Role of RIPK3-CaMKII-mPTP signaling pathway-mediated necroptosis in cardiovascular diseases (Review)

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Abstract. Necroptosis, which is distinct from apoptosis and necrosis, serves a crucial role in ontogeny and the maintenance of homeostasis. In the last decade, it has been demonstrated that the pathogenesis of cardiovascular diseases is also linked to necroptosis. Receptor interaction protein kinase (RIPK) 1, RIPK3 and mixed lineage kinase domain-like protein serve vital roles in necroptosis. In addition to the aforementioned necroptosis-related components, calcium/calmodulin-dependent protein kinase II (CaMKII) has been identified as a novel substrate for RIPK3 that promotes the opening of the mitochondrial permeability transition pore (mPTP), and thus, mediates necroptosis of myocardial cells through the RIPK3-CaMKII-mPTP signaling pathway. The present review provides an overview of the current knowledge of the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway in cardiovascular diseases, focusing on the role of the RIPK3-CaMKII-mPTP signaling pathway in acute myocardial infarction, ischemia-reperfusion injury, heart failure, abdominal aortic aneurysm, atherosclerosis, diabetic cardiomyopathy, hypertrophic cardiomyopathy, atrial fibrillation, and the cardiotoxicity associated with antitumor drugs and other chemicals. Finally, the present review discusses the research status of drugs targeting the RIPK3-CaMKII-mPTP signaling pathway.

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Abbreviations: RIPK1, receptor interaction protein kinase 1; RIPK3, receptor interaction protein kinase 3; MLKL, mixed lineage kinase domain-like protein; CaMKII, calcium/calmodulin-dependent protein kinase II; mPTP, mitochondrial permeability transition pore; AMI, acute myocardial infarction; I/R, ischemia-reperfusion; HF, heart failure; AAA, abdominal aortic aneurysm; AS, atherosclerosis; DCM, diabetic cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation; FADD, FAS-associated death domain protein; TNFR, tumor necrosis factor receptor; RHIM, RIP homotypic interaction motif; ROS, reactive oxygen species; RyR2, ryanodine receptor 2; SR, sarcoplasmic reticulum; ER, endoplasmic reticulum; MCU, mitochondrial Ca^{2+} uniporter; $\Delta\Psi m$, mitochondrial inner membrane potential; CypD, cyclophilin D; CsA, cyclosporin A; SMC, smooth muscle cell; HG, high-glucose; AGE, advanced glycation end product; BPA, bisphenol A; 3-T1AM, 3-iodothyronamine; H_2S, hydrogen sulfide; NO, nitric oxide; I1PP1, inhibitor-1 of protein phosphatase 1; DOX, doxorubicin

Key words: necroptosis, RIPK3, CaMKII, mPTP, drug target, cardiovascular diseases

Contents

1. Introduction

Cardiovascular diseases are associated with a high morbidity rate and are the leading cause of mortality, thus posing a serious threat to human health (1). Numerous factors, including genetic and environmental factors, metabolic disorders, inflammation, oxidative stress, mitochondrial dysfunction and cell death, are involved in the pathogenesis of cardiovascular disease, and myocardial cell loss caused by cell death serves a crucial role in cardiovascular disease (2,3). At present, cell death has been classified into several distinct forms, including apoptosis, autophagy, pyroptosis, ferroptosis and necroptosis (4). Necroptosis is a recently reported pathway of programmed cell necrosis regulated by the kinases receptor interaction protein kinase (RIPK)3 and mixed lineage kinase domain-like protein (MLKL) that, in contrast to apoptosis, induces a proinflammatory state (5). Necroptosis can be experimentally activated under apoptosis-deficient conditions (6,7). When RIPK1 is activated, cells with higher levels of FAS-associated death domain protein (FADD) and caspase8 than MLKL may

preferentially induce apoptosis. Conversely, when caspase8 activity is compromised, recruitment of RIPK3 to the ripoptosome complex promotes MLKL-mediated necroptosis, and necroptosis is another form of programmed cell death that closely resembles necrosis and is more proinflammatory than apoptosis (8). Table I shows the differences among necroptosis, apoptosis and necrosis.

Previous studies have reported that the RIPK3/MLKL signaling pathway is a classical necroptosis pathway and serves a critical role in cardiovascular diseases (9-12). Zhang *et al* (13) demonstrated that, in addition to MLKL as a substrate for RIPK3, calcium/calmodulin-dependent protein kinase II (CaMKII) could also act as a substrate for RIPK3 to mediate necroptosis in myocardial cells via the RIPK3-CaMKII-mitochondrial permeability transition pore (mPTP) signaling pathway. The authors also found that deficiency of RIPK3 prevented ischemia-reperfusion (I/R) injury and reduced doxorubicin (DOX)-induced CaMKII activation, which inhibited necroptosis in the myocardium (13).

With the advancement of research regarding the mechanism of necroptosis, the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway has been revealed to serve important roles in other cardiovascular diseases, such as acute myocardial infarction (AMI) (14-16), heart failure (HF) (17), abdominal aortic aneurysm (AAA) (18), atherosclerosis (AS) (19), diabetic cardiomyopathy (DCM) (20,21), hypertrophic cardiomyopathy (HCM) (22,23) and atrial fibrillation (AF) (24), and cardiotoxicity of antitumor drugs and other chemicals (13,25-27), and targeting of the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway may provide benefits for the treatment of cardiovascular diseases (13). The present review particularly focuses on novel developments regarding RIPK3-CaMKII-mPTP signaling pathway-mediated necroptosis in cardiovascular diseases, outlining the molecular mechanisms that activate the RIPK3-CaMKII-mPTP signaling pathway in different physiological and pathological contexts. With an understanding of the key components and the regulatory mechanisms of the RIPK3-CaMKII-mPTP signaling pathway, pharmacological intervention strategies targeting the pathway are examined and their therapeutic potential in the treatment of cardiovascular diseases is discussed.

2. Overview of the RIPK3-CaMKII-mPTP signaling pathway

Necroptosis can be initiated by a variety of triggers, including the tumor necrosis factor receptor (TNFR) and Toll-like receptor families, the intracellular DNA/RNA receptor Z-DNA binding protein 1, and interferon (28,29). One of the most well-studied and abundant signaling pathways is the TNF-α-mediated signaling pathway, in which TNF-α binds to TNFR1, RIPK1, DD-containing adaptor protein TNFR1-associated death domain protein (TRADD), TNF receptor-associated factors 2 and 5, and cellular inhibitor of apoptosis proteins 1 and 2, which are recruited to form complex I and initiate necroptosis (Fig. 1) (30,31). RIPK3 is a member of the serine/threonine RIPK family, whose structural components include the N-terminal kinase structural domain and the C-terminal RIP homotypic interaction motif (RHIM) (32). When RHIM-containing RIPK1 is deubiquitinated and activated, RIPK1 assembles a cytoplasmic signaling complex that contains RIPK3, FADD, caspase8 and TRADD (33-35). Under conditions of impaired caspase8 activity, RIPK1 self-phosphorylation promotes the phosphorylation and activation of RIPK3, thereby mediating the phosphorylation and activation of MLKL, which is then oligomerized and transported to the plasma membrane, where the N-terminal four-helix bundle structural domain of MLKL can mediate cell membrane disruption (36), leading to cellular micropore formation and eventually resulting in cell swelling and lysis, and the triggering of subsequent necroptosis (Fig. 1) (35). Sun *et al* (37) have demonstrated that RIPK3 and MLKL are essential for necroptosis. However, inhibition of MLKL does not completely block cardiomyocyte necroptosis, indicating that there are other signaling pathways involved in necroptosis (13).

CaMKII is a serine and threonine kinase with four isoforms encoded by four genes (α, β, γ and δ), namely CaMKIIα, CaMKIIβ, CaMKIIγ and CaMKIIδ, of which CaMKIIδ is mainly expressed in cardiomyocytes (38). CaMKII holoenzymes exist as multimers of 12 subunits, each consisting of three structural domains: The N-terminal catalytic domain, a central regulatory domain and a C-terminal association domain (39). The variable regions are in the central regulatory domain, and their composition differs according to different CaMKII splice variants (39). Normally, CaMKII can regulate various biological functions of the heart, by regulating myocardial cell membrane excitability, maintaining cell Ca^{2+} homeostasis, participating in excitation contraction coupling between myocardial cells and regulating the cell cycle, so it is an important regulator in cardiomyocytes (38,40). However, sustained CaMKII activation is considered to serve a central role in the pathogenesis of cardiovascular diseases (38,41). Emerging evidence suggests that CaMKII can act as a novel substrate for RIPK3 to mediate necroptosis (13). When RIPK3 binds to CaMKII, it can mediate the phosphorylation of the Thr287 site of CaMKII through its kinase activity, and RIPK3 can also promote the production of reactive oxygen species (ROS) by increasing the activities of glycogen phosphorylase, glutamate-ammonia ligase and glutamate dehydrogenase 1, and then oxidize the Met281/282 site of CaMKII, and both the oxidation and phosphorylation of CaMKII are involved in RIPK3-mediated necroptosis (Fig. 1) (13,42).

Ryanodine receptor 2 (RyR2) is a major calcium release channel on the sarcoplasmic reticulum (SR) of myocardial cells (43). Gui *et al* (42) indicated that RyR2 is one of the major downstream targets of CaMKII in myocardial cells and serves an important role in regulating Ca^{2+} homeostasis. When CaMKII is activated, it will phosphorylate the RyR2-Ser2814 site, leading to endoplasmic reticulum (ER) stress and subsequent Ca^{2+} leak through RyR2 from the SR (42,44,45). CaMKII can also phosphorylate the mitochondrial Ca^{2+} uniporter (MCU) and increase its ability to transport Ca^{2+} (46). Excessive Ca^{2+} in the cytoplasm will enter the mitochondria through the MCU, causing mitochondrial Ca^{2+} overload, and thus, promoting the opening of the mPTP (13,41,47,48). Recent studies have reported that the mPTP is part of the final pathway of necroptosis (49,50). The opening of the mPTP leads to loss of mitochondrial inner membrane potential ($\Delta\Psi m$) and the rapid dissipation of proton gradients across

Table I. Differences among necroptosis, apoptosis and necrosis.

Differences	Necroptosis	Apoptosis	Necrosis
Cause	Activated by death receptor ligands (4,5)	Physiological or pathological changes (4)	Pathological changes or severe injuries (4)
Gene regulation	Programmed (4,5)	Programmed (4,5)	Unprogrammed (4)
Cell morphology	Cell swelling (5)	Cell shrinkage (5)	Cell swelling (4)
Cell membrane	Membrane rupture (5)	Membrane integrity (5)	Membrane rupture (4)
Organelle	Organelle swelling and rupture (5)	No obvious changes (5)	Organelle swelling and rupture (4)
Apoptotic bodies	No apoptotic bodies (4,5)	Apoptotic bodies (4,5)	No apoptotic bodies (4)
Inflammatory response	Inflammatory response (5-8)	No inflammatory response (5-8)	Inflammatory response (4)
Key regulatory proteins	RIPK1, RIPK3, MLKL and CaMKII (5-8)	Caspase family (4-8)	Unregulated (4)

RIPK1, receptor interaction protein kinase 1; RIPK3, receptor interaction protein kinase 3; MLKL, mixed lineage kinase domain-like protein; CaMKII, calcium/calmodulin-dependent protein kinase II.

the inner membrane, disrupting mitochondrial respiratory function and leading to metabolic shutdown, thereby causing mitochondrial structural and functional disorders that result in mitochondrial damage and mitochondrial swelling; this leads to decreased ATP synthesis and increased ROS generation, ultimately causing organelle swelling, cell swelling and loss of plasma membrane integrity, which leads to the occurrence of necroptosis, ultimately triggering the innate immune inflammatory response and leading to tissue damage (10,13,38,51). The opening of the mPTP and ROS generation can further increase CaMKII activity, thus forming a positive feedback loop and accelerating necroptosis (Fig. 1) (13,40). The opening of the mPTP and the accumulation of ROS also link death receptor-mediated necroptosis to the mitochondrial-dependent necrosis pathway, suggesting that necroptosis can be induced through the mitochondrial pathway, further enriching the mechanism of necroptosis (50,52).

3. Effects of RIPK3-CaMKII-mPTP signaling pathway activation in cardiovascular diseases

AMI and I/R injury. AMI, a common disease, is caused by acute or subacute coronary artery occlusion (53). Prolonged ischemia leads to myocardial cell hypoxia and subsequent myocardial cell death, which is a serious threat to patient health and life (54,55). Reperfusion therapy is the preferred treatment strategy for patients with AMI (56). Although early reperfusion of the ischemic myocardium in coronary artery infarction can rescue the agonal cardiac muscle, it causes subsequent myocardial I/R injury, which lowers the protective effect of reperfusion therapy and restricts the development of this therapy (56,57).

With the continuous development of research, it has been revealed that necroptosis serves an important role in AMI and I/R injury (10,58-60). Luedde *et al* (61) generated a mouse AMI model by permanent ligation of the left anterior descending coronary artery and subsequently found that the RIPK3 protein level was markedly upregulated in ischemic heart tissue, while the upregulation of RIPK3 could promote myocardial damage, indicating that RIPK3 was activated during AMI. Furthermore, knockdown of RIPK3 improved cardiac function, attenuated the inflammatory response, alleviated mitochondrial injury, decreased ROS production and inhibited cardiac necroptosis (61). Zhang *et al* (62) found that upregulation of microRNA-325-3p inhibited the progression of AMI in a mouse model by preventing RIPK3 activation and subsequent necroptosis. Oerlemans *et al* (63) reported that when myocardial I/R injury occurred, RIPK3 phosphorylation was elevated, and inhibition of RIPK3-mediated necroptosis could reduce myocardial cell loss and contribute to increased resistance to I/R injury. Collectively, the aforementioned studies suggest that the RIPK3-MLKL-mediated classical necroptosis pathway serves an important role in I/R injury.

Multiple studies have demonstrated that, in a model of myocardial I/R injury, in addition to the classical RIPK3-MLKL signaling pathway, RIPK3 can mediate the necroptosis of myocardial cells through CaMKII, and inhibition of CaMKII can also prevent the necroptosis of myocardial cells induced by I/R injury (14-16). Thus, these studies suggest that CaMKII serves a crucial role in the pathogenesis of I/R injury. Similarly, studies have demonstrated that the cellular levels of RIPK1, RIPK3 and CaMKII were markedly increased in hypoxic myocardial cells (14,15). When I/R injury occurred, both RIPK3 and CaMKII were present in myocardial cells, and the binding of the two was enhanced, while the phosphorylation of CaMKII increased with the upregulation of RIPK3, indicating that RIPK3 could directly bind and phosphorylate CaMKII; however, after inhibition of CaMKII activation using KN-93, a small-molecule inhibitor of CaMKII, myocardial cell mortality was still reduced even when RIPK3 was upregulated (13). The study further identified two pathways of CaMKII activation by RIPK3: Direct phosphorylation of RIPK3 or indirect ROS-mediated oxidation could activate CaMKII, and inhibition of either CaMKII oxidation or phosphorylation markedly inhibited RIPK3-mediated necroptosis (13). Therefore, direct phosphorylation of RIPK3 and indirect ROS-mediated oxidation mediate RIPK3-induced myocardial cell necroptosis.

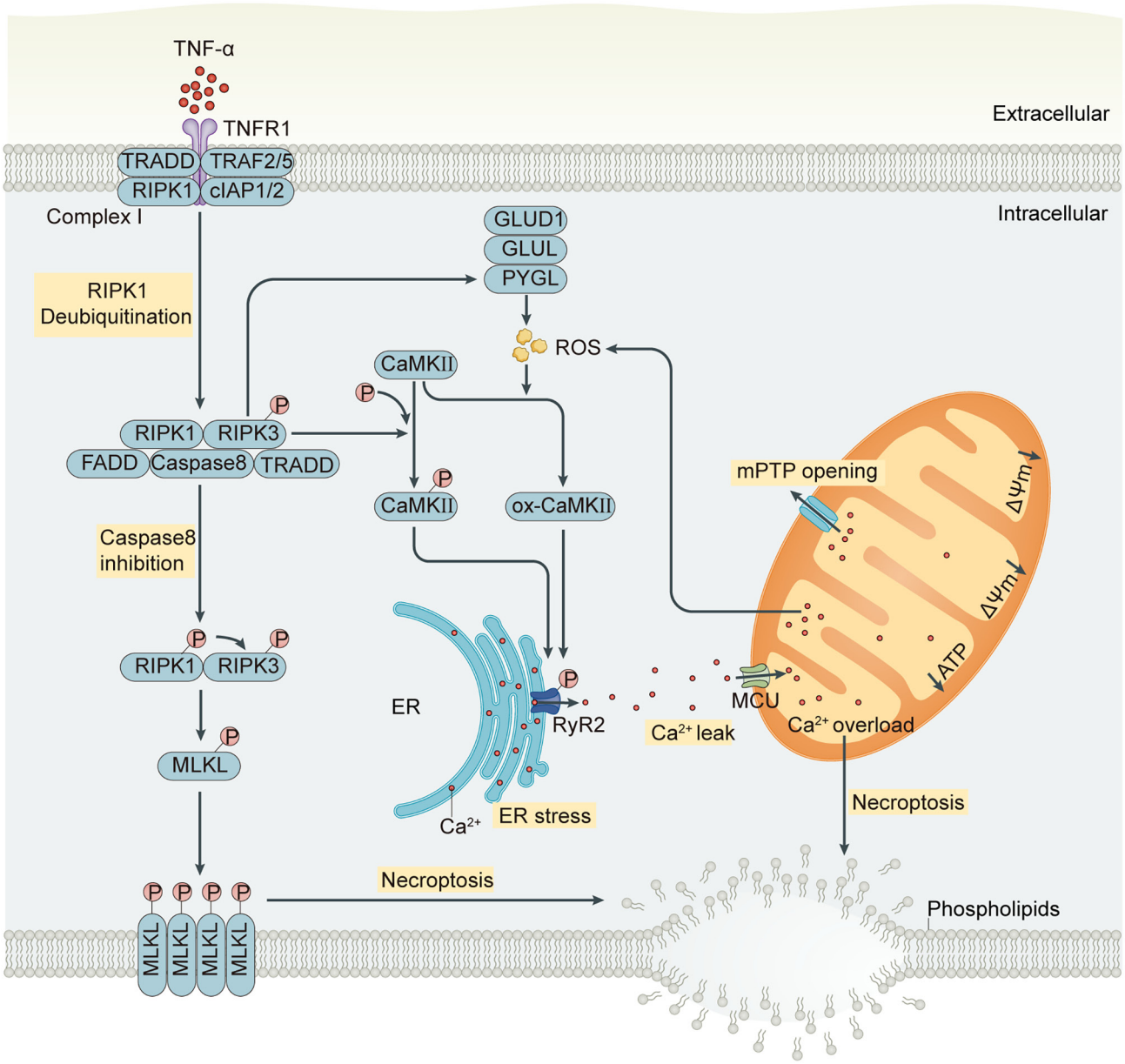

Figure 1. Overview of the RIPK3-CaMKII-mPTP signaling pathway. When TNF-α binds to TNFR1, TNFR1 recruits the adaptor protein TRADD, which recruits other proteins to form complex I. When RIPK1 is deubiquitinated and activated, RIPK1 assembles a cytoplasmic signaling complex that includes RIPK3, FADD, caspase8 and TRADD. Under conditions of impaired caspase8 activity, RIPK1 self-phosphorylation promotes the phosphorylation and activation of RIPK3, thereby mediating the phosphorylation and activation of MLKL, which is subsequently oligomerized and transported to the plasma membrane, ultimately leading to the occurrence of necroptosis. In addition, p-RIPK3 can also activate CaMKII. When CaMKII is activated, it causes ER stress, which then leads to Ca^{2+} leakage. Excess Ca^{2+} in the cytoplasm enters the mitochondria via the MCU, leading to mitochondrial Ca^{2+} overload, promoting the opening of mPTP, which leads to the loss of ΔΨm and reduced ATP synthesis, and ultimately leads to necroptosis. RIPK3, receptor interaction protein kinase 3; CaMKII, calcium/calmodulin-dependent protein kinase II; MLKL, mixed lineage kinase domain-like protein; mPTP, mitochondrial permeability transition pore; TNFR1, tumor necrosis factor receptor 1; RIPK1, receptor interaction protein kinase 1; TRADD, DD-containing adaptor protein TNFR1-associated death domain protein; TRAF2/5, TNF receptor-associated factors 2 and 5; cIAP1/2, cellular inhibitor of apoptosis proteins 1 and 2; FADD, FAS-associated death domain protein; P, phosphorylated; ROS, reactive oxygen species; PYGL, glycogen phosphorylase; GLUL, glutamate-ammonia ligase; GLUD1, glutamate dehydrogenase 1; ox-, oxidized; RyR2, ryanodine receptor 2; ER, endoplasmic reticulum; MCU, mitochondrial Ca^{2+} uniporter; ΔΨm, mitochondrial inner membrane potential.

A previous study has demonstrated that CaMKII can promote Ca^{2+} entry into mitochondria by increasing the MCU, thus causing the opening of the mPTP (47). Cyclophilin D (CypD), a peptidylprolyl cis-trans isomerase, is a key component of the mPTP complex, and its activity regulates the open state of the mPTP and is essential for mPTP-induced cell death (64). Cyclosporin A (CsA) is a specific inhibitor of CypD that binds specifically to CypD, thereby preventing mPTP opening (65). The use of CsA has been found to be clinically efficacious in myocardial I/R injury by preventing mPTP opening while reducing the occurrence of mitochondrial disruption and necroptosis (47,66). Therefore, we hypothesized that mPTP opening is part of the final pathway of necroptosis, and it is related to myocardial cell death and myocardial injury caused by I/R injury (34). In a study by Zhang *et al* (13), upregulation of RIPK3 resulted in depolarization of ΔΨm in

a CypD-dependent manner, suggesting that RIPK3 has the ability to promote mPTP opening. Inhibition of CaMKII blocks the RIPK3-induced depolarization of $\Delta\Psi m$, thus the opening of the mPTP induced by RIPK3 requires CaMKII, thereby demonstrating that the mPTP is an indispensable downstream effector in the conduction of the RIPK3-CaMKII-mediated necroptosis signaling pathway (13,45). Chronic adverse stress induced by chronic pain increases the risk of cardiovascular disease, and it is potentially associated with myocardial I/R injury; however, to the best of our knowledge, the underlying mechanisms are not known (67). In a previous study, chronic pain stimulation was simulated by constructing a reserved nerve injury model, and it was revealed that chronic pain markedly aggravated postischemic myocardial injury and increased RIPK3-dependent CaMKII and MLKL phosphorylation levels, indicating that RIPK3-mediated MLKL and CaMKII activation were two key ways to increase susceptibility to myocardial I/R injury under chronic pain conditions (68). In addition, RIPK3 serves a role in I/R injury by activating mPTP opening through the ER stress/Ca^{2+} overload/xanthine oxidase/ROS pathway (45). In conclusion, these findings suggest that the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway serves an important role in the pathogenesis of myocardial I/R injury and that inhibition of the RIPK3-CaMKII-mPTP signaling pathway may be an important therapeutic target for I/R injury.

HF. HF is the final stage in the development of several cardiovascular diseases, including ischemic cardiomyopathy and dilated cardiomyopathy, rather than an independent disease (69). The loss of cardiomyocytes and subsequent deterioration of systolic function are the main hallmarks of HF (70). Previous studies have demonstrated that the expression levels of RIPK1, RIPK3 and MLKL were markedly increased in mouse models of HF as well as human patients with HF, and were positively associated with the severity of HF (45,71). The targeting of these markers of necroptosis may improve AMI-induced cardiac remodeling, cardiac dysfunction and HF (45,61,72). Collectively, the aforementioned studies suggest that RIPK3-MLKL signaling pathway-mediated necroptosis is involved in the pathogenesis of HF.

Previous studies have demonstrated that the novel necroptosis pathway also serves an important role in HF (17,73). Compared with those in healthy mice with low CaMKII expression, in mice with congestive HF, the expression levels and activity of CaMKII were increased, and myocardial cell death was also increased, which may lead to further worsening of HF (74). A further study has demonstrated that inhibition of CaMKII reduced the levels of proteins involved in necroptosis and improved myocardial contractile function in mouse cardiomyocyte necroptosis induced by myocardial I/R injury, indicating that CaMKII serves a role in the development of HF via necroptosis (73); however, to the best of our knowledge, the upstream substrate of CaMKII remains unclear. Zhang *et al* (13) reported that CaMKII activation could lead to opening of the mPTP and induction of myocardial necroptosis, ultimately leading to cardiac remodeling and HF. Concurrently, phosphorylation and oxidation of CaMKII were markedly increased in the myocardial cells of HF mice, and this upregulation was markedly reversed in mice with knockout of RIPK3, thus suggesting that RIPK3 is essential for the activation of CaMKII, and CaMKII may be a downstream target or substrate of RIPK3-mediated myocardial injury and necroptosis in HF (17). Further in-depth study revealed that the ultrastructure of myocardial mitochondria was improved in HF mice after inhibition of RIPK3, suggesting that mitochondrial damage may be a potential mechanism for RIPK3-mediated necroptosis in HF (17). In addition, CaMKII also contributes to the development of HF by mechanisms other than necroptosis. Ca^{2+} leakage in the diastolic SR is an important pathological mechanism in the development of HF, and CaMKII can activate RyR2 on the SR and promote Ca^{2+} release from the SR, causing cytoplasmic Ca^{2+} overload, which in turn leads to cardiac diastolic dysfunction (42,75). Overall, these studies suggest that the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway is involved in the pathogenesis of HF and may represent a novel target for the treatment of HF.

AAA. AAA is defined as aneurysmal dilatation of the abdominal aorta, which manifests as abnormal dilatation of the abdominal aorta to >50% of its normal diameter (76). The majority of patients with AAA are asymptomatic, AAA is often identified incidentally on physical examination for other reasons and patients with AAA have a potentially fatal risk of rupture that would have a serious impact on the lives of patients (77,78). Currently, there are no effective drugs to slow aneurysm growth or prevent rupture (79). Depletion of smooth muscle cells (SMCs) in the medial layer of the arterial wall is the main pathological feature of AAA; however, the cause of SMC depletion is unknown (80,81). With further research, some studies have demonstrated that the expression levels of RIPK3 were increased in human and mouse atheroma tissues (82,83), and RIPK3 is a key mediator in the regulation of necroptosis, suggesting that necroptosis is involved in the pathogenesis of AAA. Previous studies have demonstrated that inhibition of RIPK3 expression inhibited SMC depletion and aneurysm formation in mice, suggesting that the RIPK3 signaling pathway may promote the progression of AAA by causing SMC necroptosis (82-84). However, to the best of our knowledge, the downstream necroptosis signaling pathway involved in RIPK3-induced SMC death remains unknown. A subsequent study has revealed that both MLKL and CaMKII were activated in mouse atheroma tissue and that inhibition of RIPK3 markedly reduced the phosphorylation of MLKL and CaMKII in aneurysms, demonstrating that the activation of MLKL and CaMKII was dependent on RIPK3 (18). Using TNF-α and Z-Val-Ala-DL-Asp-fluoromethylketone to induce necroptosis in SMCs of the mouse aorta, it was revealed that the levels of phosphorylated MLKL and CaMKII were increased in the mouse aortic aneurysm tissue compared with those in tissues from untreated mice (18). Thus, these findings suggest that both MLKL and CaMKII are involved in SMC necroptosis *in vitro* and *in vivo*. However, in the study, coimmunoprecipitation assays did not detect an interaction between RIPK3 and CaMKII during SMC necroptosis, suggesting that RIPK3 may not directly phosphorylate CaMKII, but it cannot be ruled out that the lack of interaction between RIPK3 and CaMKII was caused by the use of SMCs instead of cardiomyocytes in the immunoprecipitation experiment, while silencing MLKL inhibited CaMKII phosphorylation

in SMC necroptosis, indicating that CaMKII may be located downstream of MLKL. Further studies are still needed for confirmation (18). In conclusion, RIPK3 can induce SMC necroptosis via MLKL and CaMKII, and inhibition of the RIPK3-MLKL/CaMKII signaling pathway may be a potential way to treat AAA.

AS. AS is a chronic inflammatory disease (85). Pathologically, initial observations include the formation of fat stripes on the vascular intima, and then the fat stripes evolve into necrotic plaques that are rich in lipids and macrophages (86). In late-stage development, atherosclerotic plaques can form unstable plaques, also known as vulnerable plaques, with thin fibrous caps that are easy to rupture and lead to vascular occlusion, and unstable plaques are a leading cause of AMI, acute cerebral infarction and acute limb ischemia (87). Necroptosis can contribute to the progression of atheromatous plaques and was initially found in a mouse model of AS (88) but has not been studied in humans. Later studies have revealed that RIPK3 and MLKL expression was increased in humans with unstable carotid AS, and MLKL phosphorylation was detected in advanced atheromatous plaques (82,89). It has also been reported that RIPK3 deficiency prevented necroptosis of macrophages in atherosclerotic plaques of mice and delayed the progression of AS (88,90). These findings suggest that the necroptosis pathway mediated by RIPK3-MLKL is involved in the development of human AS. A previous study has demonstrated that CaMKII was highly expressed in atherosclerotic plaques, suggesting that CaMKII is involved in the pathogenesis of AS (19). It has been demonstrated that RIPK3 can lead to SMC necroptosis via CaMKII, leading to AAA progression (18). However, it is unclear whether CaMKII is dependent on RIPK3 to mediate necroptosis in AS, and further research is needed.

DCM. DCM is defined as ventricular dysfunction in the absence of coronary vascular disease or hypertension (91,92). DCM, one of the major cardiovascular complications of diabetes, is characterized by myocardial dysfunction in patients with diabetes (93). Cell death is considered the end point of myocardial cells in DCM (94). Myocardial cell death in diabetes mainly includes apoptosis, autophagy and necroptosis, of which necroptosis serves an important role in DCM (94-96).

Liu *et al* (97) reported that RIPK3 expression was increased in diabetic rats, suggesting that RIPK3-mediated necroptosis is involved in the development of diabetes. However, whether necroptosis also serves a role in DCM was not determined at the time. In one study, RIPK3 expression was markedly increased in H9C2 cardiomyocytes following 35 mmol/l high-glucose (HG) treatment, while the use of Nec-1, an inhibitor of necroptosis, markedly reduced RIPK3 expression in H9C2 cardiomyocytes exposed to HG and prevented HG-induced myocardial injury (98). In conclusion, the study indicated that HG treatment was a strong stimulus for the induction of necroptosis and that HG treatment may lead to myocardial cell injury via necroptosis, which in turn leads to DCM.

In DCM, the downstream substrates of the RIPK3-mediated necroptosis signaling pathway are gradually being found (20,21). Several previous studies have demonstrated that CaMKII expression is markedly increased in DCM and that HG can induce myocardial cell death via CaMKII (99,100). CaMKII can serve a central role in the pathogenesis of several cardiac diseases as a substrate for RIPK3 (13,17,18). However, it is not clear whether the RIPK3-CaMKII signaling pathway mediates necroptosis of cardiomyocytes in DCM. A previous study has demonstrated that HG increased RIPK3 and CaMKII expression but did not markedly change RIPK1 expression and phosphorylated (p-)MLKL levels, suggesting that RIPK3 and CaMKII mediate HG-induced necroptosis rather than RIPK1 or MLKL (20). Chen *et al* (21) also revealed that the phosphorylation and oxidation of CaMKII were markedly increased in cardiomyocytes from a mouse model of DCM, but this phenomenon was markedly reversed in RIPK3$^{-/-}$ mice, suggesting that CaMKII is a downstream target or substrate of RIPK3 in DCM. It was also revealed that inhibition of necroptosis by knocking down RIPK3 not only attenuated myocardial injury but also improved cardiac function in DCM mice and improved the mitochondrial ultrastructure in cardiomyocytes, suggesting that mitochondrial damage is involved in necroptosis in DCM (21). In addition, HG can indirectly activate CaMKII and trigger mPTP opening by increasing ROS, ultimately leading to necroptosis (96).

A large amount of advanced glycation end products (AGEs) are known to be present in diabetic patients (101). These AGEs are closely associated with complications in diabetic patients and have the potential to be biomarkers of diabetes and its complications (101,102). Furthermore, CaMKIIδ splicing is strictly regulated, and an imbalance in CaMKIIδ alternative splicing causes cardiomyocyte dysfunction and ultimately cardiovascular diseases (74). Recently, a study has revealed that AGEs increased RIPK3 expression, leading to an imbalance of CaMKIIδ alternative splicing, promoted CaMKII activation and ultimately induced cardiomyocyte necroptosis (103). By contrast, by downregulating RIPK3 or using the RIPK3 inhibitor GSK'872, the imbalance of CaMKIIδ alternative splicing can be corrected, CaMKIIδ activation can be inhibited, oxidative stress can be reduced, necroptosis can be reduced and myocardial cell damage can be reduced (103). Overall, these studies have demonstrated the important role of the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway in the pathogenesis of DCM and provide novel ideas for the prevention and treatment of DCM.

HCM. HCM is a myocardial disorder of unknown etiology characterized by abnormal thickening of the left ventricular walls. Its histological manifestations are mainly hypertrophy of myocardial cells (104). The clinical manifestations of HCM include palpitations, exertional dyspnea, precordial pain, syncope, HF or even sudden death, and HCM is one of the main causes of sudden cardiac death in young adults (105). A growing body of evidence has suggested that necroptosis is also involved in the development of cardiac hypertrophy and HCM (22,23).

Previous studies have demonstrated that CaMKII expression was increased in cardiac hypertrophy and have also revealed that the regulation of CaMKIIδ alternative splicing may be related to cardiac function and was involved in cardiac hypertrophy in humans and mice (74,106,107). In a recent

Table II. Cardiotoxicity of antitumor drugs and other chemicals.

First author/s, year	Drug	Target	Cardiotoxic effect	(Refs.)
Zhang *et al*, 2016	Doxorubicin	RIPK3 and CaMKII	Cardiomyocyte death	(13)
Zhou *et al*, 2021	Vincristine	RIPK1, RIPK3, MLKL and CaMKII	Cardiomyocyte death	(25)
McMullen *et al*, 2021	Sunitinib	CaMKII	Cardiac fibroblast death	(26)
Reventun *et al*, 2020	BPA	RIPK3 and CaMKII	Endothelial cell death	(27)

RIPK3, receptor interaction protein kinase 3; CaMKII, calcium/calmodulin-dependent protein kinase II; RIPK1, receptor interaction protein kinase 1; MLKL, mixed lineage kinase domain-like protein; BPA, bisphenol A.

animal study, a murine model of HCM was constructed by subcutaneous infusion of angiotensin II using osmotic mini-pumps (22). The study revealed that, compared with those in the mice without myocardial hypertrophy (infusion of phosphate buffered solution), the levels of RIPK3 in HCM mice were markedly increased, and the levels of both the oxidation and phosphorylation of CaMKIIδ were also increased (22). The study also revealed that, compared with those in untreated mice, the levels of RIPK3, RIPK1, CaMKIIδ and p-MLKL were decreased in mice treated with the RIPK3 inhibitor (GSK'872) (22). In another study by Wang *et al* (23), the oxidation and phosphorylation levels of CaMKIIδ were increased in myocardial cells in a phenylephrine (PE)-induced cardiac hypertrophy model, while downregulation of RIPK3 markedly inhibited CaMKII activation in PE-stimulated cardiomyocytes. These studies suggest that CaMKII may act as a substrate for RIPK3 and mediate necroptosis of cardiomyocytes in HCM. In addition, upregulation of RIPK3 in HCM mice resulted in CaMKIIδ alternative splicing impairment, a marked decrease in CaMKIIδA and CaMKIIδB expression, and a marked increase in CaMKIIδC expression compared with those of mice without myocardial hypertrophy, which in turn led to necroptosis of cardiomyocytes (22,23). However, compared with wild-type mice (infusion of angiotensin II), knockdown or deletion of RIPK3 inhibited CaMKII activation, corrected CaMKIIδ alternative splicing impairment, alleviated oxidative stress, reduced necroptosis and reversed myocardial injury in HCM mice (22,23). Therefore, targeting the RIPK3-CaMKII-mPTP signaling pathway may protect cardiomyocytes from necroptosis, which may be important for the prevention and treatment of HCM.

AF. AF is a common cardiac arrhythmia. The incidence rate in Chinese individuals >75 years old was 8.6% and the mortality rate of patients with AF with stroke was >50% in a cohort study (108). The pathogenesis of AF has been extensively explored, and structural remodeling of the atria is considered an important part of the pathogenesis of AF (108,109). AF is a common consequence of several diseases that lead to atrial remodeling (110). Atrial structural remodeling is characterized by atrial enlargement and atrial fibrosis (111). A previous study has demonstrated that RIPK3-mediated necroptosis contributes to cardiomyocyte death, which may result in cardiac fibrosis and cardiac remodeling in the long term (61). Sustained CaMKII activation is considered to serve an important role in atrial remodeling and may promote AF (40,112). Several studies have demonstrated that by knocking down RIPK3 or inhibiting its downstream substrate CaMKII, necroptosis signaling can be inhibited, and fibrosis can be prevented (113-115). These studies suggest that necroptosis is involved in the development of fibrosis and is potentially related to atrial fibrosis and atrial structural remodeling. In a recent study, the levels of RIPK1, RIPK3, MLKL, CaMKII and their phosphorylated forms were markedly increased in the atria of AF mice, and myocardial fibrosis and susceptibility to AF caused by acetylcholine-$CaCl_2$ were attenuated by treatment with necrostatin-1, a necrotrophic apoptosis inhibitor (24). In addition, the study has indicated that aerobic swim training of mice inhibited necroptosis signaling, thereby decreasing susceptibility to AF and atrial structural remodeling (24). In conclusion, these results suggest that necroptosis is involved in the development of atrial fibrosis and is an important pathogenesis leading to AF, which provides a novel target for the prevention and treatment of AF.

Cardiotoxicity of antitumor drugs and other chemicals. Chemotherapy is currently one of the leading treatments for cancer; however, improper treatment causes damage to a variety of organs, with the heart being one of the organs frequently affected (116). Prolonged use of antineoplastic drugs can cause cardiotoxicity, triggering excessive loss of cardiomyocytes and leading to cardiovascular complications such as HF, AMI, hypertension, thromboembolism and arrhythmias (117), which adversely affect the prognosis of patients with cancer and are a major cause of treatment termination and drug development failure (118). Excessive loss of cardiomyocytes is known to serve an important role in the cardiotoxicity of antineoplastic drugs (119). As research continues, the role of necroptosis in the cardiotoxicity of various antineoplastic drugs is emerging. The cardiotoxicity of antitumor drugs and other chemicals is summarized in Table II.

DOX is a first-line chemotherapy drug that is widely used to treat a variety of cancer types, including carcinomas, sarcomas and hematological cancers (120) It causes irreversible cardiotoxicity in 30-40% of patients, resulting in the loss of numerous myocardial cells and ultimately leading to cardiomyopathy and HF, thus severely limiting its clinical application (121). It has been reported that the mRNA and protein expression levels of RIPK3 were increased 2-3-fold in DOX-treated mouse cardiomyocytes, and the levels of phosphorylation of the Thr287 site of CaMKII and oxidation of the Met281/282 site of CaMKII were markedly increased, as was

the opening of the mPTP (13). RIPK3$^{-/-}$ mice have a certain resistance to the cardiotoxicity of DOX, which is specifically manifested by a reduction in the activation of CaMKII, a reduction in myocardial cell death, and improvement in the heart function and survival of mice (13). These results indicate that DOX is able to activate CaMKII through RIPK3, thereby promoting the opening of the mPTP and ultimately inducing necroptosis of myocardial cells (13).

Vincristine is also a first-line chemotherapy drug, and myocardial injury induced by vincristine has been reported continuously since the launch of the drug on the market (122,123). However, to the best of our knowledge, the potential mechanisms leading to myocardial injury remain unclear. A recent study has demonstrated that activation of CaMKII was also detected in myocardial necroptosis induced by vincristine, and both *in vivo* and *ex vivo* experiments with vincristine demonstrated that the levels of RIPK1, RIPK3, MLKL and p-CaMKII were all increased in cardiomyocytes, suggesting that vincristine-induced necroptosis of cardiomyocytes can be mediated not only by MLKL but also by CaMKII (25). The study has also revealed that the oxidation of the Met281/282 site of CaMKII did not markedly change after treatment of H9C2 cells with vincristine (25). By contrast, both the phosphorylation and oxidation levels of CaMKII were increased in DOX-mediated necroptosis of myocardial cells (13), indicating that different antineoplastic drugs may lead to cardiac toxicity via different mechanisms.

In addition to myocardial cells, the damage caused by antineoplastic drugs to cardiac fibroblasts in the heart may also mediate cardiotoxicity (26). A recent study has demonstrated that the oxidation of CaMKII was increased in cardiac fibroblasts after sunitinib treatment, and cell death was correspondingly increased (26). However, because the study did not evaluate the expression levels of key components of necroptosis, such as RIPK3 and MLKL, it is still unclear whether CaMKII-mediated cell death is dependent on RIPK3.

In addition to the aforementioned antitumor drugs, some studies have demonstrated that other chemicals can cause cardiotoxicity through the RIPK3-CaMKII-mediated necroptosis signaling pathway. Bisphenol A (BPA) is a chemical substance widely used in daily life. Because of its widespread use, individuals are often exposed to it, so its safety has become a focus of public attention (124,125). Epidemiological studies have demonstrated that BPA increased the incidence rate and mortality of cardiovascular diseases (126,127). In a recent study, the necroptosis of coronary artery endothelial cells was increased, and the mice also had severe complications such as coronary artery injury, vascular leakage, myocardial injury and even HF after the mice were treated with BPA (27). The study further explored the underlying mechanisms in depth and found that the activation of CaMKII in endothelial cells was increased, and inhibition of CaMKII activity with KN-93 could reduce endothelial necroptosis but did not affect the increased expression levels of RIPK3 induced by BPA treatment (27). However, knockdown of RIPK3 prevented the phosphorylation of CaMKII and the transduction of necroptosis signals (27). Therefore, these results indicated that BPA can induce necroptosis through the RIPK3-CaMKII-mPTP signaling pathway, thus leading to cardiotoxicity.

In summary, the RIPK3-CaMKII-mPTP-mediated necroptosis signaling pathway serves an important role in the cardiotoxicity of antitumor drugs and other chemicals, and targeting this necroptosis signaling pathway is important to reduce the cardiotoxicity of drugs.

4. Therapeutic targeting of the RIPK3-CaMKII-mPTP signaling pathway

At present, the therapeutic options for the treatment of cardiovascular diseases remain unsatisfactory, necessitating the development of more precise targeted therapeutic drugs. The aforementioned findings (13-27) on the involvement of RIPK3-CaMKII-mPTP-mediated necroptosis in a variety of cardiovascular diseases suggest that the RIPK3-CaMKII-mPTP pathway has high potential as a drug target. The emergence of drugs targeting RIPK3, CaMKII and mPTP enables the development of clinical candidates. The present review discusses the inhibitors that target RIPK3, CaMKII and mPTP, and these are summarized in Table III.

Inhibitors targeting RIPK3. RIPK3 kinase activity serves an important role in necroptosis, and the kinase domain of RIPK3 is an important target of small-molecule inhibitor intervention (128). Numerous RIPK3 kinase inhibitors have been reported, including GSK'840, GSK'843 and GSK'872, which can bind to the RIPK3 kinase domain with high affinity and inhibit RIPK3 kinase activity, thereby inhibiting RIPK3-induced necroptosis (129,130). GSK'840 and GSK'843 have been reported to be therapeutic interventions for viral infection, inflammatory diseases and cancer by inhibiting necroptosis (130). GSK'872 has been reported to correct the imbalance of CaMKII alternative splicing and to prevent necroptosis, thus reducing myocardial cell damage (103). A previous study has demonstrated that GSK'872 also had a marked protective effect on Alzet miniosmotic pumps (ALZA Corporation) and perfused with angiotensin II-induced cardiac hypertrophy in mice, which may make it a potential drug candidate for HCM (22). Necrostatin-1 is a classic necroptosis inhibitor. It has been demonstrated to reduce the area of myocardial infarction (131,132) and to prevent adverse cardiac remodeling after I/R injury in mice (63). A previous study has also demonstrated that necrostatin-1 reduced RIPK3 expression induced by HG, thereby increasing cardiomyocyte viability, reducing ROS production and attenuating $\Delta\Psi m$, and ultimately exerting a protective effect against DCM (98). 3-Iodothyronamine (3-T1AM) is a metabolite of decarboxylated and deiodinated thyroid hormone. Previous studies have demonstrated that 3-T1AM could attenuate cardiomyocyte apoptosis induced by I/R injury (133). A recent study has revealed that 3-T1AM could also reduce the expression levels of RIPK1, RIPK3 and CaMKII in myocardial cells with I/R injury and could alleviate necroptosis of myocardial cells, thus protecting myocardial cells from I/R injury (15). Melatonin is a hormone produced by the pineal gland that is mainly involved in the regulation of sleep and the circadian rhythm (134). A previous study has reported that melatonin could inhibit the phosphorylation of RIPK3 and the downstream molecule CaMKII, and reduced the occurrence of necroptosis, thus improving cardiac function after I/R injury (68). ZYZ-803

Table III. Summary of inhibitors of necroptosis in cardiovascular diseases.

First author/s, year	Inhibitor	Target	Cardiovascular diseases	(Refs.)
Zhang *et al*, 2022	GSK'872	RIPK3	HCM	(22)
Oerlemans *et al*, 2012; Lim *et al*, 2007; Smith *et al*, 2007	Necrostatin-1	RIPK3	AMI and I/R injury	(63,131,132)
Liang *et al*, 2017	Necrostatin-1	RIPK3	DCM	(98)
Wei *et al*, 2022	3-T1AM	RIPK1, RIPK3 and CaMKII	I/R injury	(15)
Yang *et al*, 2018	Melatonin	RIPK3 and CaMKII	I/R injury	(68)
Chang *et al*, 2019	ZYZ-803	RIPK3 and CaMKII	AMI	(136)
Feng and Anderson, 2017; Adameova *et al*, 2012	KN-93	CaMKII	I/R injury	(38,137)
Ikeda *et al*, 2019	Nifedipine	CaMKII	DOX-induced cardiomyopathy	(140)
Ikeda *et al*, 2019	Amlodipine	CaMKII	DOX-induced cardiomyopathy	(140)
Liu *et al*, 2019	CaMKIIn	CaMKII	AF	(112)
Sun *et al*, 2019	I1PP1	CaMKII	DCM	(20)
Xu *et al*, 2018; Joiner *et al*, 2012; Lim *et al*, 2012; Piot *et al*, 2008; Cung *et al*, 2015; Ottani *et al*, 2016	CsA	mPTP	I/R injury	(16,47,141-144)
Zhang *et al*, 2019	CsA@PLGA-PEG-SS31	mPTP	I/R injury	(146)
Yao *et al*, 2010	H_2S	mPTP	I/R injury	(147)

RIPK3, receptor interaction protein kinase 3; HCM, hypertrophic cardiomyopathy; AMI, acute myocardial infarction; I/R, ischemia-reperfusion; DCM, diabetic cardiomyopathy; 3-T1AM, 3-iodothyronamine; RIPK1, receptor interaction protein kinase 1; CaMKII, calcium/calmodulin-dependent protein kinase II; DOX, doxorubicin; AF, atrial fibrillation; I1PP1, inhibitor-1 of protein phosphatase 1; CsA, cyclosporin A; mPTP, mitochondrial permeability transition pore; H_2S, hydrogen sulfide.

is a novel type of hydrogen sulfide (H_2S)-nitric oxide (NO) coupling donor that can be decomposed into H_2S and NO (135). A study has reported that it could correct the imbalance in these two small gas molecules, inhibit the interaction between RIPK3 and CaMKII, and inhibit the phosphorylation of CaMKII, thereby inhibiting the RIPK3-CaMKII-mPTP signaling pathway, reducing the necroptosis of myocardial cells and having a protective effect in AMI (136).

Inhibitors targeting CaMKII. Sustained CaMKII activation has an important role in the necroptosis pathway and is implicated in the pathogenesis of several cardiovascular diseases, thus CaMKII can be an important target for the inhibition of necroptosis (38). KN-93 is a small-molecule inhibitor of CaMKII. During myocardial I/R injury, treatment of the heart with KN-93 reduces cell death under RIPK3 upregulation conditions, improves postischemic cardiac contractile function and inhibits the development of malignant arrhythmias (38,137). KN-93 could also downregulate CaMKIIδ expression, exhibiting potential therapeutic value for I/R injury (38,137). The L-type Ca^{2+} channel is the main pathway of Ca^{2+} entry into myocardial cells and is essential for myocardial contraction (138). L-Type Ca^{2+} channel blockers, such as nifedipine and amlodipine, are known to block this pathway and are commonly used to treat hypertension (139). A previous study has demonstrated that nifedipine and amlodipine could inhibit the activity of CaMKII and reduce myocardial cell death by reducing the levels of Ca^{2+} in myocardial cells, thus serving a protective role in DOX-induced cardiomyopathy (140). However, the study noted that L-type Ca^{2+} channel blockers suppressed DOX-induced cardiomyocyte injury by reducing cellular apoptosis. Nevertheless, their role in necroptosis was not investigated. CaMKIIn, a specific inhibitor of CaMKII, has been found to reduce atrial structural remodeling and to decrease the incidence of AF in an animal model of HF (112). RIPK3-mediated necroptosis in myocardial cells was markedly inhibited by inhibiting CaMKII activation or oxidation using inhibitor-1 of protein phosphatase 1 (I1PP1) (20). Furthermore, I1PP1 can reverse CaMKIIδ alternative splicing, reduce CaMKII activity and attenuate the necroptosis of cardiomyocytes induced by HG, which may provide benefits for the treatment of DCM (20).

Inhibitors targeting mPTP. Increasing evidence indicates that the opening of the mPTP is a part of the final pathway of necroptosis and is related to the death of myocardial cells and myocardial damage caused by necroptosis (49-51). CsA is an mPTP antagonist that acts mainly by inhibiting the activity of CypD, thus preventing mPTP opening, reducing $\Delta\Psi m$ damage and preventing necroptosis caused by mitochondrial damage

and I/R damage (32,48). In an animal study and a small clinical trial (141,142), CsA has been demonstrated to reduce the area of AMI, which is conducive to reducing I/R injury. However, in two large clinical trials, CsA was injected intravenously before percutaneous coronary intervention, and it was found that the clinical outcome and long-term prognosis of the experimental group were similar to those of the placebo group (143,144). Based on the aforementioned research conclusions, the clinical application of CsA has been severely limited. The potential reasons for this phenomenon may include some known confounding factors, such as age, diabetes, interference of clopidogrel and other drugs, and insufficient CsA reaching the mitochondria of the ischemic myocardium (145,146). In this regard, the CsA@PLGA-PEG-SS31 nanoparticles successfully overcome the limitations of CsA by effectively accumulating in ischemic myocardial tissue and accurately targeting the mitochondria of ischemic myocardial cells, thus exerting a protective effect on the ischemic myocardium (146). This method represents a prospective clinical strategy in which CsA can be used to treat I/R injury after AMI in the future. H_2S is a gaseous molecule. As an endogenous antioxidant, it can regulate cardiovascular function and has a potential protective effect on the heart (147). A previous study has demonstrated that H_2S could prevent the opening of the mPTP in cardiomyopathy induced by I/R injury and could prevent apoptosis of cardiomyocytes (147). Previous studies have indicated that exogenous administration of H_2S can inhibit necroptosis, alleviate the dysfunction of cardiomyocytes induced by HG and improve heart damage caused by diabetes, suggesting that the restoration of normal levels of H_2S in the body may be a potential treatment for DCM; however, it is unclear whether H_2S can also prevent the opening of the mPTP in DCM, which needs to be further confirmed (148,149).

5. Conclusions and perspectives

In the last decade, necroptosis mediated by RIPK3 and MLKL has been recognized as the classical necroptosis pathway. The necrosome components RIPK1, RIPK3 and MLKL are key regulators of necroptosis. RIPK3 activates MLKL and promotes MLKL localization to the plasma membrane, ultimately leading to plasma membrane permeabilization and cell death. CaMKII has been found to act as a novel substrate for RIPK3 in mediating necroptosis of cardiomyocytes via the RIPK3-CaMKII-mPTP signaling pathway, which causes ATP depletion by inducing mitochondrial damage, ultimately leading to necroptosis. This adds to the complexity of the necroptosis pathway by linking death receptor-mediated necroptosis with the mitochondrial necrosis pathway.

As aforementioned, RIPK3-CaMKII-mPTP pathway-mediated necroptosis serves an important role in a variety of cardiovascular diseases, including AMI, HF, AAA, DCM, HCM and AF, and cardiotoxicity of antitumor drugs and other chemicals. The expression levels of CaMKII are increased in AS and sunitinib-induced cardiotoxicity. Additionally, the oxidation levels of CaMKII are also increased in sunitinib-induced cardiotoxicity, and cell death has been demonstrated to increase with the oxidation of CaMKII. However, to the best of our knowledge, there are currently no studies evaluating the role of necroptosis components such as RIPK3 in AS and sunitinib-induced cardiotoxicity. Therefore, it remains uncertain whether CaMKII-mediated cell death is dependent on RIPK3. Further studies are needed.

Since RIPK3-CaMKII-mPTP-mediated necroptosis serves a role in the pathogenesis of several cardiovascular diseases, targeting the RIPK3-CaMKII-mPTP pathway is a potential therapeutic approach for several cardiovascular diseases. Several inhibitors of the necroptosis pathway, such as necrostatin-1, KN-93 and CsA, have been developed. However, most studies on these inhibitors are based on *in vitro* experiments or animal models, and their clinical feasibility requires validation *in vivo* and in clinical trials. Future work will focus on conducting clinical studies to verify the clinical application value of these inhibitors, as well as a more precise understanding of the RIPK3-CaMKII-mPTP signaling pathway in the pathogenesis of cardiovascular diseases. In addition, to improve the selectivity and safety of the drugs, the value of necroptosis inhibitors in clinical applications should be carefully investigated.

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Authors' contributions

JZ contributed to the conception, design and drafting of the manuscript. SC, SG, ZY, FO, SL and LL contributed to data collection and manuscript drafting. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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