# Implications of Necroptosis for Cardiovascular Diseases*

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**Summary**: Necroptosis is a non-apoptotic programmed cell death pathway, which causes necrosis-like morphologic changes and triggers inflammation in the surrounding tissues. Accumulating evidence has demonstrated that necroptosis is involved in a number of pathological processes that lead to cardiovascular diseases. However, the exact molecular pathways linking them remain unknown. Herein, this review summarizes the necroptosis-related pathways involved in the development of various cardiovascular diseases, including atherosclerosis, cardiac ischemia-reperfusion injury, cardiac hypertrophy, dilated cardiomyopathy and myocardial infarction, and may shed light on the diagnosis and treatment of these diseases.
**Key words**: cell death; death-associated molecular patterns; necroptosis; cardiovascular disease

Cardiovascular disease is a class of diseases that involve the heart or blood vessels[1]. Cardiovascular disease includes coronary artery diseases, stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis[2].

Cell death plays an important role in the occurrence and development of cardiovascular diseases. It is well known that there are two major types of cell death: apoptosis[3–5] and necrosis[6]. However, in recent years a new cell death pathway called necroptosis has been discovered, which is different from classical necrosis and apoptosis, and has also been believed to be associated with cardiovascular diseases[7]. The effect of necroptosis on cardiovascular diseases was shown to be greater than that of apoptosis and classical necrosis[8]. The role of apoptosis, necrosis, and other cell death pathways in cardiovascular diseases has been extensively studied and summarized. However, necroptosis, as a newly discovered type of cell death, remains to be further explored. In this review we have attempted to answer some specific questions related with the effect of necroptosis on cardiovascular diseases.

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## 1 CELL DEATH

Cells are the basic structural and functional unit of the body. Cell death is a basic process, which is essential for the development and homeostasis of organisms. When cells are severely damaged, various metabolic processes may be paused, cell structure may be ruined, or other irreversible changes may occur that lead to cell death. In general, there are three types of cell death: apoptosis, autophagy, and necrosis[9].

### 1.1 Apoptosis

Apoptosis (type Ⅰ programmed cell death) is a programmed cell death involved in the maintenance of cell homeostasis, which is controlled by several genes and is characterized by an orderly regulated procedure[10]. The morphological changes of apoptosis include cell shrinkage, disappearance of pseudopodia, chromatin condensation, nuclear membrane wrinkling, nucleolus splitting, and finally the formation of apoptotic bodies. It involves the activation of many genes and diverse regulatory mechanisms. Apoptosis can be initiated through one of the two pathways, the extrinsic and the intrinsic pathway. In the extrinsic pathway, apoptosis is triggered by extracellular signals, which then activate cysteinyl aspartate specific proteinases (caspases). The intrinsic pathway is activated in response to internal stimuli, such as the mitochondria, to lead to the activation of caspases. These activated caspases can lead to the degradation of

vital proteins in cells and cause apoptosis[11].

**1.2 Autophagy**

Autophagy (type Ⅱ programmed cell death) is a process of transporting intracellular damaged and degenerated or aging proteins and organelles, including mitochondria, to the autophagosomes, which then carry this cargo to the lysosomes. The autophagosome then fuses with the lysosome to form autolysosomes, where the cargo is either digested or degraded[12]. In terms of normal function, autophagy plays a key role in the maintenance of cell survival, renewal, material recycling, and homeostasis, as autophagy is the primary pathway for recycling macromolecular substances and organelles by self-digestion.

**1.3 Necrosis**

Under unfavorable conditions such as ischemic reperfusion, viral or bacterial infections, and neurodegenerative diseases, cells may acquire morphological characteristics of necrosis. Necrosis can be induced by death ligands, including Fas ligand (FasL), tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand 1 (TRAIL1), and TRAIL2[13]. It can also be initiated by pathogen receptor (PRR) family members, including the Toll-like receptors (TLRs) on plasma membrane and NOD-like receptors in cytoplasm, which are expressed primarily in cells of the immune system and bind to pathogen-associated molecular patterns (PAMPs), such as the nucleotides of viruses and bacteria, lipopolysaccharides, lipoproteins or peptidoglycans, activating inflammation and triggering cell death. Necrosis is passive and uncontrolled, and results in membranous organelle swelling or lysis, increase in cell membrane permeability and a consecutive inflammatory response in the surrounding tissues. However, in recent years, it has been revealed that apart from classic necrosis, there is another regulated non-apoptotic procedural death that induces necrotic morphological changes in cells. In 2005, researchers proposed a new term, necroptosis (programmed necrosis), to define this kind of regulated necrosis.

**1.4 Necroptotic Pathways**

It is known that programmed necrosis can be triggered by TNF death ligand family of proteins, TLRs, *etc*. Here we describe TNF pathways, as an example, to elaborate on necroptosis. When TNF binds with the extracellular death domain of TNF receptor 1 (TNFR1) on the cell membrane, the conformation of the receptor changes to recruit multiple proteins, including TNFR1-associated death domain protein (TRADD), TNF receptor associated factor 2 (TRAF2), TRAF5, *etc*., leading to the formation of supramolecular complexes defined as "TNFR1 complex Ⅰ"[14]. This complex can determine the functional consequences according to the different states of the signal or microenvironment. TRAF1 allows cellular inhibitor of apoptosis proteins (cIAP) to regulate receptor interating protein 1 (RIP1) ubiquitination at K63 by inhibiting its own ubiquitination to stabilize acid phosphatase[15]. TRAF2 also catalyzes RIP1 multi-site ubiquitination in the presence of 1-phosphate-1-lysine[16]. However, the state of RIP1 ubiquitination determines cell fate. When the K63 domain of RIP1 is ubiquitinated, TLR4 antagonist kinase (TAK1) binds to tumor necrosis factor alpha-induced protein (TAB2) and TAB3 to form TAK1-TAB2-TAB3 complex, which further leads to the expression of genes that promote cell survival[17]. But when the deubiquitinating enzymes TNFAIP3 (A20) and CYLD lead to RIP1 deubiquitination in the NF-κB pathway, the complex can also inhibit the formation of TAK1-TAB2-TAB3 complex, which inhibits the expression of growth-promoting genes.

TNFR1 is internalized from cell membrane, while TRADD and RIP1 dissociate from complex Ⅰ and provide binding sites to Fas-related death domain proteins (FADD). Then caspase-8 and receptor interacting serine/threonine kinase 3 (RIPK3) form a new intracellular supramolecular complex, usually called complex Ⅱ, which is known as the death-inducing signaling complex (DISC). Studies have shown that interfering RNA can inhibit the RIP1 deubiquitinating enzyme CYLD, which can significantly inhibit TNF-induced necroptosis by preventing RIP1 deubiquitination[18]. These results suggest that RIP1 ubiquitination is one of the key factors in determining cell fate.

When complex Ⅱ is formed, the cells will either undergo apoptosis or necroptosis. When caspase-8 is activated in complex Ⅱ, RIP1 and RIP3 will be cleaved from it and become inactivated, thereby inducing the caspase-dependent apoptotic pathway. Conversely, if the activity of caspase-8 is inhibited by drug or genetic methods, complex Ⅱ transits the signal of necroptosis. At that time, RIP3 combines with RIP1 into a complex, named necrosome[19, 20]. RIP proteins are a class of serine/threonine kinases, which have a common N-terminal domain and a different C-terminal domain that exerts great influence on the regulation of cell viability. RIP3 has an N-terminal kinase domain, which binds with the C-terminal RIP homotypic interaction motif (RHIM) of RIP1[19]. However, unlike RIP1, RIP3 does not participate in the NF-κB signaling, but it is an indispensable component of the TNFR1-induced necroptosis.

At the same time, it was also found that RIP1 enzyme inhibitors interact with RIP1 and RIP3, suggesting that the activity of RIP enzyme is one of the prerequisites for the formation of the RIP1-RIP3 necrosome[19–22]. Necrosome regulates the activity of RIP1 and RIP3 to promote a death signal, possibly by RIP3-mediated phosphorylation of RIP1 or self-phosphorylation in Serine 161[19, 20]. Mixed lineage

kinase like protein (MLKL) is believed to be a key enzyme downstream of RIP3 in the necroptosis pathway. The knockout of MLKL in HT-29 cells significantly blocks cell necrosis and reduces the production of reactive oxygen species (ROS)[23]. Sun *et al* found that MLKL is a necessary participant in RIP1 phosphorylation that could interact directly with RIP3. They pointed out that tyrosine 357 and serine 358 in the MLKL kinase domain could be phosphorylated by RIP3, leading to the conversion from a monomeric MLKL to a dimeric state. Then, the polymerized MLKL is combined with phosphoinositol and diphosphatidyl glycerol, transferred from cytoplasm to cell membrane or organelle membrane, and then forms permeability transition pore leading to the release of cell contents, so that it breaks down the balance of ion concentration in-and-out of cells, ultimately leading to cell necrosis[24, 25].

Studies have shown that in the process of RIP1/RIP3 complex-induced necrosis, glycogen phosphorylase (PYGL), glutamate-ammonia ligase (GLUL) and glutamate dehydrogenase 1 (GLUD1) are important factors. PYGL can catalyze the decomposition of α (1-4) glyosidic linkages and decompose glycogen into glucose-1-phosphate, which plays an important role in the process of using glycogen. GLUL exists in the cytoplasm, where it catalyzes the formation of glutamine from glutamate and ammonia, which is regarded as a major source of energy. GLUD1, is an enzyme located in the mitochondrial matrix, which catalyzes the conversion of glutamate to α-ketoglutarate and plays a key role in glutamate metabolism. Phosphorylated RIP3 interacting with PYGL, GLUL and GLUD1 leads to enhanced ROS production by the catabolic metabolism of glucose, glutamic acid and glutamine[26]. More interestingly, Zhang *et al* pointed out that there may be a positive feedback in the necroptosis pathway. They found that ROS could subsequently activate RIP1 autophosphorylation on serine 161 through oxidizing three crucial cysteines (C257, C268 and C586) in RIP1, and thereby promote the formation of necrosome and the occurrence of necroptosis. Furthermore, the generation of ROS was dependent on the function of RIP3 in necrosome, so ROS may regulate necroptosis in a positive feedback loop[27] (fig. 1). Finally, we have summarized the possible necroptotic pathway in cardiovascular diseases at different time points, which may reveal a clear sight between them[8, 28–30] (table 1).

## 2 NECROPTOSIS AND CARDIOVASCULAR DISEASES

### 2.1 Necroptosis, Inflammation and Atherosclerosis

Atherosclerosis is a progressive disease characterized by the accumulation of lipid and fibrous elements in the aorta[31]. It is a chronic disease with a long asymptomatic phase, where effective early diagnoses and treatments are lacking, thus people are often diagnosed after they have suffered from secondary acute cardiovascular diseases, such as thrombosis. At present, a close relationship is thought to exist between atherosclerosis and endothelial cell dysfunction[32],

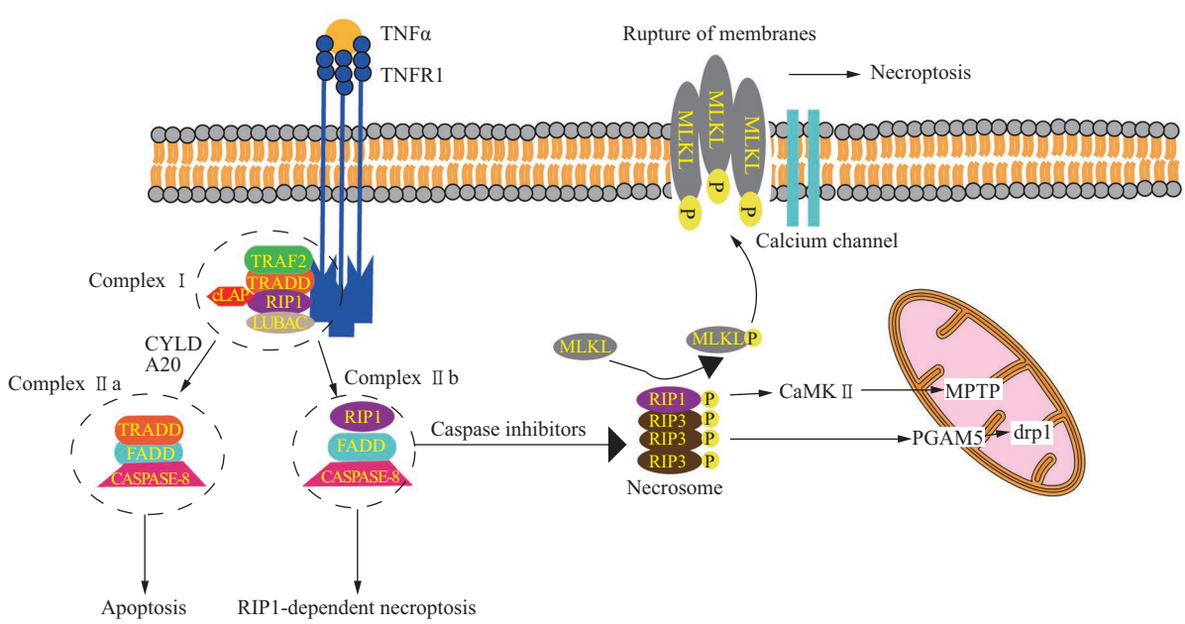


**Fig. 1** The pathway of TNFR1-mediated apoptosis, RIP1-dependent necrosis, and necroptosis
Association of TNFR1 with TNF leads to the formation of complex I consisting of TRAF2, TRADD, RIP1, and cIAP1 at the cell membrane. In the absence of cIAP1 or cFLIP, TRADD, FADD, and caspase-8 form complex IIa to induce apoptosis. When caspase-8 in complex II is activated, RIP1, FADD, and caspase-8 form complex IIb to induce RIP1-dependant necrosis. Conversely, RIP1 interacts with RIP3 to form the necrosome, which is involved in necroptosis. RIP3 and MLKL are phosphorylated and translocated to the plasma membrane, where the complex mediates membrane permeabilization.

**Table 1 The relationship between cardiovascular diseases and necroptosis (important time points)**

| Diseases | RIPK1 | RIPK3 | MLKL | Else pathway | Cell types | Induced factors |
|---|---|---|---|---|---|---|
| Atherosclerosis | Upregulated (2016) | Upregulated (2013) | Upregulated (2016) | | Macrophages | Ox-LDL (2016) |
| MIR | | Upregulated (2016) | Upregulated (2016) | CaMK Ⅱ (2016) | MC | ROS Inflammation (2017) |
| HCM | Upregulated (2014) | Upregulated (2014) | | mTOR (2016) | MC | PA (2014) |
| DCM | Upregulated (2017) | Upregulated (2017) | Upregulated (2017) | | MC | Viral infection (2017) |
| MI | | Upregulated (2014) | | | MC | PEDF (2014) |

MIR: myocardial ischemia-reperfusion disease; HCM: hypertrophic cardiomyopathy; DCM: dilated cardiomyopathy; MI: myocardial infarction; MC: myocardial cells

while inflammation is also considered to play an important role in atherosclerosis[33]. Researchs have gradually shown the fundamental role of inflammation in all stages of atherosclerosis, from the early stages to the formation of a thrombus, which is regarded as a cornerstone in atherosclerosis. Evidence has shown that necroptosis can promote the development of atherosclerosis by inducing inflammation, and this suggests that targeting necroptosis may be used in the diagnosis and treatment of atherosclerosis[34]. Thus, we summarize the molecular mechanisms underlying the necroptosis-induced inflammation which then leads to atherosclerosis, hoping to identify possible targets of necroptosis that are involved in this disease and also provide new ideas for the diagnosis and treatment of atherosclerosis.

Many hypotheses exist regarding the mechanism of atherosclerosis formation among which the most recognized one is the lipid infiltration hypothesis[35]. In order to remove deposited low-density lipoprotein (LDL) accumulated in endothelium matrix, endothelial cells attract the mononuclear cells into the arterial wall, then mononuclear cells change into macrophages and phagocytose LDL deposited in the intima. During this process, macrophages internalize a certain amount of oxidized-LDL (ox-LDL) and form foam cells. If these foam cells are not able to process ox-LDL in time or not recruited by high-density lipoprotein (HDL) to remove fat, rupture of arterial plaque occurs, leading to thrombosis. In this process, the deposition and oxidation of LDL is considered to be the initiating factor of atherosclerosis[33]. Available evidence also suggests that necroptosis may also contribute to the development of inflammation[36]. It has been suggested that ox-LDL can promote the adhesion of monocytes and endothelin secretion (ET), thereby damaging the vascular endothelial cells[37, 38]. In fact, this may also be related to necroptosis. Some studies have suggested that ox-LDL could increase the expression levels of RIPK3 in macrophages and induce phosphorylation of RIPK3 and MLKL, which makes macrophages more fragile and prone to necroptosis[39–41] (fig. 2). Compared to macrophages undergoing apoptosis or autophagy, macrophages undergoing necroptosis will release their contents, lipids, *etc*., which will attract more mononuclear cells and trigger arterial inflammation or atherosclerosis.

Similarly, there is evidence that during development of atherosclerosis, necroptosis is a major type of foam cell death, and these foam cells undergoing necroptosis can induce inflammation and promote the development of atherosclerosis. Recent studies have found that nutrition supply of foam cells was reduced in atheromatous plaque which may result in death of foam cells[42]. The death of foam cells is often related with necroptosis, classical necrosis and apoptosis. However, over half of them are dead because of necroptosis. Lack of nutrition has been linked to the formation of the RIPK1-RIPK3 complex and necroptosis through MLKL[28]. Similarly, compared to the autophagic foam cells or apoptotic foam cells, necroptotic foam cells will release their contents, lipid, *etc*., to attract more monocytes repeatedly, which may promote arterial inflammation and the development of atherosclerosis.

Some researches have shown that lipoprotein E promotes the development of atherosclerosis[43–45]. Recently, researchers discovered that necroptosis plays a major role in the progress of atherosclerosis caused by apolipoprotein E (APOE)[46]. Meng *et al* found that the lifespan of APOE/RIP3 double knockout mice was remarkably extended compared to APOE single knockout mice fed on a high cholesterol diet[46]. Compared to APOE single knockout mice, inflammatory monocytes and lymphocyte antigen 6C were reduced significantly in APOE/RIP3 double knockout mice. The phosphorylation of RIP3, the biomarker of necroptosis, was observed in atherosclerotic plaques of APOE single knockout mice. Also, IL-1α mRNA levels were significantly downregulated in atherosclerotic plaques of RIP3 knockout mice. These findings indicate that necroptotic cells release cytokines during APOE-

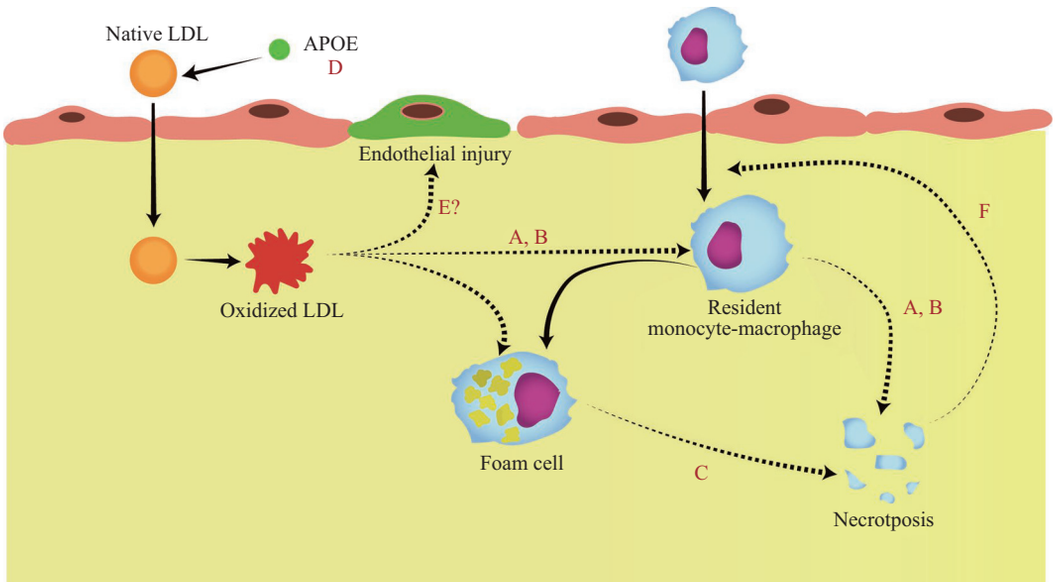


**Fig. 2** Necroptosis, inflammation, and atherosclerosis
A: ox-LDL increases the expression levels of RIPK3. B: ox-LDL induces phosphorylation of RIPK3 and MLKL. C: formation of the complex of RIPK1-RIPK3 necroptosis through MLKL. D: APOE plays an important role in the progress of the necroptosis during atherosclerosis. E: necroptosis participates in the endothelial injury caused by endothelial dysfunction. F: macrophages with necroptosis release the cell contents, and repeatedly attract more mononuclear cells, triggering atherosclerosis.

mediated progression of atherosclerosis, allowing monocytes to accumulate at the atherosclerotic lesion and aggravate the progression of the disease.

Necroptosis is now proved to promote the inflammatory response and the development of atherosclerosis. ox-LDL deposited in the endothelium can upregulate the expression of RIPK3 and ox-LDL-related genes in macrophages to mediate phosphorylation of RIPK3 and MLKL, resulting in the necroptosis of macrophages[41]. Finally, it leads to an inflammatory response and induces atherosclerosis. In process of the disease development, some cytokines are released to make the monocytes assembling in lesion and exacerbate the inflammation accumulating in site. On the other hand, a large number of foam cells die of necroptosis, exacerbating the progress of the disease.

Karunakaran *et al* have suggested that it is desirable to identify high-risk patients with disease-rupture by targeting necroptosis[41]. It has also been shown that 5-aminolevulinic acid-mediated sonodynamic therapy can protect foam cells by activating caspase-8, inhibiting the formation of RIPK1-RIPK3 necrosome and reducing MLKL aggregation, which may reduce necroptosis of foam cells and the risk of atherosclerotic plaque rupture and the occurrence of acute cardiovascular events.

It is also believed that endothelial injury caused by endothelial dysfunction is also a key step in atherosclerosis. Dysfunctional endothelial cells not only lose the ability to secrete nitric oxide (NO), but they also accelerate the production of ROS, and induce inflammation in the vascular system which can lead to rupture of the atherosclerotic plaque and thrombosis[47]. Do necroptotic endotheliocytes influence the development of the disease? The related mechanism of necroptosis in endotheliocytes needs to be further examined.

### 2.2 Necroptosis and Myocardial Ischemia-Reperfusion Disease

For patients with cardiac ischemia, timely unclogging the infarcted arteries and restoring blood supply is the key to maintaining normal heart function. However, it has been found that restoring blood perfusion after myocardial ischemia may damage the heart tissue, leading to myocardial stunning, impaired cardiac function, and malignant arrhythmia, sometimes called cardiac ischemia/reperfusion injury. The existence of ischemic/reperfusion injury poses a greater challenge in treating myocardial ischemic disease[48]. A study found that cardiac ischemia/reperfusion was often related with increased ROS levels[49], calcium overload[50, 51], disorders in myocardial energy metabolism[50], neutrophil infiltration[51], endothelial injury *etc*. Recent studies have found that myocardial ischemia-reperfusion injury may be also associated with necroptosis. A lot of evidence suggests that the relationship between necroptosis, ROS and inflammation is very complex, and can promote the development of this disease. More interestingly, during the development of myocardial ischemia and reperfusion, autophagy can regulate necroptosis in a certain extent. These findings suggest that necroptosis

plays an important role in myocardial ischemia-reperfusion.

After ischemia-reperfusion, the activated endothelium produces more ROS than in the ischemic stage, but the amount of NO produced decreases. The imbalance between the superoxide and NO in endothelial cells induced by inflammatory mediators makes the adhesion of white blood cells and endothelial cells. At the same time, these increased inflammatory mediators can also activate endothelial cells that are not exposed to the initial ischemic injury in distal organs, causing white blood cell-dependent vascular injury, further inflammation and multiple organ dysfunction syndrome (MODS)[52]. Similar to atherosclerotic disease, inflammation also plays an important role in ischemia-reperfusion. There is evidence showing that weakening necroptosis can make the expression of myocarditis inflammatory factors IL-6 and TNF-α decrease after myocardial ischemia-reperfusion to protect cariocytes. Interestingly, RIP3-mediated cardiac inflammation is dependent on the activation of calmodulin-dependent protein kinase II (CaMKII) rather than RIP1. More interestingly, researchers have found that the RIP1-RIP3-MLKL pathway is not necessary, but the protagonist is RIP3-induced activation of CaMKII which leads to mPTP opening. This is undoubtedly a major discovery, which reveals a new target for necroptosis[53].

At the same time, necroptosis and ROS levels have a delicate relationship. Some evidence suggests that production of ROS can cause necroptosis during myocardial hypoxia-reoxygenation injury. ROS produced by mitochondria cause intracellular protein oxidation and lipid damage, leading to necroptosis. Furthermore, necroptosis can also aggravate the production of ROS[27].

More interestingly, it has been suggested that autophagy can affect the occurrence of necroptosis during myocardial ischemia-reperfusion. Lu *et al* have reported that this process is regulated by phosphoglycerate mutase family member 5 (PGAM5). PGAM5 is recognized as the downstream of RIP1/RIP3 in the progress of necroptosis[54]. However, PGAM5 can promote the dependence of phosphatase and tensin homolog deleted on chromosome ten (PTEN) to induce putative kinase 1 (PINK1) on mitochondrial autophagy, so that reducing the production of ROS leads to reduced necroptosis[54]. However, Liu *et al* found that heat shock protein 70 (HSP70) plays an important role in connecting autophagy and necroptosis during ischemia-reperfusion. They have found that HSP70 can effectively inhibit apoptosis and autophagy of cardiomyocytes, and autophagy can regulate necroptosis[55]. Thus, autophagy can affect necroptosis in myocardial ischemia, and either PGAM5 or HSP70 is a promising pharmaceutical target for solving myocardial ischemia-reperfusion problems.

More importantly, there is evidence suggesting that the incidence of myocardial ischemia and reperfusion injury can be reduced by targeting necroptosis. It has been found that miR-223-5p/3p duplex can inhibit necroptosis of myocardial cells induced by ischemia and reperfusion by regulating DR6, TNFR1 death receptor protein signaling, and inflammatory signals of NLRP3. At the same time, miR-223 can reduce the expression of IL-1β and TNF-α and thus reduce inflammation. These effects of miR-223-5p/3p duplex protect myocardial cells from ischemia-reperfusion injury. Dong *et al* have suggested that pre-miR-223 may serve as a prophylactic drug for the treatment of myocardial ischemia-reperfusion[56]. Meanwhile, it has been shown that Traf2 can regulate TNF-induced necroptosis, at least in the acute phase, through an NF-κB-independent mechanism and suggested that this may serve as a novel therapeutic target for heart failure[57]. It has also been found that hypoglycemic agents can protect cardiomyocytes that have been damaged by ischemia-reperfusion, through activation of the AMP-activated protein kinase (AMPK) pathway, but the specific molecular mechanisms are not clear[58]. Some researchers have shown that AMPK can inhibit RIP1/3 phosphorylation-induced necroptosis and TNF-α-induced activation of caspases[59]. This may explain why hypoglycemic agents can protect the cardiomyocytes of ischemia-reperfusion through the AMPK pathway (fig. 3).

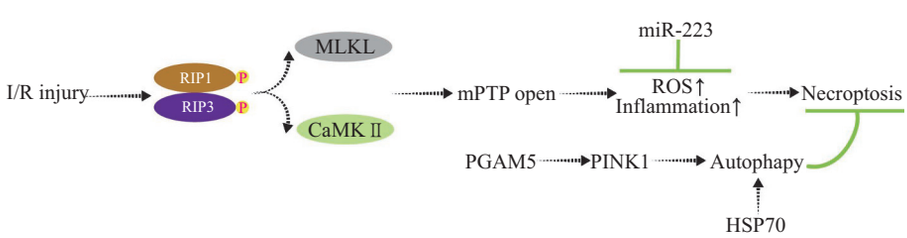


**Fig. 3** Necroptosis and myocardial ischemia-reperfusion disease
Necroptosis plays a role in cardiac ischemia/reperfusion (I/R) injury through the RIP1-RIP3-CaMKII pathway and induces the formation of reactive oxygen species (ROS) and inflammation. PGAM5 and HSP70 may induce RIPK1 and RIPK3, affecting necroptosis in myocardial ischemia. miR-223 may regulate inflammation to inhibit necroptosis of myocardial cells caused by IR injury.

**2.3 Necroptosis and Hypertrophic Cardiomyopathy**

Pathological cardiac hypertrophy is a type of adaptation of myocardial cells to stimulation within the participation of myocardium mesenchyme and fibroblasts, such as hormone disorder, increase of cardiac preload or afterload and abnormal energy metabolism *etc*[60]. Cardiac hypertrophy is associated with decompensated heart failure which leads to patients' death. So, delaying the occurrence and development of cardiac hypertrophy is considered as a key step in the fight against cardiovascular disease. Excessive consumption of saturated fatty acids can lead to heart failure[61] due to lipid toxicity. Lipid overload in non-adipose tissue may also induce hypertrophy and even cell death[62], suggesting that people who eat too much saturated fatty acids have a high risk of cardiac hypertrophy. Zhao *et al* found that necroptosis was closely related to palmitic acid (PA)-mediated cardiac hypertrophy. They found that PA could cause endoplasmic reticulum stress of myocardial cells, which may lead to increased expression of RIPK1 and RIPK3 and the induction of necroptosis of myocardial cells. More interesting, the endoplasmic reticulum stress of cardiomyocytes can be suppressed if RIPK1 is inhibited. Also, mTOR, which was thought to be one of the PA-induced hypertrophic genes, may be the downstream signaling molecule of RIPK1[63] (fig. 4).

**2.4 Necroptosis and Dilated Cardiomyopathy (DCM)**

When the load of myocardia exceeds the capacity of myocardial hypertrophy, ventricular dilatation and DCM result.

DCM is a primary cardiomyopathy with unknown etiology, characterized by left, right or bilateral ventricular enlargement, and ventricular systolic dysfunction, with or without congestive heart failure. Various arrhythmia may occur during its development. The disease may lead to death with high mortality at any stage.

Many scientists have shown that the death of cardiomyocytes in DCM is associated with apoptosis. In biopsies from 7 cases of DCM with left ventricular dysfunction, a typical DNA ladder was detected, which indicated that there may be a relationship between myocardial cell apoptosis and DCM[64]. But, Szobi *et al* found that necroptosis of cardiomyocytes may also occur in patients with DCM, and the proportion of cells undergoing necroptosis may exceed that of apoptosis. They pointed out that the levels of apoptosis-related molecules Bcl-2, BAX and caspase-7 were downregulated, which indicated a reduction in apoptosis in DCM patients. Contrary to this, the cytoplasm and membrane compartments of cardiomyocytes in these patients were reported to have increased levels of RIP3. Increased levels of RIP3, pSer227-RIP3, RIP1 and pSer358-MLKL suggest the presence of necroptosis[8].

Moreover, another study found that DCM patients had a persistent viral infection in the myocardium[65]. Histological examination showed that experimental viral myocarditis presented myocardial fibrosis and eventually developed into DCM. However, the mechanism of virus-induced dilated myocardium has not yet been conclusive. It is thought to be related to direct attack of the virus, as well as autoimmune damage. Interestingly, scientists have found that a variety of viruses can mediate necroptosis, including the Coxsackie B group 3 virus. Whether the virus induces necroptosis and then causes DCM remains to be investigated (fig. 4).

**2.5 Necroptosis and Myocardial Infarction (MI)**

MI is an acute coronary syndrome (ACS), where blood supply to the heart suddenly stops or reduces. Effective and timely treatment is required for MI patients. Nitroglycerin, opioids or aspirin are currently used for MI treatment, but they cannot improve the overall effects. Seeking a more effective treatment strategy is still a major research hotspot.

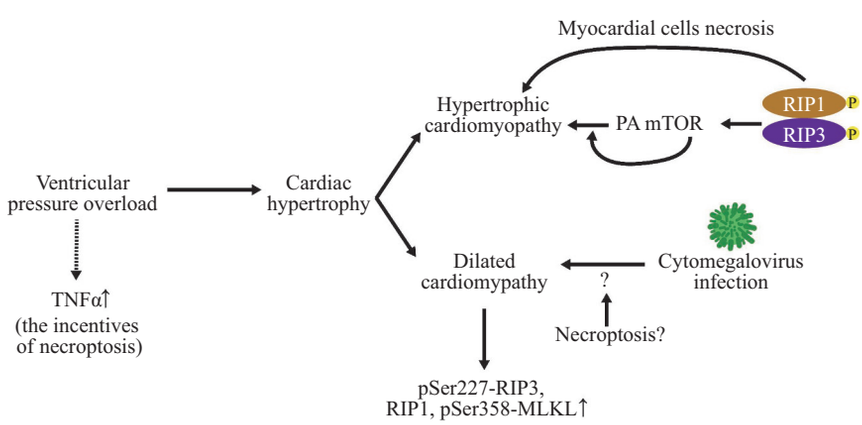


**Fig. 4** Necroptosis in hypertrophic cardiomyopathy and dilated cardiomyopathy

PA-induced hypertrophic cardiomyopathy and viral infection-induced dilated cardiomyopathy are associated with the increases of RIP1, RIP3 and MLKL, and necroptosis of myocardial cells.

Acute MI (AMI) due to reduced oxygen supply, decreases the production of adenosine triphosphate (ATP) from cardiomyocytes, which results in accumulation of ROS and mitochondrial dysfunction followed by a variety of cellular damages and activation of apoptosis and necroptosis[66, 67]. Gao *et al* found that pigment epithelium-derived factor (PEDF) and its functional 44mer peptide were able to reduce the death of cardiomyocytes following AMI by protecting against apoptosis and necroptosis by reducing oxidation. PEDF and its functional 44mer peptide can reduce the amount of ROS, increase the activity of SOD, GPx and CAT, and inhibit the levels of activated-caspase 3 and RIP3. Meanwhile, their study also demonstrated that the individual 44mer and PEDF protein had similar antioxidant functions, and a small chance to cause an immune response. This discovery, no doubt, provides a direction for future treatment of AMI[67].

At present, the adverse effects of myocardial ischemia-reperfusion limit the effectiveness of surgical coronary artery recanalization after MI and of thrombolytic drugs or other treatments. It has been suggested that HSP70 could protect the myocardium from ischemia-reperfusion injury, thereby improving the efficacy of these treatments *via* inhibition of necroptosis. Evidence suggested that after myocardial ischemia/reperfusion *in vivo*, HSP70 inhibited necroptosis and autophagy of cardiomyocytes to protect cardiomyocytes from ischemia-reperfusion injury after myocardial infarction, where autophagy may have a regulatory effect. Necrostatin-1 (Nec-1) is a small molecule based on tryptophan. It has been found that Nec-1 can reduce $H_2O_2$-induced H9C2 cell death. In the presence of caspase inhibitors, Nec-1 may delay the opening of mitochondrial permeability pore, while lowering plasma calcium levels to protect cardiomyocytes. It has been shown that intraventricular injection of Nec-1 can reduce the infarct volume of the middle cerebral artery and protect the heart from ischemia-reperfusion injury. It has also been found that Nec-1 was protective at 30 μmol/L but it increased infarct size at 100 μmol/L. This suggests that Nec-1 can also produce non-specific toxic effects at higher concentration[68].

In addition, there is evidence showing that recombinant adenovirus human growth factor (Ad-HGF) can improve the cardiac remodeling after myocardial infarction in mice by upregulating autophagy and necroptosis and inhibiting apoptosis. Liu *et al* found that Ad-HGF could induce Beclin-1 from Beclin-1/Bcl-2 complex, activate Beclin-1 to form the Beclin1-Vps34-Atg14L complex to promote autophagy. At the same time, Ad-HGF also inhibited apoptosis by inducing the chelation of Bcl-2 and Bax thus inactivating Bax and inhibiting apoptosis. Furthermore, Ad-HGF resulted in a significant reduction in caspase-8 activity, which resulted in necroptosis of cardiomyocytes, thereby reducing cardiac remodeling after MI[69] (fig. 5).

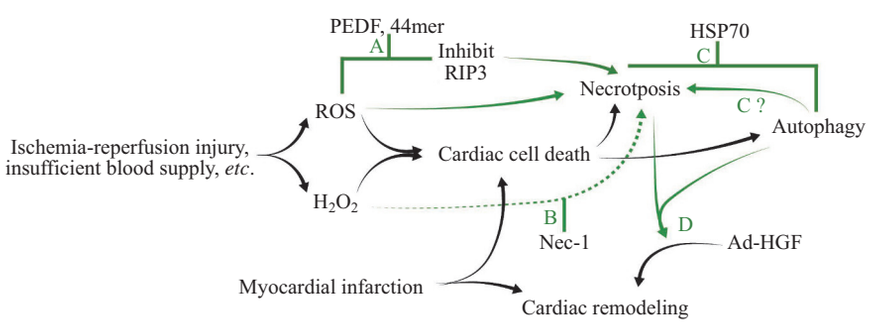


**Fig. 5** Necroptosis in myocardial infarction

A: Pigment epithelium-derived factor (PEDF) and 44mer reduce the necroptosis of cardiomyocytes. B: Nec-1 reduces $H_2O_2$-induced H9C2 cell death. C: HSP70 inhibits the necroptosis and autophagy of cardiomyocytes. D: Recombinant adenovirus (Ad-human growth factor, Ad-HGF) upregulates autophagy and necroptosis and inhibites apoptosis.

## 3 CONCLUSIONS

This article summarizes recent studies on necroptosis related to cardiovascular disease. RIP1, RIP3 and MLKL have been considered as important molecules in necroptosis pathway and have been found to be involved in many cardiovascular diseases. Meanwhile, the progress of cardiovascular diseases can be inhibited by using an inhibitor of necroptosis or inducing other ways of cell death. RIP1-CaMKII-mPTP pathway, a pathway inducing necroptosis, has been found to affect the development of ischemia-reperfusion injury.

**Conflict of Interest Statement**

The authors declare no potential conflicts of interest.

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