Molecular Therapy
Nucleic Acids
Review

Regulation of pyroptosis in cardiovascular pathologies: Role of noncoding RNAs

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Cardiovascular disease (CVD) is one of the most important diseases endangering human life. The pathogenesis of CVDs is complex. Pyroptosis, which differs from traditional apoptosis and necrosis, is characterized by cell swelling until membrane rupture, resulting in the release of cell contents and activation of a strong inflammatory response. Recent studies have revealed that inflammation and pyroptosis play important roles in the progression of CVDs. Noncoding RNAs (ncRNAs) are considered promising biomarkers and potential therapeutic targets for the diagnosis and treatment of various diseases, including CVDs. Growing evidence has revealed that ncRNAs can mediate the transcriptional or posttranscriptional regulation of pyroptosis-related genes by participating in the pyroptosis regulatory network. The role and molecular mechanism of pyroptosis-regulating ncRNAs in cardiovascular pathologies are attracting increasing attention. Here, we summarize research progress on pyroptosis and the role of ncRNAs, particularly microRNAs (miRNAs), long ncRNAs (lncRNAs), and circular RNAs (circRNAs), in the regulation of pyroptosis in CVD pathologies. Identifying these disease-related ncRNAs is important for understanding the pathogenesis of CVDs and providing new targets and ideas for their prevention and treatment.

INTRODUCTION

Cardiovascular disease (CVD) is one of the most important diseases endangering human life. According to the official statistics of the Global Burden of Disease collaboration, the prevalence of CVDs nearly doubled from 271 million in 1990 to 523 million in 2019. In addition, the number of CVD-related deaths steadily increased from 12.1 million in 1990 to 18.6 million in 2019.[1] The characteristics of high morbidity, high mortality, high disability, high recurrence rate, and multiple complications make CVD the most fatal disease.[2] The increasing incidence and mortality of CVDs have become a major public health problem that urgently needs to be addressed through effective interventions.

The pathogenesis of CVDs is complex. Adult cardiomyocytes are terminally differentiated cells with limited proliferative capacity. Untimely or inappropriate myocardial cell loss as a result of excessive cell death may contribute to an irreversible loss of cardiac function, ultimately causing myocardial infarction, malignant arrhythmia, heart failure, and sudden cardiac death.[3] Apoptosis is the first widely recognized mode of programmed cell death. Many nonapoptotic cell death mechanisms, including necroptosis, pyroptosis, ferroptosis, and autophagy-dependent cell death, have been identified in the past few decades.[4] Pyroptosis, which differs from traditional apoptosis and necrosis, is a pro-inflammatory type of cell death. It is characterized by cell swelling until membrane rupture, resulting in a massive release of cell contents and a strong inflammatory response. The nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is activated in response to multiple stimuli and subsequently induces cardiomyocyte pyroptosis; this also occurs in many other cell types, including macrophages, vascular smooth muscle cells (SMCs), endothelial cells (ECs), and fibroblasts, finally contributing to the progression of CVDs.[5] The suppression of the NLRP3 inflammasome through the direct knockout of *Nlrp3* or through the downregulation of its expression by negative regulators inhibits cellular pyroptosis and alleviates cardiac damage.[6–9] Therefore, NLRP3-inflammasome-mediated pyroptosis plays a crucial role in the pathogenesis of CVDs and provides novel therapeutic strategies.

Noncoding RNAs (ncRNAs) account for up to 98%–99% of the human genome and participate in regulating the expression of protein-coding genes.[10,11] The interaction between ncRNAs and protein-coding genes forms a highly complex RNA regulatory network. Abnormalities in ncRNAs are closely related to numerous diseases. Recent efforts to identify the molecular mechanisms underlying the pathogenesis of CVDs have revealed a significant regulatory role of ncRNAs in the occurrence and development of major CVDs, such as myocardial infarction, coronary heart disease, atrial fibrillation, atherosclerosis, cardiomyopathy, and heart failure.[12–14] The main classes of ncRNAs are microRNAs (miRNAs), long ncRNAs

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(lncRNAs), and circular RNAs (circRNAs); these classes possess different lengths and characteristics and unique functional mechanisms. miRNAs are single-stranded RNA molecules that are approximately 22 nucleotides long and can negatively regulate gene expression at the posttranscriptional level.[15] lncRNAs are transcripts longer than 200 nucleotides and are involved in almost every stage of gene expression regulation.[11] circRNAs, a new type of endogenous RNA with a covalent closed loop structure, are abundantly expressed in mammalian cells and are involved in the transcriptional or posttranscriptional regulation of gene expression.[16] lncRNAs and circRNAs can act as competing endogenous RNAs (ceRNAs) to regulate miRNAs and subsequently interact with their mRNA targets. Notably, the short length of miRNAs and the circular structure of circRNAs endow them with excellent stability, resistance to enzymatic degradation, and the ability to circulate in bodily fluids for an extended duration.[17] Thus, ncRNAs are promising biomarkers and potential therapeutic targets for the diagnosis and treatment of CVDs.

In recent years, growing evidence has revealed that ncRNAs mediate the transcriptional or posttranscriptional regulation of pyroptosis-related genes by participating in the pyroptosis regulatory network. The role and molecular mechanism of pyroptosis in cardiovascular pathologies are attracting increasing attention. Identifying disease-related ncRNAs is important for understanding their pathogenesis of CVDs and providing new targets and ideas for prevention and treatment. This review summarizes research progress on pyroptosis and recent findings on the role of ncRNAs in the regulation of pyroptosis-related CVDs, such as diabetic cardiomyopathy, atherosclerosis, myocardial infarction, ischemia-reperfusion (I/R) injury, cardiac hypertrophy and heart failure, uremic cardiomyopathy (UCM), and sepsis-induced myocardial dysfunction. Moreover, the potential clinical implications of pyroptosis and ncRNAs in CVDs, current limitations, and future research directions are discussed.

PYROPTOSIS

The history of knowledge about pyroptosis

As early as 1992, Zychlinsky et al.[18] discovered that macrophages infected by *Shigella flexneri* undergo programmed cell death. Based on cytological observations using electron microscopy and DNA fragmentation using electrophoresis, it was believed that these cells had undergone apoptosis. Later, in 1994, Zychlinsky et al.[19] further identified that macrophages undergoing apoptosis induced by *S. flexneri* infection rapidly and strongly release the inflammatory cytokine interleukin-1 (IL-1). In 1996, Chen et al.[20] reported that IL-1β-converting enzyme (ICE; caspase-1) is activated during *S. flexneri* infection but that specific inhibitors of caspase-1 prevent *S.-flexneri*-induced apoptosis. At this time, studies all used "apoptosis" to describe this type of inflammatory cell death.[20,21]

Nevertheless, further studies pointed out that *Salmonella-typhimurium*-induced macrophage cell death exhibits the feature of significant DNA fragmentation but that the membrane swelled and ruptured; furthermore, this type of death was not accompanied by activation of caspase-3 and PARP, but was dependent on caspase-1 activity.[22,23] Because traditional apoptosis does not cause inflammation, it was confirmed that the programmed cell death that depends on caspase-1 and promotes the inflammatory response is a new form of cell death, which was named "pyroptosis" by Cookson and Brennan[24] in 2001, based on the Greek words "pyro-" (meaning fire) and "-ptosis" (meaning failing). Moreover, in 2014, Shi et al.[25] reported that caspase-4/5/11 can be activated via direct binding to cytoplasmic lipopolysaccharide (LPS) without binding to intracellular receptors to trigger pyroptosis. Subsequently, gasdermin D (GSDMD) was identified as another key effector substrate for inflammatory caspases in addition to pro-IL-1β/pro-IL-18.[26–28] Caspase-1 and caspase-4/5/11 cleave GSDMD to relieve the autoinhibitory activity of the C-terminal domain and release the pore-forming activity of the N-terminal domain (GSDMD-NT), which binds to membrane lipids and lyses cells by forming pores.[29–33] Other gasdermin family members, such as GSDMA3 and GSDME, have similar functions.[29,34] Thus, gasdermin family proteins are the principal executioners of pyroptosis, and Shi et al.[35] defined pyroptosis as programmed necrotic cell death mediated by the gasdermin family. In 2018, the Nomenclature Committee on Cell Death updated the definition of pyroptosis to "a type of regulated cell death (RCD) that critically depends on the formation of plasma membrane pores by members of the gasdermin protein family, often (but not always) as a consequence of inflammatory caspase activation."[36]

The characteristic features of pyroptosis

Intracellular protein complexes referred to as inflammasomes form through the innate immune system in cells infected by pathogens or in response to endogenous danger signals.[37] Next, caspases are activated to cleave gasdermin protein family members, and the released N-terminal domain recognizes and punches holes in the cell membrane, forming many honeycomb-shaped pores with a diameter of approximately 10–15 nm.[29] This pore-forming activity destroys the osmotic pressure of the cell; the imbalance of electrolytes inside and outside of the cell causes the cell to form bubble-like protrusions (pyroptotic bodies) and finally rupture, releasing a large amount of pro-inflammatory cytoplasmic contents, recruiting immune cells to trigger the inflammatory cascade, and resulting in pyroptosis.[26]

Although pyroptosis and apoptosis share some characteristics, such as chromatin condensation, DNA damage, and positive TdT-mediated dUTP nick end labeling (TUNEL) staining, the former is lytic cell death accompanied by rapid cell membrane rupture, while the latter is non-lytic cell death accompanied by the formation of apoptotic bodies at later stages.[38] Similar to necroptosis, pyroptosis results in cell bursting, cellular content release, and inflammation. However, pyroptosis is driven by non-selective gasdermin pores, while necroptosis is mediated by iron channels formed by the mixed lineage kinase domain-like (MLKL) protein.[31] Ferroptosis is an iron-dependent form of cell death characterized by the excessive accumulation of reactive oxygen species (ROS) and lipid peroxides.[39] The characteristic morphological changes associated with ferroptosis include smaller mitochondria with increased membraned density,

Table 1. The main features of pyroptosis and other RCD processes

RCD	Pyroptosis	Apoptosis	Necroptosis	Ferroptosis	Autophagy
Inducement	infection by certain pathogens and pathological stimuli	gene regulation under physiological conditions, pathological stimuli	extracellular cytokines, pathogens, and pathological stimuli	iron-dependent accumulation of intracellular lipid ROS	hypoxia, nutritional deficiency, ROS, and pathological stimuli
Morphological characteristics	cell swelling with many bubble-like protrusions, plasma membrane disruption	cell shrinking and blebbing, chromatin condensation, apoptotic bodies formation	cell swelling, plasma membrane disruption, necrosome formation	cell swelling, smaller mitochondrial size and disorganized cristae	double-membrane vacuoles (autolysosomes)
Biochemical characteristics	inflammasome formation, caspase and gasdermin dependent, pro-inflammatory factors release	DNA fragmentation of genomic DNA into multiples of 180–200 bp units	RIPK3-MLKL-dependent, intracellular contents spill out	iron accumulation and lipid peroxidation	increased lysosomal activity
Cell membrane	disruption	intact	disruption	disruption	intact
Inflammatory response	yes	no	yes	yes	no

reduced or absent cristae, and outer membrane rupture, making it quite different from other modes of RCD, including pyroptosis.[40] Autophagy is characterized by the formation of double-membraned autolysosomes for digesting damaged cell inclusions or organelles. Excessive autophagy can cause autophagy-dependent cell death, a type of RCD that relies on autophagic machinery.[36] The typical feature of autophagy cannot be observed during pyroptosis. The main features that distinguish pyroptosis from other forms of RCD are listed in Table 1, and the different molecular pathways are provided in Figure 1.

Mechanisms of pyroptosis

Pyroptosis is essentially a cascade of inflammatory responses that play an important role in immune defense to fight infections.[41] To date, pyroptosis can be triggered by the caspase-1-mediated canonical inflammasome and caspase-4/5/11-mediated noncanonical inflammasome pathways. Notably, proapoptotic caspases, such as caspase-3 and caspase-8, can also induce pyroptosis (Figure 1).

The caspase-1-mediated canonical inflammasome signaling pathway

Caspase-1-dependent pro-inflammatory cell death is the canonical pathway of pyroptosis. Caspase-1, the first discovered member of the caspase family, is activated by inflammasomes and is one of the most important pathways in cellular innate immunity.[42,43] Cells recognize exogenous pathogen-associated molecular patterns (PAMPs) or endogenous damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs) that then bind corresponding ligands to form multiprotein complexes to stimulate the innate immune response. PRRs can be divided according to their localization into membrane-bound PRRs, including C-type lectin receptors and Toll-like receptors, and cytoplasmic PRRs, including absent in melanoma 2 (AIM2)-like receptors, retinoic acid-inducible gene-I-like receptors, and NOD-like receptors (NLRs). The PRR proteins in the canonical pyroptosis pathway contain the NLR family, such as NLRP1, NLRP3, NLR-containing a caspase recruitment domain 4 (NLRC4), NLRP6, and NLRP9, which can be activated by a variety of exogenous and endogenous danger signals.[44,45] NLR family proteins have the following: a C-terminal leucine-rich repeat (LRR) domain that mainly mediates self-regulation and recognition signals; a central nucleotide-binding (NACHT) domain that is required for NLR activation and mediated self-oligomerization; and an N-terminal caspase recruitment (CARD) or pyrin (PYD) domain that interacts with adaptor molecules or downstream effector proteins to mediate signal transduction.[46] After recognizing a signal, the NLR transmits it to apoptosis-associated speck-like protein containing a ASC, a special adaptor protein containing an N-terminal PYD domain and a C-terminal CARD domain.[47] To respond to PAMP and DAMP stimuli, NLRs interact with ASCs via the PYD domain; the CARD domain of ASCs recruits the CARD domain of caspase-1 precursor (pro-caspase-1), forming the multiprotein signaling complex known as the inflammasome.[37,48] NLRP1, NLRP3, NLRC4, AIM2, and pyrin comprise the five major inflammasomes, of which NLRP3 is the most studied inflammasome receptor critical for immune defense to date.[49,50] Assembly of inflammasomes can mediate autoproteolysis of inactive pro-caspase-1 to its p20/p10 heterodimer mature form, which promotes the cleavage, maturation, and release of cytokine pro-IL-1β and pro-IL-18 into biologically active IL-1β and IL-18, respectively, to expand the inflammatory response. Moreover, activated caspase-1 can shear and activate GSDMD, and the active GSDMD-NT is transferred to the plasma membrane to form pores, causing water influx, cell swelling, and lysis during pyroptosis.[26,27,32]

The caspase-4/5/11-mediated noncanonical inflammasome signaling pathway

In the noncanonical pathway of pyroptosis, caspase-4/5/11 (caspase-4 and caspase-5 in humans and caspase-11 in mice) directly bind LPS from gram-negative bacteria through their CARD domains, promoting caspase-4/5/11 oligomerization and activation.[51,52] Activated caspase-4/5/11 cleaves GSDMD at Asp276 to produce GSDMD-NT that then self-assembles in the plasma membrane to form pores and stimulate the NLRP3 inflammasome to activate caspase-1-dependent IL-1β and IL-18 maturation, resulting in pyroptosis.[25,26] Furthermore, activated caspase-4/5/11 can also cleave the pannexin-1 channel protein, resulting in the release of ATP to induce P2X7 activation, which directly mediates K^+ efflux to activate the NLRP3 inflammasome.[41,53]

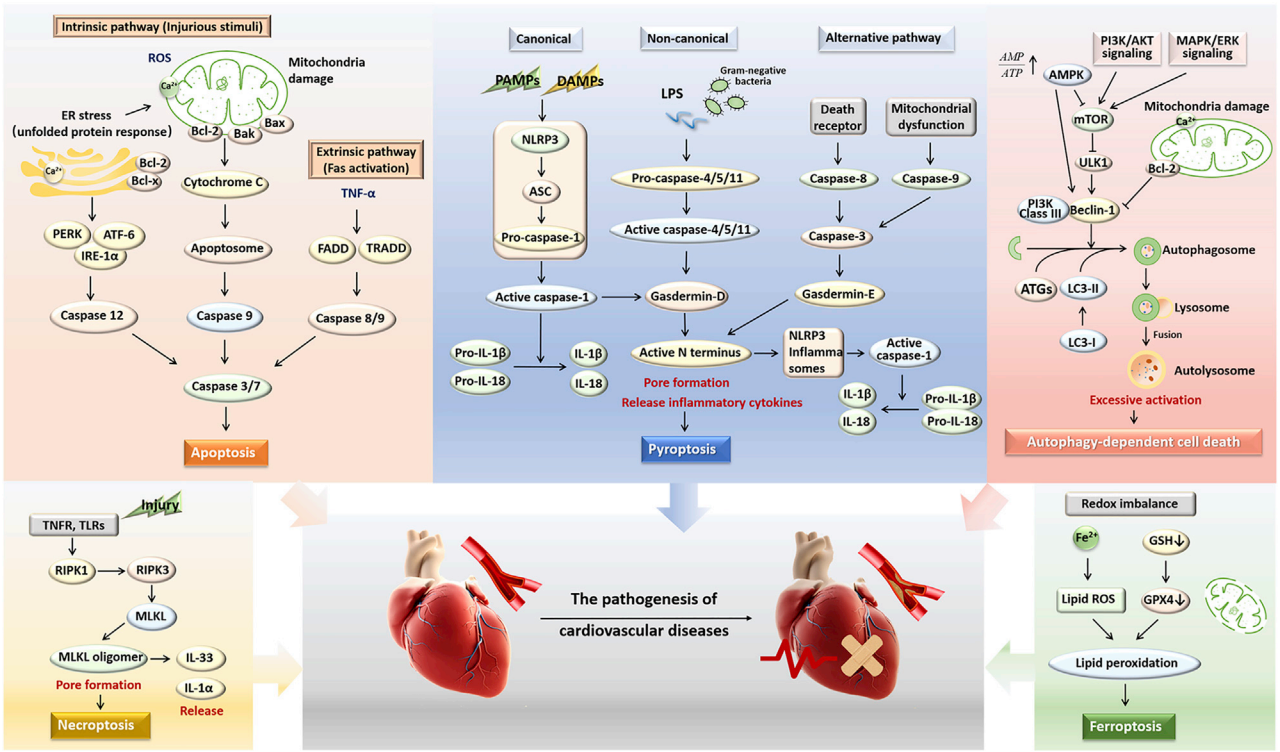

Figure 1. The molecular signaling mechanisms of pyroptosis and other forms of RCD that mediate pathogenesis of CVDs

Pyroptosis can be triggered by the caspase-1-mediated canonical inflammasome pathway, caspase-4/5/11-mediated noncanonical inflammasome pathway, and caspase-3- and caspase-8-mediated alternative pathways. Apoptosis mainly occurs through three signal transduction pathways: death receptor pathway, mitochondrial pathway, and endoplasmic reticulum pathway. Necroptosis can be triggered by the activation of receptor-interacting protein kinase 1 (RIP1) and RIP3. RIP1 binds and activates RIP3 to form the necrosome that then recruits and activates the mixed lineage kinase domain-like (MLKL) protein to translocate to the plasma membrane. The mTOR kinase is a critical modulator of autophagy induction and UNC-51-like kinase-1 (ULK1) acts downstream of the mTOR complex. AMPK can either inhibit mTOR activity or directly phosphorylate ULK1 to activate the class III PI3K complex (containing hVsp34, beclin-1, p150, and ATG-14 or UVRAG) to activate autophagy. Ferroptosis can be triggered indirectly by blocking glutathione synthesis, and directly by inhibiting glutathione peroxidase 4 (GPX4) activity, or by intracellular iron (mostly Fe^{2+}) accumulation that can effectively catalyze lipid ROS production, thereby initiating and propagating lipid peroxidation.

The caspase-3- and caspase-8-mediated pyroptosis

Thus far, 14 caspases have been implicated in apoptotic (caspase-2, -3, -6, -7, -8, -9, and -10) and inflammatory (caspase-1, -4, and -5) pathway cascades.[54] Of these apoptotic caspases, caspase-8 is involved in the initiation process and caspase-3 is the main executioner protease of apoptosis.[55] However, Wang et al.[34] revealed that caspase-3 can recognize and cleave GSDME (previously known as DFNA5) to generate a pore-forming GSDME-NT fragment that induces pyroptosis. Because of the faster progression of pyroptosis than apoptosis, caspase-3 is activated to cleave GSDME in cell lines with high GSDME expression, leading to pyroptosis upon chemotherapy drug treatment. Recently, Xu et al.[56] have revealed that persistent mitochondrial permeability transition elicited by bile acids drives assembly of an Apaf-1-caspase-4/11 pyroptosome that triggers caspase-3/GSDME-dependent pyroptosis. Nevertheless, caspase-3 can still induce apoptosis by activating Apaf-1 in GSDME-deficient or GSDME-low-expressing cells.

Sarhan et al.[57] used *Yersinia pseudotuberculosis* with costimulation of (5Z)-7-oxozeaenol (a small-molecule inhibitor of transforming growth factor β [TGF-β]-activated kinase 1 [TAK1]) and LPS to induce death in murine macrophages. The researchers observed that the ensuing cell death occurred rapidly, with IL-1β release and a morphology similar to that in pyroptosis, demonstrating that apoptotic caspase-8 can trigger pyroptosis through the cleavage of GSDMD and GSDME.[57] Loss of GSDMD delays membrane rupture, reverting the cell death morphology to that of apoptosis.[57] In tumor cells under hypoxia, PD-L1 can promote the expression of GSDMC, which is specifically cleaved by caspase-8, generating a GSDMC-NT that forms pores on the cell membrane and induces pyroptosis.[58] Thus, caspase-3 and caspase-8 act as regulators in pyroptosis, and caspase-3/GSDME and caspase-8/GSDMC/GSDMD/GSDME may be the signaling pathways involved in the mechanism of pyroptosis regulation.

Executioners of pyroptosis: gasdermin proteins

Activation of pyroptosis differs in response to diverse stimuli, but the downstream signaling pathways are similar. Gasdermin family proteins are the key executioners of pyroptosis and include GSDMA, GSDMB, GSDMC, GSDMD, GSDME, and DFNB59. The released

GSDMD-NTs form pores in the plasma membrane, a crucial step of pyroptosis, inducing a net increase in osmotic pressure, water influx, and cell swelling that eventually results in osmotic lysis and release of inflammatory intracellular contents.[59] As key executioners, the cleavage and activation of gasdermin proteins at specific site are the effect mechanism of caspase-induced pyroptosis. Thus, the form of cell death is not entirely determined by caspases in apoptotic or inflammatory pathways, but rather by the substrates recognized and hydrolyzed.

Detection of pyroptosis

The notable features of pyroptosis include the loss of plasma membrane integrity and release of cellular contents into the extracellular environment. As the morphological characteristic of pyroptosis are similar to those of necroptosis, it is necessary to detect pyroptosis from multiple aspects using the techniques described below.[60]

Cell viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell counting kit-8 (CCK-8) assay are commonly used for assessing cell viability and are suitable for detecting pyroptosis. The ATP-based CellTiter-Glo luminescent cell viability assay can also be used to assess cell viability.

Cell morphology

Transmission electron microscopy can be used to directly observe membrane pores. As propidium iodide (PI) can enter a dying cell with membrane disruption, changes in the integrity of the cell membrane can be assessed by PI fluorescent staining. PI-positive cells undergo lytic cell death. Membrane permeability changes can also be monitored using the lactate dehydrogenase (LDH) release assay. In addition, phase-contrast imaging and high-content imaging-based assays can be performed to observe dynamic changes in cell morphology and the cell death process.[34,61] Cells undergoing pyroptosis swell and eventually rupture. This is accompanied by increased PI uptake and LDH release.

Pyroptosis rate

Flow cytometry following annexin V-fluorescein isothiocyanate (FITC)/PI or annexin V-Alexa 647/PI double staining is a classical method for determining the apoptotic status. It can also be used to analyze the percentage of pyroptotic cell death. Annexin V^+/PI^+ cell populations represent pyroptotic cells, annexin V^-/PI^+ populations represent apoptotic cells, annexin V^+/PI^- populations represent necrotic cells, and annexin V^-/PI^- populations represent live cells.[34,61]

Cytokine levels

Levels of the inflammatory cytokines IL-1β and IL-18 in cell supernatants can be assessed using enzyme-linked immunosorbent assay (ELISA).

Protein biomarker levels

Based on previously described mechanisms, changes in pyroptosis-associated proteins (including caspase-1, caspase-4/5/11, caspase-3, NLRP3, ASC, GSDMD, GSDME, IL-1β, and IL-18) can be determined by western blot analysis or immunostaining.

ncRNAs IN THE REGULATION OF PYROPTOSIS AND CARDIOVASCULAR PATHOLOGIES

ncRNAs, including miRNAs, lncRNAs, and circRNAs, are involved in the regulation of pyroptosis and are important for the occurrence and development of CVDs. The identification and functional study of these ncRNAs may provide specific molecular targets for inhibiting pyroptosis and preventing CVDs, as well as a theoretical basis for the clinical development of new therapeutic methods. The following sections summarize the roles of reported ncRNAs in pyroptosis and the likely molecular mechanisms involved in the pathogenesis of various CVDs (Figure 2; Table 2).

DCM

Diabetic cardiomyopathy (DCM), independent of hypertensive cardiopathy and coronary artery disease, is one of the most common chronic complications of diabetes characterized by myocardial structure changes and cardiac systolic and diastolic function abnormalities.[62] DCM is a global disease and is an essential risk factor for later development of clinical heart failure and cardiogenic sudden death.[63] Although the etiology of DCM has not been fully understood, factors such as myocardial energy metabolism disorder, microvascular changes, collagen structure changes, increased myocardial fibrosis, and cardiac autonomic neuropathy may be the causes of DCM.[64] As hyperglycemia-induced ROS overproduction is a major contributor to the NLRP3 inflammasome, NLRP3-dependent pyroptosis may be an important mediator of inflammation and cardiomyocyte death during DCM progression.[65] In a recent study, Zeng et al.[66] demonstrated that cardiomyocyte pyroptosis triggered by NLRP3 inflammasome activation via caspase-1 causally contributes to myocardial dysfunction progression and DCM pathogenesis. Moreover, increasing evidence has revealed that ncRNAs participate in the pathogenesis of DCM via pyroptosis.

miRNAs

Levels of miR-30d are increased in both streptozotocin (STZ)-induced diabetic rats and in high-glucose (HG)-treated cardiomyocytes.[67] Overexpression of miR-30d can enhance caspase-1 activity and release of the pro-inflammatory cytokines IL-1β and IL-18, thus promoting cardiomyocyte pyroptosis in DCM. Mechanistically, Foxo3a, a crucial transcription factor involved in diverse cell activities, is a direct target of miR-30d, and apoptosis repressor with caspase recruitment domain (ARC) is a transcriptional downstream target of Foxo3a. miR-30d reduces expressions of Foxo3a and ARC. Furthermore, cardiomyocytes transfected with small interfering RNA (siRNA) against ARC show increased caspase-1 and enhanced pyroptosis. Taken together, miR-30d is pro-pyroptotic in DCM and under hyperglycemic conditions and acts through the Foxo3a, ARC, and caspase-1 pathways.[67] In cardiac fibroblasts (CFs), miR-21-3p is pro-pyroptotic and aggravates STZ-induced diabetic cardiac fibrosis by targeting androgen receptor (AR), which regulates CF collagen deposition and pyroptosis through caspase-1 signaling.[68]

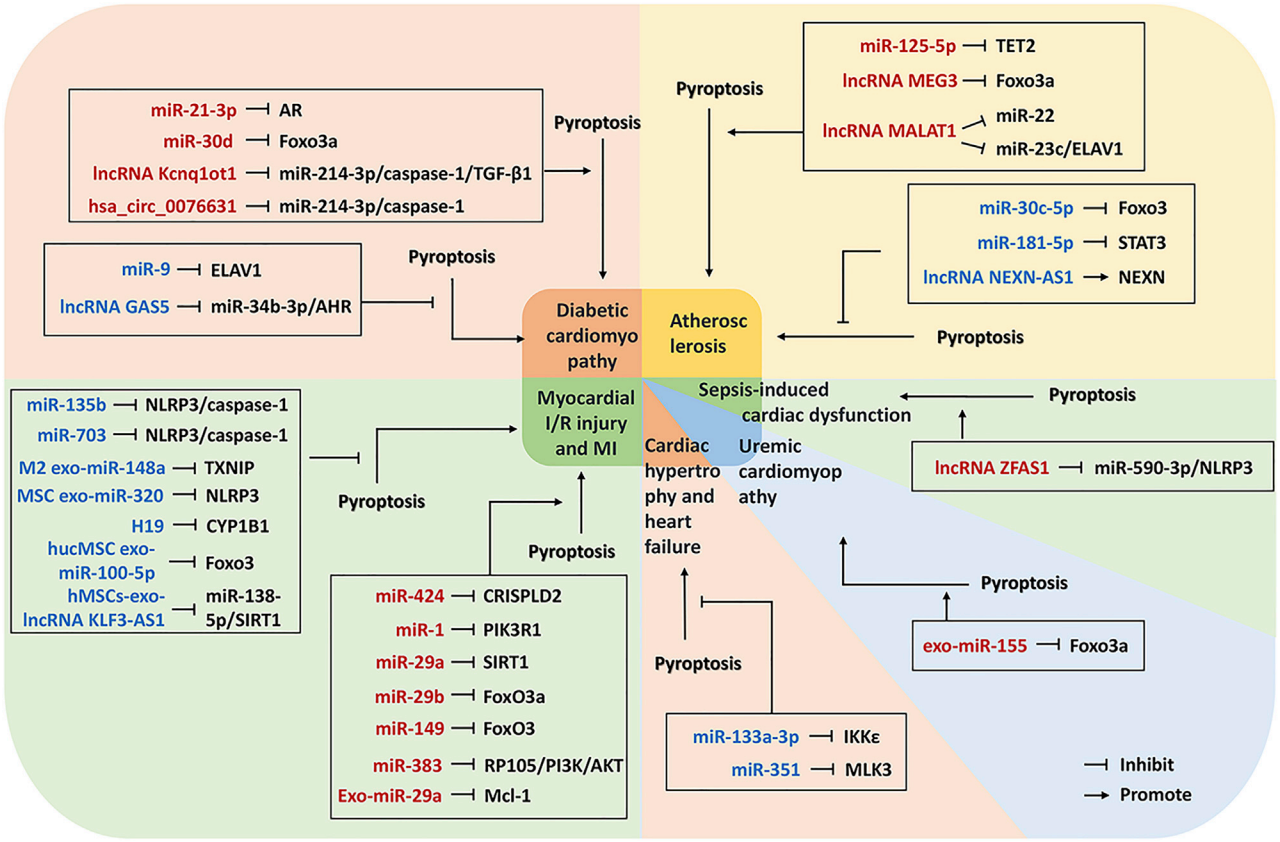

Figure 2. A summary of the roles of miRNAs, lncRNAs, and circRNAs in regulating pyroptosis and related cardiovascular pathologies

The molecular mechanisms and downstream targets of the aforementioned ncRNAs are shown in the figure. Arrows indicate a promoting effect, whereas T bars indicate a suppressing effect. Red indicates positive regulation of pyroptosis, whereas blue indicates negative regulation of pyroptosis.

By contrast, miR-9 expression is significantly reduced in human diabetic hearts and HG-treated ventricular cardiomyocytes.[69] miR-9 directly targets the 3′ UTR of ELAV-like protein 1 (ELAVL1), a vital regulator that is involved in the progression of inflammation and heart failure. Inhibition of miR-9 increases ELAVL1 expression, caspase-1 activation, and IL-1β secretion, whereas cardiomyocyte treatment with miR-9 mimics attenuates hyperglycemia-induced ELAVL1 and inhibits pyroptosis.[69] Thus, miR-9 is a potential therapeutic target against cardiomyocyte pyroptotic cell death during heart failure in patients with diabetics.

lncRNAs

Yang et al.[70] found that the lncRNA Kcnq1ot1 is upregulated in diabetic mouse hearts and activated in CFs under hyperglycemic stress. *In vivo* analysis demonstrated that Kcnq1ot1 regulates myocardial pyroptosis and that silencing Kcnq1ot1 alleviates cardiac function and fibrosis in diabetic mice.[70] Further investigations have revealed that Kcnq1ot1 functions as a ceRNA to antagonize miR-214-3p function, leading to the liberation of caspase-1. Knockdown of Kcnq1ot1 decreases NLRP3 levels, GSDMD cleavage, and IL-1β secretion, thus repressing the TGF-β1/Smad pathway in HG-treated CFs. Downregulating Kcnq1ot1 ameliorates pyroptosis and fibrosis through the miR-214-3p/caspase-1/TGF-β1 signaling pathway.[70] By regulating cell proliferation, apoptosis, the cell cycle, and other cellular processes, the lncRNA growth arrest-specific transcript 5 (GAS5) is involved in the occurrence and development of a variety of acute and chronic diseases. Xu et al.[71] reported that lncRNA GAS5 can improve cardiac function and myocardial hypertrophy in DCM mice by repressing NLRP3 inflammasome activation-mediated pyroptosis via miR-34b-3p/aryl hydrocarbon receptor (AHR) targeting.

circRNAs

More recently, one research group sought to determine whether circRNA is involved in DCM pyroptosis, and they identified that hsa_circ_0076631, named caspase-1-associated circRNA (CACR), is elevated both in HG-treated AC16 cells (a human cardiac cell line) and in the serum of patients with diabetes.[72] Silencing CACR suppresses cardiomyocyte inflammation and death by sponging miR-214-3p and regulating caspase-1. Additionally, miR-214-3p inhibition partially abolishes the beneficial effects of CACR silencing on pyroptosis in cardiomyocytes, further demonstrating the potential therapeutic role of CACR in the treatment of DCM via the miR-214-3p/caspase-1 pathway.[72]

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the arterial wall caused by lipid metabolism disorder, and cell death and inflammation play key roles in all stages of the disease. Atherosclerosis is the common pathological basis of coronary heart disease, cerebral infarction, hypertension, and many other CVDs. Death of various types of cells, including ECs, monocytes/macrophages, and SMCs, can typically be observed in the pathogenic process.[73] Of note, caspase-1-dependent pyroptosis, a programmed cell death mode accompanied by the release of pro-inflammatory factors, is closely related to atherosclerosis.[74] Expression of the NLRP3 inflammasome signaling-pathway-relevant components ASC, caspase-1, IL-1β, and IL-18 is significantly higher in atherosclerotic plaques than in normal arteries, and upregulation of their expression is closely related to plaque stability, demonstrating that NLRP3 inflammasome activation mediates the progression of atherosclerotic lesions.[75]

miRNAs

miR-125-5p is a pro-pyroptotic factor that triggers oxidized low-density lipoprotein (ox-LDL)-induced pyroptosis in human umbilical vein ECs (HUVECs) by directly targeting tet methylcytosine dioxygenase 2 (TET2), resulting in abnormal DNA methylation levels and enhanced ROS generation; the result is activation of nuclear factor κB (NF-κB) signaling and sequentially the NLRP3 inflammasome, contributing to pyroptosis and ultimately promoting atherosclerosis.[76] Furthermore, inhibition of miR-125-5p reduces expression of pyroptosis-related proteins (NLRP3, caspase-1, and IL-1β) and significantly attenuates HUVEC pyroptosis triggered by ox-LDL treatment.[76] Nevertheless, forced expression of miR-30c-5p reverses ox-LDL-induced pyroptosis by downregulating FOXO3 and inactivating the NLRP3 inflammasome in human aortic ECs (HAECs).[77] In addition to ox-LDL, shear stress changes caused by abnormal blood flow are an important factor in atherosclerosis and low fluid shear stress is conducive to the occurrence of atherosclerosis and plate growth. Xu et al.[78] reported that low shear stress can downregulate mechanosensitive miR-181-5p expression and induce pyroptosis in HUVECs. Moreover, miR-181-5p mimic transfection can suppress signal transduction and transcriptional activation factor 3 (STAT3) gene expression by directly binding to its 3′ UTR, resulting in reduced NLRP3 transcription through lysine deacetylation and amelioration of NLRP3 inflammasome-dependent pyroptosis.[78]

lncRNAs

Intragastric administration of melatonin decreases expression of the pyroptosis-associated genes NLRP3, ASC, cleaved caspase-1, NF-κB/GSDMD, and GSDMD N-termini and markedly reduces atherosclerotic plaques in high-fat diet-treated ApoE$^{-/-}$ mouse aortas.[79] Mechanistically, melatonin downregulates lncRNA MEG3, high expression of which enhances pyroptosis in HAECs. Furthermore, MEG3 can suppress expression of miR-223 and its endogenous molecular sponge activity and subsequently increase NLRP3 expression and promote EC pyroptosis. Thus, the MEG3/miR-223/NLRP3 axis mediates the protective actions of melatonin against pyroptosis in atherosclerosis.[79] MALAT1 is another pro-pyroptotic cell death-related lncRNA that was found to be upregulated in HG-treated EA.hy926 human ECs (EA.hy926 cells). It was demonstrated that knockdown of MALAT1 significantly reduced NLRP3 expression by competitively binding to miR-22 and inhibited EC pyroptosis in EA.hy926 cells under HG stress, which may critically influence atherosclerosis.[80] Similarly, in a study reported by Han et al.,[81] MALAT1 levels were robustly increased in the bone-marrow-derived macrophages of diabetic atherosclerosis rats, and low-dose sinapic acid administration suppressed MALAT1-induced pyroptosis in HG-oxLDL-treated macrophages through the miR-23c/ELAVL1 axis.

Hu et al.[82] revealed that NEXN-AS1 acts as a protective lncRNA against atherosclerosis progression by upregulating its cognate protein-coding gene NEXN through interactions with the chromatin remodeler BAZ1A, thus inhibiting endothelial activation and monocyte recruitment. Later, this group found that NEXN-AS1 and NEXN can be significantly induced by the cholesterol-lowering agent atorvastatin, which decreased expression of pyroptosis-related proteins, including NLRP3, caspase-1, GSDMD, IL-1β, and IL-18, in human vascular ECs (HVECs).[83] Knockdown of NEXN-AS1 or RNA interference of NEXN abrogates the inhibitory effect of atorvastatin on pyroptosis, indicating the critical regulatory role of NEXN-AS1/NEXN in transferring the anti-pyroptotic signal from atorvastatin to the NLRP3 inflammasome.[83]

Myocardial I/R injury and myocardial infarction

Acute myocardial infarction is myocardial necrosis caused by acute and persistent ischemia and hypoxia in the coronary arteries, which seriously endangers human health. However, reperfusion therapy may aggravate cardiac ultrastructural, metabolic, and functional damage when restoring myocardial blood supply and oxygen supply, even leading to irreversible myocardial I/R injury. The pathogenesis of I/R injury is complex. There are interrelated and synergistic effects among ROS eruption, calcium overload, mitochondrial dysfunction, inflammatory mechanisms, energy metabolism disorders, and other related mechanisms.[84] Of note, increasing evidence indicates that the NRLP3 inflammasome-caspase axis is a key factor in modulating inflammation-induced cell death during I/R. Indeed, it was found that the infarct sizes of Langendorff-perfused rat hearts were significantly reduced by treatment with VX-765, an inhibitor of caspase-1 that is clinically available.[85] Furthermore, studies have shown that administration of VX-765 at reperfusion in $P2Y_{12}$ receptor antagonist-treated rats can not only downregulate inflammation and pyroptosis-related factors but also reduce infarct size, attenuate left ventricular remodeling, and improve heart function.[86] In addition to pathway inhibitors, nucleic-acid-mediated therapy, especially upstream ncRNA regulators, is a promising approach for myocardial I/R treatment.

miRNAs

miR-424 expression is substantially increased in I/R heart tissue and hypoxia/reoxygenation (H/R)-challenged H9c2 cells, and miR-424 mimics elevate expression of caspase-1 and the pro-inflammatory cytokines IL-1β and IL-18 by directly targeting cysteine-rich secretory protein LCCL domain-containing 2 (CRISPLD2), thereby contributing

Table 2. Summary of ncRNAs that are currently reported to be involved in regulating pyroptosis in various CVDs

ncRNA	Source	*In vitro* study cell type	Target	Role in pyroptosis	Function	References
miRNA						
miR-1		H9c2 myocardial cells	PIK3R1	pro-	promote cardiomyocytes pyroptosis injury	Yao et al.[88]
miR-9		human ventricular cardiomyocytes	ELAVL1	anti-	inhibit hyperglycemia-induced pyroptosis in DCM	Jeyabal et al.[69]
miR-21		neonatal rat cardiac fibroblasts	AR	pro-	enhance cardiac fibroblasts pyroptosis in diabetic cardiac fibrosis	Shi et al.[68]
miR-29a		H9c2 myocardial cells	SIRT1	pro-	aggravate myocardial I/R injury	Ding et al.[93]
miR-29a	M1 macrophage-derived exosomes	neonatal mouse cardiomyocytes	Mcl-1	pro-	promote cardiomyocyte pyroptosis	Wang et al.[102]
miR-29b		neonatal rat cardiomyocytes	FoxO3a	pro-	enhance cardiomyocyte pyroptosis in H/R injury	Zhong et al.[94]
miR-30c-5p		human aortic endothelial cells	FOXO3	anti-	inhibit ox-LDL-induced endothelial cell pyroptosis in AS	Li et al.[77]
miR-30d		neonatal rat cardiomyocytes	FoxO3a/ARC	pro-	promote cardiomyocyte pyroptosis in DCM	Li et al.[67]
miR-100-5p	human umbilical cord MSC-derived exosomes	AC16 cells	FOXO3	anti-	protect against H/R induced cardiomyocyte pyroptosis and injury	Linag et al.[100]
miR-125-5p		vascular endothelial cells	TET2	pro-	promote oxLDL-induced pyroptosis and AS	Zhaolin et al.[76]
miR-133a-3p		human myocardial cell line	IKKε	anti-	alleviate Ang-II-induced cardiac hypertrophy	Zhu et al.[110]
miR-135b		neonatal mice ventricular cardiomyocytes	NLRP3/caspase-1/IL-1β pathway	anti-	restore impaired cardiac function caused by MI	Li et al.[96]
miR-148a	M2 macrophage-derived exosomes	neonatal rat cardiomyocytes	TXNIP	anti-	alleviate myocardial I/R injury	Dai et al.[99]
miR-149		H9c2 myocardial cells	FoxO3	pro-	aggravate pyroptosis in myocardial I/R damage	Lin et al.[95]
miR-155	macrophage-derived exosomes	neonatal rat cardiomyocytes	FoxO3	pro-	promote pyroptosis and uremic cardiomyopathy changes in uremic mice	Wang et al.[114]
miR-181b-5p		human umbilical vein endothelial cells	STAT3	anti-	alleviate low shear stress-induced pyroptosis in AS	Xu et al.[78]
miR-320	MSC-derived exosomes	neonatal rat cardiomyocytes	NLRP3	anti-	inhibit pyroptosis and protect myocardium against I/R injury	Tang et al.[101]
miR-351			MLK3	anti-	inhibit pyroptosis and alleviate heart failure in response to TAC	Wang et al.[111]
miR-383			RP105/AKT signaling	pro-	facilitate pyroptosis and myocardial I/R injury	Guo et al.[89]
miR-424		H9c2 myocardial cells	CRISPLD2	pro-	promote cardiac I/R injury	Lou et al.[87]
miR-590-3p		neonatal mice cardiomyocytes	AMPK/mTOR signaling	anti-	inhibit LPS-induced cardiomyocyte pyroptosis	Liu et al.[115]
miR-703		mouse cardiomyocytes	NLRP3/caspase-1 pathway	anti-	protect against H/R-induced cardiomyocyte injury	Wei et al.[97]
lncRNA						
GAS5		HL-1	miR-34b-3p/AHR	anti-	improve cardiac function and myocardial hypertrophy in DCM mice	Xu et al.[71]

(*Continued on next page*)

Table 2. *Continued*

ncRNA	Source	*In vitro* study cell type	Target	Role in pyroptosis	Function	References
H19		H9c2 myocardial cells	PBX3/CYP1B1 signaling	anti-	suppress cardiomyocyte pyroptosis and attenuate myocardial infarction	Han et al.[104]
Kcnq1ot1		cardiac fibroblasts	miR-214/caspase-1	pro-	exacerbate pyroptosis and fibrosis in DCM	Yang et al.[70]
KLF3-AS1	human MSC-derived exosomes	H9c2 myocardial cells	miR-138-5p/Sirt1	anti-	ameliorate pyroptosis and attenuate MI progression	Mao et al.[105]
MALAT1		bone-marrow-derived macrophages	miR-23c/NLRP3	pro-	aggravate pyroptosis in rats with diabetic AS	Han et al.[81]
MALAT1		EA.hy926 cells	miR-22/NLRP3	pro-	promote HG-induced pyroptosis in AS	Song et al.[80]
MEG3		human aortic endothelial cells	miR-223/NLRP3	pro-	promote ox-LDL-induced pyroptosis in AS	Zhang et al.[79]
NEXN-AS1		human vascular endothelial cells	NEXN	anti-	inhibit pyroptosis and mediate the protective role of atorvastatin against AS development	Wu et al.[83]
ZFAS1		neonatal mice cardiomyocytes	miR-590–3p/NLRP3	pro-	aggravate pyroptosis and sepsis-induced cardiac dysfunction	Liu et al.[115]
circRNA						
hsa_circ_0076631/ CACR		AC16 cells	miR-214/caspase-1	pro-	promote pyroptosis in DCM	Yang et al.[72]

AS, atherosclerosis; MI, myocardial infarction.

to cardiac I/R injury.[87] miR-1 aggravates cardiomyocyte pyroptosis and promotes the release of inflammatory factors by downregulating phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) expression.[88] miR-383 is also pro-pyroptotic and the downregulation of which by piperine treatment reduces cardiomyocyte pyroptosis and alleviates myocardial I/R injury in a RP105/phosphatidylinositol 3-kinase (PI3K)/AKT-dependent manner.[89] It has been demonstrated that silent information regulator factor 2-related enzyme 1 (SIRT1) is cardioprotective and can regulate cardiomyocyte pyroptosis by inhibiting the intracellular ROS generation modulator NAPDH oxidase 4 (NOX4), thereby diminishing ROS production following ischemia.[90] The miR-29 family, which is involved in the regulation of CVDs through diverse pathways, is composed of miR-29a, miR-29b, and miR-29c, and their target genes mainly participate in biologically related processes such as cell proliferation, cell differentiation, apoptosis, migration, and immune regulation.[91,92] Ding et al.[93] revealed that expression of miR-29a was significantly upregulated in I/R and that inhibition of miR-29a ameliorated myocardial I/R injury by directly targeting SIRT1 through suppressing oxidative stress and NLRP3-mediated pyroptosis. Dexmedetomidine (DEX) can downregulate miR-29b expression to activate FoxO3a and its downstream protein ARC, thus inhibiting H/R-induced pyroptosis in myocardial cells and protecting the heart against I/R injury.[94] Taken together, miR-29a/b is pro-pyroptotic and negatively targets SIRT1/FoxO3a to suppress their protective effects in I/R injury. Furthermore, FoxO3a can be silenced by miR-149, and overexpression of miR-149 inhibits cell viability and promotes pyroptosis in I/R-treated H9c2 cells. However, knockdown of FoxO3a alleviates the effect of miR-149 mimics on cell proliferation and pyroptosis.[95] Thus, miR-149 exacerbates pyroptosis and is a potential therapeutic target against cardiac I/R injury.[95]

There are also anti-pyroptotic miRNAs that exert protective roles in myocardial I/R injury. Through *in vivo* and *in vitro* analyses, miR-135b was demonstrated to be downregulated after injury, and overexpression of miR-135b remarkably mitigated the myocardial infarction surgery- and hydrogen peroxide (H_2O_2) treatment-caused pyroptosis enhancement, decreased NLRP3/caspase-1/IL-1β protein levels, and restored cardiac function impairment.[96] Similarly, miR-703 protects against H/R-induced cardiomyocyte injury by suppressing NLRP3/caspase-1-mediated pyroptosis.[97]

Exosomes are vesicles released by many kinds of cells, including macrophages, and are natural carriers of many signaling molecules, such as DNA, RNA (miRNA, lncRNA, and circRNA), and proteins. Once released, exosomes fuse with the membrane of receptor cells, and the signaling molecule cargo is released to mediate cell-cell communication, affecting the physiological and pathological processes of the receptor cells.[98] Treatment with anti-inflammatory M2 phenotype macrophage-derived exosomes (M2-exos) significantly reduces cardiomyocyte pyroptosis after H/R stimuli and infarct size in a myocardial I/R rat model.[99] Further investigations of the mechanism of action revealed that miR-148a carried by M2-exos directly suppresses thioredoxin-interacting protein (TXNIP) levels and inactivates the TRL4/NF-κB/NLRP3 inflammasome pathway to reduce

pyroptosis and enhance cell viability, thus alleviating myocardial I/R injury.[99] Enriched miR-100-5p in human umbilical cord mesenchymal stem cells (MSCs)-derived exosomes protects against H/R-induced cardiomyocyte pyroptosis and injury through suppressing FOXO3 expression, which inhibits the activation of NLRP3 inflammasome and the release of LDH/cytokines.[100] Exosomal miR-320b derived from MSCs directly targets NLPR3 protein to inhibit pyroptosis and protect myocardium against I/R injury.[101] However, under HR treatment, exosomal miR-29a derived from M1 macrophages promotes cardiomyocyte pyroptosis by targeting Mcl-1.[102]

lncRNAs

H19 is a well-studied muscle-enriched lncRNA that participates in heart pathogenesis.[103] A recent study revealed that H19 can suppress cardiomyocyte pyroptosis to alleviate myocardial infarction progression and improve survival mainly through regulating pre-B cell leukemia homeobox 3 (PBX3) and its target gene cytochrome P450 1B1 (CYP1B1).[104] Mao et al.[105] reported that lncRNA KLF3-AS1 in exosomes secreted from human MSCs acts as a ceRNA to upregulate SIRT1 expression by directly sponging miR-138-5p, thus decreasing cardiomyocyte pyroptosis and attenuating myocardial infarction progression. Similarly, transfection of a miR-138-5p inhibitor reduced pyroptosis and infarct size with KLF3-AS1 exosome incubation both *in vitro* and *in vivo*, whereas knockdown of SIRT1 upregulated NLRP3, ASC, cleaved caspase-1, IL-1β, and IL-18 expressions and reversed the protective effect of exosomal KLF3-AS1 in hypoxic cardiomyocytes. Hence, lncRNA H19 and exosomal KLF3-AS1 might serve as promising sources of novel therapeutic targets for myocardial I/R injury and myocardial infarction.

Cardiac hypertrophy and heart failure

Cardiac hypertrophy in response to sustained pressure overload leads to adverse cardiac remodeling, including left ventricular cavity enlargement and wall thinning, cardiac dysfunction, and ultimately progression to heart failure. Heart failure is associated with persistent inflammation, the degree of which correlates with disease severity. The NLRP3 inflammasome has direct effects on calcium homeostasis and myocardial contractility through IL-1β and plays a central role in modulating the inflammation that promotes cardiac dysfunction in a calcineurin transgenic mouse model of hypertrophic cardiomyopathy.[106,107] In a transverse aortic constriction (TAC) mouse model, levels of the NLRP3 inflammasome were significantly increased, and triptolide treatment inhibited NLPR3 activation-mediated pyroptosis and alleviated pressure overload-induced myocardial remodeling.[108] The IκB kinase (IKK)/NF-κB signaling pathway is a key regulator of the inflammatory response, and the IKKε suppressor negatively regulates cardiac hypertrophy.[109] In a recent study, Zhu et al.[110] revealed that miR-133a-3p attenuates angiotensin II (Ang-II)-induced cardiomyocyte hypertrophy via inhibition of pyroptosis by directly targeting IKKε. Transfection of miR-133a-3p mimics mitigates activation of pyroptosis-associated proteins (caspase-1, NLRP3, IL-1β, IL-18, GSDMD, and ASC) and the increasement of hypertrophic markers (ANP, BNP, and β-MHC) in Ang-II-treated cardiomyocytes.[110] miR-351 can inhibit the expression of mixed lineage kinase 3 (MLK3), a positive regulator of pyroptosis through the NF-κB/NLRP3 signaling pathway, and significantly improve cardiac function in TAC-induced heart failure mice.[111] Thus, miR-133a-3p and miR-351 upregulation may be a feasible therapeutic strategy for controlling cardiac hypertrophy and heart failure by inhibiting inflammatory processes.

UCM

UCM is the myocardial damage caused by hypertension, volume overload, and uremic toxins in chronic kidney disease, which manifests as cardiac hypertrophy and fibrosis and often progresses to malignant arrhythmia and heart failure.[112] Macrophages are important components in the uremic microenvironment, and macrophage infiltration affects the development and outcome of CVD to a great extent.[113] Wang et al.[114] reported that infiltrated macrophage-derived exosomal miR-155 promotes cardiomyocyte pyroptosis by directly targeting FoxO3a in UCM mice. In uremic hearts, FoxO3a was found to be downregulated, and overexpression of FoxO3a with AAV-FoxO3a-GFP delivery reduced pyroptosis and improved cardiac function in UCM. miR-155, identified as an upstream regulator of FoxO3a, is most highly expressed in macrophages. It was further demonstrated that after injection with macrophage-derived exosomes, uremic miR-155$^{-/-}$ mice exhibited enhanced pyroptosis with aggravated myocardial damage; by contrast, treatment with GW4869 to block exosome secretion attenuated pyroptosis and ameliorated abnormal heart function upon uremic challenge.[114] This suggests that sequence-specific inhibitors targeting miR-155 may serve as novel therapeutic avenues for pyroptosis-related UCM.

Sepsis-induced myocardial dysfunction

Patients with sepsis are prone to infective endocarditis and septic shock, and severe infection can cause hypotension, systemic inflammatory response, and multiple organ failure, especially heart failure. In both a cecal ligation and puncture (CLP)-induced sepsis mouse model and LPS-challenged cardiomyocytes, expression levels of lncRNA ZFAS1 were significantly elevated, and knockdown of ZFAS1 ameliorated sepsis-induced cardiac dysfunction.[115] Mechanistically, ZFAS1 downregulates miR-590-3p to modulate AMPK/mTOR signaling and affect NLRP3-mediated cardiomyocyte pyroptosis. Moreover, ZFAS1 is directly transcriptionally activated by SP1 to exacerbate the pathogenesis of sepsis-induced cardiac dysfunction.[115]

CLINICAL IMPLICATIONS OF PYROPTOSIS AND ncRNAs IN CVDs

Pyroptosis as a therapeutic target in CVDs

Cell death is an indispensable and important facet of numerous life processes. It orchestrates the end of life of cells, removes damaged cells from the body in time, and maintains normal metabolic function. Pyroptosis is a pro-inflammatory form of RCD that helps prevent infection. However, excessive pyroptosis can induce pathological inflammation. The NLRP3 inflammasome is an important regulator of pyroptosis. Hence, the attenuation of the NLRP3 inflammasome may be a promising strategy for the prevention and treatment of pyroptosis-related CVDs. Because of its complex signaling cascade,

various blockade sites can be used to inhibit the NLRP3 inflammasome and alleviate pyroptosis. The inhibition of NLRP3 inflammasome priming by NF-κB inhibitors, suppression of upstream signals by P2X7 receptor antagonist, prevention of inflammasome assembly by NLRP3 inhibitors, inhibition of caspase-1 activation by caspase-1 inhibitors, blockade of pore-forming protein cleavage by gasdermin inhibitors, and neutralization of inflammatory cytokines by an IL-1 receptor antagonist or IL-1β blocker are examples of potential pharmacological interventions.[116]

So far, researchers have identified many drugs that can target the NLRP3 inflammasome and regulate pyroptotic pathways to reduce the pathological progression of or ameliorate cardiac dysfunction. These drugs include rosuvastatin,[117,118] atorvastatin,[83] colchicine,[119] liraglutide,[90] cholecalciterol cholesterol emulsion,[120] metformin,[121,122] and melatonin.[79,123] Some other commonly used traditional Chinese medicines and synthetic derivatives of natural compounds can also exert anti-oxidant and anti-inflammatory effects to inhibit pyroptosis in major heart cells (cardiomyocytes and ECs) and macrophages. These include gastrodin,[124] emodin,[125] piperine,[89] thymoquinone,[126] apigenin,[127] salidroside,[128] sinapic acid,[81] triptolide,[108] skimmin,[129] and Kanglexin.[130] MCC950, a potent selective NLRP3 inhibitor, acts by directly binding to the Walker B motif in the NLRP3 NACHT domain, blocking ATP hydrolysis, and preventing inflammasome formation.[131,132] MCC950 has been studied for its protective role against the NLRP3 inflammasome in many CVDs models, such as diabetes-associated atherosclerosis, sepsis-induced myocardial dysfunction, and acute myocardial infarction.[9,133–135] In addition, other selective NLRP3 inhibitors, such as CY-09 and the newly identified covalent NLRP3 inhibitor oridonin, have effects similar to those of MCC950 and can preserve cardiac function.[136,137] However, study on these small-molecule NLRP3 inhibitors has not progressed to an overt clinical stage. On the other hand, OLT1177 (dapansutrile), an orally active β-sulfonyl nitrile compound, is the most clinically advanced NLRP3 inhibitor. Phase 1b clinical trials on OLT1177 have been completed in patients with systolic heart failure (Clinical Trials ID: NCT03534297), although its mechanism has not been elucidated as clearly as that of MCC950.[138,139] The inhibition of caspase-1 activity through the caspase-1 inhibitor VX-765 (belnacasan) is another effective way of alleviating pyroptosis-related CVDs.[85,86,140] VX-765 is a promising clinical compound. However, further studies and clinical trials are warranted to ensure sufficient efficacy and safety. Canakinumab, a new anti-inflammatory drug developed by Novartis, is a human anti-IL-1β monoclonal antibody used for IL-1β blockade. It has achieved remarkable clinical efficacy in treating autoinflammation and CVDs. Data from the completed large-scale phase III clinical trial CANTOS published in 2017 indicated that canakinumab can significantly reduce the incidence of recurrent cardiovascular events and decrease cancer mortality rates in patients with atherosclerosis.[141,142] In addition, IL-1β signaling can be blocked by a US Food and Drug Administration (FDA)-approved IL-1 receptor antagonist, anakinra, which has been tested in multiple clinical trials to assess its efficacy and safety in treating patient with various CVDs, including heart failure, acute myocardial infarction, and atherosclerosis (https://clinicaltrials.gov/). Nevertheless, the pharmacological inhibition of IL-1β signaling may still be limited in clinical practice because other inflammatory cytokines, such as IL-18 and high mobility group box 1 (HMGB1), are also involved in the pathogenesis of CVDs.

ncRNAs as biomarkers and therapeutic targets in CVDs

In the past few decades, significant progress has been made in the field of ncRNAs, particularly with regard to their types, structures, and functions. Some research achievements have successfully translated into clinical applications. For instance, the lncRNA prostate cancer antigen 3 (PCA3) is the first tumor biomarker to be approved by the FDA for the routine clinical diagnosis of patients with prostate cancer, while Onpattro (patisiran) is the first siRNA drug to be approved by the FDA for the treatment of polyneuropathy in patients with hereditary transthyretin amyloidosis.[143,144] A growing body of evidence reveals the critical roles of ncRNAs in the pathogenesis and progression of CVDs, and many preclinical studies and clinical trials are ongoing to assess the diagnostic and prognosis potential of ncRNAs and evaluate the effect of ncRNA-based therapies on the treatment of CVDs.

The use of good early diagnostic markers can assist doctors in identifying high-risk CVDs as early as possible; this is important for reducing mortality and improving prognosis. Creatine kinase MB isoenzyme (CK-MB) and cardiac troponin I (cTnI) are the most commonly used clinical markers of cardiac injury; however, some limitations still exist. Therefore, it is urgent to identify new disease markers with high clinical sensitivity and specificity, stability, and ease of detection. Compared with traditional biomarkers, ncRNAs have the following four unique advantages: (1) Abnormal expression levels of ncRNAs are closely related to the occurrence and progression of diseases. (2) The expression of ncRNA is specific to cells, tissues, and organs, and it shows high sensitivity and specificity for the diagnosis of some diseases. (3) ncRNAs can be secreted into the blood and body fluids and are easy to obtain and detect. (4) ncRNAs can be carried by exosomes, as a result of which they are relatively stable and are able to disseminate signals either locally or at distances.[145] Thus, ncRNAs are expected to surpass the diagnostic efficiency of traditional protein biomarkers and become new clinical biomarkers for the diagnosis, risk stratification, and prognosis of CVDs. For example, miR-499 is mainly expressed in the myocardium, and its concentration is very low in the plasma of healthy individuals. However, significantly elevated miR-499 levels can be detected in the plasma of patients within 1–3 h after acute myocardial infarction. Further studies have also confirmed that compared with healthy controls, increased plasma miR-499 levels can be detected in patients with acute myocardial infarction 1 h after the onset of chest pain and that the levels continue to increase with the progression of the disease.[146,147] miR-499 is considered a candidate biomarker for the early diagnosis of acute myocardial infarction; it may compensate for clinical limitations, such as the low sensitivity of CK-MB and insufficient specificity of cTnI. In addition, the combined application of miR-499

and traditional diagnostic markers can definitely improve the diagnosis accuracy with high sensitivity and specificity.

Moreover, given their pivotal role in gene expression and disease progression, ncRNAs are emerging as promising therapeutic targets. An example would be miRNA, the most thoroughly studied ncRNA. On one hand, the overexpression of miRNAs, the downregulation of which caused diseases by increasing their target genes, can be achieved using synthetic miRNA duplex approaches (miRNA mimic or agomirs) or cardiac-specific virus-mediated miRNA transfer; on the other hand, for the pathogenic upregulation of miRNAs, knockdown approaches, such as the use of single-stranded oligonucleotides complementary to miRNA (miRNA inhibitor or antagomirs) or cardiac-specific vector-based knockdown (miRNA sponges and miRNA erasers), can be implemented.[148] However, the mechanisms of lncRNA and circRNA are more complex than that of miRNA. Hence, the strategies targeting them as therapeutics need to be studied further. In addition, the recent clinical relevance of ncRNAs in CVDs has been discussed in detail in a published report.[149] Although the overall understanding of ncRNAs is in its infancy, it is believed that with continuous exploration of the mechanism by which ncRNAs regulate pyroptotic pathways, ncRNAs could serve as a promising therapeutic strategy for pyroptosis-related CVDs.

CURRENT LIMITATIONS AND FUTURE INSIGHTS

Recent studies have revealed the critical roles of pyroptosis and ncRNAs in CVDs; however, some issues still need to be addressed. First, more in-depth studies on the mechanism of pyroptosis in CVDs are needed. The current reported literature is mostly related to the caspase-1-mediated canonical pathway. However, the caspase-4/5/11-mediated noncanonical pathways and whether the apoptosis-related caspase-3 and caspase-8 regulate the occurrence and development of CVDs through the pyroptosis pathway remain unclear. In addition, the role of gasdermin proteins in CVDs deserves further investigation. Second, the heart is naturally death resistant; however, under extreme conditions, myocardial cells adopt different cell death mechanisms. Full exploration of the decision-making processes regarding pyroptosis is necessary. Moreover, the common involvement of mitochondrial dysfunction provides evidence for and arouses research interest in the crosstalk between pyroptosis and other necrotic cell death forms, such as necroptosis, apoptosis, and ferroptosis.[150] Third, a direct method for monitoring pyroptosis levels *in vivo* needs to be developed. In the past few years, great efforts have been made to elucidate the definitive mechanism and have revealed that gasdermin family proteins are key effectors of pyroptosis.[27,29–31,33] The caspase proteins activated by inflammasomes cleave GSDMD to release the N terminus and C terminus; the former gets transferred and interacts with the plasma membrane to form pores, resulting in cell swelling and membrane rupture and consequently induces pyroptosis. The invention of a molecular probe that specifically recognizes the activated form of GSDMD will help identify and detect the occurrence and levels of pyroptosis in living animals and can help develop useful clinical diagnostic reagents. For this purpose, Ji et al.[151] have recently developed a genetically encoded pyroptotic reporter for the noninvasive and real-time monitoring of pyroptosis in both cultured cells and animal blood. Through molecular mutagenesis, these researchers placed secreted Gaussia luciferase (Gluc) in the p30-p20-tolerated junction of GSDMD and verified the natural pyrophosphorylation and live imaging functions of this fused Gluc-GSDMD reporter.[151] However, optimized methods that can be applied to humans and detect other gasdermin proteins are required. Fourth, compared with miRNAs and lncRNAs, there are few studies on circRNAs in pyroptosis-related CVDs. In addition, the field of piwi-interacting RNAs (piRNAs) is attracting great interest. piRNAs are abundantly expressed in germline cells, and a more recent study by Gao et al.[152] demonstrated their critical roles in regulating cardiac hypertrophy by controlling the N6-methyladenosine methylation of downstream mRNA targets. Atrial fibrillation is a vital contributor to cardiac morbidity and mortality and ncRNAs, particularly miRNAs and lncRNAs, are emerging as significant modulators.[14,145,153] Recently, inflammasomes have been identified to be the root cause of atrial fibrillation;[154] however, whether ncRNAs can target pyroptosis pathways to regulate the pathogenesis of atrial fibrillation remains unknown. All these factors provide new ideas for future research. And fifth, the current studies mainly rely on *in vitro* experiments and small animal models. Translating basic research findings into clinical applications will help decipher the molecular mechanism by which ncRNAs regulate pyroptosis and offer the basis for applying ncRNAs as new targets for the generation of novel cardiovascular drugs. Accelerating the transformation of basic research findings and generating valuable tools to benefit patients with CVDs will be the focus of researchers and clinicians in the future.

Conclusions

Cell death is necessary for tissues to maintain normal physiological functions and morphology, and it is the main cause of clinical diseases and severe pathological injuries. The reduction of terminally differentiated myocardial cell damage and death has been the main goal of CVD treatment. Pyroptosis, a pro-inflammatory form of RCD, is closely related to various diseases, including CVDs, and is currently a focus of clinical research. Inflammasome activation is the key factor involved in pyroptosis, and gasdermin proteins are the key executioners. ncRNAs are emerging as significant regulators that target pyroptotic signaling pathways to affect the occurrence and development of CVDs. Thus, revealing the detailed mechanism of pyroptosis will help clarify the pathogenesis of CVDs. Moreover, understanding the role of ncRNA-regulated pyroptosis in CVDs will help identify new diagnostic markers and therapeutic targets.

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AUTHOR CONTRIBUTIONS

K. Shao and J.N. Gao conceived this article; X.T. Chen collected the related papers; J.N. Gao wrote the manuscript. P.C. Wei drew the figures; X.T. Chen, Y. Wang, and P.F. Li helped to revise the manuscript and do the final editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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