Review

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Metabolism-Gated Cell Death Switching in Myocardial Ischemia-Reperfusion Injury: From Regulated Death Networks to Precision Cardioprotection

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Myocardial ischemia–reperfusion injury (MIRI) is driven by dynamic metabolic disturbances that extend beyond adenosine triphosphate depletion and encompass mitochondrial dysfunction, redox imbalance, calcium overload, iron dysregulation, lipid peroxidation, and inflammatory activation. These alterations regulate multiple forms of programmed cell death, including apoptosis, necroptosis, ferroptosis, pyroptosis, and mitochondrial permeability transition pore-dependent necrosis. However, these pathways are frequently investigated in isolation, despite sharing common upstream metabolic triggers and interacting through overlapping signaling networks and execution mechanisms. This conceptual narrative review summarizes current evidence regarding how metabolic states influence the activation thresholds, temporal dynamics, and relative contributions of regulated cell-death pathways during myocardial ischemia and reperfusion. We propose a metabolism-gated framework in which cellular energy status, mitochondrial integrity, redox homeostasis, calcium burden, iron availability, lipid composition, and inflammatory priming collectively determine context-dependent susceptibility and dominance of specific death pathways. Autophagy and mitophagy are considered regulators of metabolic adaptation and mitochondrial quality control rather than independent cell-death modalities. Current evidence indicates extensive pathway co-activation and crosstalk, with the predominant mechanisms varying according to reperfusion stage, myocardial region, cell type, metabolic background, and experimental model. In contrast, definitive switching between terminal death pathways within the same cell population remains insufficiently established. Functional pathway switching should therefore be distinguished from concurrent expression of pathway-associated markers or shared upstream signaling events and should be demonstrated through time-resolved multi-pathway analyses, selective genetic or pharmacological inhibition of an initially activated pathway, compensatory activation of an alternative execution pathway, and greater protection achieved through combined inhibition compared with single-pathway targeting. This framework further emphasizes the limitations of attributing myocardial injury to a single death mechanism based solely on pathway-associated biomarkers. Integrating metabolic profiling with cell-type-specific and spatially resolved assessments of death-pathway execution may enable more precise mechanistic classification and support the development of rational combination therapies. Thus, metabolism-gated pathway selection and interpathway crosstalk represent a testable framework for elucidating the heterogeneity of MIRI and for designing context-dependent cardioprotective strategies.

Keywords: MIRI; metabolism-gated cell death switching; regulated cell death; metabolic reprogramming; ferroptosis; precision cardioprotection

Introduction

Timely reperfusion through percutaneous coronary intervention (PCI) remains the most effective strategy for salvaging ischemic myocardium after acute myocardial infarction (AMI); however, the restoration of oxygenated blood flow can paradoxically trigger myocardial ischemia–reperfusion injury (MIRI) [1,2]. MIRI is clinically significant because it contributes to infarct expansion, microvascular obstruction (MVO), arrhythmogenesis, adverse ventricular remodeling, heart failure development, and unfavorable long-term outcomes. Reperfusion-induced damage may account for up to 50% of the final infarct size in experimental studies [3,4]. MVO occurs in approximately 40–60% of patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI, while intramyocardial hemorrhage (IMH) is observed in a substantial proportion of these patients [5]. The reported incidence varies depending on the imaging techniques and diagnostic criteria applied. Importantly, the extent of MVO has been independently associated with adverse clinical outcomes, including mortality (adjusted hazard ratio [HR], 1.14 per 1% absolute increase in MVO; 95% confidence interval [CI], 1.09–1.19) and hospitalization for heart failure (adjusted HR, 1.08 per 1% increase; 95% CI, 1.05–1.12) at 1 year [5]. Despite advances in contemporary

reperfusion strategies, the pooled 1-year mortality rate following PCI for STEMI remains approximately 7–12% [6]. The underlying pathophysiology begins during ischemia, when oxygen deprivation suppresses oxidative phosphorylation and shifts cardiomyocytes toward inefficient anaerobic glycolysis, resulting in adenosine triphosphate (ATP) depletion, lactate accumulation, intracellular acidosis, and ion-pump dysfunction. Reperfusion subsequently restores oxygen and substrate availability abruptly to metabolically compromised cells, leading to excessive reactive oxygen species (ROS) generation, calcium overload, pH normalization, mitochondrial dysfunction, endothelial barrier disruption, sterile inflammation, and cell death [2,7].

Cell death during MIRI was previously regarded primarily as a passive necrotic process; however, it is now recognized as a regulated and highly interconnected network phenomenon. Ferroptosis, pyroptosis, necroptosis, apoptosis, autophagy-associated injury, and mitochondrial permeability transition pore (mPTP)-dependent necrosis all occur in the reperfused myocardium and share common upstream stressors, including ROS accumulation, calcium dyshomeostasis, mitochondrial injury, endoplasmic reticulum stress, lipid remodeling, iron dysregulation, ATP depletion, and inflammatory danger signals [8,9]. This extensive overlap helps explain why inhibition of a single terminal pathway may produce substantial protective effects in young, healthy animal models but fail to achieve consistent clinical benefits [10,11]. Furthermore, metabolic reprogramming provides a unifying framework for understanding this interconnected cell-death network. The ATP/adenosine diphosphate (ADP) ratio, xidized nicotinamide adenine dinucleotide $(NAD)^+$/reduced nicotinamide adenine dinucleotide (NADH) balance, glutathione availability, iron bioavailability, lipid peroxidation substrate abundance, mitochondrial membrane potential, AMP-activated protein kinase (AMPK)–mechanistic target of rapamycin (mTOR) signaling activity, and inflammatory priming collectively influence whether oxidative and ionic stress remains reversible or surpasses the threshold for activation of ferroptosis, pyroptosis, necroptosis, apoptosis, or mitochondrial necrosis [12,13]. Therefore, the predominant cell-death modality is not predetermined but is instead governed by the metabolic state of individual cell types at specific stages following reperfusion.

This framework is particularly relevant for translational research. Experimental MIRI studies are often conducted in young, healthy animals without comorbid conditions, whereas patients with AMI are typically older and frequently present with diabetes, obesity, hypertension, dyslipidemia, endothelial dysfunction, chronic inflammation, and exposure to multiple medications [11,14]. These clinical factors profoundly reshape cardiac metabolism and modify the thresholds governing regulated cell death (RCD) execution. Diabetes may reduce the threshold for ferroptosis activation by increasing mitochondrial ROS production and disrupting antioxidant capacity as well as iron–lipid homeostasis; aging may enhance susceptibility to mPTP opening, mitochondrial DNA release, inflammasome activation, and impaired mitophagy; and dyslipidemia may increase the availability of peroxidizable lipid substrates [14,15]. Furthermore, cellular heterogeneity within the myocardium adds additional complexity to therapeutic targeting, as cardiomyocytes, endothelial cells, immune cells, platelets, and fibroblasts respond differently to metabolic and inflammatory signals [16]. Post-transcriptional and epigenetic mechanisms introduce an additional regulatory layer in determining cell-death pathway activation and potential switching. MicroRNAs, circular RNAs, long non-coding RNAs, m6A modifications, selective translation, ubiquitination, and deubiquitination regulate key processes, including glutathione peroxidase 4 (GPX4) stability, NOD-like receptor family pyrin domain-containing 3 (NLRP3) activation, receptor-interacting protein kinase (RIPK) signaling, autophagic flux, and mitochondrial quality control [17,18]. In parallel, metabolites such as acetyl-coenzyme A (acetyl-CoA), α-ketoglutarate, S-adenosylmethionine (SAM), NAD^+, and lactate function as substrates or cofactors for chromatin- and RNA-modifying enzymes, thereby linking metabolic stress to sustained RCD-related gene expression and epigenetic memory [19,20].

Here, we reinterpret major RCD pathways in MIRI through the concept of metabolism-gated cell-death switching (Fig. 1). Unlike previous reviews that primarily catalogue individual cell-death pathways, this review presents an integrative framework to explain how shared metabolic checkpoints regulate the activation thresholds, relative dominance, and potential transitions among distinct death programs during ischemia and reperfusion. Specifically, we identify ATP reserves, mitochondrial redox status, calcium burden, iron availability, lipid peroxidation capacity, antioxidant defenses, and inflammatory priming as key upstream determinants that govern the activation of ferroptosis, pyroptosis, necroptosis, apoptosis, autophagy-associated injury, and mPTP-dependent necrosis. We further emphasize that cell-death switching is spatially, temporally, and cell-type dependent, involving cardiomyocytes, endothelial cells, immune cells, fibroblasts, and the microvascular compartment, and is substantially influenced by comorbid conditions such as diabetes, obesity, dyslipidemia, aging, and endothelial dysfunction. Finally, we extend this framework toward precision cardioprotection by integrating biomarker-defined death states, comorbidity-associated susceptibility, therapeutic timing, cell-type-specific targeting, and rational combination strategies with dominant injury phenotypes.

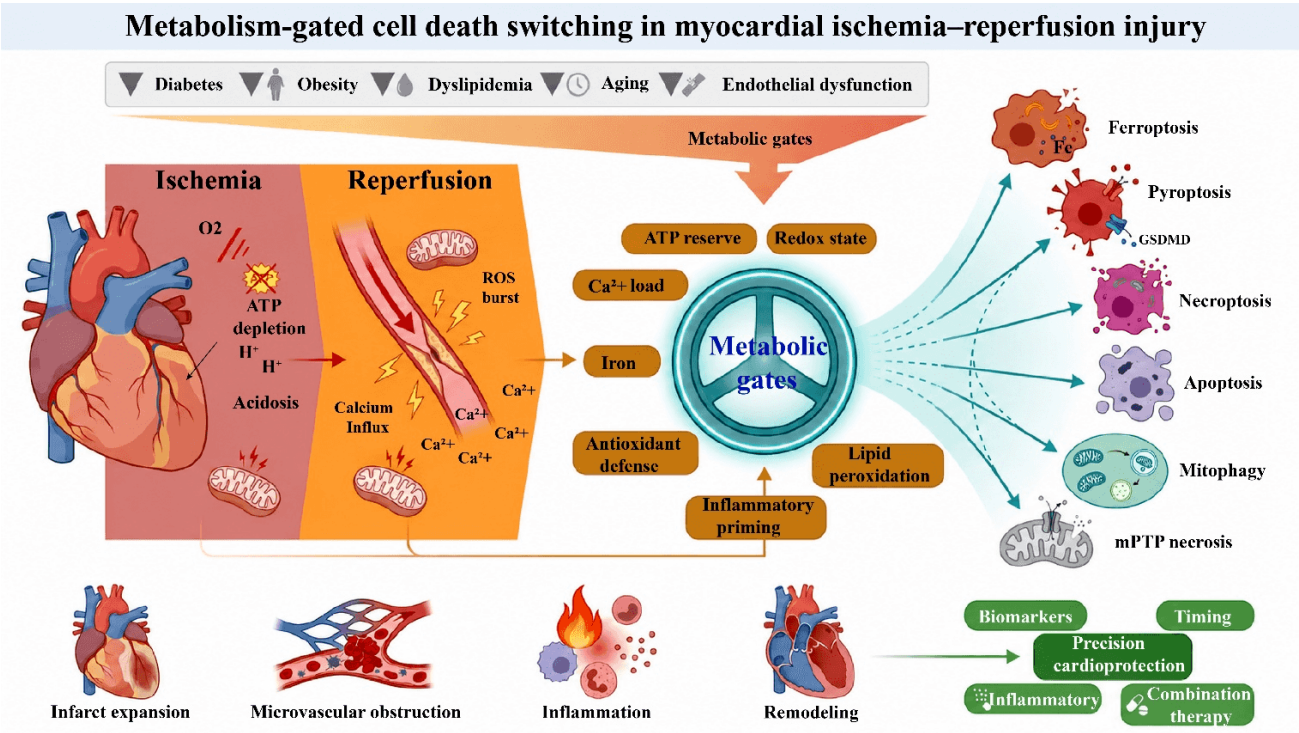

Fig. 1. Overall framework of metabolism-gated cell death switching in MIRI. During ischemia, interruption of coronary blood flow results in oxygen deprivation, suppression of mitochondrial oxidative phosphorylation, ATP depletion, intracellular acidosis, Ca^{2+} accumulation, and progressive mitochondrial dysfunction. Subsequent reperfusion restores oxygen delivery but simultaneously triggers a rapid burst of reactive oxygen species (ROS), Ca^{2+} overload, lipid peroxidation, inflammatory activation, and mitochondrial permeability transition pore (mPTP) opening. These ischemic and reperfusion-induced stresses converge on key metabolic checkpoints, including ATP reserves, mitochondrial redox status, Ca^{2+} burden, iron availability, lipid peroxidation susceptibility, antioxidant capacity, and inflammatory priming. The integrated state of these metabolic checkpoints determines the activation threshold, relative dominance, and potential transition among distinct regulated cell-death programs, including ferroptosis, pyroptosis, necroptosis, apoptosis, and mPTP-dependent necrosis. Mitophagy acts as a context-dependent regulatory mechanism that may either eliminate damaged mitochondria and limit injury or, when impaired or excessively activated, reshape the balance among competing cell-death pathways. Comorbidities such as diabetes, obesity, dyslipidemia, aging, and endothelial dysfunction further modify the metabolic landscape and reset cell-death thresholds, thereby contributing to infarct expansion, MVO, inflammatory injury, and adverse ventricular remodeling. Biomarker-guided patient stratification, optimized therapeutic timing, cell-type-specific targeting, and rational combination therapies may facilitate precision cardioprotection tailored to the predominant metabolic and cell-death phenotype. The figure was created using Adobe Illustrator 2025 (version 29.0) (Adobe Inc., San Jose, CA, USA). ATP, adenosine triphosphate; Ca^{2+}, calcium ion; MIRI, myocardial ischemia–reperfusion injury; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; MVO, microvascular obstruction.

Metabolic Stress Axes That Gate Regulated Cell Death in MIRI

Ischemic Metabolic Collapse as a Priming Phase

During ischemia, interruption of coronary blood flow deprives the myocardium of oxygen and metabolic substrates, resulting in rapid inhibition of mitochondrial oxidative phosphorylation. Anaerobic glycolysis provides limited ATP generation but leads to lactate accumulation, intracellular acidosis, depletion of high-energy phosphates, and progressive impairment of Na^+/K^+-ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase function [10,21]. Activation of the Na^+/H^+ exchanger and reverse-mode Na^+/Ca^{2+} exchange further promotes intracellular sodium and calcium accumulation. These alterations do not immediately determine a specific cell-death pathway; rather, they prime the reperfused myocardium by reducing ATP reserves, increasing calcium burden, weakening antioxidant capacity, and sensitizing mitochondria to mPTP opening after pH normalization [22,23]. The extent of ischemic priming influences subsequent cell-death pathway activation. Severe ATP depletion and phosphate accumulation favor mPTP-dependent necrosis, whereas preserved ATP availability with moderate mitochondrial injury may permit caspase-dependent apoptosis. In contrast, glutathione depletion and disrupted lipid–iron metabolism promote ferroptosis, while mitochondrial damage-associated molecular patterns (DAMPs) contribute to inflammatory cell-death priming.

Thus, ischemia establishes a metabolic vulnerability landscape that is subsequently revealed during reperfusion.

Lactate accumulation during ischemia may directly contribute to cell-death pathway regulation through epigenetic reprogramming, in addition to its established role in bioenergetic failure. Histone and non-histone protein lactylation, a recently identified post-translational modification that links metabolic status to transcriptional and functional reprogramming, utilizes lactate as its substrate [24,25]. Emerging studies mapping the lactylation landscape in the ischemic heart suggest that lactate-driven lactylation may alter cell-death thresholds even before reperfusion begins. In hypoxic cardiomyocytes, lactylation of phosphofructokinase platelet type at lysine 688 (PFKP-K688la) enhances PFKP enzymatic activity and glycolytic flux while suppressing mitochondrial respiration. This adaptive lactate–PFKP–glycolysis feedback loop improves cardiomyocyte survival and contractile function under hypoxic conditions, indicating that specific lactylation events may transiently maintain energy production during ischemia. Conversely, other lactylation events may exacerbate ionic imbalance, mitochondrial dysfunction, ferroptotic stress, and inflammatory injury. In cardiomyocytes subjected to hypoxia/reoxygenation, enhanced glycolysis promotes histone lactylation at the transient receptor potential melastatin 7 (TRPM7) promoter, increasing TRPM7 transcription and contributing to Ca^{2+} overload and cell death [26,27]. Furthermore, lactate induces mitochondrial calcium uptake family member 3 (MICU3) expression and promotes mitochondrial Ca^{2+} uptake, mitochondrial dysfunction, and neutrophil extracellular trap formation, thereby aggravating microvascular injury through histone H3 lysine 18 (H3K18) lactylation at the MICU3 promoter in neutrophils [28]. Several key cell-death regulators are directly modulated by protein lactylation. acyl-CoA synthetase long-chain family member 4 (ACSL4) lactylation at lysine 83 increases its protein stability and promotes ferroptosis; GPX4 lactylation at K218 and K228 suppresses its antioxidant activity, thereby lowering the threshold for ferroptotic activation; and NLRP3 lactylation at K245 enhances its stability, facilitating inflammasome assembly and pyroptosis [29,30,31]. In contrast, Serpina3k lactylation at lysine 351 increases protein stability, and Serpina3k secreted by cardiac fibroblasts protects cardiomyocytes against reperfusion-induced cell death through paracrine signaling [32].

Together, these findings demonstrate that ischemia-induced lactate accumulation extends beyond ATP depletion and actively reshapes the transcriptional and post-translational landscape through lactylation, establishing a metabolic–epigenetic memory that may alter the thresholds for mitochondrial injury, ferroptosis, pyroptosis, and apoptosis during reperfusion. By targeting specific lactylation events rather than lactate itself, the lactate–lactylation network represents an additional layer of metabolic regulation that operates upstream of classical cell-death pathways and may provide new therapeutic opportunities. Representative lactylation events that may influence metabolic gating and RCD thresholds in MIRI are summarized in **Supplementary Table 1**.

Reperfusion ROS and Redox Disequilibrium

Reoxygenation is essential for tissue salvage but simultaneously induces an early ROS burst originating from the dysfunctional electron transport chain, reverse electron transport at complex I, xanthine oxidase, NADPH oxidases, uncoupled nitric oxide synthase, and activated inflammatory cells [33]. ROS can damage lipids, proteins, nucleic acids, and mitochondrial membranes; however, its role extends beyond direct cytotoxicity. Instead, ROS functions as a signaling hub that connects metabolic stress with ferroptosis, pyroptosis, necroptosis, apoptosis, and mitochondrial necrosis [34,35,36,37]. The consequences of ROS accumulation are highly context dependent. In iron-enriched, polyunsaturated fatty acid (PUFA)-rich, GPX4-deficient cells, ROS promotes lipid peroxidation and ferroptotic cell death. In inflammasome-primed cells, ROS cooperates with potassium efflux, lysosomal disruption, mitochondrial DNA release, and cardiolipin exposure to activate NLRP3 signaling and pyroptosis [35,38]. In cells characterized by calcium overload and RIPK3 activation, ROS promotes calcium/calmodulin-dependent protein kinase II (CaMKII) oxidation and mPTP opening, thereby linking necroptotic signaling to mitochondrial collapse [36]. Thus, the same reperfusion-induced ROS burst can generate distinct cell-death phenotypes depending on antioxidant capacity, iron–lipid status, mitochondrial integrity, and inflammatory priming.

Calcium Overload, Mitochondrial Dysfunction, and mPTP Opening

Calcium overload represents a central metabolic–ionic checkpoint in MIRI. During ischemia, ATP depletion and intracellular acidosis impair calcium homeostasis; upon reperfusion, pH normalization and sodium–calcium exchange promote rapid calcium influx. Mitochondria subsequently sequester excess calcium through the mitochondrial calcium uniporter, and when buffering capacity is exceeded, calcium interacts with ROS, phosphate accumulation, and adenine nucleotide depletion to induce mPTP opening [22]. Sustained mPTP opening dissipates mitochondrial membrane potential, disrupts oxidative phosphorylation, suppresses ATP production, promotes osmotic swelling, and facilitates the release of pro-death and pro-inflammatory signals [22,39]. The threshold for mPTP opening is dynamic and influenced by NAD^+/NADH balance, ATP/ADP ratio, phosphate concentration, calcium burden, redox status, and mitochondrial membrane lipid integrity. Therefore, mPTP is not simply a terminal mitochondrial lesion; rather, it functions as a metabolic decision node that determines whether cells recover, undergo

apoptosis, or progress toward necrotic rupture. Detailed methodological considerations for evaluating mPTP opening, unresolved questions regarding its molecular identity, and translational challenges associated with mPTP-targeted cardioprotection are provided in **Supplementary Note 1** and **Supplementary Table 2**.

Lipid, Iron, Antioxidant, and Inflammatory Gates

Ferroptosis is strongly regulated by lipid metabolism, iron homeostasis, and antioxidant capacity. Reperfusion supplies oxygen required for lipid peroxidation, while ischemia–reperfusion stress disrupts lipid remodeling and increases the vulnerability of PUFA-containing phospholipids to oxidative damage. ACSL4 and lysophosphatidylcholine acyltransferase 3 (LPCAT3) promote the enrichment of membranes with oxidizable PUFA phospholipids, whereas labile iron accelerates radical generation through Fenton chemistry [40,41,42]. When system Xc^- activity, glutathione synthesis, GPX4 function, or compensatory antioxidant mechanisms become insufficient, lipid peroxide accumulation drives ferroptotic membrane damage. Sterile inflammation and microvascular injury further extend these death networks beyond cardiomyocytes. Injured cardiomyocytes and mitochondria release DAMPs, including mitochondrial DNA, high-mobility group box 1 (HMGB1), ATP, cardiolipin, and extracellular matrix fragments, thereby activating pattern-recognition receptors and engaging complement pathways, endothelial cells, platelets, neutrophils, macrophages, and fibroblasts [43,44]. Endothelial pyroptosis or ferroptosis compromises barrier integrity and contributes to no-reflow, whereas neutrophil-derived ROS and proteases further amplify oxidative injury [42]. Once microvascular perfusion is impaired, regional hypoxia persists despite restoration of epicardial blood flow, creating a vicious cycle that reinforces metabolic stress and RCD activation.

Together, ischemic metabolic collapse, reperfusion-induced ROS generation, calcium overload, mitochondrial dysfunction, lipid–iron imbalance, antioxidant failure, sterile inflammation, and microvascular injury constitute an integrated stress-axis network. The predominant cell-death pathway is determined by the relative intensity and interaction of these metabolic stress axes. Severe ATP depletion and sustained mPTP opening favor mitochondrial necrosis; GPX4 dysfunction, iron overload, PUFA enrichment, and lipid peroxidation promote ferroptosis; DAMP release, NLRP3 activation, and gasdermin cleavage facilitate pyroptosis; loss of caspase-8-mediated restraint accompanied by RIPK1/RIPK3 activation promotes necroptosis; and moderate mitochondrial injury with preserved ATP availability supports apoptosis [13,45]. The major metabolic stress axes that regulate RCD activation in MIRI are summarized in Table 1 (Ref. [10,12,22,33,37,42,43,45,46,47,48,49,50,51,52,53,54,55,56,57,58]), and Fig. 2.

Regulated Cell Death Modules and Switching Boundaries

Ferroptosis

Ferroptosis is an iron-dependent form of regulated cell death driven by lipid peroxidation. In the reperfused heart, ferroptotic execution becomes metabolically enabled when three conditions coexist: expansion of the labile iron pool, accumulation of oxidizable PUFA-containing phospholipids, and insufficient detoxification of lipid peroxides by the GSH–GPX4 axis or compensatory antioxidant systems [46,47,59]. The same ROS burden may remain reversible in a redox-buffered cell but become lethal within an iron-enriched, GPX4-deficient microenvironment. Ferroptosis therefore represents a prototypical example of metabolism-gated cell-death regulation. However, the presence of enhanced lipid peroxidation or a single molecular signal alone is insufficient to establish ferroptosis as the dominant death mechanism in MIRI [48,60]. Accordingly, ferroptosis should be designated as the mode of cell death only when pathway-specific molecular, morphological, temporal, cell-type-specific, and functional lines of evidence converge—ideally supported by genetic or pharmacological rescue [49]. Changes in GPX4, ACSL4, iron levels, or lipid peroxidation products, when observed in isolation, should be interpreted as supportive rather than definitive evidence of causal ferroptotic death. Detailed evidence requirements are summarized in **Supplementary Table 3**.

Pyroptosis

Pyroptosis is a lytic inflammatory cell-death program characterized by inflammasome activation, gasdermin cleavage, membrane pore formation, and the release of IL-1β and IL-18. In MIRI, mitochondrial ROS, mitochondrial DNA, extracellular ATP, potassium efflux, lysosomal damage, cardiolipin exposure, and nuclear factor kappa B (NF-κB) priming collectively determine whether NLRP3 activation reaches the required threshold [35,61]. Pyroptosis serves as a link between organelle stress and multicellular sterile inflammation across cardiomyocytes, endothelial cells, macrophages, neutrophils, and fibroblasts. Robust identification of pyroptotic execution requires convergent evidence of gasdermin-mediated membrane injury, inflammasome activation, appropriate temporal and cellular localization, and pathway-specific rescue. Changes in NLRP3, caspase-1, IL-1β, IL-18, or other individual inflammatory markers alone should not be interpreted as definitive evidence of causal pyroptotic cell death [62,63]. Detailed evidence requirements are summarized in **Supplementary Table 3**.

Necroptosis

Necroptosis is primarily regulated by RIPK1, RIPK3, and mixed lineage kinase domain-like protein (MLKL). When caspase-8 remains active, death-receptor or in-

Table 1. Metabolic stress axes gating regulated cell death in MIRI.

Axis	Events	Gating	Phenotypes	Readouts	References
Ischemic collapse	ATP depletion; lactate accumulation; acidosis; Na^+/Ca^{2+} overload.	Lowers energy reserve; sensitizes mitochondria to mPTP opening.	mPTP necrosis; apoptosis; ferroptosis; inflammatory death.	ATP/ADP; phosphocreatine; lactate; pH; Na^+; Ca^{2+}.	[10,12,22,51]
Reperfusion ROS	Reoxygenation; RET; oxidase activation; mitochondrial ROS burst.	Outcome depends on redox, iron, mitochondria, and inflammation.	Ferroptosis; pyroptosis; necroptosis; mPTP necrosis.	mtROS; lipid ROS; 4-HNE; MDA; protein carbonyls.	[33,37,52,53]
Calcium/pH	Na^+ accumulation; Ca^{2+} influx; MCU uptake; rapid pH recovery.	Lowers mPTP threshold; accelerates mitochondrial collapse.	mPTP necrosis; necroptosis; apoptosis.	Cytosolic Ca^{2+}; mitochondrial Ca^{2+}; p-CaMKII; CypD; ΔΨm.	[22,50,52,54]
Lipid-iron imbalance	Labile iron; ferritinophagy; ACSL4/LPCAT3; PUFA peroxidation.	Oxidizable membranes exceed lipid-peroxide detoxification capacity.	Ferroptosis; endothelial injury; inflammatory amplification.	Labile iron; ACSL4; LPCAT3; PUFA-PLs; 4-HNE; MDA.	[47,49,55,58]
Antioxidant exhaustion	GSH depletion; GPX4 failure; impaired compensatory defenses.	Converts reversible oxidation into irreversible membrane damage.	Ferroptosis; mitochondrial injury; inflammatory death.	GSH/GSSG; GPX4; SLC7A11; FSP1; CoQ10; DHODH; Nrf2.	[46,48,49,56]
Inflammation/microvascular stress	DAMP release; complement activation; leukocyte and endothelial activation.	Amplifies inflammasomes, no-reflow, hypoxia, and secondary oxidative stress.	Pyroptosis; necroptosis; endothelial death; PANoptosis-like convergence.	IL-1β; IL-18; NLRP3; GSDMD; p-MLKL; mtDNA; MVO.	[42,43,45,57]

ATP, adenosine triphosphate; ADP, adenosine diphosphate; ROS, reactive oxygen species; RET, reverse electron transport; mtROS, mitochondrial reactive oxygen species; Na^+, sodium ion; Ca^{2+}, calcium ion; MCU, mitochondrial calcium uniporter; mPTP, mitochondrial permeability transition pore; ΔΨm, mitochondrial membrane potential; ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; PUFA, polyunsaturated fatty acid; GSH, reduced glutathione; GSSG, oxidized glutathione; GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; FSP1, ferroptosis suppressor protein 1; CoQ10, coenzyme Q10; DHODH, dihydroorotate dehydrogenase; Nrf2, nuclear factor erythroid 2-related factor 2; DAMP, damage-associated molecular pattern; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; CaMKII, calcium/calmodulin-dependent protein kinase II; IL-1β, interleukin-1 beta; NLRP3, NOD-like receptor family pyrin domain-containing 3; GSDMD, gasdermin D; p-MLKL, phosphorylated mixed lineage kinase domain-like protein; mtDNA, mitochondrial DNA.

nate immune signaling generally favors apoptosis or cell survival; however, when caspase-8-mediated restraint is disrupted, RIPK1/RIPK3 signaling promotes MLKL-dependent membrane rupture [64,65,66]. In cardiomyocytes, necroptosis is further linked to mitochondrial injury through RIPK3–CaMKII–mPTP signaling, positioning this pathway at the interface between inflammatory receptor signaling and metabolic collapse [36,67]. Evidence beyond the detection of phosphorylated RIPK3 or MLKL alone is required to establish necroptosis as a functional mechanism in MIRI. Functional attribution of necroptosis requires convergent evidence of RIPK1/RIPK3/MLKL execution, appropriate temporal and cellular localization, and protection following pathway-selective genetic or pharmacological intervention [9,68,69,70]. Changes in RIPK1, RIPK3, or MLKL abundance alone are insufficient to establish causal necroptotic death. Detailed evidence requirements are summarized in **Supplementary Table 3**.

Apoptosis

Apoptosis is a caspase-dependent form of regulated cell death characterized by mitochondrial outer membrane permeabilization, cytochrome c release, apoptosome formation, and activation of caspase-9 and caspase-3 [71,72]. Its execution requires adequate ATP availability and relatively preserved mitochondrial integrity. In contrast, severe ATP depletion, calcium overload, and sustained mPTP opening shift cellular fate toward mitochondrial-dependent necrosis [73]. Causal attribution of apoptosis requires convergent morphological and molecular evidence of apoptotic execution together with temporal, cell-type-specific, and functional validation [74,75]. TUNEL positivity, an altered BAX/BCL-2 ratio, or changes in a single caspase-associated marker alone are insufficient to establish apoptosis as the dominant mechanism of myocardial injury. Detailed evidence requirements are summarized in **Supplementary Table 3**.

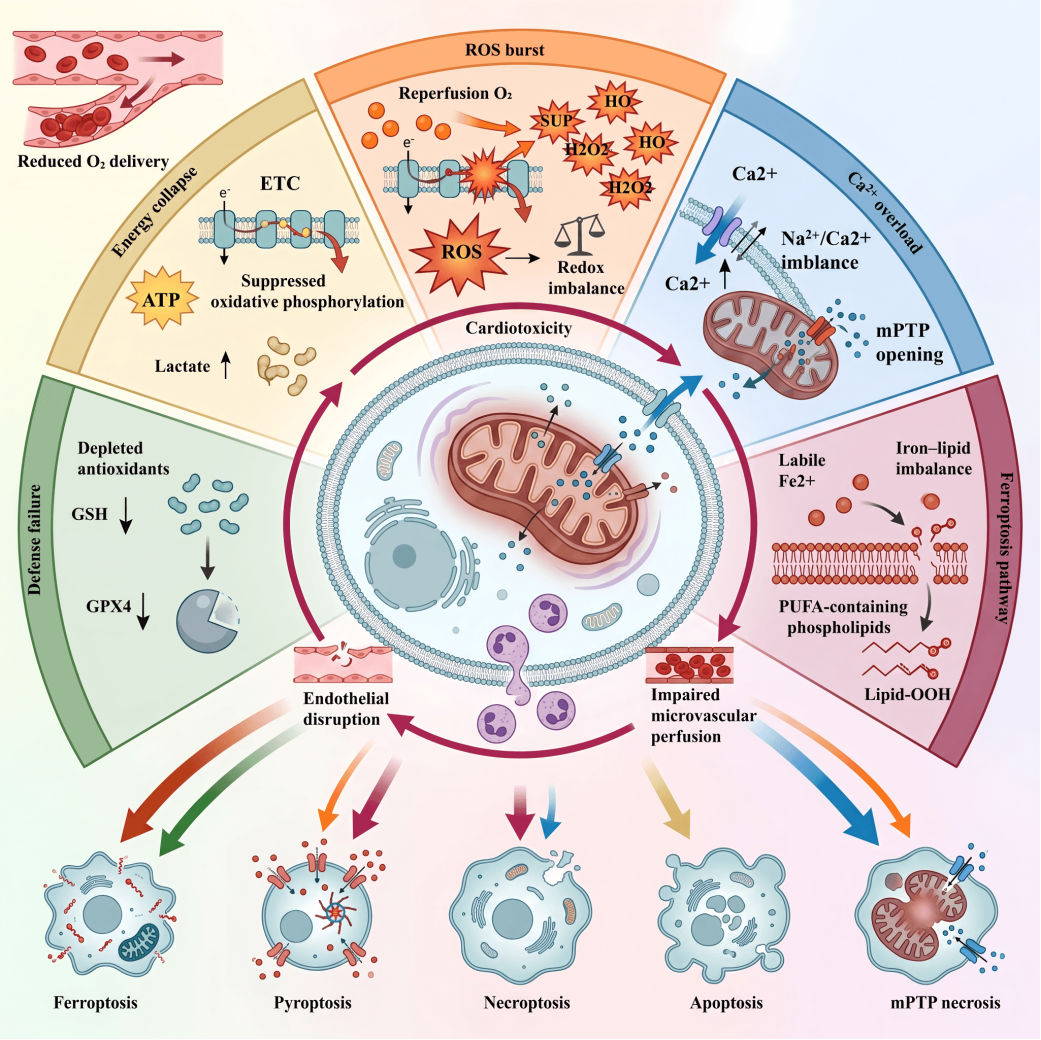

Fig. 2. Metabolic stress axes that gate regulated cell death in MIRI. During ischemia, reduced oxygen delivery suppresses mitochondrial electron transport and oxidative phosphorylation, leading to ATP depletion, lactate accumulation, and progressive energy failure. Upon reperfusion, rapid reoxygenation promotes electron leakage from the mitochondrial electron transport chain and triggers a burst of reactive oxygen species (ROS), thereby disrupting cellular redox homeostasis. Concurrent Na^{+}/Ca^{2+} imbalance induces cytosolic and mitochondrial Ca^{2+} overload, which further exacerbates mitochondrial dysfunction and facilitates mPTP opening. In parallel, glutathione depletion and reduced glutathione peroxidase 4 (GPX4) activity impair antioxidant defenses, whereas increased labile Fe^{2+} availability and enrichment of polyunsaturated fatty acid (PUFA)-containing phospholipids promote lipid hydroperoxide accumulation and ferroptotic injury. These interconnected metabolic disturbances converge to drive mitochondrial damage, cardiomyocyte injury, endothelial dysfunction, inflammatory-cell recruitment, and impaired microvascular perfusion. The relative intensity, temporal dynamics, and interaction of these metabolic disturbances determine the activation thresholds and relative dominance of ferroptosis, pyroptosis, necroptosis, apoptosis, and mPTP-dependent necrosis during myocardial ischemia–reperfusion injury. In the figure, ↑ and ↓ indicate increased/upregulated and decreased/downregulated levels or activity, respectively, whereas other arrows indicate the direction of the depicted processes or pathways. The figure was created using Adobe Illustrator 2025 (version 29.0) (Adobe Inc., San Jose, CA, USA). ATP, adenosine triphosphate; Ca^{2+}, calcium ion; ETC, electron transport chain; Fe^{2+}, ferrous iron; GPX4, glutathione peroxidase 4; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; Lipid-OOH, lipid hydroperoxide; MIRI, myocardial ischemia–reperfusion injury; mPTP, mitochondrial permeability transition pore; O_2, molecular oxygen; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species.

Autophagy and Mitophagy as Modulators

Autophagy and mitophagy are not considered independent cell-death modalities in this review; rather, they are discussed as context-dependent regulators of survival and RCD switching. These processes modulate the balance between cellular adaptation and injury during MIRI. Efficient and selective mitophagy limits ROS accumulation, DAMP release, and mPTP opening, whereas impaired autophagic flux, excessive mitochondrial elimi-

nation, or ferritinophagy-mediated iron release can amplify downstream cell-death pathways [76]. Determining the modulatory role of autophagy or mitophagy in MIRI requires evidence extending beyond alterations in microtubule-associated protein 1 light chain 3-II (LC3-II) or p62 abundance. Their functional contribution should be assessed by combining autophagic-flux measurements with cell-type-specific, genetic, pharmacological, and functional evidence [76,77]. Changes in LC3-II, p62, or other individual autophagy-associated markers alone cannot determine whether autophagy or mitophagy is protective, detrimental, or causally involved in MIRI. Detailed assessment criteria are summarized in **Supplementary Table 3**.

mPTP-Dependent Necrosis

Sustained mPTP opening induces a form of regulated necrosis in which mitochondrial inner membrane permeabilization dissipates ΔΨm, disrupts ATP synthesis, promotes osmotic swelling, and ultimately leads to plasma membrane rupture [22]. This pathway becomes predominant when ischemic priming is severe, ATP depletion is profound, and calcium overload exceeds the threshold required for pore opening. Unlike apoptosis, mPTP-dependent necrosis does not require caspase activation; instead, bioenergetic failure and disruption of ion homeostasis drive rapid cellular disintegration. Cyclosporin A and related cyclophilin D (CypD)-binding ligands can delay or attenuate this form of cell death in experimental models, supporting its pharmacological modifiability [22]. Establishing mPTP-dependent necrosis in MIRI requires evidence beyond ΔΨm loss or protection by cyclosporin A alone. Robust attribution of mPTP-dependent necrosis requires convergent evidence of sustained permeability transition, bioenergetic collapse, necrotic membrane failure, appropriate temporal and cellular localization, and protection following validated genetic or pharmacological manipulation of pore-regulatory mechanisms. Mitochondrial depolarization or a single molecular readout alone is insufficient to establish mPTP-dependent necrosis as a causal death mechanism [78]. Detailed evidence requirements are summarized in **Supplementary Table 3**.

Crosstalk and PANoptosis-Like Convergence

Because ROS, DAMPs, iron dyshomeostasis, lipid peroxidation, calcium overload, and ATP depletion can activate multiple cell-death pathways simultaneously, tissues frequently exhibit overlapping RCD signatures. GPX4 dysfunction may initiate ferroptotic signaling while oxidized lipids promote inflammasome activation; NLRP3 activation and gasdermin pore formation can further exacerbate ionic imbalance and mitochondrial injury; RIPK3–CaMKII signaling can link necroptosis with mPTP opening; and impaired caspase-8 activity can redirect apoptotic signaling toward necroptosis [64,66]. The concept of PANoptosis describes coordinated pyroptotic, apoptotic, and necroptotic signaling assembled through multiprotein PANoptosome complexes [64,79]. However, direct evidence demonstrating canonical PANoptosis in MIRI remains limited; therefore, the term "PANoptosis-like convergence" provides a more cautious interpretation. This concept acknowledges the co-activation and functional interplay among inflammatory cell-death programs without presuming the formation of a single unified molecular complex (Fig. 3). The major RCD modules, their metabolic switching boundaries, and potential therapeutic intervention nodes are summarized in Table 2 (Ref. [13,36,49,55,62,64,71,75,77,78,80,81,82,83,84,85]). Detailed pathway-specific evidence requirements are summarized in **Supplementary Table 3**. Representative evidence supporting or constraining the proposed metabolism-gated switching framework is summarized in **Supplementary Table 4**. Spatial and temporal contexts, together with cell-type-specific and comorbidity-associated susceptibility patterns, are further summarized in **Supplementary Tables 5,6**.

Ferroptosis as a Metabolically Licensed Death Program

In MIRI, ferroptosis is determined by a permissive metabolic state in which redox-active iron availability, oxidizable phospholipids, and insufficient lipid-peroxide detoxification converge, rather than by a single initiating trigger [59]. Because multiple RCD pathways share reperfusion-induced ROS accumulation, elevated lipid ROS levels, malondialdehyde, 4-hydroxynonenal, iron accumulation, and altered GPX4 expression, these individual changes may indicate ferroptosis-associated stress but do not establish ferroptosis as a primary driver of myocardial injury. Therefore, ferroptosis in MIRI should be evaluated by integrating evidence of pathway-specific execution with the underlying metabolic context and its demonstrable contribution to cardiac injury.

Integrated Iron–Lipid–Antioxidant Gating

Iron handling, membrane lipid composition, and antioxidant capacity collectively regulate the ferroptotic threshold. The labile iron pool can be expanded through nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy, while local oxidative stress is further exacerbated by mitochondrial iron dysregulation and impaired iron–sulfur cluster metabolism [46]. Concurrently, peroxidation-prone PUFA-containing phospholipids are enriched in cellular membranes through the actions of ACSL4 and LPCAT3 [30]. When the system Xc^-–GSH–GPX4 axis fails to eliminate phospholipid hydroperoxides, these pro-ferroptotic signals collectively become lethal [41,86]. Additional protective buffering is provided by GPX4-independent defense systems, including FSP1–CoQ10, DHODH, GCH1–BH4, and Nrf2-regulated antiox-

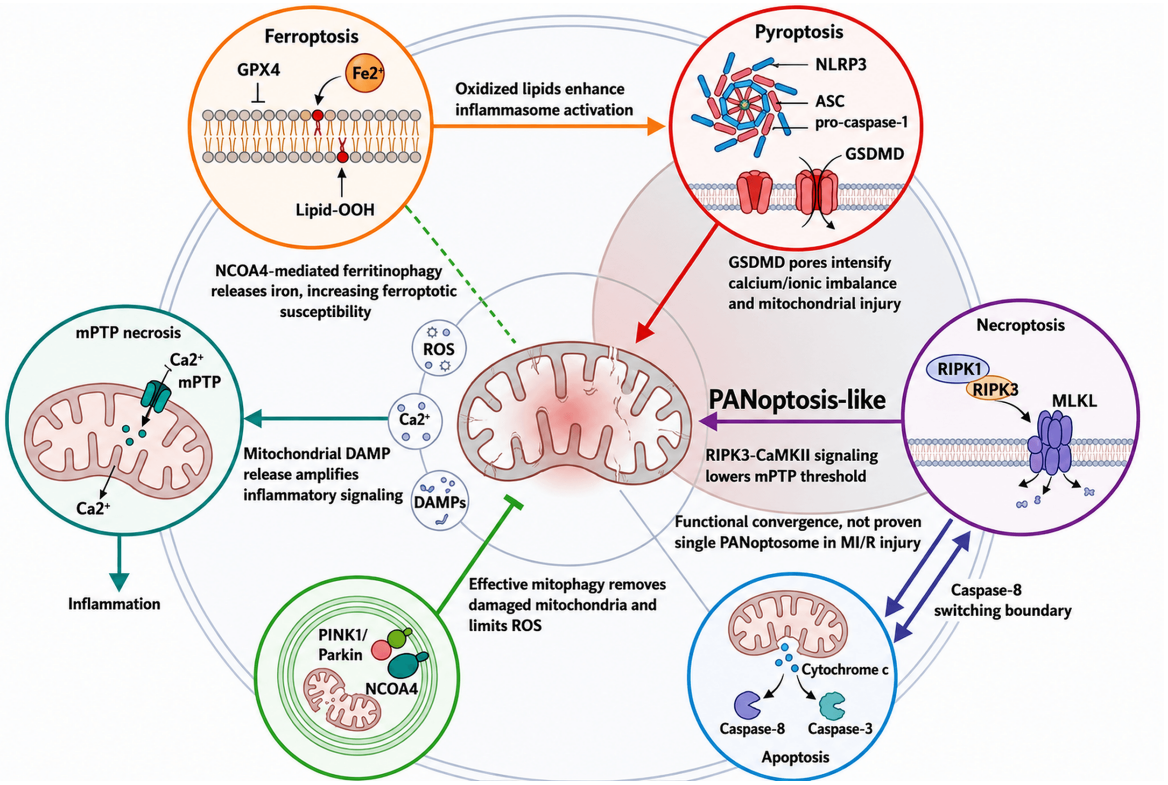

Fig. 3. Crosstalk among ferroptosis, pyroptosis, necroptosis, apoptosis, mitophagy, and mitochondrial permeability transition pore-dependent necrosis in myocardial ischemia–reperfusion injury. Reperfusion-induced reactive oxygen species (ROS), Ca^{2+} overload, mitochondrial injury, and damage-associated molecular pattern (DAMP) release serve as shared upstream signals that connect multiple regulated cell-death pathways. During ferroptosis, increased redox-active Fe^{2+} availability, glutathione peroxidase 4 (GPX4) dysfunction, and lipid hydroperoxide accumulation promote oxidative membrane damage, whereas oxidized lipids may further enhance NLRP3 inflammasome activation and facilitate pyroptotic signaling. Assembly of the NLRP3–ASC–pro-caspase-1 inflammasome promotes gasdermin D-mediated membrane pore formation, thereby aggravating ionic imbalance, Ca^{2+} overload, and mitochondrial injury. Necroptotic signaling mediated by RIPK1, RIPK3, and MLKL interacts with mitochondrial injury pathways, and RIPK3–CaMKII signaling may reduce the threshold for mitochondrial permeability transition pore opening. Caspase-8 serves as a critical regulatory boundary between apoptosis and necroptosis, whereas mitochondrial cytochrome c release and downstream caspase-3 activation contribute to apoptotic execution. mPTP opening induces mitochondrial depolarization and swelling, followed by the release of mitochondrial DAMPs and amplification of inflammatory signaling. Efficient PINK1/Parkin-mediated mitophagy eliminates damaged mitochondria and limits ROS accumulation; conversely, NCOA4-mediated ferritinophagy promotes intracellular iron release and may increase susceptibility to ferroptosis. The overlapping signaling region involving pyroptotic, apoptotic, and necroptotic pathways is termed "PANoptosis-like" to indicate functional convergence and pathway interaction rather than definitive evidence of a single canonical PANoptosome in myocardial ischemia–reperfusion injury. The figure was created using Adobe Illustrator 2025 (version 29.0) (Adobe Inc., San Jose, CA, USA). ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; Ca^{2+}, calcium ion; CaMKII, calcium/calmodulin-dependent protein kinase II; DAMPs, damage-associated molecular patterns; Fe^{2+}, ferrous iron; GPX4, glutathione peroxidase 4; GSDMD, gasdermin D; Lipid-OOH, lipid hydroperoxide; MLKL, mixed lineage kinase domain-like pseudokinase; mPTP, mitochondrial permeability transition pore; NCOA4, nuclear receptor coactivator 4; NLRP3, NOD-like receptor family pyrin domain-containing 3; PINK1, PTEN-induced kinase 1; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; ROS, reactive oxygen species.

idant and iron-handling pathways [46]. Therefore, rather than being determined by alterations in a single molecule, ferroptosis reflects the net balance between the availability of oxidizable substrates and multilayered detoxification capacity.

Mitochondrial, Inflammatory, and Clinical Context

Although ferroptotic membrane damage may further impair respiratory-chain function and reduce the threshold for mPTP opening, mitochondrial ROS production, calcium overload, and bioenergetic failure can also promote lipid peroxidation in cardiomyocytes [87,88]. Sim-

Table 2. Regulated cell death modules and intervention nodes in MIRI.

Module	Mechanism	Biomarkers	Targets	Crosstalk	Evidence	References
Ferroptosis	Iron-dependent phospholipid peroxidation with failed GPX4 defense.	GPX4; ACSL4; LPCAT3; labile iron; lipid peroxides.	Iron control; GPX4 restoration; lipid-peroxidation blockade.	Mitochondrial ROS and ferritinophagy amplify inflammasome activation.	III	[49,83]
Pyroptosis	Inflammasome activation drives caspase-1-GSDMD pore formation.	NLRP3; ASC; caspase-1; GSDMD; IL-1β; IL-18; mtDNA.	NLRP3/NEK7; caspase-1; GSDMD; IL-1 signaling; DAMP control.	GSDMD pores intensify ionic, mitochondrial, and inflammatory stress.	III	[62,84]
Necroptosis	RIPK1-RIPK3-MLKL activation after failed caspase-8 restraint.	p-RIPK1; p-RIPK3; p-MLKL; caspase-8; p-CaMKII.	RIPK1/RIPK3/MLKL; CaMKII; mPTP stabilization.	RIPK3-CaMKII lowers mPTP threshold; caspase-8 restrains necroptosis.	III	[36,80,81]
Apoptosis	Mitochondrial permeabilization with ATP-dependent caspase activation.	BAX/BAK; BCL-2/BCL-XL; cytochrome c; caspase-9/3.	Mitochondrial protection; BCL-2 balance; ATP recovery; efferocytosis.	ATP loss favors lytic death; caspase-8 restrains necroptosis.	II	[71,75]
Autophagy/mitophagy	Selective quality control becomes harmful when flux fails.	LC3 flux; p62; PINK1; Parkin; BNIP3; NCOA4.	Restore flux; promote mitophagy; limit ferritinophagy.	Mitophagy limits ROS; ferritinophagy increases labile iron.	III	[55,77]
mPTP necrosis	Sustained mPTP opening causes irreversible energetic collapse.	ΔΨm loss; CypD sensitivity; ATP/NAD^+; mtDNA; swelling.	Raise mPTP threshold; stabilize Ca^{2+}; preserve NAD^+/redox.	Lipid peroxidation and RIPK3-CaMKII amplify mitochondrial collapse.	II	[78,82,85]
PANoptosis-like	Concurrent pyroptotic, apoptotic, and necroptotic signaling.	NLRP3/GSDMD; caspase-3/8; RIPK3/MLKL; mtDNA; redox stress.	Combined inflammatory, mitochondrial, and redox-lipid targeting.	Canonical PANoptosome assembly remains unproven in MIRI.	IV	[13,64]

Evidence levels: Level II, translational evidence combining supportive human findings with reproducible *in vivo* validation; Level III, causal preclinical evidence based on pathway-selective genetic or pharmacological manipulation with functional outcomes; and Level IV, exploratory or conceptual evidence derived mainly from in vitro studies, isolated biomarkers, cross-disease extrapolation, or mechanistic integration without direct *in vivo* validation in MIRI. These levels indicate the directness, causal strength, and translational maturity of the evidence and do not represent a formal GRADE assessment. PINK1, PTEN-induced kinase 1; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma 2.

ilarly, membrane rupture caused by pyroptosis or necroptosis may generate pro-oxidant signals that enhance secondary ferroptotic injury, whereas oxidized phospholipids and lipid aldehydes can compromise endothelial integrity and further amplify inflammasome activation [89]. Therefore, ferroptosis may function as a secondary amplifier under certain conditions and as a dominant execution pathway in others. This distinction is particularly important in the context of myocardial injury with multiple concurrent stressors. The ferroptotic threshold can be reduced by alterations in iron availability, lipid composition, antioxidant capacity, and mitochondrial quality control associated with diabetes, obesity, dyslipidemia, aging, hypertension, and chronic inflammation [90,91]. Accordingly, patients whose injury is primarily driven by calcium–mPTP collapse or inflammasome activation may derive limited benefit from ferroptosis-targeted therapies compared with patients exhibiting convergent evidence of iron dyshomeostasis, phospholipid peroxidation, and impaired GPX4/GSH or compensatory antioxidant defenses. Thus, therapeutic development should consider target engagement, reperfusion timing, myocardial delivery, and the potential for compensatory pathway activation or switching following ferroptosis inhibition. The operational criteria for distinguishing pathway association, functional execution, dominance, and true death-pathway switching are discussed in the "Evidence Standards for Distinguishing Pathway Activation From Functional Cell-Death Execution" and further detailed in **Supplementary Note 2**. A detailed comparison of cancer-associated metabolic vulnerabilities and cardiomyocyte ferroptosis is provided in **Supplementary Note 3**, whereas the multicellular and microenvironmental dimensions of ferroptosis in the reperfused myocardium are discussed in **Supplementary Note 4**.

Inflammatory Death Switching: Pyroptosis, Necroptosis, and PANoptosis-Like Convergence

Pyroptosis and necroptosis are lytic inflammatory RCD subtypes that translate intracellular stress signals into extracellular cytokine release, DAMP signaling, leukocyte recruitment, endothelial dysfunction, and tissue-level injury. Unlike apoptosis, which generally maintains plasma membrane integrity until efferocytosis, these pathways directly disrupt membrane integrity and amplify sterile inflammation [43,45]. Although post-ischemic inflammation is often considered detrimental, it also plays essential roles in recruiting phagocytes, clearing necrotic cardiomyocytes and extracellular debris, initiating tissue repair, and promoting the formation of a stable infarct scar. Therefore, the pathological and therapeutic consequences of inflammatory cell death depend on its magnitude, timing, cellular origin, and relationship with the transition from acute injury toward inflammation resolution and tissue repair.

NLRP3 Inflammasome and Gasdermin Execution

NLRP3 is a major sterile-danger sensor in MIRI. Its activation requires an initial priming step, commonly mediated by NF-κB-dependent induction of NLRP3 and pro-IL-1β, followed by activation signals including mitochondrial ROS production, potassium efflux, calcium overload, lysosomal rupture, mitochondrial DNA release, cardiolipin exposure, and extracellular ATP accumulation [35,38,61]. Reperfusion simultaneously provides many of these activating stimuli. Therefore, NLRP3 activation represents not only an inflammatory response but also an indicator of mitochondrial dysfunction and metabolic failure. Caspase-1 activation cleaves gasdermin D (GSDMD), generating an N-terminal fragment that forms plasma membrane pores and enables the release of IL-1β, IL-18, and DAMPs [35]. Sustained pore formation promotes osmotic swelling, calcium influx, mitochondrial stress, and lytic cell death. The consequences of NLRP3–GSDMD activation are multicellular: cardiomyocyte pyroptosis reduces contractile cell mass, endothelial pyroptosis exacerbates vascular permeability and no-reflow, macrophage and neutrophil activation amplifies cytokine production, and fibroblast inflammatory signaling influences subsequent extracellular matrix remodeling [43,92]. At the tissue level, activation of the NLRP3–GSDMD axis may contribute to infarct expansion by promoting cardiomyocyte loss, disrupting endothelial barrier integrity, recruiting inflammatory leukocytes, and inducing microvascular dysfunction. Endothelial GSDMD pore formation may increase vascular permeability and tissue edema, facilitating neutrophil and platelet accumulation and thereby worsening MVO and no-reflow despite restoration of epicardial coronary blood flow. Persistent IL-1β and IL-18 signaling may further influence fibroblast activation, extracellular matrix turnover, and subsequent ventricular remodeling. These effects provide a key clinical rationale for targeting inflammasome-mediated injury in MIRI.

However, current human evidence remains largely associative. Circulating inflammatory biomarkers, including IL-1β, IL-18, IL-6, extracellular mitochondrial DNA, and related mediators, may reflect the severity of myocardial injury and systemic inflammation but do not conclusively demonstrate pyroptotic death in cardiomyocytes or endothelial cells. Their circulating levels can also be influenced by immune-cell activation, renal dysfunction, infection, metabolic comorbidities, and the extent of necrotic tissue injury. Therefore, elevated circulating cytokine levels should not be interpreted as direct evidence that pyroptosis represents the primary mechanism underlying myocardial cell death. Future clinical studies should integrate serial biomarker measurements with cardiac magnetic resonance imaging to assess infarct size, MVO, IMH, myocardial edema, ventricular remodeling, arrhythmia, heart failure hospitalization, and long-term cardiac function. When myocardial tissue samples are available, detailed analyses of inflammasome assembly, gasdermin cleavage, and cell membrane injury should be performed alongside evaluation of therapeutic responses targeting these pathways. The potential of selectively inhibiting NLRP3, caspase-1, or GSDMD to reduce reperfusion injury in humans without impairing subsequent tissue repair requires investigation in well-designed controlled studies. From the perspective of death-pathway switching, GSDMD represents an inflammatory execution node whose consequences depend on cellular metabolic capacity. Transient pore formation may remain partially reversible when membrane repair mechanisms and ATP availability are preserved; however, sustained pore formation under conditions of severe ROS accumulation, calcium overload, and mitochondrial injury can exceed the reversibility threshold and culminate in irreversible pyroptosis.

RIPK1/RIPK3/MLKL Necroptosis and Cardiac Mitochondrial Coupling

Necroptosis is primarily regulated by RIPK1, RIPK3, and MLKL. In canonical signaling, formation of the RIPK1/RIPK3 necrosome induces MLKL phosphorylation, oligomerization, membrane translocation, and eventual membrane rupture [65]. In MIRI, TNF signaling, TLR activation, DAMP release, ROS accumulation, and metabolic stress can activate this pathway. Its inflammatory consequences arise from the release of nucleic acids, ATP, mitochondrial components, and intracellular proteins, which stimulate immune-cell activation and endothelial responses [36]. The heart exhibits a distinctive necroptosis–mitochondria interface mediated through RIPK3–CaMKII–mPTP signaling [36,67]. Reperfusion-induced calcium overload activates CaMKII, while RIPK3 can further enhance CaMKII activity, aggravating calcium dysregulation and reducing the threshold for mPTP open-

ing. RIPK3 signaling through MLKL may promote a necroptotic phenotype, whereas enhanced RIPK3 coupling with CaMKII and sustained mPTP opening may shift injury toward mitochondrial bioenergetic collapse [80]. However, these downstream branches have not yet been demonstrated to represent mutually exclusive or quantitatively dominant death states *in vivo*. Their relative contribution is likely determined by calcium burden, ROS intensity, ATP availability, and mitochondrial stability. Caspase-8 represents another critical regulatory node. Active caspase-8 suppresses RIPK1/RIPK3 necrosome formation while permitting apoptotic signaling under conditions of sufficient bioenergetic support. Loss or inhibition of caspase-8 can promote RIPK3–MLKL activation in experimental systems [81]. However, in the reperfused heart, direct evidence demonstrating that caspase-8 manipulation can precisely convert apoptosis into necroptosis within the same cardiomyocyte population remains limited. Therefore, caspase-8 should be considered a branching regulator rather than a validated binary switch in MIRI. The clinical significance of necroptosis remains less clearly established than its experimental relevance. Because early reperfusion myocardial tissue is rarely accessible in humans, circulating RIPK3 or MLKL measurements alone cannot determine the cellular origin, temporal dynamics, or functional significance of necroptotic signaling [93]. Therefore, translational studies should not rely solely on elevated RIPK3 or MLKL levels as evidence of necroptosis but should incorporate phosphorylation status, membrane translocation, temporal analyses, pathway-specific interventions, imaging findings, and clinically relevant cardiac outcomes.

PANoptosis-Like Convergence and Therapeutic Implications

Severe reperfusion stress may simultaneously activate pyroptotic, apoptotic, and necroptotic signaling pathways. However, mechanistic interpretation of such overlap requires caution. The concurrent detection of NLRP3, caspases, RIPK3, MLKL, and gasdermins indicates the presence of overlapping RCD signals but does not, by itself, establish canonical PANoptosis. Demonstration of PANoptosome assembly and functional interdependence among these death pathways is required before PANoptosis can be definitively assigned [64,79]. In MIRI, the term PANoptosis-like convergence is more appropriate because it describes interconnected inflammatory death networks driven by mitochondrial ROS, DAMP release, caspase dysregulation, and RIPK signaling without presuming the existence of a unified molecular complex. Inflammatory death pathways are tightly regulated by metabolic conditions. ROS promotes NLRP3 activation and RIPK signaling; ATP depletion impairs apoptotic execution and membrane repair while extracellular ATP enhances inflammasome activation; calcium overload aggravates GSDMD-mediated membrane injury and the RIPK3–CaMKII axis; and lipid peroxidation provides a mechanistic link between ferroptosis and inflammatory cell death [79,94]. Therefore, therapeutic intervention in post-ischemic inflammation should aim to limit excessive inflammatory amplification rather than completely suppress inflammatory responses [95]. Uncontrolled activation of the NLRP3–GSDMD axis, necroptotic membrane disruption, cytokine release, and endothelial injury during early reperfusion may aggravate infarct expansion and MVO [62]. Short-term inhibition of specific pathways, including NLRP3, caspase-1, GSDMD, RIPK1, RIPK3, or MLKL, may reduce acute lytic injury when these pathways represent major contributors to tissue damage. However, inflammation also plays essential roles in the clearance and repair of infarcted myocardium. Neutrophils and inflammatory macrophages contribute to the removal of necrotic cells, damaged extracellular matrix, and cellular debris, whereas the transition toward pro-resolving and reparative macrophage states supports efferocytosis, angiogenesis, fibroblast activation, collagen deposition, and scar maturation. Conversely, excessive or poorly timed suppression of inflammatory signaling may delay debris clearance, impair resolution of inflammation, and disrupt reparative matrix organization, resulting in the formation of a structurally immature infarct scar. Although early anti-inflammatory intervention may reduce acute inflammatory injury, prolonged or inappropriate suppression may interfere with debris removal, macrophage-state transitions, and scar maturation, thereby affecting infarct expansion, ventricular wall stability, cardiac rupture, and long-term remodeling. Accordingly, anti-inflammatory strategies for MIRI require careful optimization of therapeutic timing, duration, and tissue specificity. Short intervention windows during early reperfusion may provide greater benefit than continuous systemic inhibition, particularly for agents targeting inflammasomes or necroptotic pathways. To minimize injury to cardiomyocytes and endothelial cells while preserving the essential functions of phagocytic and reparative immune populations, targeted delivery of anti-inflammatory agents to the myocardium or specific cell types may represent a more effective approach. Therapeutic selection should also consider whether inflammatory cell death represents a primary driver of injury, a secondary consequence of irreversible necrosis, or a component of a broader PANoptosis-like network.

Future preclinical studies should directly compare different therapeutic inhibition schedules, including pretreatment, early-phase, delayed, intermittent, and continuous intervention strategies. Outcome assessments should extend beyond infarct size and acute cardiac function to include necrotic-debris clearance, efferocytosis, macrophage phenotypic transition, fibroblast and myofibroblast activation, angiogenesis, collagen content and organization, scar thickness, infarct expansion, cardiac rupture, and long-term ventricular remodeling. Combination therapies should be tailored to distinct phases of injury, with early interven-

tions aimed at limiting excessive lytic signaling followed by strategies that promote inflammation resolution and tissue repair. Therefore, therapeutic approaches should focus on modulating inflammatory cell death rather than completely suppressing it. Effective cardioprotection requires a balance between restraining early pyroptotic and necroptotic amplification and preserving the immune and stromal responses essential for necrotic-debris clearance, tissue repair, and the formation of a stable infarct scar.

Mitochondrial Injury Thresholds and Death-Mode Selection in MIRI

Mitochondria play a central role in the transition from reversible cellular stress to irreversible cardiomyocyte death by integrating multiple determinants, including calcium burden, redox status, substrate availability, membrane integrity, and ATP production [1]. The mechanisms underlying mitochondrial ROS generation, calcium overload, mPTP activation, lipid peroxidation, and inflammatory signaling have been extensively discussed in previous sections. Therefore, this section focuses on how the severity, duration, and cellular context of mitochondrial injury determine the relative contributions of apoptosis, mPTP-dependent necrosis, ferroptosis-associated membrane damage, and inflammatory lytic death to MIRI. The central concept is that mitochondrial injury does not inevitably activate a single predetermined cell-death pathway. Mild and potentially reversible mitochondrial dysfunction may preserve sufficient bioenergetic capacity to support ATP-dependent apoptosis or mitochondrial quality-control processes [96]. In contrast, severe calcium overload, sustained permeability transition, rapid ATP depletion, and loss of membrane integrity favor necrotic or inflammatory cell-death outcomes. Intermediate states characterized by iron-dependent lipid peroxidation and inflammatory signaling may lower the threshold for membrane disruption and reshape the ultimate cell fate. Thus, mitochondria should be regarded as regulators of cell-death susceptibility and pathway selection rather than as evidence for the universal dominance of any single mitochondrial death mechanism.

Relative Contribution of Apoptosis During Acute Reperfusion Injury

Although these processes represent important components of MIRI, their presence does not definitively establish apoptosis as the primary mechanism responsible for cardiomyocyte loss following acute reperfusion. Apoptotic execution requires adequate ATP availability to support apoptosome assembly, caspase activation, chromatin condensation, cytoskeletal reorganization, and controlled cellular dismantling [97]. This energy requirement restricts effective apoptosis largely to cells with moderate mitochondrial injury and partially preserved bioenergetic capacity. In contrast, cardiomyocytes exposed to prolonged ischemia or severe early-reperfusion stress frequently undergo profound ATP depletion, calcium overload, sustained mPTP opening, ionic dysregulation, and rapid membrane disruption [98]. Under these conditions, mPTP-dependent necrosis and other lytic death pathways are more compatible with the prevailing metabolic environment than complete caspase-dependent apoptotic execution.

The spatial and temporal distribution of injury is also a critical determinant. Apoptosis is more likely to occur in the infarct border zone, in partially injured cardiomyocytes that retain mitochondrial integrity and glycolytic ATP production, or during delayed reperfusion and post-infarction remodeling [99]. In the severely ischemic core, abrupt bioenergetic failure and membrane disruption may occur before the complete apoptotic program can be executed [100]. Ferroptotic lipid damage, pyroptotic pore formation, and necroptotic signaling may further restrict the time and energy available for apoptotic progression. Therefore, apoptosis should be considered one component of a heterogeneous and spatially variable death response rather than a universal explanation for cardiomyocyte loss during acute MIRI. The interpretation of apoptosis also depends on the detection methods employed. TUNEL staining identifies DNA fragmentation but may also label cells undergoing other forms of cell death, including late-stage necrosis or inflammatory lytic processes. Similarly, increased BAX/BCL-2 ratios, cytochrome c release, or cleaved caspase-3 indicate apoptotic activation but do not directly quantify the extent of tissue loss attributable to apoptosis [101]. Conversely, broad caspase inhibition may suppress apoptotic features while redirecting death-receptor or mitochondrial stress signals toward alternative lytic pathways, including necroptosis, mPTP-dependent necrosis, or other forms of regulated cell death. Therefore, reductions in apoptotic markers should not automatically be interpreted as evidence of reduced overall cardiomyocyte death or smaller infarct size.

Direct comparative studies are required to evaluate apoptosis inhibition alongside interventions targeting ferroptosis, pyroptosis, necroptosis, and mPTP opening within the same experimental models and under comparable ischemic durations and reperfusion conditions. Furthermore, combination inhibition studies are necessary to determine whether apoptosis functions as an independent death pathway, a secondary response, or a pathway that becomes prominent only after suppression of more rapidly executing lytic mechanisms.

Sustained mPTP Opening as an Early Bioenergetic Endpoint

When ATP levels decline below the threshold required for controlled apoptotic execution, cells tend to shift toward regulated necrosis, characterized by osmotic failure, plasma membrane rupture, and DAMP release. This bioenergetic transition explains the central role of mPTP-dependent

necrosis in severe reperfusion injury during the initial minutes to hours after reperfusion [82,102]. It also defines a mechanistic boundary between apoptosis and necrosis: under conditions of mitochondrial stress, apoptosis may proceed when ATP production is partially preserved, whereas necrosis emerges when sustained permeability transition results in irreversible bioenergetic collapse [103]. The critical determinant is not merely the presence of mPTP-associated signaling but rather the magnitude and duration of pore opening that drive irreversible mitochondrial failure. These interactions further highlight that ferroptotic or inflammatory signaling may amplify mitochondrial energy failure without implying that mPTP opening is exclusively attributable to ferroptosis or necroptosis.

Mitophagy Modifies Mitochondrial Death Thresholds Rather Than Constituting a Separate Death Program

Although mitophagy is not an independent RCD modality, it plays a critical role in MIRI by determining whether mitochondrial injury remains reversible or progresses toward apoptosis, mPTP-dependent necrosis, ferroptosis, or pyroptosis. Impaired mitophagy results in the accumulation of dysfunctional mitochondria, leading to increased oxidative stress, reduced calcium-retention capacity, persistent inflammasome activation, and enhanced susceptibility to apoptotic and lytic cell death. In contrast, appropriately timed mitophagy provides protection by removing severely damaged organelles while preserving a functional mitochondrial network. However, excessive or prolonged mitochondrial clearance may become detrimental when mitochondrial biogenesis and substrate availability are insufficient to compensate for organelle loss. Under these conditions, reduced mitochondrial mass can further aggravate ATP depletion and impair contractile recovery. Thus, the effects of mitophagy are nonlinear and depend on timing, magnitude, cell type, and the balance between mitochondrial elimination and regeneration.

Accordingly, studies investigating mitophagy in MIRI should assess mitophagic flux using lysosomal inhibition approaches or validated reporter systems, evaluate mitochondrial turnover and biogenesis, and determine whether interventions preserve ATP production and contractile function. Cell-type specificity is particularly important because cardiomyocytes, endothelial cells, fibroblasts, and immune cells exhibit distinct metabolic requirements and may require different levels of mitochondrial clearance. Mitophagy also influences RCD pathway selection. Efficient removal of damaged mitochondria may reduce cytochrome c release, mPTP sensitivity, dissemination of oxidized lipids, and mitochondrial DAMP signaling simultaneously. Conversely, defective mitochondrial clearance may permit amplification and interaction among multiple death pathways. This broader role in modulating cellular thresholds explains why alterations in mitophagy can affect multiple death-associated markers without necessarily indicating the predominance of any single downstream pathway [104].

An Integrative Framework for Assigning Mitochondrial Death-Mode Contribution

The relative contribution of each death pathway should be evaluated in the context of injury severity, ATP availability, myocardial region, and reperfusion duration [8]. Severe injury within the ischemic core during early reperfusion is most consistent with sustained mPTP opening, profound ATP depletion, and necrotic membrane failure. In contrast, cells experiencing moderate injury with preserved bioenergetic capacity may undergo apoptosis, particularly within the infarct border zone or during later stages of reperfusion. The likelihood of ferroptotic involvement increases when iron availability, oxidizable phospholipid abundance, and antioxidant failure converge, whereas pyroptosis and necroptosis may become more prominent following activation of inflammatory sensors, gasdermin pore formation, RIPK signaling, and membrane disruption [105]. These patterns are not mutually exclusive and may differ substantially among cardiac cell types. Therefore, assigning pathway dominance based solely on a single biomarker panel is inappropriate. Comprehensive investigations should first define the temporal dynamics and cellular localization of candidate death pathways, then demonstrate pathway-specific rescue using genetic approaches and complementary pharmacological interventions. Subsequently, the protective effects of inhibiting apoptosis, mPTP-dependent necrosis, ferroptosis, pyroptosis, and necroptosis should be directly compared under equivalent experimental conditions. Furthermore, studies should determine whether combined pathway inhibition provides additional protection or induces compensatory activation of alternative death mechanisms. Assessment of overall membrane integrity, infarct size, MVO, cardiac function, and long-term remodeling should be integrated with pathway-specific molecular analyses.

The therapeutic implications of these findings suggest that mitochondrial preservation should extend beyond conventional anti-apoptotic strategies. During severe early reperfusion injury, prioritizing ATP restoration, limiting calcium-dependent permeability transition, and maintaining membrane integrity may provide greater benefit than solely inhibiting caspase activation. In settings of moderate injury, preventing irreversible mitochondrial outer-membrane permeabilization may help preserve viable cardiomyocytes. Furthermore, targeting ferroptotic and inflammatory cascades may require pathway-specific interventions when these mechanisms substantially contribute to functional impairment [106]. Treatment timing is critical, as prolonged suppression of apoptosis or mitochondrial stress responses may temporarily preserve damaged cells without restoring long-term cellular function. Overall, mitochondria act as dynamic regulators that shape cel-

lular susceptibility to different death outcomes. The contribution of apoptosis to acute MIRI is likely context dependent, being less dominant in cells undergoing rapid bioenergetic collapse but more relevant in metabolically preserved border-zone cardiomyocytes or populations exposed to delayed injury. Distinguishing apoptosis from necrotic, ferroptotic, and inflammatory forms of cell death requires direct comparative interventions and evaluation of overall tissue preservation rather than reliance on conventional apoptotic markers alone.

Context-Dependent Heterogeneity and Clinically Relevant Modifiers of Regulated Cell Death in MIRI

The contribution of RCD pathways to MIRI is highly variable and depends on reperfusion duration, myocardial region, cell type, and patient-specific characteristics. This variability is not merely descriptive; it is essential for determining the predominant injury mechanisms, interpreting biomarkers, and identifying potential therapeutic targets within specific experimental or clinical contexts. A pathway detected across the entire myocardium at a single time point may represent a transient response within a limited cell population rather than the primary driver of tissue injury. Therefore, experimental models and clinical study designs should incorporate temporal, spatial, cellular, and patient-related heterogeneity to achieve a more comprehensive assessment. In this review, a "pathway-enriched state" refers to a defined temporal, anatomical, and cellular context in which pathway-specific terminal execution markers are accompanied by selective genetic or pharmacological rescue. This concept does not imply that a given pathway functions independently or remains universally dominant throughout the reperfused myocardium. A pathway should be considered dominant only when its contribution has been directly compared with plausible alternative death mechanisms under appropriately matched experimental conditions.

Temporal Specificity: Death Pathways Evolve Across Reperfusion

The initial minutes to hours after reperfusion are characterized by abrupt restoration of oxygen availability and pH normalization, mitochondrial calcium overload, ROS generation, mPTP opening, ATP depletion, and membrane disruption [50]. In severely injured cardiomyocytes, these events favor mPTP-dependent necrosis and other rapidly destructive forms of cell death. Concurrently, ferroptotic lipid peroxidation and inflammatory signaling may be initiated; however, their contribution depends on whether they exceed the threshold required to induce irreversible membrane damage [107]. Apoptosis is more likely to occur in cells with preserved energy availability and may therefore be more prominent in the infarct border zone or during later stages of reperfusion rather than during the initial phase of bioenergetic collapse. Over the subsequent hours to days, the injury landscape evolves. Persistent oxidative stress, altered iron homeostasis, mitochondrial dysfunction, endothelial injury, leukocyte recruitment, inflammasome activation, and cytokine signaling can further amplify ferroptotic, pyroptotic, necroptotic, and apoptotic processes [108]. Current evidence supports the existence of temporally and cell-type-enriched death-pathway activities but does not establish ferroptosis-prone and inflammasome-dominant states as mutually exclusive or stable phenotypes. *In vivo* studies indicate that ferroptosis-associated lipid peroxidation is preferentially enhanced during reperfusion rather than ischemia alone and that iron chelation or ferroptosis inhibition can attenuate myocardial injury [108]. Similarly, genetic and pharmacological studies targeting the NLRP3–caspase-1–GSDMD pathway support a causal role for inflammasome-associated pyroptotic injury. However, these mechanisms have generally been investigated in separate experimental systems and have not yet been directly compared through parallel assessment of terminal execution events within the same cardiac cell population. Human evidence remains limited. In human ischemic cardiomyopathy samples, reduced activity of the GUCY1A1–LDHA–GPX4 axis has been associated with increased lipid peroxidation and cardiac fibrosis; however, inflammasome activation and gasdermin-mediated execution were not assessed simultaneously [109]. Therefore, current evidence supports the existence of ferroptosis-enriched or inflammasome-enriched injury contexts rather than definitively distinct and mutually exclusive dominant states.

In advanced stages of MIRI, fibroblast activation, immune-cell phenotypic transitions, extracellular matrix remodeling, and clearance of damaged cells become increasingly important determinants of tissue repair and functional recovery. Consequently, a therapy that provides benefit when administered at reperfusion may become ineffective or even detrimental during the repair phase. Similarly, prolonged suppression of inflammatory signaling may reduce early injury but impair debris clearance, angiogenesis, scar formation, or structural stabilization. Future studies should prospectively define sampling and intervention windows rather than relying on a single arbitrary endpoint. Assessment of pathway activity should encompass early reperfusion, the inflammatory expansion phase, and the remodeling phase. Sequential analyses should distinguish pathway initiation from terminal execution and determine whether therapeutic inhibition results in durable tissue preservation or merely delays cell death. Explicit evaluation of time-by-treatment interactions is essential, and nonsignificant outcomes should be interpreted within the context of the intervention window rather than regarded as evidence that a pathway lacks biological relevance.

Spatial Specificity: The Ischemic Core, Border Zone, and Microvasculature are Biologically Distinct

MIRI exhibits substantial spatial heterogeneity [110,111]. The ischemic core typically experiences profound ATP depletion, calcium overload, mitochondrial dysfunction, and plasma membrane disruption, thereby favoring necrotic forms of cell death. In contrast, the infarct border zone contains partially perfused and metabolically stressed cells that may retain viability and exhibit greater susceptibility to apoptosis, reversible mitochondrial dysfunction, adaptive autophagy, or delayed ferroptotic injury [15]. The microvascular compartment is characterized by endothelial swelling, oxidative stress, leukocyte adhesion, platelet activation, barrier disruption, and the no-reflow phenomenon, creating an environment in which endothelial and inflammatory cell death can indirectly amplify cardiomyocyte injury despite restoration of epicardial coronary flow. Analysis of whole-heart homogenates may mask these regional differences and contribute to inconsistent mechanistic interpretations. For example, increased expression of a death-associated biomarker originating primarily from infiltrating leukocytes or injured endothelial cells may be incorrectly attributed to cardiomyocyte death [58]. Conversely, a strong signal restricted to the infarct core may have limited relevance to the viable and therapeutically accessible border zone. Future studies should incorporate predefined anatomical sampling strategies, histological spatial alignment, and region-specific molecular profiling. Emerging approaches, including spatial transcriptomics, multiplex imaging, laser-capture microdissection, and region-targeted proteomic and lipidomic analyses, should be integrated with infarct mapping and microvascular assessment whenever feasible. Functional recovery should also be evaluated separately within the ischemic core, border zone, and microvascular compartment rather than inferred solely from averaged whole-heart measurements.

Cell-Type Specificity: Pathway Activation and Therapeutic Consequences Differ Among Cardiac Cell Populations

Cardiomyocytes represent the major contractile cell population lost during infarction; however, they are not the only biologically relevant targets of RCD. Endothelial-cell injury contributes to barrier dysfunction, edema formation, impaired vasodilation, MVO, and leukocyte recruitment. Both resident and infiltrating immune cells can undergo pyroptotic or other lytic forms of cell death, releasing cytokines and DAMPs that influence injury amplification, inflammation resolution, and subsequent tissue repair [16]. Although fibroblasts exhibit relative resistance to certain acute stressors, they are essential regulators of extracellular matrix deposition and scar formation; excessive loss or prolonged suppression of fibroblast activity may impair effective healing. Pericytes, vascular smooth muscle cells, and conduction-system cells also contribute to tissue perfusion, arrhythmia susceptibility, and structural recovery after injury [2]. The biological consequences of a specific molecular pathway depend strongly on the affected cell type. For example, inhibiting inflammasome activation in infiltrating myeloid cells may reduce inflammatory amplification, whereas excessive suppression of inflammatory signaling in reparative macrophages may delay debris clearance and tissue healing. Similarly, enhancing mitophagy may protect cardiomyocytes with sufficient mitochondrial reserve but could worsen energetic insufficiency when mitochondrial mass is already critically reduced. These cell-type-specific effects cannot be adequately resolved using bulk tissue analyses alone. Future studies should integrate cell-type-specific genetic manipulation, lineage tracing, conditional knockout models, single-cell or single-nucleus sequencing, flow cytometry, and spatial validation approaches. Pathway-associated markers should be assigned to specific cellular populations and correlated with cell-type-specific survival, tissue perfusion, contractile function, and remodeling outcomes. A pathway should not be considered predominant in MIRI unless its inhibition rescues the relevant cell population, improves tissue-level outcomes, and does not induce compensatory injury in other cellular compartments.

Patient-Level Modifiers: Comorbidities, Sex, Renal Dysfunction, and Prior Medication Use

Patient-specific factors influence mitochondrial reserve, redox homeostasis, iron metabolism, endothelial function, inflammatory responses, immune-cell activity, and therapeutic responsiveness. Comorbid conditions such as diabetes, obesity, dyslipidemia, and aging can increase oxidative stress, impair mitochondrial adaptability, alter lipid composition, promote chronic inflammation, and reduce endogenous cardioprotective mechanisms. These changes may increase susceptibility to multiple cell-death pathways and modify responses to targeted pathway inhibition. Therefore, conclusions derived solely from young, metabolically healthy animal models may not adequately represent the biological heterogeneity of the clinical population.

Sex should be considered a biological variable rather than merely a demographic descriptor because it influences multiple physiological processes, including sex hormone signaling, mitochondrial metabolism, calcium handling, antioxidant capacity, immune activation, endothelial function, platelet reactivity, and body composition [112]. These factors may affect MIRI severity and modify the activation thresholds of apoptotic, ferroptotic, and inflammatory lytic pathways. Research studies should include both male and female subjects whenever appropriate, report sex-disaggregated data, and evaluate sex-by-treatment interactions rather than relying on pooled analyses alone [113]. Furthermore, age and reproductive status should be

considered, as these factors may modify the biological effects associated with sex across different life stages and reproductive states. Renal dysfunction represents another important clinical determinant. It is associated with uremic toxin accumulation, systemic oxidative stress, chronic inflammation, endothelial dysfunction, anemia, disturbed calcium–phosphate homeostasis, altered iron metabolism, electrolyte imbalance, and reduced drug clearance [114]. These alterations may increase susceptibility to mitochondrial dysfunction, lipid peroxidation, inflammasome activation, arrhythmogenesis, and microvascular injury. Renal impairment may also influence drug pharmacokinetics, toxicity profiles, and therapeutic efficacy, particularly in patients with cardiorenal disease compared with those with preserved renal function. Therefore, translational studies should incorporate renal function assessment, utilize chronic kidney disease models when appropriate, and evaluate drug pharmacokinetics and safety under conditions of impaired renal clearance.

Prior medication exposure introduces substantial heterogeneity into clinical settings. Patients with AMI frequently receive multiple therapies, including statins, antiplatelet agents, renin–angiotensin system inhibitors, beta-blockers, glucose-lowering medications, diuretics, anticoagulants, and other cardiovascular drugs before reperfusion. These treatments may influence mitochondrial metabolism, oxidative stress, endothelial function, platelet activity, inflammatory responses, iron homeostasis, and ischemic preconditioning, thereby modifying baseline pathway activity and altering the observed effects of subsequent interventions. Failure to account for background therapy may partly explain why treatments that demonstrate efficacy in treatment-naive animal models often show reduced benefits in contemporary clinical populations. Medication history should therefore be systematically documented and analyzed as a potential effect modifier rather than treated solely as a confounding variable. Preclinical studies designed to improve clinical translation should evaluate candidate interventions in the presence of clinically relevant background therapies and investigate potential pharmacological interactions. In clinical trials, predefined subgroup analyses or interaction assessments for major medication classes should be incorporated whenever sample size permits. Similar considerations should be applied to ischemic preconditioning, previous myocardial infarction, chronic inflammatory conditions, and other exposures that may alter the baseline state and responsiveness of RCD pathways.

From Descriptive Heterogeneity to a Testable Stratification Framework

Future studies should establish a multidimensional stratification matrix before data collection to move beyond descriptive analyses. Key variables incorporated into this framework should include ischemic duration, reperfusion time, myocardial region, cell type, sex, age, metabolic status, renal function, and prior medication exposure. Candidate death pathways should then be evaluated using orthogonal molecular markers, pathway-selective genetic or pharmacological interventions, and tissue-level functional outcomes. The efficacy of rescue strategies should be directly compared under standardized experimental conditions, and combined inhibition approaches should be employed to identify compensatory pathway activation or cooperative interactions. Direct manipulation of metabolic gates can provide evidence for pathway routing and functional cooperation; however, it has not yet demonstrated a definitive reciprocal switch between terminal death modalities in the intact heart. Experimental modulation of calcium loading, mitochondrial permeability transition, oxidative stress, and lipid peroxidation indicates that these factors influence the relative contribution and interaction of mitochondrial and lipid-peroxidative injury mechanisms. Greater protection achieved through combined inhibition of mPTP opening and lipid peroxidation compared with either intervention alone is more consistent with parallel or cooperative mechanisms than with complete replacement of one pathway by another [88]. Similarly, endothelial GUCY1A1 deletion or overexpression alters susceptibility within the GUCY1A1–LDHA–GPX4 axis and selectively modulates endothelial ferroptosis; however, reciprocal activation of pyroptosis, necroptosis, apoptosis, or mPTP-dependent necrosis has not been demonstrated [109]. Therefore, no cardiac study has yet established that manipulation of a single metabolic gate can suppress one fully executed death pathway while inducing a reciprocal increase in another pathway within the same cell population. Within the framework proposed here, “switching” should be considered a testable hypothesis, whereas context-dependent pathway selection, altered pathway weighting, and metabolic routing are more directly supported by current evidence.

A practical experimental workflow should involve several key steps. First, the timing and spatial distribution of pathway-specific execution should be defined, together with identification of the affected cell population. Second, causality should be established through selective intervention and demonstration of improvements in overall cell survival, infarct size, microvascular perfusion, or cardiac function. Third, therapeutic strategies should be validated in models incorporating clinically relevant modifiers, including advanced age, diabetes, obesity, renal dysfunction, sex differences, and background cardiovascular therapies. Fourth, interaction analyses should be performed to determine whether treatment efficacy varies across these clinical contexts. Finally, delayed remodeling and repair processes should be assessed to exclude the possibility that acute protection is offset by impaired inflammation resolution, defective scar formation, or compromised tissue stability. Within this framework, research should aim to define the causal and therapeutically actionable role of each cell-death pathway within specific cell populations, myocar-

dial regions, reperfusion windows, and patient subgroups. This stratified approach can reconcile apparently conflicting findings, identify context-dependent therapeutic targets, and improve the translational relevance of preclinical evidence.

Biomarker-Guided Identification of Death-Switching Phenotypes

Clinical Context and Serial Biospecimen Collection

Defining the clinical context in which biomarker signals are generated requires consideration of multiple variables, including ischemic duration, reperfusion strategy, infarct location, MVO, sex, age, renal function, metabolic comorbidities, and prior medication exposure. These factors can influence biomarker release, distribution, metabolism, clearance, and the activation patterns of different cell-death pathways [115]. Serial sampling is essential because pathway activity evolves dynamically during reperfusion. Therefore, specimens should ideally be collected before or at the time of reperfusion, during early reperfusion, throughout the subsequent inflammatory phase, and during early recovery. A single measurement cannot distinguish pre-existing pathway activation from reperfusion-induced execution or determine transitions between different death programs. Circulating biomarkers should be interpreted alongside conventional indicators of myocardial injury and tissue-level outcomes, including cardiac troponin release, infarct size, MVO, ventricular function, and remodeling. Such integrated assessment can help quantify injury severity and clinical consequences but cannot independently identify a specific cell-death pathway. These conventional measures primarily provide a framework for linking pathway-associated biomarker changes with clinically meaningful myocardial injury.

Multidimensional Biomarker Panels and Cell-of-Origin Assignment

A comprehensive panel for clinical application should incorporate markers that represent multiple stages of each respective pathway. Apoptosis-related assessments may include caspase activation, nucleosomal fragmentation products, and mitochondrial apoptotic signaling. Ferroptosis-related evaluations may involve oxidized phospholipids, lipid-peroxidation products, iron-status indicators, and alterations in GPX4-associated antioxidant capacity [116]. Pyroptosis-related analyses may comprise cleaved gasdermins, IL-1β, IL-18, and inflammasome-associated proteins, whereas necroptosis-related investigations may include markers of RIPK3 and MLKL activation. Circulating mitochondrial DNA, mitochondrial proteins, severe bioenergetic impairment, and markers of membrane rupture may reflect mitochondrial necrotic injury. These assessments should be interpreted as components of an integrated biological phenotype rather than as independent diagnostic biomarkers. Before assigning a major role to a specific pathway, at least two mechanistically distinct indicators associated with that pathway should demonstrate consistent temporal alterations. Interpretation should also account for factors including renal clearance, hepatic metabolism, systemic inflammation, hemolysis, skeletal muscle injury, and potential extracardiac sources. Identification of the cellular origin is critical because circulating markers may originate from cardiomyocytes, endothelial cells, leukocytes, platelets, or noncardiac tissues. Improved source attribution may be achieved through approaches such as cardiomyocyte-enriched extracellular vesicles, cell-specific surface markers, tissue-specific methylation signatures, and spatially resolved imaging. Without such evidence, an increase in an inflammatory death-associated marker should not be automatically interpreted as evidence of pyroptotic cardiomyocyte loss.

Classification of Death-Switching Phenotypes

Phenotypes suggestive of death switching should be derived from serial, multidimensional datasets rather than from fixed thresholds based on individual biomarkers. A pathway-dominant phenotype requires consistent alterations in multiple pathway-associated measurements, appropriate temporal dynamics, a plausible cellular origin, and an association with tissue injury or functional deterioration. Evidence supporting a switching phenotype would arise when longitudinal analyses demonstrate a coordinated decline in one pathway-related signature accompanied by an increase in another, together with corresponding changes in cellular injury, myocardial damage, or therapeutic response [117]. Rather than assigning each patient to a single-pathway category, mixed phenotypes should be retained because multiple death programs may operate simultaneously across different myocardial regions or cell populations. Approaches such as composite scoring systems, latent-class analysis, trajectory clustering, and other prespecified multivariable methods may facilitate the identification of apoptosis-associated, ferroptosis-associated, inflammatory-lytic, mitochondrial-collapse, and mixed phenotypes. Model development should include internal validation, adjustment for clinically relevant covariates, evaluation of calibration and discrimination performance, and validation in independent external cohorts. The proposed biomarker-defined phenotypes and corresponding therapeutic strategies are summarized in Table 3 (Ref. [5,13,36,42,45,48,49,57,58,62,64,77,78,79,80,82,83,84,95,107,116,117,118,119,120,121]).

Functional Validation and Prospective Clinical Evaluation

Biomarker-defined phenotypes require functional validation before they can be used to guide therapeutic decisions. Prior to clinical application, candidate signatures should be evaluated in experimental systems in which the

Table 3. Proposed biomarker-defined phenotypes and matched strategies.

Phenotype	Signature	Strategy	Caution	Evidence	References
Ferroptosis-prone	Lipid peroxides; labile iron; low GPX4/GSH; high ACSL4.	Control iron and lipid peroxidation before reperfusion.	Single markers lack specificity and causal dominance.	IV	[48,49,83,107,116]
Inflammasome-dominant	IL-1β/IL-18; NLRP3; caspase-1; GSDMD; mitochondrial DAMPs.	Target inflammasome signaling during acute-to-subacute inflammation.	Over-suppression may impair clearance, angiogenesis, and repair.	III	[45,62,84,118]
Necroptosis/mitochondrial collapse	RIPK-MLKL/CaMKII activation; mPTP sensitivity; ATP/NAD^+ depletion.	Combine necroptosis blockade with mitochondrial stabilization at reperfusion.	Late single-pathway inhibition may fail after mitochondrial collapse.	III	[36,78,80,82]
Microvascular/no-reflow	MVO; hypoperfusion; endothelial injury; thrombo-inflammation; hypoxia.	Protect endothelium, barriers, and microvascular perfusion early.	Systemic targeting risks bleeding and off-target effects.	III	[5,42,57,58,119]
PANoptosis-like mixed	Concurrent GSDMD, caspase, RIPK3/MLKL, DAMP, and redox signals.	Stage mitochondrial protection before selective inflammatory modulation.	Require PANoptosome assembly before claiming canonical PANoptosis.	IV	[13,64,79,117]
Remodeling/failed resolution	Persistent cytokines, DAMPs, endothelial activation, fibrosis, matrix turnover.	Support resolution, endothelial repair, mitophagy, and antifibrotic control.	Over-suppression may weaken scar stability and repair.	III	[77,95,118,120,121]

corresponding pathways are selectively inhibited through genetic approaches and orthogonal pharmacological interventions. A valid signature should demonstrate reduced activity following blockade of functional execution and should be associated with improvements in overall cell survival, infarct size, microvascular perfusion, or cardiac function [116]. Assessment of patient-derived plasma, extracellular vesicles, or immune cells in human cardiomyocyte, endothelial-cell, or multicellular cardiac models may help determine whether the identified biological state recapitulates pathway-specific injury and whether such injury can be selectively reversed. These studies provide mechanistic evidence linking circulating signatures to myocardial tissue damage but cannot replace prospective clinical validation. Translation into clinical practice should proceed through sequential stages of biomarker discovery, internal validation, external validation, and evaluation of treatment interactions. A biomarker panel achieves clinical relevance only when it reliably identifies patient subgroups with distinct prognostic profiles or differential responses to pathway-targeted interventions. Therefore, biomarker classification should ultimately support therapeutic stratification and outcome prediction rather than merely improve the detection of molecular abnormalities.

Challenges in Real-Time Assessment of Metabolic States

A key limitation of the metabolism-gated switching framework is the difficulty in directly quantifying the complex concept of the "metabolic state". This state encompasses multiple interacting factors, including ATP availability, phosphocreatine reserves, substrate utilization, mitochondrial redox balance, intracellular pH, calcium burden, antioxidant capacity, labile iron levels, membrane lipid composition, and inflammatory activation. These variables change at different rates and may exert nonlinear or threshold-dependent effects on regulated cell death pathways. Consequently, no single metabolite, imaging parameter, or circulating biomarker can comprehensively represent the metabolic state of the ischemic or reperfused myocardium. Temporal resolution represents another major challenge. During coronary occlusion and early reperfusion, intracellular pH, ATP levels, mitochondrial membrane potential, calcium concentration, ROS generation, and substrate flux can fluctuate within seconds to minutes. Measurements obtained before reperfusion, immediately after reperfusion, or several hours later may therefore capture substantially different biological states. Many clinical sampling and imaging approaches integrate signals over relatively broad time windows, potentially missing transient metabolic thresholds that determine irreversible commitment to cell death. Moreover, the metabolic profile detected after injury may represent a consequence of cell death rather than the initiating event, further complicating the distinction between causal drivers and downstream biomarkers.

Spatial and cellular heterogeneity further complicate the assessment of metabolic state. The ischemic core, peri-infarct border zone, MVO region, and remote myocardium differ substantially in residual perfusion, oxygen availability, substrate delivery, mitochondrial in-

tegrity, and inflammatory-cell infiltration. Even within the same anatomical region, cardiomyocytes, endothelial cells, fibroblasts, resident macrophages, recruited leukocytes, platelets, and vascular smooth muscle cells may exist in distinct metabolic states. Consequently, bulk myocardial measurements represent an average of signals derived from viable, reversibly injured, irreversibly injured, and infiltrating cells, potentially masking highly vulnerable metabolically distinct subpopulations. Conversely, peripheral blood measurements cannot reliably determine the myocardial location or cellular source of a metabolite or death-associated signal. Current experimental and clinical technologies address only selected aspects of this challenge. Phosphorus-31 magnetic resonance spectroscopy enables non-invasive assessment of myocardial high-energy phosphate metabolism and intracellular pH [122]. However, its clinical utility is constrained by low sensitivity, limited spatial resolution, motion artifacts, and prolonged acquisition times, which restrict its ability to capture rapid and region-specific metabolic changes during acute reperfusion. Hyperpolarized carbon-13 magnetic resonance imaging provides dynamic information regarding pyruvate metabolism and substrate flux; however, transient signal duration, dependence on tracer delivery, technical requirements, motion sensitivity, and limited availability currently restrict its widespread clinical application [123]. Positron-emission tomography provides valuable information regarding substrate uptake and inflammatory-cell activity but lacks sufficient temporal resolution to accurately characterize rapid reperfusion-associated metabolic transitions and may be influenced by systemic metabolic conditions. Research approaches including myocardial microdialysis, optical redox imaging, fluorescent metabolic probes, and genetically encoded metabolic sensors offer higher temporal resolution in experimental models. Nevertheless, their invasiveness, restricted spatial coverage, or difficulties in translation to human cardiac studies limit their application in intact human myocardium. Similarly, tissue metabolomics, lipidomics, spatial metabolomics, and single-cell analyses generate comprehensive molecular profiles but are commonly obtained at discrete time points, thereby providing only indirect information regarding dynamic metabolic trajectories. These limitations require careful interpretation when considering precision cardioprotection. At present, metabolic-state-guided therapy should not be interpreted as the immediate classification of each patient into a distinct, directly measurable death state. A more realistic near-term strategy involves probabilistic and multimodal inference of metabolic vulnerability. Such inference may integrate ischemic duration, reperfusion timing, angiographic flow, MVO, cardiac magnetic resonance features, metabolic status, comorbid conditions, medication history, and serial circulating biomarkers reflecting energy depletion, oxidative stress, lipid injury, mitochondrial dysfunction, and inflammation. Rather than applying a universal threshold, longitudinal changes and combinations of partially independent biomarkers may be used to estimate the likelihood that a specific metabolic–death pathway contributes substantially to therapeutic response. This approach acknowledges the dynamic and heterogeneous nature of myocardial injury while providing a more practical framework for developing metabolism-informed cardioprotective strategies.

The precision aspect of this framework should initially focus on patient enrichment and stratification rather than relying exclusively on deterministic bedside selection of a single pathway inhibitor. Prospective clinical studies are required to determine whether composite signatures can accurately predict target engagement and therapeutic response. It is also essential to establish whether these signatures retain predictive performance across different infarct territories, ischemic durations, comorbidity profiles, and reperfusion conditions. Whenever feasible, signatures derived from circulating biomarkers and imaging modalities should be correlated with myocardial tissue findings, spatially resolved experimental datasets, or pathway-specific pharmacodynamic responses. Although real-time, cell-type-specific mapping of metabolic states represents an important long-term objective, current precision cardioprotection approaches must explicitly account for measurement uncertainty, spatial sampling limitations, and temporal variability.

Evidence Standards for Distinguishing Pathway Activation From Functional Cell-Death Execution

Evidence Continuum From Pathway Association to Functional Dominance

Association with a specific pathway is supported by alterations in gene expression, total protein abundance, upstream signaling activity, or circulating metabolites. Although such findings indicate biological involvement, they are insufficient to establish the occurrence of cell death. Definitive pathway activation requires engagement of the relevant execution machinery together with corresponding morphological, biochemical, or membrane-associated changes. Demonstrating a causal role further requires selective inhibition of the proposed pathway and evidence of improved overall cell survival or organ-level function. To determine the relative contribution of a specific death pathway, direct comparison with alternative cell-death mechanisms under equivalent experimental conditions is essential, together with assessment of potential compensatory pathway activation or transition.

Pathway-Specific Minimum Evidence Requirements

To establish apoptotic dominance, reliance solely on increased BAX expression, reduced BCL-2 expression, cytochrome c release, TUNEL positivity, or cleaved caspase-3 is insufficient. Stronger evidence should include as-

sessment of caspase activity, confirmation of apoptosis-compatible morphology, and verification of appropriate mitochondrial signaling events [8,71]. Importantly, interventions should demonstrate a reduction in overall cell loss rather than merely altering apoptotic-associated markers or morphology. For ferroptosis, increased ACSL4 expression, reduced GPX4 levels, iron accumulation, or elevated malondialdehyde and 4-hydroxynonenal alone cannot establish ferroptotic execution. Functional validation requires demonstration of iron-dependent phospholipid peroxidation within an appropriate metabolic context, together with rescue experiments using mechanistically distinct ferroptosis-targeted interventions or genetic manipulation of key regulatory factors. Evidence of tissue protection should also be incorporated, along with evaluation of competing apoptotic and inflammatory lytic pathways. Similarly, increased NLRP3, caspase-1, IL-1β, or IL-18 levels indicate inflammasome-associated signaling but do not confirm pyroptotic execution. Robust evidence requires demonstration of gasdermin cleavage, membrane pore formation, membrane rupture, cytokine maturation and release, as well as pathway-specific rescue [124]. Identification of the affected cell type is particularly important because inflammasome activation in infiltrating immune cells has distinct biological consequences compared with gasdermin-mediated cardiomyocyte death. For necroptosis, changes in RIPK1 or RIPK3 abundance alone are insufficient to establish pathway activation [125]. Functional execution requires evidence of MLKL activation, membrane translocation, or oligomerization, together with compatible lytic morphology and rescue following selective genetic or pharmacological targeting of RIPK3 or MLKL. In addition, assessment of caspase activity and death-receptor signaling is necessary to distinguish necroptosis from overlapping apoptotic or inflammatory death programs.

Mitochondrial depolarization or calcium overload alone is insufficient to establish pore-dependent execution in mPTP-mediated necrosis. Evidence supporting this mechanism should include sustained permeability transition, loss of mitochondrial membrane potential, ATP depletion, necrotic membrane failure, and protection achieved through validated manipulation of pore-regulatory mechanisms. Importantly, transient mitochondrial depolarization must be distinguished from irreversible permeability transition leading to cell death. In clinical investigations, it may not always be feasible to fulfill all mechanistic criteria directly. Therefore, human biomarker findings should be described as consistent with, associated with, or supportive of a specific death phenotype unless supported by functional intervention studies or validated tissue-level evidence. Terms such as “pathway dominance” and “functional execution” should be reserved for studies demonstrating selective rescue of the implicated pathway together with meaningful preservation of tissue structure or organ function.

Evaluation of Multi-Target Compounds and Natural Products

Multi-target compounds are particularly relevant to MIRI because multiple RCD pathways coexist and may undergo compensatory interactions. Although natural products and bioactive compounds derived from traditional medicinal systems represent potential therapeutic candidates, their translational value should be evaluated according to the same mechanistic and clinical criteria applied to synthetic agents. Compounds should be classified based on validated molecular targets and demonstrated functional effects rather than their therapeutic origin or botanical source [126]. Changes in oxidative stress markers, inflammatory cytokines, BAX/BCL-2 ratios, or pathway-associated protein expression alone are insufficient to confirm regulation of a specific cell-death program. Robust evidence requires demonstration of target engagement, pathway-specific rescue, pharmacokinetic characterization, chemical consistency, dose-response relationships, and improvements in tissue-level outcomes. Multi-component preparations present additional challenges, including batch-to-batch variability, uncertainty regarding active constituents, limited exposure data, and difficulties in distinguishing direct regulation of death pathways from nonspecific antioxidant or anti-inflammatory effects. Unless the active components, myocardial exposure profiles, molecular targets, and functional contributions of these interventions are clearly established, they should be regarded as hypothesis-generating or multi-target candidates. Applying a unified evaluation framework avoids the use of different evidentiary standards based on therapeutic origin and allows natural products to be incorporated into the broader framework of RCD switching and translational validation.

Conclusions

Metabolism-gated cell death switching provides a hypothesis-driven framework for understanding the complex activation patterns of multiple cell-death pathways during MIRI. Factors including ATP reserve, mitochondrial redox state, calcium burden, iron and phospholipid availability, antioxidant capacity, mitochondrial integrity, and inflammatory priming may regulate the activation thresholds and relative contributions of distinct cell-death pathways in a spatially, temporally, and cell-type-specific manner. Current experimental evidence supports context-dependent susceptibility, pathway-specific functions, and convergence at shared metabolic and mitochondrial regulatory nodes. However, definitive evidence for exclusive dominant death states or complete transitions between terminal death modalities within the intact reperfused heart remains insufficient.

The translational significance of this framework does not lie in assigning each patient to a single predetermined death phenotype, but rather in generating testable hypothe-

ses for identifying therapeutically relevant injury patterns and metabolic vulnerabilities. Because the metabolic state of the reperfused human heart cannot currently be measured comprehensively, continuously, or with cell-type resolution, near-term precision cardioprotection will depend on probabilistic inference from multimodal and serial surrogate measurements rather than deterministic real-time classification. Future studies should incorporate time-resolved, multi-pathway assessments, cell-type-specific genetic and pharmacological interventions, targeted manipulation of defined metabolic gates, factorial inhibition strategies, and validation in aged, comorbid, large-animal, and human models. Prospective investigations should further determine whether composite signatures integrating ischemic duration, reperfusion timing, imaging characteristics, comorbidities, systemic metabolic status, and pathway-associated biomarkers can reliably predict target engagement and therapeutic response. Such evidence will be essential for establishing whether metabolism-informed stratification can guide the timing, targeting, and combination of cardioprotective interventions in patients with myocardial infarction.

Availability of Data and Materials

Not applicable.

Author Contributions

XNJ conceptualized the study, designed the methodology, performed the investigation, and wrote the initial draft of the manuscript. HJC contributed to the conceptualization and methodology development, participated in the validation of results and formal analysis, participated in the manuscript writing and critical revision for important intellectual content, and acquired the funding for this study. Both authors gave final approval of the version to be published. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

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