PANoptosis: Novel insight into regulated cell death and its potential role in cardiovascular diseases (Review)

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Abstract. PANoptosis, a complex form of proinflammatory programmed cell death, including apoptosis, pyroptosis and necroptosis, has been an emerging concept in recent years that has been widely reported in cancer, infectious diseases and neurological disorders. Cardiovascular diseases (CVDs) are an important global health problem, posing a serious threat to individuals' lives. An increasing body of research shows that inflammation has a pivotal role in CVDs, which provides an important theoretical basis for PANoptosis to promote the progression of CVDs. To date, only sporadic studies on PANoptosis in CVDs have been reported and its role in the field of CVDs has not been fully explored. Elucidating the various modes of cardiomyocyte death, the specific molecular mechanisms and the links among the various modes of death under various stressful stimuli is of notable clinical significance for a deeper understanding of the pathophysiology of CVDs. The present review summarizes the molecular mechanisms of apoptosis, pyroptosis, necroptosis and PANoptosis and their prospects in the field of CVDs.

Contents

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1. Introduction

PANoptosis is a newly defined programmed cell death (PCD) involving apoptosis, pyroptosis and necroptosis, presenting an innovative, integrated view of cellular death mechanisms. This paradigm underscores the intricate manner in which cells, at a molecular level, orchestrate diverse signaling pathways to elicit a comprehensive cell death response. PANoptosis has a pivotal role in modulating immune responses and inflammatory processes, and in determining cell fate (1). For instance, it was recently shown that PANoptosis can integrate disparate cell death pathways through the interplay of molecular mechanisms, such as absent in melanoma 2, Pyrin and Z-DNA binding protein 1 (ZBP1). This insight offers a novel perspective in comprehending host defense mechanisms (2). Furthermore, the involvement of PANoptosis in viral infections, particularly in cases of influenza and herpes simplex virus type 1, has attracted considerable scholarly interest, and its significant function in the mechanisms of viral infection is coming to light (3). The occurrence of PANoptosis in ischemic brain injuries highlights it as a potential therapeutic target for managing central nervous system disorders (4). The discernible patterns of PANoptosis have demonstrated their potential in predicting the survival rates and responses to immune therapies in patients with gastric cancer, thereby underscoring its profound impact on the progression of this disease (5). The role of PANoptosis in regulating the tumor microenvironment and advancing cancer immunotherapy has been particularly highlighted, providing novel avenues for cancer treatment research (6). Collectively, these findings not only enhance our comprehension of the multifaceted nature of PANoptosis but also pave the way for innovative approaches to treating and preventing diseases.

Cardiovascular diseases (CVDs) represent a significant global health concern, encompassing a spectrum of conditions such as coronary artery disease, heart failure (HF) and

hypertension. Atherosclerosis (AS), myocardial infarction (MI), as well as myocardial ischemia-reperfusion, are often accompanied by cell death and acute/chronic inflammatory reactions. An increasing number of studies have demonstrated that apoptosis, necroptosis or pyroptosis participate in CVD progression and may thus have promising therapeutic implications (7-9). However, only one study has linked PANoptosis to CVDs, to the best of our knowledge; Bi *et al* (10) found that FUN14 domain containing 1 (FUNDC1), a mitophagy receptor, protects against doxorubicin-induced cardiomyocyte PANoptosis by stabilizing mitochondrial DNA via interaction with Tu translation elongation factor mitochondrial (TUFM). The present study revealed that it is of notable significance to further study the role of PANoptosis in CVDs. Little is understood regarding the function of PANoptosis in CVDs. Therefore, there remains considerable room for research on the specific mechanism of action of PANoptosis in CVDs, and thus, PANoptosis may have a significant impact in this field.

In the present review, the role of PANoptosis in CVDs is explored, focusing on its potential as a novel therapeutic target. The definition and molecular mechanisms of apoptosis, pyroptosis, necroptosis and PANoptosis are outlined, emphasizing their pathophysiological impact on CVD progression. In addition, the challenges and future directions in this emerging field are discussed, aiming to provide a concise yet comprehensive overview of the current body of knowledge and potential advancements in CVD treatment.

2. Programmed cell death

Cell death, a meticulously regulated process, is strongly influenced by the cellular environment and numerous stimuli. PCD is executed through a suite of genetically encoded mechanisms. PCD includes apoptosis, necroptosis and pyroptosis, and it is an irreversible phenomenon (11). Broadly speaking, PCD pathways are classified into lytic and non-lytic categories. The lytic forms, such as pyroptosis, necroptosis and PANoptosis, are characterized as 'violent' cell death types (12,13), as they are accompanied by a violent inflammatory response. They entail the loss of membrane potential and cellular swelling, leading to disruption of cellular integrity and the consequent release of potent inflammatory inducers. Conversely, non-lytic forms of PCD, characterized by apoptosis, involve the systematic disintegration of dying cells into smaller entities, termed apoptotic bodies. This process ensures the containment of cellular contents, thereby averting inflammatory responses (14). It is particularly noteworthy that PANoptosis, a relatively newly discovered form of cell death, uniquely combines aspects of various PCD pathways including apoptosis, necroptosis and pyroptosis, and is thus a unique, comprehensive form of cell death. Its distinctiveness lies in the interplay of multiple signaling pathways and molecular mechanisms, activated under a range of conditions, leading to cell death. This versatile cell death approach enhances cellular adaptability to stress and injury. Research into PANoptosis is vital for understanding cellular responses to death signals in inflammatory and infectious diseases and cancer. Thus, studying PANoptosis may improve our understanding of cell death and unveil novel therapeutic targets for the management of these diseases. The present review examines the intricate dynamics between various forms of cell death, apoptosis, pyroptosis and necroptosis, and their consequential roles in CVDs. The molecular mechanisms driving PANoptosis are methodically outlined, emphasizing crucial components such as ZBP1, receptor-interacting protein kinase 1 (RIPK1), RIPK3, mixed lineage kinase domain-like pseudokinase (MLKL), TNF and toll-like receptor (TLR) (Table I).

Apoptosis, the prototypical PCD pathway, can be initiated via intrinsic and extrinsic mechanisms. When a mitochondrion is compromised, the mitochondrial outer membrane exhibits increased permeability, facilitating the release of various molecules, including cytochrome C. Cytoplasmic cytochrome C is sensed by the apoptotic protease activating factor-1, leading to the formation of the apoptosome with initiator caspase-9. This mature caspase-9 subsequently activates the downstream effector caspase-3. In addition, endoplasmic reticulum (ER) stress induces endogenous apoptosis by activating caspase-12 and caspase-3 in turn by releasing Ca^{2+} in the ER cavity. Extrinsic apoptosis is induced by death receptors such as Fas and TNF-α receptors, with pro-apoptotic molecules such as Fas-associated protein with death domain and caspase-8 identified downstream. Consequently, caspase-9 and caspase-8 are initiator caspases for the intrinsic and extrinsic apoptosis pathways, respectively. These pathways converge on activating the same effector enzymes, caspase-3 and caspase-7, culminating in apoptosis (11) (Fig. 1).

Pyroptosis, a caspase-1-dependent form of PCD, is activated by the Nod-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome, comprising a sensor protein, the adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) and caspase-1. Caspase-1 activation cleaves its substrates, inducing the maturation of inflammatory cytokines pro-IL-1β and pro-IL-18. The understanding of pyroptosis has evolved with the discovery of various inflammasomes that activate different inflammatory caspases (caspase-1, -4, -5 and -11) (15). Recent studies have broadened this understanding by identifying additional mechanisms that lead to pyroptosis. These include the non-canonical inflammasome pathway, which involves caspase-1-independent cleavage of the protein gasdermin D (GSDMD), as well as the cleavage of GSDMD by caspase-8. In addition, the cleavage of GSDMA by Streptococcal pyrogenic exotoxin B (SpeB) protease, the cleavage of GSDMB by caspase-3/6/7 or Granzyme A, the cleavage of GSDMC by caspase-8 and the cleavage of GSDME by caspase-3, have been recognized, expanding the definition of pyroptosis beyond the traditional caspase-1-dependent pathway (16-18) (Fig. 2).

Necroptosis, an adjustable form of cell necrosis that operates independently of caspases (non-caspase-dependent), shares several characteristics with both apoptosis and necrosis. This process is predominantly characterized by notable cell swelling and the compromise of cellular membrane integrity, which may precipitate an inflammatory response (19). Necroptosis is initiated by death receptors and is triggered by ligands attached to these receptors. Crucial molecules in the necroptosis pathway, particularly those participating in the tumor necrosis factor (TNF)-α/TNF receptor-induced pathway, include RIP1, RIP3 and MLKL. This RIP1-RIP3-MLKL cascade is recognized as the classical pathway of necroptosis. Various drugs and compounds effectively inhibit this

Table I. Cell death pathways: From programmed cell death to PANoptosis.

Disease	Death mode	Target/intervention	Mechanism	(Refs.)
Atherosclerosis	Apoptosis	Ox-LDL	Activates the Fas/Fasl pathway	(26)
		Hyperglycemia	Triggers inflammatory cytokines and initiates a caspase cascade through NF-κB pathway activation	(27)
		Paeonol	The inhibitory effect of Paeonol on apoptosis in VEC induced by high glucose/high pressure is mediated through the regulation of the SIRT1/FOXO3a/NF-κB pathway	(28)
		Geniposide + Notoginsenoside R1	Activates AMPK/mTOR/Nrf2 signaling pathway, inhibiting the NLRP3 inflammasome and Bax/Bcl2/caspase-3 pathway	(29)
		M-CSF	CSF1 deficiency reduces macrophage proliferation and promotes macrophage apoptosis (activated caspase-3)	(31)
	Pyroptosis	Nicotine	Elevates caspase-1 expression	(52)
		TMAO	Activates NF-κB and MAPK signaling pathways, worsening endothelial dysfunction and promoting AS plaque accumulation	(53)
		High-fat diet + TMAO	Increases caspase-1 and NLRP3 expression, accelerating AS progression	(54)
		HDAC6	Key role of ROS/NF-κB/NLRP3 axis and mitochondria in macrophage pyroptosis	(56,57)
		Eatp	Caspase-11/GSDMD pyroptosis pathway	(58)
		Irisin	Inhibition of NLRP3-mediated pyroptotic cell death	(60)
		Caspase-1 inhibitor VX-765	Significantly reduces ox-LDL-induced endothelial cell pyroptosis	(61)
	Necroptosis	RIP3	RIP3 signaling pathway	(77,78)
		MLKL	MLKL directly contributes to lesion development and necrotic core formation	(82)
		Oxidative stress	Exacerbating oxidative stress, activates HIF-1α	(83)
Myocardial infarction	Apoptosis	miR-182-5p	Suppressed Bcl-2 expression, and increased Bax, Bnip3, and caspase-3/7 activity levels	(32)
		IGF-1	AKT/SFRP2/β-catenin pathway	(33)
		miR-338	Inhibits cardiomyocyte apoptosis in MI through the MAP3K2/JNK signaling pathway	(34)
		MEG3 (lncRNA)	Promotes apoptosis (the expression of Caspase-3 and Bcl-2)	(35)
		miR-146b	Mediates vascular inflammation and apoptosis in MI, potentially through the PI3K/Akt/NF-κB pathway	(36)
		Gm18840	Gm18840 drives cardiomyocyte apoptosis	(37)
	Pyroptosis	Calpain	NLRP3/ASC/caspase-1 axis	(62)
		SIRT1	Targeting oxidative stress and NLRP3-mediated cell pyroptosis	(63)
		Caspase-1	Promotes pyroptosis	(64,65)
	Necroptosis	RIP3	Via the RIP1-RIP3-MLKL and RIP3-camkii pathways	(84,85)
		TAK1	Traf2 critically regulates RIP1, RIP3 and MLKL necroptotic signaling with TNFR1-associated death domain protein as an upstream regulator and TAK1 as a downstream effector	(86)
		TRAF2	RIP1-mediated necrosis	(87)
		S-allyl cysteine sulfoxide	Inhibits the expression of RIP1, RIP3 and TRAF2	(89)

Table I. Continued.

Disease	Death mode	Target/intervention	Mechanism	(Refs.)
		miR-325-3p	RIPK1/RIPK3/p-MLKL	(88)
		Ad-HGF	Ad-HGF significantly decreases the caspase 8 protein and activity levels, which urges the cell to undergo necroptosis under hypoxia and block of apoptosis	(90)
Heart failure	Apoptosis	miR-182	miR-182 inhibits cardiomyocyte apoptosis induced by non-ischemic HF via downregulating PDCD4 and PACS2	(38)
		BYD	Ameliorates myocardial apoptosis via the P38 MAPK-CRYAB pathway	(39)
		Shenfu	Shenfu formula can regulate the initiative factors Fas/Fas-L in the intrinsic pathway and Bcl-2/Bax in the extrinsic apoptosis pathway to suppress apoptosis	(40)
	Pyroptosis	NLRP3	Promotes collagen synthesis and activates caspase-1, leading to IL-1β and IL-18 release, inducing pyroptosis	(66,67)
		Caspase-1	Regulates Ang II-induced cardiac hypertrophy through IL-1β regulation	(68)
		BMP-7	BMP-7 attenuates pyroptosis (caspase-1, IL-1β, IL-18 and gasdermin-D)	(69)
	Necroptosis	Hsp90	Hsp90 inhibitors may limit RIP1-RIP3-MLKL pathway activation	(93)
		CML	CML activates RIP3 through its receptor RAGE	(94)
		RIP3	RIP3-camkii	(95)
		Combined use of Necrostatin-1 (necroptosis inhibitor) and Z-VAD	RIP1-mediated necrosis	(96)
Pulmonary hypertension	Apoptosis	MFF	Drp1 activation	(41)
		Notch1 signaling pathway	Inhibits apoptosis via Bcl-2 and survivin	(42)
		PGE1	HIF pathway	(43)
		SOX2-OT (lncRNA)	Modulates the miR-455-3p/SUMO1 axis	(44)
		miR-30d-5p	Regulates PASMC toxicity and apoptosis, potentially through the Notch-3 signaling pathway	(45)
		Dec1-PPARγ axis	Changes in the Bax/Bcl-2 ratio and cleaved caspase 3 expression	(46)
		BMPR2	Loss of functional BMPR2 signaling leads to increased PAEC apoptosis and cell proliferation through augmentation of TGF-β responses	(47)
		KCNA5	Decreased apoptosis (Annexin V-PI)	(48)
		YM155	Suppresses PASMC proliferation and promotes PASMC apoptosis by inhibiting survivin expression	(49)
		Qiliqiangxin	Attenuates RV myocardial apoptosis (Caspase-3, Bcl-2, BAX)	(50)
	Pyroptosis	GLI1	Orchestrates PASMC pyroptosis by inducing ASC expression, targeting the ASC promoter region	(70)
		STAT1/PD-L1	Induces caspase-1 expression	(72)

Table I. Continued.

Disease	Death mode	Target/intervention	Mechanism	(Refs.)
		Caspase-1	Catalyzes PASMC proliferation via the caspase-1/IL-18/IL-6/STAT3 pathway	(73)
		NLRC3	Downregulates the production of pro-inflammatory cytokines and induces pyroptosis by inhibiting the inflammasome	(74)
		miR-155	Promotes inflammation and induces PH through the c-Fos/NLRP3/caspase-1 pathway	(75)
		Tannic acid	Ameliorates MCT-induced PH through antioxidative properties by inhibiting the NLRP3 inflammasome signaling pathway	(76)
	Necroptosis	TLR & NLR pathways	Activation of TLR and NLR pathways is associated with the upregulation of DAMPs. RIPK3- mediated necroptosis promotes DAMPs generation in MCT-induced PH	(97)
		RIP3	Increased pthr231/Ser232-RIP3 levels leading to various necrosis-like cell deaths may contribute to PH pathomechanisms	(98)
Influenza virus	PANoptosis	ZBP1	ZBP1 recruits RIPK3 and caspase-8 to activate the ZBP1-NLRP3 inflammasome	(102)
COVID-19	PANoptosis	ZBP1	ZBP1 induced during coronavirus infection limits the efficacy of IFN therapy by driving inflammatory cell death and lethality	(103)
Microbial infections, cancers, ALI/ARDS, ischemia-reperfusion and organic failure	PANoptosis	ZBP1	These PANoptosomes further induce caspase-3/7 activation, GSDMD and GSDME cleavage, and MLKL phosphorylation, resulting in membrane pore formation and PANoptosis progression	(105-106)
Ischemic stroke	PANoptosis	RIPK1	RIPK1/RIPK3/MLKL pathway	(107)
Psoriatic Inflammation	PANoptosis	RIPK1	RIPK1/RIPK3/MLKL pathway	(108)
Cerebral ischemic injury	PANoptosis	MLKL	RIPK1/RIPK3/MLKL pathway	(114)
Depression	PANoptosis	MLKL	RIPK1/RIPK3/MLKL pathway	(115)
COVID-19	PANoptosis	TNF signaling pathway	The JAK/STAT1/IRF1 axis activated by TNF-α and IFN-γ co-treatment induced iNOS for the production of nitric oxide. Pharmacological inhibition and genetic deletion of this pathway inhibited pyroptosis, apoptosis and necroptosis in macrophages	(116)
The innate immune system	PANoptosis	TLR signaling pathway	The TLR signaling pathway may indirectly modulate cell death mechanisms associated with PANoptosis by activating NF-κB	(118)
Neurodegenerative diseases	PANoptosis	TLR signaling pathway	Although existing studies did not establish a direct link between the TLR signaling pathway and PANoptosis, they underscore the latent role of this pathway in cellular death and inflammation	(119, 120)
Inflammation and ischemic stroke	PANoptosis	Intracellular stress pathways	ER stress-autophagy axis	(121, 122)
Myocardial injury	PANoptosis	Mitochondrial signaling pathways	FUNDC1 stabilizes mitochondrial DNA by binding to TUFM, thereby protecting cardiomyocytes from DOX-induced PANoptosis	(10)

Table I. Continued.

Disease	Death mode	Target/intervention	Mechanism	(Refs.)
Pancreatic ductal adenocarcinoma	PANoptosis	Mitochondrial signaling pathways	SEP may enter cancer cells effectively, then damage nuclear DNA, boost mitochondrial superoxide anion radicals and affect various signaling pathways related to redox homeostasis and tumor metabolism	(124)
Sepsis and HLH	PANoptosis	Mitochondrial signaling pathways	PANoptotic stimulation induces RET and ROS in mitochondria, while 1-methoxy PMS and dimethyl fumarate can inhibit PANoptosis by suppressing RET-mediated oxidation of mitochondrial DNA	(125)
Ischemic stroke and CI/RI	PANoptosis	Mitochondrial signaling pathways	Exposure to stimuli during CI/RI can lead to the initiation of the apical sensors, such as ZBP1, which then induces the activation of proteins involved in pyroptosis, apoptosis and necroptosis to form the ZBP1-PANoptosome and mediate PANoptosis	(126)

ox-LDL, oxidized low-density lipoprotein; AMPK, adenosine 5'-monophosphate activated protein kinase; mTOR, mechanistic target of rapamycin; Nrf2, nuclear factor erythroid 2-related factor 2; HF, heart failure; AS, atherosclerosis; MI, myocardial infarction; CSF1, colony-stimulating factor 1; M-CSF, macrophage colony-stimulating factor; TMAO, trimethylamine N-oxide; HDAC6, histone deacetylase 6; eATP, extracellular adenosine triphosphate; RIP3, receptor-interacting serine-threonine kinase 3; ZBP1, Z-DNA binding protein 1; RIPK1, receptor-interacting protein kinase 1; MLKL, mixed lineage kinase domain-like pseudokinase; HIF, hypoxia-inducible factor; BNIP3, Bcl-2 19-kDa interacting protein 3; IGF-1, insulin-like growth factor-1; SFRP2, secreted frizzled-related protein 2; SIRT1, silent information regulator sirtuin 1; MEG3, maternally expressed gene 3; ER, endoplasmic reticulum; FUNDC1, FUN14 domain containing 1; DOX, doxorubicin; RET, reverse electron transport; PMS, phenazinium methyl sulphate; TAK1, transforming growth factor β-activated kinase 1; TRAF2, tumor necrosis factor receptor-associated factor 2; HSP90, heat shock protein 90; CML, chronic myeloid leukemia; Mff, mitochondrial fission factor; DRP1, dynamin-related protein 1; PGE1, prostaglandin E1; SOX2-OT, SOX2 overlapping transcript; Dec1-PPARγ, differentiated embryo chondrocyte expressed gene 1-peroxisome proliferative-activated receptor-γ; BMPR2, bone morphogenetic protein receptor 2; KCNA5, potassium voltage-gated channel subfamily A member 5; GLI1, glioma-associated oncogene homologue 1; NLRC3, nucleotide-oligomerization domain-like receptor subfamily C3; BMP, bone morphogenetic protein; CI/RI, cerebral ischemia/reperfusion injury; HLH, hemophagocytic lymphohistiocytosis; SFRP2, secreted frizzled-related protein 2; miR, microRNA; lncRNA, long non-coding RNA; ROS, reactive oxygen species.

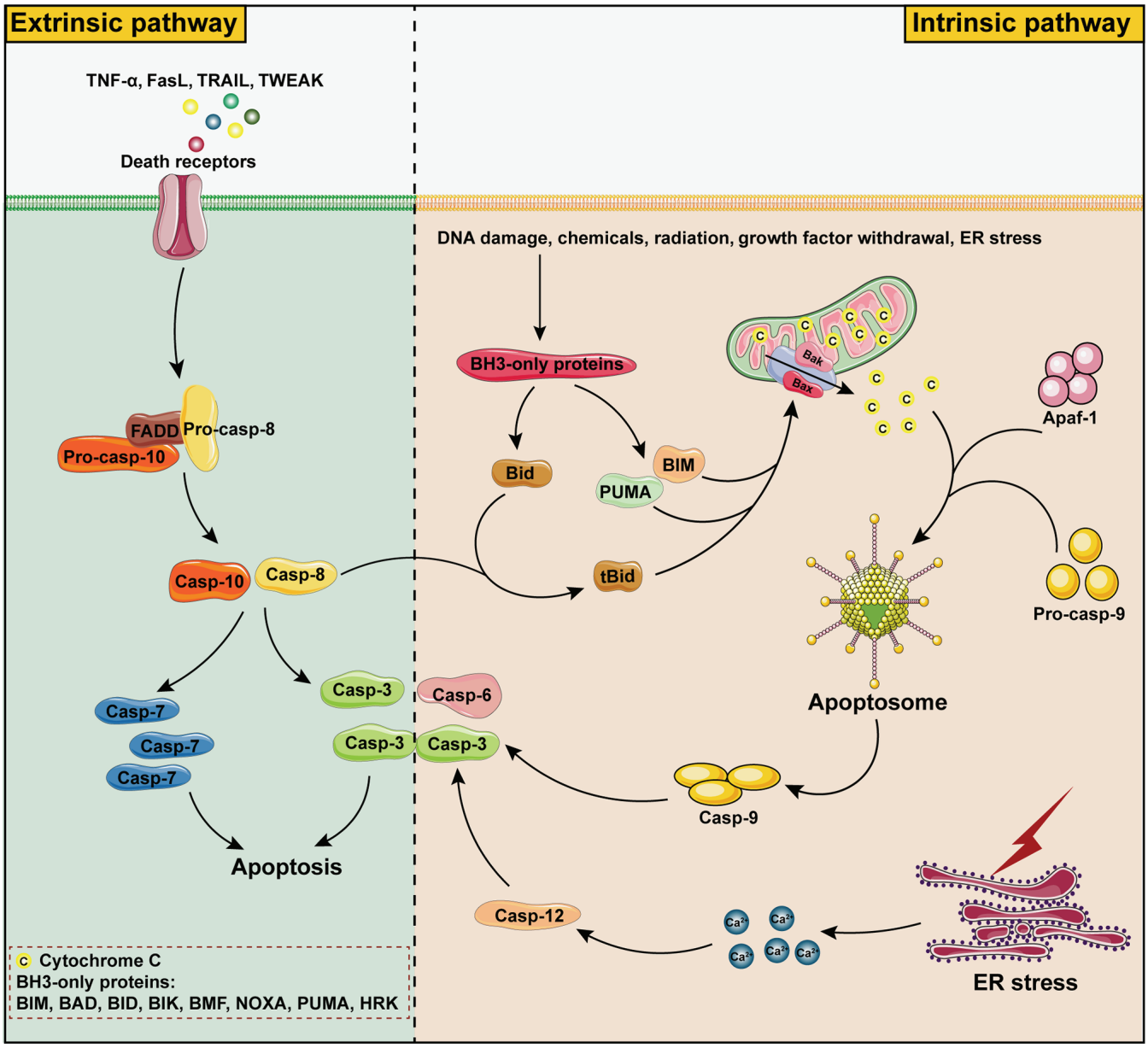

Figure 1. Mechanisms of apoptosis. Apoptosis can be caused by endogenous and exogenous factors. Exogenous apoptosis is mainly triggered by death receptors and then FADD binds to pro-caspase-8 or pro-caspase-10 and induces apoptosis by activating caspase-8/10 and then caspase-3/7 sequentially. Endogenous apoptosis is divided into the mitochondrial pathway and the ER pathway, which is mainly caused by activation of pro-apoptotic proteins BH3-only proteins by factors such as DNA damage, chemical stimuli, radiation, growth factor withdrawal and ER stress, etc. BH3-only proteins mainly include BAD, BID, BIK, BIM, BMF, HRK, NOXA and PUMA, of which BIM, PUMA and tBID are potent apoptosis initiators, which form channels in mitochondria by shearing BAX and BAK proteins. Cytochrome C in mitochondria is released into the cytoplasm through BAX/BAK channels and binds to APAF1 and pro-caspase-9 to form the apoptosome, which in turn causes cascade activation of caspase-9 and caspase-3/6 to induce apoptosis. In addition, apoptosis can also be induced by promoting Ca^{2+} release from the ER after ER stress, which leads to caspase-12-dependent activation of caspase-3. BH3, BCL-2 homologous region 3; BAD, BCL-2 associated agonist of cell death; BID, BH3-interacting domain death agonist; BIK, BCL-2 interacting killer; BIM, BCL-2 interacting mediator of cell death; BMF, BCL-2 modifying factor; HRK, Harakiri; NOXA, phorbol-12-myristate-13-acetate induced protein 1; PUMA, p53 upregulated modulator of apoptosis; APAF1, apoptotic peptidase activator 1; ER, endoplasmic reticulum; Casp, caspase; TNF, tumor necrosis factor; TLR, Toll-like receptor; FADD, Fas-associated death domain; FASL, factor-related apoptosis ligand; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TWEAK, tumor necrosis factor-like weak inducer of apoptosis.

programmed necrosis by targeting key necroptotic molecules such as RIP1, RIP3 and MLKL (20). Elucidating the molecular mechanisms underlying cardiomyocyte necroptosis and its implications in the pathophysiology of CVDs holds substantial clinical significance. In-depth research into necroptosis may reveal novel interventional strategies and therapeutic targets, thereby potentially lowering CVD-associated mortality rates and improving the prognosis of patients (Fig. 3).

PCD is integral to organismal development, maintaining homeostasis, and a critical component of innate immune responses. However, over-activation of PCD pathways can be deleterious, contributing to acute injuries and chronic degenerative diseases (19). Its involvement is noted in conditions such as acute spinal cord injuries (21) and chronic degenerative diseases such as AS and vascular calcification (22). Despite the diversity of PCD types, the CVD-associated signaling

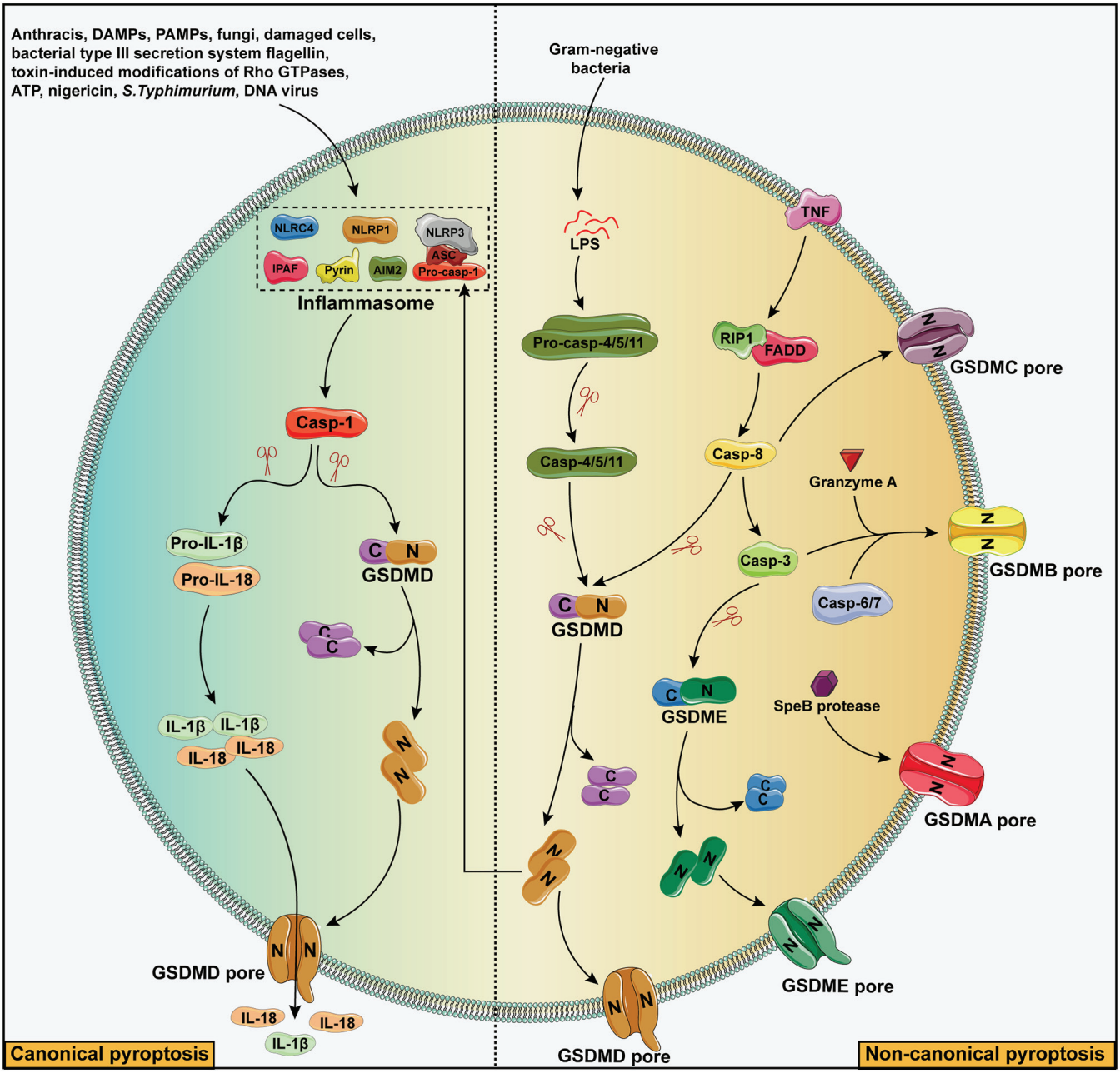

Figure 2. Mechanisms of pyroptosis. Depending on the type of stressor, pyroptosis can be induced by canonical or non-canonical pathways. Stressors that activate the classical pathway include Anthracis, PAMPs, DAMPs, fungi, damaged cells, bacterial type III secretion system flagellin, toxin-induced modifications of Rho GTPases, ATP, nigericin, *S. Typhimurium*, double-stranded DNA viruses, etc., and the stressors that activate the non-canonical pathway are mainly gram-negative bacteria. Inflammasomes that activate the canonical pathway mainly include six types, namely NLRP1-inflammasome, NLRP3-inflammasome, NLRC4-inflammasome, IPAF-inflammasome, AIM2-inflammasome and pyrin-inflammasome. After the assembly of the inflammasome, caspase-1 can be activated. The activated caspase-1 can shear the GSDMD to obtain N-terminal GSDMD with active domain peptide on the one hand, inducing cell membrane perforation, then cell swelling and rupture, and releasing the contents to cause inflammation. On the other hand, activated caspase-1 cuts the precursors of IL-1β and IL-18 to form active IL-1β and IL-18, which are released outside the cell to recruit inflammatory cells and further amplify the inflammatory response. For the non-canonical pathway of cellular pyroptosis, signals such as gram-negative bacteria, granzyme A and SpeB protease induce cell membrane perforation and ultimately cellular pyroptosis by activating caspase-3/4/5/6/7/8/11 through the formation of active N-terminal peptides such as GSDMA, GSDMB, GSDMC, GSDMD and GSDME. Casp, caspase; PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; AIM2, absent in melanoma 2; NLRP1, nucleotide-binding domain-containing, leucine-rich repeat-containing and pyrin domain-containing protein 1; IPAF, ICE-protease activating factor; ASC, adapter protein apoptosis-associated speck-like protein containing a caspase recruitment domain; NLRC4, NLR-family CARD-containing protein 4; GSDM, gasdermin; LPS, lipopolysaccharide; RIP1, receptor-interacting serine/threonine protein kinase 1; pro-IL-1β, pro-interleukin-1β; pro-IL-18, pro-interleukin-18; ATP, adenosine triphosphate.

pathways function as a coordinated system with highly interconnected pathways capable of mutual compensation (23). Apoptosis, pyroptosis and necroptosis represent distinct PCD pathways, intricately interwoven in determining the cell fate and governing physiological processes, thus establishing a dynamic equilibrium system. These three pathways are activated in various cell death mechanisms in response to various external and internal cues. Apoptosis is the primary

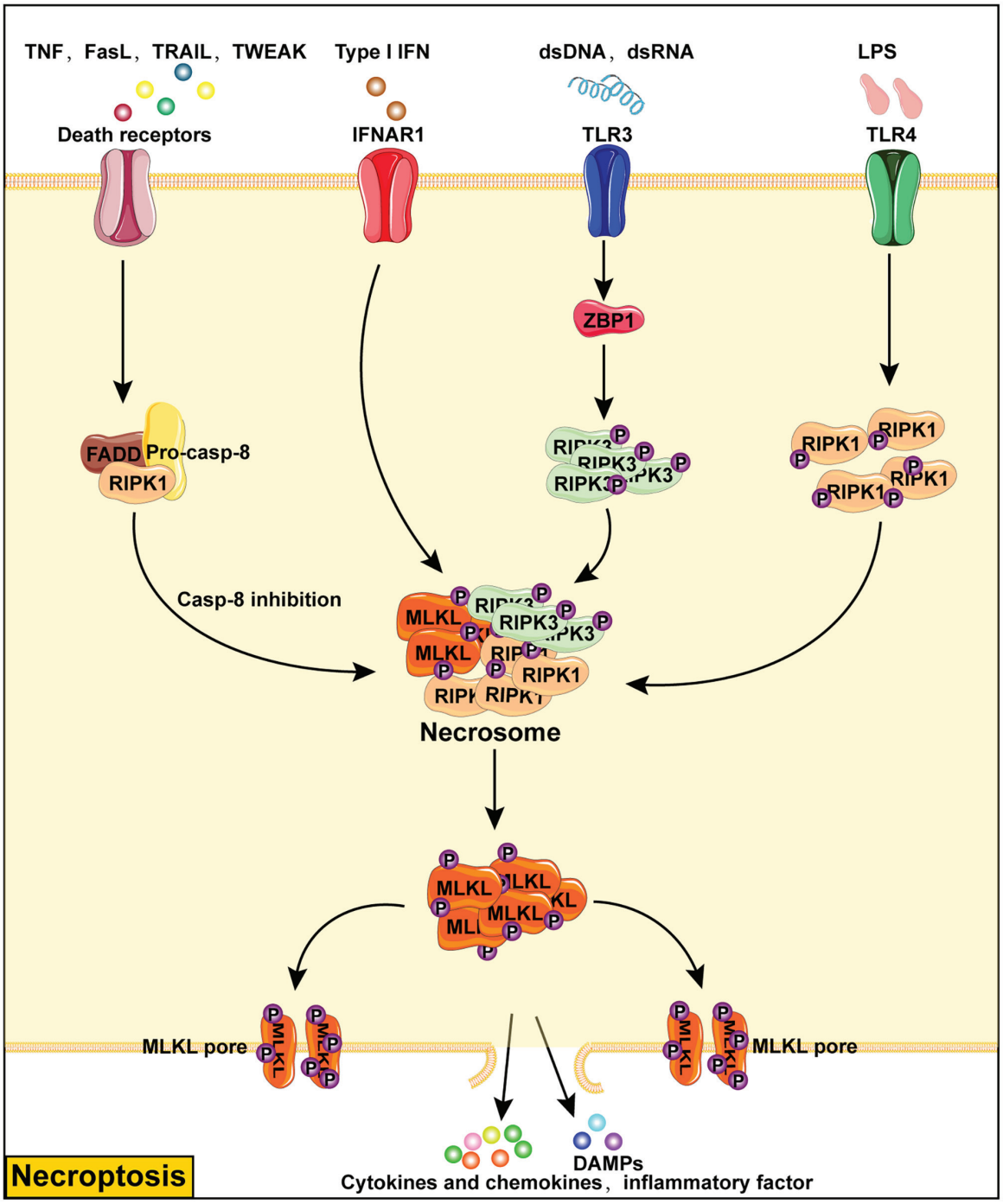

Figure 3. Mechanisms of necroptosis. Necroptosis is a mode of programmed cell death that is not dependent on caspase activity and is caused by phosphorylated MLKL punching holes in the cell membrane causing the release of intracellular associated antigens, DAMP and ultimately cell lysis and death. Necrotic apoptosis can be triggered by the stimulation of death receptors, IFNAR1, TLR3, TLR4 and other receptors. In particular, upon death receptor stimulation, necroptosis can be induced only if caspase-8 activity is inhibited, which promotes the assembly of necrosome through the binding of FADD to RIPK1 and further leads to the phosphorylation and activation of the necroptosis executor MLKL. The ligand type I IFN promotes the assembly of necrosome by activating IFNAR1 to cause necroptosis. Endogenous or exogenous dsDNA and dsRNA activate ZBP1 by TLR3 receptor and cause phosphorylation of RIPK3 to promote necrosome assembly. By contrast, LPS mediates the assembly of necrosome through TLR4 receptor, which in turn promotes the phosphorylation of RIPK1. IFN, type I interferon; IFNAR1, IFN receptor; RIPK1, receptor-interacting serine/threonine protein kinase 1; RIPK3, receptor-interacting protein kinase 3; TLR, Toll-like receptor; LPS, lipopolysaccharide; DAMP, damage-associated molecular pattern; MLKL, mixed lineage kinase domain-like protein; ZBP1, Z-DNA binding protein 1; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA.

mechanism for maintaining tissue homeostasis and eliminating damaged cells, while pyroptosis is closely associated with immune regulation and the orchestration of inflammatory responses. By contrast, necroptosis signifies a non-apoptotic and non-pyroptotic mode of cell death, which can replace apoptosis or pyroptosis in specific circumstances. Despite their unique characteristics, these pathways interact, adapting to cellular demands in a range of environmental contexts, thereby conferring upon the biological organism the versatility and adaptability required for determining cell fate.

3. Apoptosis and its relationship with CVDs

Cardiomyocytes, characterized as terminally differentiated cells, have traditionally been understood to exclusively succumb to necrosis. This long-held perspective, however, has been challenged by emerging debates over the potential role of apoptosis in these cells. Significantly, apoptosis is not merely associated with cardiac pathologies; it is a pivotal process in the broader spectrum of an organism's vital functions. Essential for preserving both the physiological integrity and structural morphology of the body, apoptosis must be carefully balanced. Deviations, manifesting as excessive or deficient apoptotic activity, can result in a spectrum of morphological and functional aberrations within the organism. Research has highlighted the role of cardiomyocyte apoptosis in a range of CVDs, including AS, MI, HF and pulmonary hypertension (PH), underscoring the importance of apoptosis in both the pathogenesis and progression of CVDs.

Role of apoptosis in AS. In the early stages of AS development, the apoptosis of vascular endothelial cells (VECs) induced by oxidized low-density lipoprotein (ox-LDL) leads to endothelial dysfunction, a critical factor in the formation of AS. This results in the accumulation of VECs, vascular smooth muscle cells (VSMCs), macrophages and their derivative foam cells within the vessel, and thus the formation of atherosclerotic plaques (24,25). Research has shown that ox-LDL induces VEC apoptosis by activating the Fas/FasL pathway (26). At the same time, hyperglycemic conditions trigger the production of inflammatory cytokines and initiate a caspase cascade through the activation of the NF-κB pathway, exacerbating VEC apoptosis (27). In addition, the inhibitory effect of Paeonol on apoptosis in VEC induced by high glucose/high pressure is mediated through the regulation of the SIRT1/FOXO3a/NF-κB pathway (28). The combination of geniposide and notoginsenoside R1 has been shown to effectively inhibit inflammation and apoptosis in AS. This effect is mediated through the activation of the adenosine 5'-monophosphate activated protein kinase/mechanistic target of rapamycin (mTOR)/nuclear factor erythroid 2-related factor 2 signaling pathway, which subsequently inhibits the NLRP3 inflammasome and the Bax/Bcl2/caspase-3 pathway, which are pivotal in regulating apoptosis within atherosclerotic plaques (29).

VSMC apoptosis is crucial in the formation and progression of atherosclerotic plaques, particularly during the later stages, where ongoing apoptosis can thin the fibrous cap, increasing plaque instability (30). Furthermore, the role of macrophage colony-stimulating factor (CSF) in regulating macrophage proliferation and apoptosis has been highlighted. Local production of CSF1 by smooth muscle cells and endothelial cells, rather than circulating CSF1, is identified as the primary driver of macrophage expansion in atherosclerotic lesions. This finding underscores the significance of local cellular interactions in modulating apoptosis within the plaque microenvironment (31).

Apoptosis and MI. MI, a leading cause of mortality worldwide, is primarily characterized by the death of cardiac tissue due to ischemia. A critical aspect of MI pathophysiology is apoptosis, or PCD, which has a pivotal role in the progression of cardiac damage and the subsequent remodeling of the myocardium. Understanding the mechanisms and implications of apoptosis in MI is crucial for developing targeted therapeutic strategies. The present review elucidates the current understanding of apoptosis in MI, referencing key research findings.

Apoptosis in cardiac cells post-MI is a double-edged sword. While it eliminates damaged cells, excessive apoptosis can lead to the detrimental loss of functional myocardium, contributing to HF. Recent studies have highlighted various molecular pathways and factors involved in regulating apoptosis during MI. For instance, the role of microRNA (miR)-182-5p in apoptosis regulation during MI has been explored, offering insight into potential therapeutic targets (32).

In addition, the insulin-like growth factor-1 mediated enhancement of bone marrow stem cell viability and anti-apoptotic effects in MI through the secreted frizzled-related protein 2 pathway has been demonstrated, suggesting a novel approach to stem cell therapy in cardiac repair (33). Furthermore, the role of miR-338 in inhibiting cardiomyocyte apoptosis in MI through the MAP3K2/JNK signaling pathway has been identified, highlighting the therapeutic potential of miRNA-based interventions (34).

Long non-coding (lnc)RNA Maternally expressed gene 3 has been found to regulate cardiomyocyte apoptosis post-MI, suggesting a novel avenue for gene therapy (35). Furthermore, miR-146b has been shown to mediate vascular inflammation and apoptosis in patients who have experienced MI, potentially via the PI3K/Akt/NF-κB signaling pathway (36). Finally, the novel non-coding transcriptional regulator Gm18840 has been identified as a promoter of myocardial cell apoptosis in MI, highlighting novel potential targets for therapeutic intervention (37).

Thus, apoptosis has a multifaceted role in the pathogenesis of MI. Understanding the mechanisms underlying apoptosis in MI can provide insight into potential therapeutic strategies for this debilitating disease.

Apoptosis and HF. HF is a complex clinical syndrome characterized by a reduced cardiac output, originating from alterations in myocardial energy metabolism, excitation-contraction coupling and cardiac structural changes stemming from various etiologies. This decline in cardiac output triggers several compensatory mechanisms, both intrinsically within the heart and systemically. These include an increased heart rate, enhanced myocardial tension, augmented myocardial contraction and significant ventricular remodeling. However, prolonged activation of these compensatory responses eventually leads to progressive ventricular remodeling, exacerbating HF.

Apoptosis of cardiac cells contributes to the deterioration of cardiac function in HF. Various molecular pathways and external stimuli influence the regulation of apoptosis in cardiomyocytes. For instance, miR-182 has been identified as a regulator that inhibits cardiomyocyte apoptosis induced by non-ischemic HF, highlighting the therapeutic potential of targeting specific miRNAs in the treatment of HF (38). In addition, oxidative stress-induced myocardial apoptosis is ameliorated by Baiyangdian via the P38 MAPK-αB crystallin signaling pathway, highlighting a novel target for the management of acute MI (39). The Shenfu formula has been found

to reduce cardiomyocyte apoptosis in a rat model of HF, suggesting the potential of Traditional Chinese Medicines in managing HF (40).

Apoptosis and PH. PH is increasingly being recognized as a progressive and debilitating condition, characterized by the constrictive remodeling of pulmonary arteries, such as the abnormal proliferation of pulmonary arterial smooth muscle cells (PASMCs) and apoptosis of endothelial cells (PAECs). This pathological transformation exacerbates pulmonary vascular resistance, precipitating right HF and ultimately mortality. The pathogenesis of PH is complex, encompassing a multifaceted interplay of genetic, molecular and environmental factors. Of note, the dysregulation of apoptosis has been identified as a pivotal factor in the progression of PH. Recent research has illuminated the molecular underpinnings of dysregulation of apoptosis in PH. The mitochondrial fission factor has emerged as a crucial element, with its midzone fission process being increased in PH-affected PASMCs. This increase results in increased cellular proliferation and reduced apoptosis, signifying a potential target for therapeutic intervention (41). In addition, the Notch1 signaling pathway has been shown to modulate endothelial proliferation and apoptosis in PH, highlighting Notch inhibitors as a prospective therapeutic avenue (42).

The synergy between apoptosis and other cellular processes, including inflammation, migration and metabolic reprogramming, in PH has also attracted attention. Prostaglandin E1, for example, has been shown to influence mesenchymal stem cell properties via the hypoxia-inducible factor (HIF) pathway, thereby impacting apoptosis in PH (43). Furthermore, the long non-coding (lnc)RNA SOX2-OT, can positively promote the transcription of SOX2 gene (one of the major regulators of pluripotency), has been highlighted as a potential diagnostic biomarker for PH, affecting PASMC proliferation, migration, anti-apoptotic activities and inflammation (44). miRNAs have garnered recognition for their pivotal role in regulating apoptosis in PH. Specifically, the miR-30 family, and more notably miR-30d-5p, were identified as regulators of PASMC toxicity and apoptosis, potentially via the Notch-3 signaling pathway. This regulatory role is significant, as evidenced in both blood samples from patients with PH and animal models, in contrast to control groups (45). The differentiated embryo chondrocyte expressed gene 1-peroxisome proliferative-activated receptor-γ axis has also been delineated as a critical element in hypoxia-modulated signaling, integral to the imbalance between proliferation and apoptosis in PAECs. This finding underscores the complexity of the cellular milieu in PH and the nuanced equilibrium required for vascular homeostasis (46).

Genetic influences are also of paramount importance in the dysregulation of apoptosis observed in PH. Mutations in bone morphogenetic protein receptor 2, have been associated with metabolic aberrations and dysregulated signaling pathways, influencing apoptosis and contributing to PH pathogenesis (47). Furthermore, newly identified loss-of-function variants in potassium voltage-gated channel subfamily A member 5 in patients with PH suggest a potential causative or contributing role through altered channel functionality (48). Emerging therapeutic strategies targeting apoptosis have shown potential in PH management. For instance, The survivin inhibitor YM155 has demonstrated efficacy in suppressing PASMC proliferation and promoting apoptosis by inhibiting survivin expression, thereby mitigating pulmonary vascular remodeling in PH (49). Similarly, Qiliqiangxin has been shown to inhibit PH-induced right ventricular remodeling by decreasing mitochondrial-associated apoptotic pathways and improving metabolic reprogramming (50).

In summary, the role of apoptosis in PH is multifaceted and critical to its pathogenesis. Delving into the mechanisms of apoptosis in PH may highlight novel therapeutic targets for the management of PH. The current research landscape presents exciting opportunities for further investigation and the development of targeted interventions.

4. Pyroptosis and its relationship with CVDs

Pyroptosis represents a distinct, pro-inflammatory paradigm of PCD, characterized by an intricate amalgamation of morphological changes reminiscent of both apoptosis and necrosis. Central to this process is the formation of pores within the cytoplasmic membrane, precipitating acute cellular swelling, rupture and subsequent discharge of pro-inflammatory agents and intracellular components. Crucially, pyroptosis exerts a profound influence on the onset and progression of a range of pathologies, including AS, MI, HF and PH. This influence is primarily exerted through the modulation of inflammatory responses and cell death. Furthermore, the molecular entities associated with pyroptosis have a significant role in dictating the CVD trajectory and clinical outcomes, underscoring their potential as therapeutic targets.

Pyroptosis and AS. Pyroptosis, a pro-inflammatory form of cell death, has a pivotal role in the pathogenesis of AS. Pyroptosis significantly exacerbates the instability of atherosclerotic plaques, leading to plaque rupture and thrombus formation. This process is a crucial trigger for acute cardiovascular events, primarily through the extensive release of pro-inflammatory cytokines (51).

Endothelial cells are at the forefront of AS development and progression, serving as a critical determinant in its pathogenesis. Several risk factors, such as hyperlipidemia, hyperglycemia, hypertension, smoking and inflammation, precipitate endothelial cell pyroptosis via the activation of the caspase-1 pathway. This activation not only triggers the release of pro-inflammatory cytokines but also compromises the structural integrity of the cell membrane. A study by Wu *et al* (52) underscored the aggravating effect of nicotine on arterial atherosclerotic lesions in apolipoprotein E $(ApoE)^{-/-}$ mice, concomitantly elevating caspase-1 expression. Furthermore, trimethylamine N-oxide (TMAO), a byproduct of the phosphatidylcholine metabolism from the gut microbiota, exacerbates endothelial dysfunction by interacting with the NF-κB and MAPK signaling pathways, thus promoting the accrual of AS plaques (53). Under a high-fat diet, $ApoE^{-/-}$ mice exhibit a pronounced oxidative stress response in vascular endothelial cells attributed to TMAO, which also upregulates caspase-1 and NLRP3 expression, thereby promoting AS progression (54).

Macrophage-derived foam cells significantly contribute to the instability of atherosclerotic plaques (55). The

ROS/NF-κB/NLRP3 axis and mitochondria have crucial roles in macrophage pyroptosis during AS (56,57). The non-classical caspase-11/GSDMD pyroptosis pathway has also been associated with AS. In addition, the observed reduction in the area of atherosclerotic plaques in ApoE and GSDME double-knockout mice under a high-fat diet underscores the significance of GSDME within macrophages (58).

VSMCs are also pivotal in AS development. VSMC apoptosis not only reduces the content of extracellular matrix (ECM), rendering the fibrous cap susceptible to rupture, but it may also intensify inflammation within the vascular wall, thereby elevating the risk of plaque instability. It has been shown that ox-LDL accumulation is intricately associated with VSMC apoptosis. Intriguingly, while low concentrations of ox-LDL promote a phenotypic shift in these cells from contractile to synthetic, facilitating the secretion of inflammatory cytokines; higher concentrations of ox-LDL are implicated in directly inducing apoptosis (59). Subsequent studies have further delineated the profound impact of ox-LDL, revealing its capacity to transform VSMCs into foam cells and increase the expression of NLRP3, ASC, caspase-1 and GSDMD. This underscores the mechanism by which ox-LDL, via the activation of the caspase-1-dependent pathway, promotes VSMC apoptosis and the subsequent release of IL-1β and IL-18. Such events contribute to the diminution of the ECM, the attenuation of the fibrous cap, the amplification of local inflammatory responses and the promotion of AS (60). The caspase-1 inhibitor VX-765 significantly reduces ox-LDL-induced endothelial cell pyroptosis, thereby slowing the progression of AS (61).

Pyroptosis and MI. The key complication of MI therapy is myocardial ischemia/reperfusion injury (MI/RI). A growing body of evidence indicates a strong association between cell pyroptosis and MI/RI. The crucial role of NLRP3 and caspase-1-mediated cell pyroptosis in MI/RI is highlighted below.

The silencing of calpain can mitigate myocardial dysfunction caused by MI/RI, primarily through the inhibition of the NLRP3/ASC/caspase-1 axis (62). In addition, targeting oxidative stress and NLRP3-mediated cell pyroptosis by miR-29a-targeted silencing of sirtuin 1 has also been demonstrated to alleviate MI/RI (63). Further research has established that mice with caspase-1 overexpression show an increased MI area (64). Conversely, VX-765, a selective caspase-1 inhibitor, effectively reduces MI in MI/RI injury models (65).

Pyroptosis and HF. HF represents the terminal stage in the progression of CVDs, characterized by a complex pathology that encompasses myocardial fibrosis, hypertrophy and excessive inflammation, among which pyroptosis has a pivotal role. This phenomenon profoundly impacts cardiac structure and function and offers novel perspectives for therapeutic strategies against HF.

Myocardial fibrosis, the thickening of the myocardial interstitium due to excessive deposition of ECM proteins, is primarily driven by activated fibroblasts, the main producers of ECM proteins. Zhang *et al* (66) discovered that the activation of the NLRP3 inflammasome in fibroblasts promotes the synthesis of collagen and activates caspase-1, leading to the release of IL-1β and IL-18, thereby inducing pyroptosis. Furthermore, IL-1β and IL-18 can exacerbate HF progression by activating the release of TNF-α and promoting the efflux of Ca^{2+} from the sarcoplasmic reticulum. Myocardial hypertrophy is crucial for maintaining cardiac ejection function. However, pathological myocardial hypertrophy induced by disease can precipitate HF. Zeng *et al* (67) reported that in patients with dilated cardiomyopathy, NLRP3 inflammasome activation is increased, accompanied by myocardial cell pyroptosis, and this is inversely correlated with cardiac ejection function. These findings underscore the role of the NLRP3 inflammasome, primarily through IL-1β, in promoting structural cardiomyopathic changes associated with myocardial dysfunction. This is further validated by the fact that caspase-1 modulates Ang II-induced cardiac hypertrophy via the regulation of IL-1β (68). Conversely, the anti-inflammatory role of bone morphogenetic protein-7 partially alleviates myocardial hypertrophy and fibrosis, highlighting its potential therapeutic value in managing HF (69).

Pyroptosis and PH. PH is a CVD closely associated with structural abnormalities and functional dysregulation of the pulmonary vasculature. The progression of this disease is influenced by genetic and environmental factors, involving numerous vasoactive molecules and signaling pathways. Damage to pulmonary arterial endothelial and smooth muscle cells leads to abnormal vasoconstriction, remodeling, thrombosis, inflammatory responses and aberrant proliferation and apoptosis of vessels, thereby triggering PH.

Under hypoxic conditions, the relationship between cell pyroptosis and the formation of PH has become increasingly apparent. Research indicates that the activation of Gli zinc finger transcription factor 1 (GLI1) promotes cell pyroptosis in PASMCs under hypoxia. GLI1 orchestrates PASMC pyroptosis by inducing ASC expression, targeting the ASC promoter region. Inhibition of GLI1 reverses *in vivo* PH symptoms and pyroptosis (70). Furthermore, a study by Cero *et al* (71) found that mice lacking ASC exhibited significantly reduced symptoms of PH and right ventricular remodeling under hypoxic conditions, suggesting that the activation of inflammasomes has a crucial role in the development of PH. Further investigation revealed that signal transducer and activator of transcription (STAT1) facilitated PD-L1 upregulation in hypoxia-induced PH models, initiating caspase-1-dependent pyroptosis in PASMCs, accelerating pulmonary vascular fibrosis and ultimately leading to PH (72). Udjus *et al* (73) employed a caspase-1 knockout mouse model and caspase-1 inhibitors to demonstrate that in hypoxia-induced PH models, caspase-1 increased PASMC proliferation via the caspase-1/IL-18/IL-6/STAT3 pathway. Additional evidence from Zha *et al* (74) showed a significant reduction of NLRC3 in patients with PH, proposing it as a potential diagnostic biomarker and prognostic indicator. Chai *et al* (75) found that increased expression of miR-155, which promotes inflammation and induces PH through the c-Fos/NLRP3/caspase-1 pathway, was observed in monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH) mouse models. Inhibition of c-Fos and NLRP3 mitigated the inflammatory impact of miR-155 in these models. Tang *et al* (76) demonstrated that tannic acid ameliorated MCT-induced PAH via its antioxidative properties through the inhibition of the NLRP3 inflammasome signaling pathway in a rat model.

5. Necroptosis and its relationship with CVDs

The preceding chapters highlighted that PCD, an inherently genetic and systematic form of active cell death, consists of processes such as apoptosis, pyroptosis and necroptosis. Of note, necroptosis has a pivotal role in the etiology of a spectrum of CVDs, including but not limited to AS, MI, HF and PH. In recent years, there has been a notable surge in research focusing on the intricate signaling pathways and molecular mechanisms that underpin necroptosis. These studies have provided profound insight into the complex interplay of factors governing this form of cell death, offering novel perspectives on potential therapeutic strategies.

Necroptosis and AS. Necroptosis has a pivotal role in the progression of AS, with its hallmark proteins RIP3 and MLKL being empirically demonstrated in human carotid atherosclerotic plaque. Studies have shown that, while knockout of RIP3 in an ApoE$^{-/-}$ mouse model had minimal impact on early-stage AS, it significantly alleviated late-stage atherosclerotic lesions, revealing the connection between necroptosis and plaque stability (77). Furthermore, elevated plasma levels of RIP3 are positively associated with the severity of coronary artery disease, suggesting its potential as an indicator for assessing the severity of coronary artery disease (78).

The death of macrophages, particularly necroptosis driven by ox-LDL, is crucial for forming the necrotic core. This process releases inflammatory cytokines such as IL-6 and IL-1α, triggering further inflammatory responses (79-81). Evidence indicates that the absence of MLKL can reduce macrophage necroptosis and the necrotic core within plaques, highlighting the pivotal role of MLKL in the development of AS (82). In addition, exacerbating oxidative stress promotes this process, activating HIF-1α and intensifying macrophage necroptosis through enhanced ROS production (83).

Necroptosis and MI. MI, the most severe manifestation of acute coronary syndrome, has seen a significant improvement in patient survival rates through interventional treatments. However, efficiently addressing cardiac remodeling and dysfunction resulting from the loss of a substantial number of myocardial cells remains a significant challenge in its treatment.

The increase in RIP3 protein expression is closely associated with MI. Mice lacking RIP3, following ligation of the left anterior descending coronary artery, exhibit improved cardiac function and reduced myocardial hypertrophy, highlighting the central role of necroptosis in MI and the subsequent cardiac remodeling (84). Research by Yang *et al* (85) showed that both MLKL and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) were involved in the pathogenesis of chronic chest pain associated with myocardial ischemia induced by RIP3, positing that MI/RI could be co-mediated by the RIP1-RIP3-MLKL and RIP3-CaMKII pathways. TGF-β-activated kinase 1 and TNF receptor-associated factor 2 have been shown to counteract necroptosis and regulate cardiac remodeling, offering novel potential targets for treating ventricular remodeling and HF (86,87). Studies have shown that S-allyl cysteine sulfoxide, found in garlic, and miR-325-3p can alleviate cardiac damage post-MI by inhibiting necroptosis (88,89). In addition, recombinant adenovirus hepatocyte growth factor improved cardiac remodeling by promoting autophagy and necroptosis and inhibiting apoptosis (90), presenting a novel avenue for intervention in cardiac remodeling post-MI via the regulation of cell death processes.

Necroptosis and HF. Necroptosis has been identified as a critical factor in the progression of HF and has a substantial role in ventricular remodeling. Early research focused on necrosis and apoptosis as the primary mechanisms of MI/RI, while recent findings have highlighted the critical role of necroptosis in cell death triggered by MI/RI (91). The groundbreaking study by Szobi *et al* (92) revealed that in samples from patients with HF, pivotal necroptosis mediators such as RIP1, RIP3 and MLKL, alongside their phosphorylated alternatives, were notably upregulated compared to those in healthy individuals. This is accompanied by a marked decrease in the expression of activated caspase-8, suggesting the potential suppression of apoptosis in the milieu of HF, thereby paving the way for necroptosis.

In addition, the post-TAC elevation in RIP1, RIP3, MLKL, and their phosphorylated counterparts can be effectively reversed by applying inhibitors targeting the molecular chaperone heat shock protein (HSP). These inhibitors also significantly reduce TNF-α-induced necroptosis by disrupting the RIP1-RIP3-MLKL interaction. This finding posits that inhibition of HSP90 could yield therapeutic benefits by reducing the activation of the RIP1-RIP3-MLKL pathway in compromised cardiac tissue (93). Research has also revealed that carboxymethyllysine, a late glycation end product derived from neutrophils, activates RIP3 through its receptor RAGE, leading to necroptotic death of myocardial cells (94). Furthermore, RIP3-activated CaMKII promotes cell death, highlighting novel therapeutic targets for treating cardiac injury and HF (95). The combined use of Nec-1 and a caspase inhibitor, zVAD-FMK, demonstrated the occurrence of multiple forms of cell death during MI/RI, providing novel therapeutic avenues for the recovery of cardiac function (96).

Necroptosis and PH. Xiao *et al* (97) demonstrated that inflammation and immunity are critical factors in developing pulmonary vascular remodeling and PH. They discovered the enrichment of TLR and NLR pathways and NF-κB-mediated inflammatory and immune profiling in MCT-induced PAH. Furthermore, they found that the activation of TLR and NLR pathways was associated with the upregulation of damage-associated molecular patterns (DAMPs) and that RIPK3-mediated necroptosis contributed to DAMP generation in MCT-induced PAH.

A study by Jarabicová *et al* (98) investigated the role of cell loss due to necroptosis and its association with pyroptosis in organ damage during PH. They found increased levels of pThr231/Ser232-RIP3, leading to various necrosis-like cell death types, which may have contributed to the pathological mechanisms occurring during PH. In addition, plasma RIP3 may serve as a novel diagnostic and prognostic marker for cardiac injury in PH.

6. PANoptosis

PANoptosis, an acronym embodying pyroptosis, apoptosis and necroptosis, is a unique PCD paradigm within innate immunity elicited by innate immune triggers. This complex process

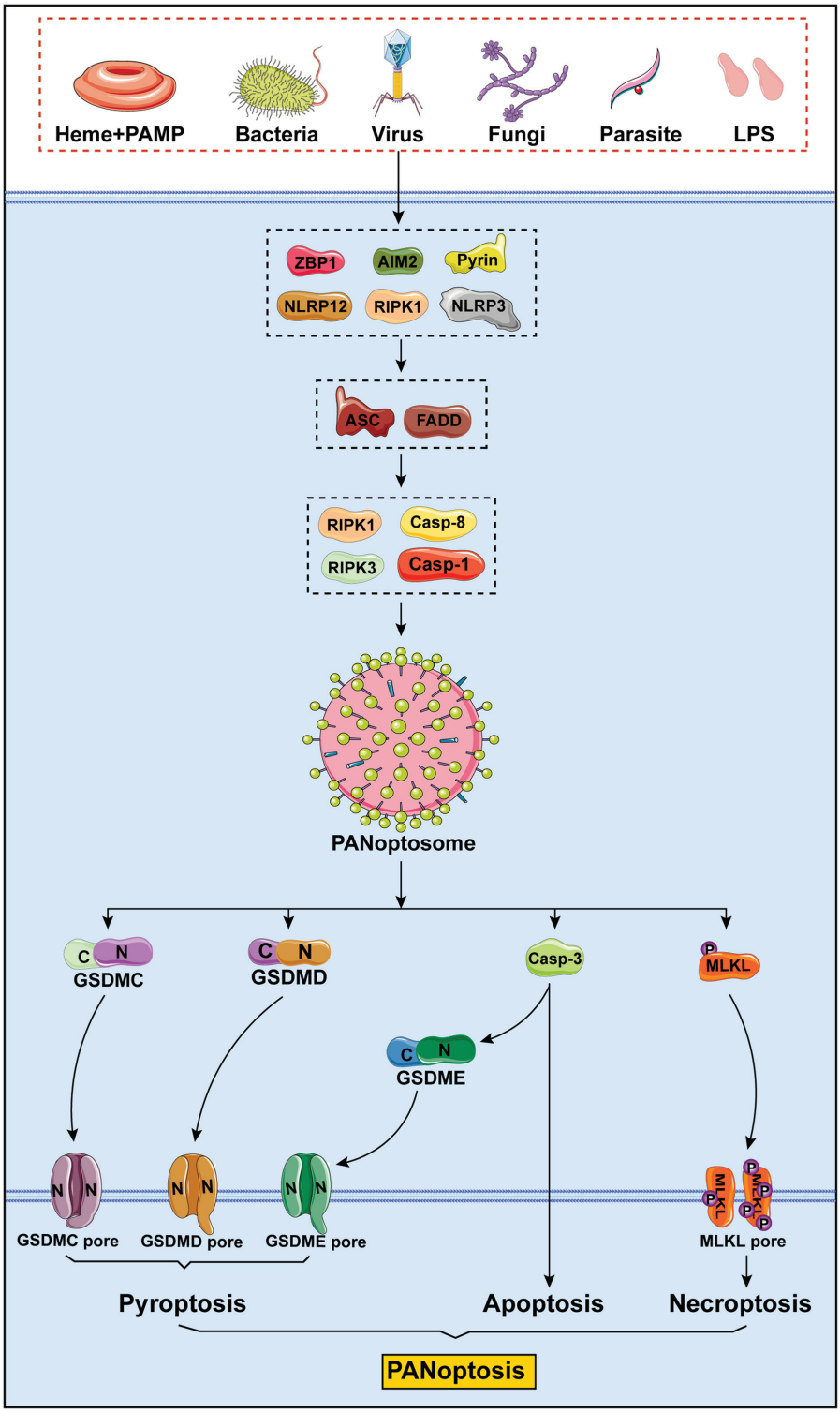

Figure 4. Mechanisms of PANoptosis. PANoptosis is an innate immune, lytic and inflammatory cell death pathway driven by caspases and RIPKs and regulated by the PANoptosome, which possesses the characteristics of apoptosis, pyroptosis and necroptosis. Different triggering factors can induce the formation of different PANoptosomes to cause PANoptosis. The main sensors that have been recognized to date are ZBP1, AIM2, pyrin, NLRP12, RIPK1 and NLRP3. These sensors bind to the adapters ASC or FADD, which then form the PANoptosome under the action of catalytic effectors, such as RIPK1, caspase-8, RIPK3 and caspase-1, thereby causing PANoptosis of the cells. PAMP, pathogen-associated molecular pattern; LPS, lipopolysaccharide; ZBP1, Z-DNA binding protein 1; AIM2, absent in melanoma 2; NLRP12, nucleotide-binding leucine-rich repeat-containing receptor 12; NLRP3, the nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3; RIPK1, receptor-interacting serine/threonine protein kinase 1; RIPK3, receptor-interacting serine/threonine protein kinase 3; ASC, adapter protein apoptosis-associated speck-like protein containing a caspase recruitment domain; GSDM, gasdermin; MLKL, mixed lineage kinase domain-like protein.

involves the PANoptosome, a sophisticated multi-protein assembly adept at amalgamating elements from various PCD modalities. The biological ramifications of PANoptosis are characterized by an interplay and regulatory overlap among pyroptosis, apoptosis and necroptosis, surpassing the specific limitations of each pathway (Fig. 4).

The PANoptosome, central to this process, is not merely a conglomerate of inflammasome components, including the NLRP3 inflammasome and the ASC, but also a nexus for apoptotic and necroptotic molecules such as caspase-8, caspase-3, RIPK1, RIPK3 and MLKL (99). The activation of PANoptosis underscores a crucial insight: The singular inhibition of any one programmed necrotic pathway, such as impeding the NLRP3 inflammasome or curtailing the production of GSDMD, is insufficient to abrogate cell death by PANoptosis (100).

In essence, PANoptosis epitomizes an inflammatory PCD pathway governed by specific activators and regulated under the supervision of the PANoptosome (Fig. 2), which also suggests a molecular framework. This pathway encapsulates the quintessential characteristics of pyroptosis, apoptosis and necroptosis. However, its complexity and multifaceted nature defy reductionist explanations limited to any singular PCD mechanism.

Potential molecular mechanisms of PANoptosis. Recent advancements in cellular biology have delineated three distinct classes of molecules integral to the composition of the (101) PANoptosome: i) Sensor molecules, exemplified by ZBP1, adept at identifying DAMPs and pathogen-associated molecular patterns (PAMPs); ii) structural molecules, encompassing caspase-8, NLRP3 inflammasome, caspase-1 and RIPK3; and iii) effector molecules, including caspase-3, GSDMD and MLKL, among others. ZBP1, which exhibits robust innate immune sensing capabilities, has been recognized as a pivotal signal initiator in innate immune responses and PANoptosis (102). The role of ZBP1-mediated PANoptosis is twofold; it facilitates the eradication of invasive pathogens and uncontrolled tumor cells, yet anomalously, it can also induce excessive inflammatory responses in a range of contexts, infectious and non-infectious alike (103).

Of note, during PANoptosis, there is a concurrent activation of caspase-1, caspase-8 and caspase-3, inducing cell death through the intricate interplay within the caspase system. Caspase-8, initially identified as a harbinger of apoptosis, has emerged as a seminal regulatory node bridging apoptotic and necrotic pathways. It serves as a precursor in NLRP3 inflammasome activation, thereby inducing pyroptosis (104). Hence, caspase-8 assumes a central role in PANoptosis, orchestrating the delicate balance between apoptosis, necroptosis and pyroptosis, and ultimately influencing the cell's fate by activating specific death signals.

The advent of PANoptosis and the concept of the PANoptosome represent a paradigm shift in our comprehension of innate immune responses and PCD mechanisms. This conceptual framework unravels the extensive molecular crosstalk inherent to various innate immune pathways. Identifying novel triggers and upstream targets is imperative for improving our understanding of this distinct form of cell death, potentially paving the way for groundbreaking therapeutic interventions.

ZBP1. ZBP1-mediated PANoptosis has a critical role in eliminating invasive pathogens and uncontrolled tumor cells. However, aberrant cell death mediated by ZBP1 can also induce excessive inflammatory responses in both infectious and non-infectious environments (102,103). In addition, during the process of PANoptosis, caspase-1, caspase-8 and caspase-3 are concurrently activated, relying on additional interconnections within the caspase systems to promote cell death (102). Notably, caspase-8, an initiator of apoptosis, was one of the earliest discovered intermediaries in both the apoptotic and necrotic pathways. Caspase-8 also acts upstream of NLRP3 inflammasome activation, facilitating the occurrence of pyroptosis (105). Therefore, as a central regulatory factor in PANoptosis, caspase-8 influences the interplay between apoptosis, necroptosis and pyroptosis. It determines the type of cell death through the activation of death signaling molecules, playing a key role in the context of PANoptosis (106).

RIPK1. RIPK1 was identified as a pivotal serine/threonine kinase, instrumental in regulating cell survival, apoptosis and necrosis. Within PANoptosis, RIPK1 engages in critical interactions with proteins such as RIPK3 and MLKL, thereby modulating an array of cell death pathways, encompassing apoptosis, necrosis and pyroptosis (107,108). A notable instance of the regulatory role of RIPK1 was observed in cerebral ischemic injury, where it governs cell death via the RIPK3/MLKL pathway. A study investigated the effect of polymyxin B, a polypeptide antibiotic, on necroptosis in a rat model of stroke (109). The results indicated that polymyxin B could enhance the endosomal sorting complex required for transport III machinery and suppress the RIPK1/RIPK3/MLKL pathway, leading to reduced necroptosis and reduced brain injury in the rat model of stroke. This effect was also observed in hypoxia-treated HT22 cells, suggesting the potential of polymyxin B in treating necroptosis-related brain damage.

Furthermore, the significance of RIPK1 extends to its involvement in skin inflammation and keratinocyte necrosis. A study by Duan *et al* (110) highlighted the critical role of necroptosis in psoriasis, a common autoimmune and chronic inflammatory skin disorder. They found that RIPK1 and MLKL were significantly upregulated in human psoriatic lesions, and the increased tendency of necroptosis in imiquimod-induced psoriasiform skin in mice highlighted the involvement of necroptosis in the pathogenesis of psoriasis. Of note, the study demonstrated that inhibiting necroptosis using the inhibitor of RIPK1 R-7-Cl-O-Necrostatin-1 (Nec-1s) and MLKL-inhibitor necrosulfonamide could effectively block inflammatory responses and reduce the production of key inflammatory factors in both cell and mouse psoriasis models.

RIPK3. RIPK3, functioning synergistically with RIPK1, drives cells towards necroptosis. The activation of RIPK3 catalyzes the phosphorylation of MLKL (111), culminating in the disruption of the cell membrane and subsequent cell death. Central to promoting PANoptosis, RIPK3 has been found to induce necroptotic death in tumor cells via the RIPK1/MLKL pathway. The study by Alaaeldin *et al* (112) was primarily centered on the impact of a novel ciprofloxacin derivative on RIPK3 and its implications for PANoptosis in cancer therapy. The findings revealed that the ciprofloxacin derivative effectively bound to and inhibited topoisomerases I and II, significantly reducing the proliferation, migration and colony formation of the cancer cells. Crucially, the study highlighted the derivative's ability to increase the proportion of apoptotic cells and activate the necro-apoptotic pathway, primarily by stimulating RIPK3,

RIPK1 and MLKL proteins. This research provides valuable insight into the role of RIPK3 in PANoptosis and underscores the potential of targeting this pathway as a novel approach to cancer treatment. The ability of the ciprofloxacin derivative to modulate RIPK3 and induce PANoptosis opens novel avenues for the development of anticancer therapies, particularly for HepG2 and A549 cancer types, and contributes to the broader understanding of the molecular mechanisms underlying cancer cell death. Furthermore, recent research identified the modulatory role of RIPK3 in cell death and inflammation, particularly via its interactions with the RIPK1 and MLKL pathways. It underscores the pivotal function of RIPK3 in initiating and regulating necroptotic processes, as well as its diverse and complex activation in response to various cellular stressors (113).

MLKL. MLKL is a critical effector in pyroptosis and PANoptosis. Phosphorylated by RIPK3, MLKL triggers the rupture of the cell membrane, leading to cell death. The activation of MLKL is closely associated with pathological processes in various disease models. For instance, in cerebral ischemic injury, MLKL is a crucial regulator of cell death, operating through the RIPK1/RIPK3 pathway. Zhang *et al* (114) investigated the role of MLKL in PANoptosis, particularly in ischemic brain injury following stroke, focusing on the effects of ligustroflavone, a compound with anti-inflammatory properties. They showed that ligustroflavone reduced brain injury and decreased the levels of necroptosis-associated proteins, including MLKL, in a rat model of ischemic stroke. However, the study noted an inconsistency in the regulation of RIPK1 levels, suggesting a need for further exploration of a ligustroflavone selective mechanism on necroptosis-related proteins. While the study identified potential targets of ligustroflavone, including RIPK1, RIPK3 and MLKL using the Molecular Operating Environment program, the unaltered levels of RIPK1 call for a more detailed investigation into its interaction with MLKL in PANoptosis. The study highlighted the potential of ligustroflavone as a therapeutic agent for ischemic brain injury. Thus, there is a need for a deeper understanding of the molecular mechanisms concerning MLKL in PANoptosis.

The role of MLKL extends to the pharmacodynamics of antidepressants, where it regulates the necroptotic death of neuronal cells via the RIPK1-RIPK3-MLKL pathway. Yan *et al* (115) focused on the antidepressant effects of Xiaoyaosan, in particular examining its influence on MLKL-mediated necroptosis in the context of depression. They revealed that Xiaoyaosan impacted the MLKL pathway in a mouse model of depression. This suggested that the therapeutic effect of Xiaoyaosan on depression may involve modulating MLKL and the related necroptotic processes, offering a novel perspective for treating depression by targeting specific aspects of PANoptosis.

TNF signaling pathway and PANoptosis. TNF-α, by interacting with TNF receptors on the cell surface, triggers a complex cascade of downstream signals. These signals lead to various cell fates, including apoptosis, necrosis or other forms of cell death, covering the range of PANoptosis. The activation of RIPK1 and RIPK3 is central to this signaling cascade. These kinases have a key role in determining cellular outcomes, decisively influencing a cell's fate toward survival, apoptosis or necrosis. The complex interactions within the TNF signaling pathway, especially between RIPK1 and RIPK3, underscore the complexity of cellular responses to pathological stimuli.

Karki *et al* (116) discovered that in COVID-19, the combined production of TNF-α and IFN-γ by innate immune cells induced PANoptosis. They also found that deleting individual mediators of pyroptosis, apoptosis or necroptosis was insufficient to prevent cell death. Ma *et al* (117) explored the role of lncRNA SPRY4 intronic transcript 1 (SPRY4-IT1) in hepatocellular carcinoma (HCC), focusing on its role in the TNF signaling pathway and potential implications for PANoptosis. Although the study offered valuable insight into the role of SPRY4-IT1 in HCC and its link to the TNF signaling pathway, it raised questions regarding the direct link between SPRY4-IT1 and PANoptosis. Further research is required to explore how SPRY4-IT1, through its interaction with the TNF signaling pathway, may influence PANoptosis in HCC, thereby contributing to tumor progression and metastasis.

TLR signaling pathway in PANoptosis. The TLR signaling pathway, a cornerstone in immunological research, has garnered extensive attention for its critical role in recognizing PAMPs and DAMPs. This pathway, activating adaptor proteins, such as myeloid differentiation factor 88 and Toll-interleukin 1 receptor domain-containing adapter inducing IFN-β, leads to the subsequent activation of NF-κB and other transcription factors, orchestrating a cascade of inflammatory responses and cell death (118). While the implications of the TLR signaling pathway in conventional cell death modalities such as apoptosis and necrosis have been thoroughly investigated, its direct contribution to PANoptosis remains undetermined. Considering the regulatory influence of the TLR signaling pathway over the traditional cell death modes, it is plausible to hypothesize its potential involvement in PANoptosis. Of note, the TLR signaling pathway may indirectly modulate cell death mechanisms associated with PANoptosis by activating NF-κB.

Contemporary research endeavors have predominantly concentrated on delineating the role of the TLR signaling pathway within specific pathological contexts, such as neurodegenerative diseases, cancer and inflammatory disorders. Recent studies demonstrated the therapeutic potential of polyphenolic compounds in neurodegenerative diseases via modulation of the TLR signaling pathway, indicating the regulatory effects of probiotic-derived metabolites on the TLR signaling pathway in inflammatory responses (119,120). Although these studies do not establish a direct link between the TLR signaling pathway and PANoptosis, they underscore the latent role of this pathway in cellular death and inflammation, laying a foundational framework for future investigations into its role in PANoptosis.

In summary, the lack of direct evidence linking the TLR signaling pathway to the regulation of PANoptosis, juxtaposed with its pivotal role in immune responses and cell death, paves the way for future research. Future studies may unravel the potential of this pathway in PANoptosis, an exploration that promises to yield novel insight into the pathogenesis and therapeutic approaches for diseases associated with PANoptosis. This emerging field of study stands at the forefront of expanding our understanding of complex cellular death mechanisms and their implications for human health and disease.

Intracellular stress pathways in PANoptosis. In the study of PANoptosis, the roles of intracellular stress pathways, particularly ER stress and autophagy, have not been directly confirmed to have a clear association with PANoptosis. However, the roles of these pathways in responding to prolonged stress and maintaining cellular homeostasis may imply their potential impact on PANoptosis. ER stress is a cellular response to abnormal protein folding or accumulation, typically activated through the protein kinase RNA-like endoplasmic reticulum kinase (PERK) and IRE1α pathways. Autophagy, on the other hand, is an intracellular degradation process that maintains cellular homeostasis by digesting and recycling cellular components.

Although current research has not directly linked ER stress and autophagy with the activation of PANoptosis, their roles in responding to stress and regulating cell death are undeniable. For example, it has been suggested that the interaction between ER stress and autophagy can contribute to the development of novel therapeutic strategies to alleviate cellular stress and inflammation associated with various pathologies (121). In addition, the regulation of the ER stress-autophagy axis may be a potential strategy for treating neurodegeneration and neurological deficits following ischemic stroke (122). While these studies have not directly demonstrated the role of ER stress and autophagy in PANoptosis, they reveal the significance of these pathways in cellular stress responses and the regulation of cellular fate.

Mitochondrial signaling pathways in PANoptosis. In the intricate landscape of PANoptosis regulation, mitochondrial signaling pathways emerge as central players, particularly in orchestrating cell death processes. Mitochondria, quintessential for energy metabolism, also serve as pivotal determinants of cellular fate. Dysfunctions in these organelles can pivotally steer cells toward PANoptosis. Emerging research has elucidated a profound association between alterations in mitochondrial function and the initiation of PANoptosis. A notable example is the increased permeability of the mitochondrial outer membrane, culminating in the release of cytochrome C (123). This event, pivotal in apoptosis, may also be a critical trigger of PANoptosis. Furthermore, the generation of ROS by mitochondria is implicated in amplifying cell death pathways, including PANoptosis. Consequently, nuanced shifts in mitochondrial functionality are increasingly recognized as determinants in the decision between survival and PANoptosis.

Shi *et al* (124) explored the roles of mitochondrial ROS (mtROS) and reverse electron transport (RET) in the induction of PANoptosis and showed that anti-RET reagents such as 1-methoxy PMS (a stable electron transfer mediator in CCK-8 kits) and dimethyl fumarate can effectively inhibit PANoptosis by blocking mtROS production. In a related context, Yuan *et al* (125) used a multi specific platinum complex SEP, which was constructed by conjugating a quinone derivative seratrodast to a prodrug of cisplatin, as the first metal complex capable of inducing PANoptosis in KRAS-mutant pancreatic ductal adenocarcinoma cells, offering a novel strategy to overcome apoptotic resistance. In doxorubicin (DOX)-induced myocardial injury, FUNDC1 stabilizes mitochondrial DNA by binding to TUFM, thereby protecting cardiomyocytes from DOX-induced PANoptosis (10). Finally, She *et al* (126) also emphasized the significance of mitochondrial dysfunction in ischemic stroke and cerebral ischemia/RI. This links them to PANoptosis and other types of PCD, suggesting neuroprotective agents targeting mitochondrial dysfunction as promising treatments for related brain injuries.

These studies highlight the pivotal role of mitochondrial signaling in PANoptosis, underscoring the significance of mitochondrial dysfunction and enhanced outer membrane permeability in cell death processes. However, they also highlight the gaps in our understanding of mitochondrial involvement in PANoptosis. Present research predominantly concentrates on the impact of the mitochondria on traditional cell death pathways; however, there is a pressing need for more comprehensive studies on their specific roles within PANoptosis.

These insights show the multifaceted roles of mitochondrial signaling in cell death and inflammatory responses and pave the way for novel explorations into the regulation of PANoptosis. Future research endeavors should focus on elucidating the direct interplay between functional shifts of mitochondria and PANoptosis and its consequent effects on cell fate. Unraveling these connections holds substantial promise for developing innovative therapeutic strategies aimed at targeting PANoptosis, and would thus mark a significant stride in cellular and molecular biology.

7. Comparison of PANoptosis with other forms of cell death

PANoptosis, as an encompassing cell death mechanism, uniquely bridges and diverges from conventional modalities such as apoptosis, necroptosis and pyroptosis in molecular mechanisms. Apoptosis, a caspase-dependent PCD, is characterized by distinctive nuclear condensation and DNA fragmentation. Necroptosis is usually 'violent' and is characterized by phosphorylated MLKL creating holes in the cell membrane, leading to membrane rupture and a subsequent inflammatory response. Pyroptosis is another type of PCD that involves major members of the caspase family, such as caspases 1, 3, 4, 5, 8 and 11, and is mediated by cellular membrane pores via the GSDM family of proteins, which ultimately leads to membrane disruption and the release of cellular components. PANoptosis amalgamates these varied mechanisms, yielding a multifaceted and intricate cellular fate-determination process.

Within the PANoptosis pathway, apoptotic proteins, notably caspases, assume pivotal roles. Caspase-8, for example, extends beyond its traditional role in the execution of apoptosis, potentially catalyzing PANoptosis via interplay with molecules from other cell death pathways. Pioneering research by Zheng *et al* (102) delineates the integral role of ZBP1 in innate immune responses against viral RNA and endogenous nucleic acids. During influenza virus infection, ZBP1-mediated sensing of Z-RNA induces PANoptosis, a synchronized cell death pathway orchestrated by the PANoptosome complex. This pathway includes the assembly of the ZBP1-NLRP3 inflammasome, activated upon ZBP1 recruitment of RIPK3 and caspase-8 in response to Z-RNA. This assembly is further modulated by various host factors, including those involved in the type I interferon signaling

pathway, caspase-6 and influenza viral proteins such as M2, NS1 and PB1-F2. In addition, the necrotic attributes in PANoptosis are underpinned by its integration of key molecules from a range of cell death pathways, such as RIPK1 and RIPK3, with MLKL playing a crucial role as a necroptosis executor (127).

Autophagy is a type II cell death process in which autophagosomes are formed under the action of autophagy-related genes and degrade aging or damaged organelles and protein aggregates by binding with lysosomes to meet the metabolic needs of cells and renewing certain organelles. Autophagy is involved in the development of tumors, autoimmune, renal, neurological, cardiovascular, cerebrovascular, metabolic and several other diseases (128-130). Since autophagy is a multistep process, it can be studied using autophagy inducers and inhibitors specific to the different stages of this process. Classical autophagy inhibitors include not only PI3K inhibitors that inhibit the initiation phase of autophagy such as 3-methyladenine, LY294002 and wortmannin, but also drugs that act on the activity of lysosomal enzymes in the terminal phase of autophagy, such as leupeptin, pepstatin A, E-64d, bafilomycin A1, concanamycin A, concanamycin B, methyl- or propylamine, chloroquine and neutral red. Autophagy inducers include rapamycin, Torin1, PP242, KU-0063794, PI-103 and NVP-BEZ235, which inhibit mTOR activity (131). In contrast to PANoptosis, autophagy is not dependent on caspase enzymes and RIPKs but occurs solely based on the actions of lysosomes.

ER stress is caused by the accumulation of misfolded proteins within the ER and activates the unfolded protein response (UPR). ER stress is present in a wide range of diseases, such as cancer, neurodegenerative diseases, metabolic disorders, hepatic and renal diseases and CVDs (132-134). There are three primary ER stress sensors, including inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 and PERK, which are not only involved in the modulation of ER function but also regulate the rate of protein synthesis. Certain reagents that stabilize protein folding intermediates, the drug ISRIB that regulates the phosphorylation state of the α-subunit of eukaryotic translational initiation factor 2, PERK inhibitor GSK2606414 and IRE1 inhibitor MKC8866, can all be used to study ER stress (135). The most important difference between ER stress and PANoptosis is that *in vivo* accumulation of protein aggregates induces the UPR, which leads to ER stress, and further ER stress can initiate apoptosis. In this regard, ER stress and PANoptosis are related.

Anoikis is a form of apoptosis observed in tissue cells (except blood cells), and is a form of PCD triggered by the loss of cell adhesion to the ECM or inappropriate cell adhesion, with the aim of removing misplaced or dislodged cells under physiological or pathological conditions. It has an important role in organismal development, tissue homeostasis and tumor metastasis. Anoikis is closely associated with several types of cancer, liver disease, cardiomyopathy and other diseases (136-138). Of note, anoikis is commonly activated during tumorigenesis, which is one of the body's self-protective means of preventing tumor metastasis. In metastatic cancer, cancer cells acquire anoikis resistance through autocrine or paracrine activation of survival signals, alterations of integrin expression patterns and inhibition of death pathways, to regain the ability to attach and spread, which ultimately leads to invasion and metastasis (139-141). From an anti-anoikis perspective, normalizing ECM remodeling by targeting matrix-related enzymes (matrix metalloproteinases, lysyl oxidase and lysyl oxidase-like proteins) or ECM-associated cell-surface receptors (integrin, discoidin domain receptors and CD44), and the use of vitamin D may have significant therapeutic anti-tumor potential (138,142-144). Anoikis uses caspases as key signaling molecules and primarily involves the PI3K/Akt, integrin, MAPK, Wnt/β-catenin, TGF-β, Smad and NF-κB pathways, and is thus both distinct and related to PANoptosis.

8. Conclusions and future perspectives

PANoptosis has emerged as a novel and distinct form of PCD, integrating elements from apoptosis, pyroptosis and necroptosis. This convergence is epitomized in the PANoptosome complex, a sophisticated entity that coordinates the synchronous activation of these diverse cell death pathways, thereby enriching the contemporary understanding of cellular demise. In delineating the specific pathways of apoptosis, pyroptosis and necroptosis, PANoptosis stands out for its amalgamation of these mechanisms. It presents a dynamic interplay involving caspases, GSDMs, RIPKs and other molecular actors, highlighting a complex network of interactions. This understanding highlights novel avenues for therapeutic interventions, particularly in diseases characterized by immune responses and inflammation.

However, the current understanding of PANoptosis in the context of CVDs is still limited and much remains to be explored regarding the molecular intricacies and clinical applications. Therefore, the promise of PANoptosis in CVDs is substantial. It offers a comprehensive framework for understanding complex pathologies such as MI and PH, where multiple forms of cell death coexist. Importantly, PANoptosis underscores the inflammatory aspects of CVDs, such as AS, providing potential anti-inflammatory strategies. The PANoptosis pathway involves apoptotic proteins, inflammasomes and necroptotic elements, hinting at a complex regulatory network that could be pivotal in cardiovascular pathologies. For example, the pro-inflammatory aspects of PANoptosis, as observed in the recruitment of RIPK3 and caspase-8, may contribute to the characteristic inflammatory milieu observed during atherosclerotic plaque formation or in the response to infarction in myocardial tissues. Similarly, the involvement of the type I interferon signaling pathway components within the PANoptosis mechanism may highlight a role in the modulation of immune responses in HF or PH. Future research should focus on delineating the roles of PANoptosis in these states, its dynamic interplay with traditional cell death pathways, and its regulatory mechanisms. Understanding PANoptosis in CVDs could open new avenues for therapeutic interventions. As an emerging field, PANoptosis has the potential to significantly impact biomedical research, offering deeper insight into the complexities of cell death and novel therapeutic prospects for various diseases, including those affecting the cardiovascular system.

In conclusion, PANoptosis represents a significant advance in our understanding of cell death, it is essential to

acknowledge certain limitations within this review. The interplay between PANoptosis and other forms of cell death, such as ferroptosis and autophagy, is currently unclear. Recent findings (145) suggest that the ferroptosis inhibitor liproxstatin-1 may have a protective role against steatosis and steatohepatitis in metabolic dysfunction-associated fatty liver disease mice by potentially regulating PANoptosis, implying a plausible link between ferroptosis and PANoptosis. Considering these recent discoveries, further studies could investigate the potential connections between PANoptosis and these additional mechanisms, elucidating whether they intersect or operate independently in various disease contexts. Consequently, advancing our comprehension of cell death mechanisms, including the interplay between different forms, stands as a crucial direction for future research in the field. Its potential to revolutionize diagnostics, inspire innovative therapeutic strategies and tailor treatments to individual needs positions it as a pivotal area for future exploration.

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Authors' contributions

WL and XG conceived and designed the review. XG wrote the manuscript. XG, SL and CM assisted with the drawings. MC, SL, CM, YH and WL reviewed and edited the manuscript. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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