical research have commenced, which could provide exciting results in the near future.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding this article.

Author contributions

TH and TZ wrote the manuscript; YF, CW, and YZ critically reviewed the manuscript; YZ supported the study and completed the manuscript. All authors made significant contributions and approved the final manuscript.

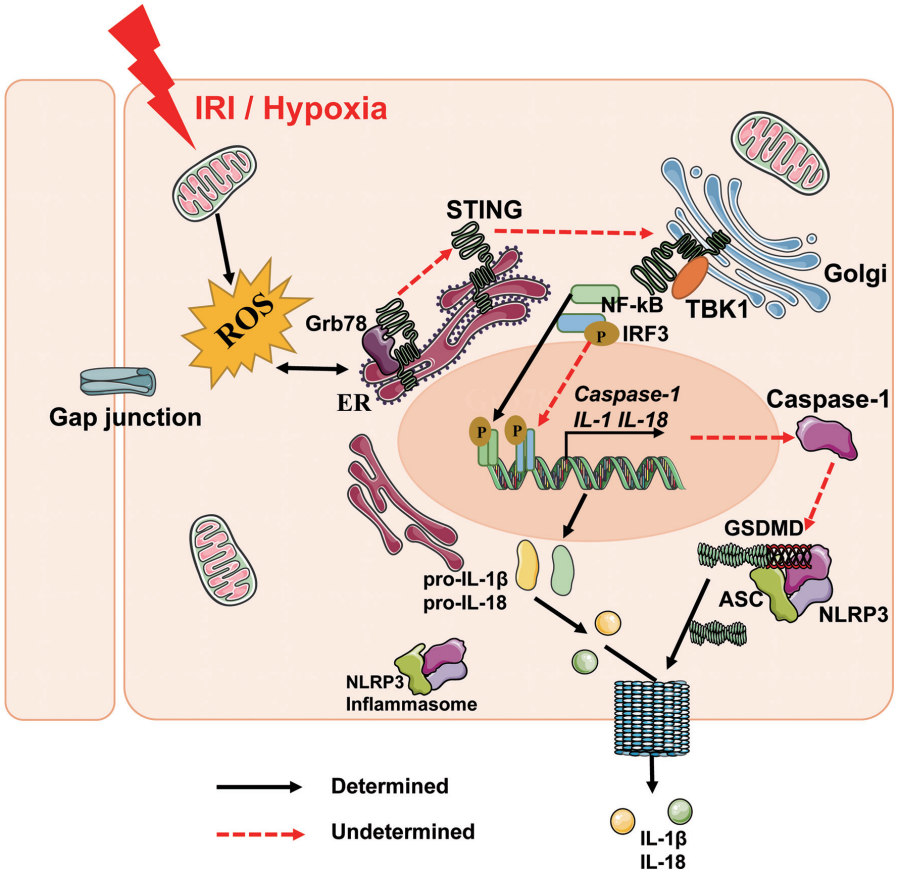

Fig. 1. Schematic model of the role of ER stress and STING/IRF3 in pyroptosis in myocardial I/R injury. Under stress states, such as myocardial I/R, ER stress was activated and the binding of Grp78 was removed, which resulted in the release and activation of STING and the subsequent phosphorylation of IRF3. Bioinformatics analysis revealed that a potential IRF3 binding site exists in the promoter region of the caspase-1 gene. Therefore, myocardial I/R aggravates ischemic cell injury, in which ER stress and pyroptosis activated by the STING-IRF3 pathway might exert great impacts. I/R, ischemia/reperfusion; ROS, reactive oxide species; ER, endoplasmic reticulum; Grp78, 78-kDa glucose-regulated protein; STING, stimulator of interferon genes; IRF3, interferon regulatory factor 3; TBK1, TANK-binding kinase 1; NLRP3, NOD-like receptor protein 3; ACS, apoptosis-related speckle-like protein; GSDMD, gasdermin D.

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