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REVIEW

Molecular Mechanisms and Signaling Pathways of Myocardial Ischemia: A Multidimensional Analysis from Energy Metabolism to Cell Death

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ABSTRACT: Myocardial ischemia, a core pathological process underlying diverse cardiovascular diseases such as coronary artery disease, poses a severe threat to global human health by frequently leading to acute myocardial infarction, heart failure, and even sudden cardiac death. A comprehensive understanding of its intricate underlying pathogenic mechanisms is not only crucial for developing effective therapeutic strategies but also essential for accelerating the translation of basic research findings into clinical practice. However, the complex regulatory networks that drive myocardial ischemia remain to be systematically clarified. These networks encompass the intricate interactions among multiple pathological processes, including energy metabolism disorder, intracellular calcium overload, mitochondrial structural and functional dysfunction, excessive oxidative stress, persistent inflammatory response, cardiomyocyte apoptosis, and autophagic imbalance. While existing research has laid a preliminary foundation by exploring individual mechanisms, it lacks an integrated overview of how these pathological processes synergistically or sequentially intertwine to induce progressive myocardial cell injury and irreversible cardiac dysfunction. Therefore, this review aims to systematically summarize the latest research advancements on the key molecular mechanisms and critical signaling pathways involved in myocardial ischemia. It will specifically focus on dissecting the dynamic crosstalk between different pathological processes, with the ultimate objective of providing a solid theoretical basis for the development of multi-targeted precision therapy. This work is expected to offer new insights into the pathological progression of myocardial ischemia and further guide the design and development of innovative clinical intervention strategies.

KEYWORDS: Myocardial ischemia; molecular mechanism; inflammation; oxidative stress; mitochondria; autophagy

1 Introduction

Myocardial ischemia, as a central issue in the cardiovascular field, has long been a focal point and hotspot in medical research. The heart, serving as the body's "power pump", continuously supplies oxygen- and nutrient-rich blood to all tissues and organs [1]. Myocardial ischemia occurs when pathological changes, such as atherosclerosis, spasm, embolism, or other lesions in the coronary arteries lead to insufficient blood supply to the myocardium. It not only constitutes the primary pathological basis of coronary heart disease

but is also closely associated with various other cardiovascular conditions, including cardiomyopathy and arrhythmias [2].

Without timely and effective treatment, the progression of myocardial ischemia may result in myocardial infarction, heart failure, or even sudden cardiac death, posing a severe threat to patients' lives and health [2]. According to the World Health Organization (WHO), cardiovascular diseases have become the leading cause of mortality worldwide, with myocardial ischemia playing a significant role. In China, driven by an aging population and lifestyle changes, the incidence of myocardial ischemia has been rising annually, imposing substantial burdens on both society and families [3]. Therefore, an in-depth exploration of the molecular mechanisms underlying myocardial ischemia holds critical importance for developing effective therapeutic strategies and improving patient prognosis.

This article comprehensively and thoroughly analyzes the molecular mechanisms of myocardial ischemia. It systematically elucidates the roles of key signaling pathways—such as energy metabolism dysfunction, oxidative stress, calcium overload, inflammatory responses, apoptosis and necrosis, autophagy imbalance, and mitochondrial dysfunction—in the pathogenesis and progression of myocardial ischemia (Table 1). The aim is to provide a robust theoretical foundation and novel research perspectives for the prevention and treatment of myocardial ischemia.

Table 1: Comparison of core pathological mechanisms and key features of myocardial ischemia

Pathological mechanism	Core inducing factors	Key molecules/Pathways	Major damaging effects
Energy metabolism disorder [4,5]	Insufficient oxygen supply during ischemia, abnormal fatty acid oxidation during reperfusion	Enhanced glycolysis, AMPK/ACC/CPT-1 pathway	ATP depletion, lactic acid accumulation, and exacerbation of oxidative stress
Calcium overload [6,7]	Ion pump dysfunction caused by ATP reduction, activation of H^+-Na^+ exchange	Na^+/Ca^{2+} exchanger, PI3K-Akt/MAPK/CaN pathway	Mitochondrial damage, enzyme activation, and disruption of cellular structure
Mitochondrial dysfunction [8]	Ischemia-hypoxia, ROS attack, calcium overload	Respiratory chain complexes, MPTP, cyt C	Insufficient energy production, activation of apoptotic pathways, and ROS release
Oxidative stress [9]	Mitochondrial electron leakage, neutrophil respiratory burst	Nrf2/HDAC3 pathway, ROS (O_2^-, ·OH)	Lipid peroxidation, protein/DNA damage, amplification of inflammation
Inflammatory response [10]	Release of DAMPs from damaged cells, immune cell infiltration	NF-κB, JAK2-STAT3, NLRP3 inflammasome	Release of pro-inflammatory cytokines (TNF-α, IL-1β), tissue edema

(Continued)

Table 1 (continued)

Pathological mechanism	Core inducing factors	Key molecules/Pathways	Major damaging effects
Apoptosis/Necrosis [11,12]	Mitochondrial damage, activation of death receptors	Bcl-2 family, caspase family, p53	Reduction in cardiomyocyte number, decreased contractile function
Autophagic imbalance [13]	Energy deficiency, oxidative stress, and calcium overload	AMPK/mTOR/ULK1 pathway	Excessive degradation of organelles or accumulation of toxic substances

Note: Abbreviation: Adenosine 5′-monophosphate-activated protein kinase (AMPK), Acetyl-CoA carboxylase (ACC), Carnitine palmitoyltransferase 1 (CPT-1), Phosphoinositide 3-kinase (PI3K), Protein kinase B (Akt), Mitogen-activated protein kinase (MAPK), Calcineurin (CaN), Mitochondrial permeability transition pore (MPTP), Cytochrome c (cyt C), Nuclear factor erythroid 2-related factor 2 (Nrf2), Histone deacetylase 3 (HDAC3), Reactive oxygen species (ROS), Nuclear factor kappa B (NF-κB), Janus kinase 2 (JAK2), Signal transducer and activator of transcription 3 (STAT3), NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), B-cell lymphoma-2 (Bcl-2), Tumor protein p53 (p53), mammalian target of rapamycin (mTOR), Unc-51 like autophagy activating kinase 1 (ULK1), Tumor necrosis factor-alpha (TNF-α), Interleukin-1 beta (IL-1β).

2 Overview of Myocardial Ischemia

2.1 Definition and Classification

Myocardial ischemia is caused by reduced coronary blood flow due to atherosclerotic plaques, spasms, or emboli, leading to insufficient oxygen supply and impaired cardiac function. This forces the myocardium to shift to anaerobic metabolism [14], disrupting energy balance and resulting in the accumulation of metabolites such as lactate [15]. Its clinical manifestations include angina pectoris (the most common), myocardial infarction, ischemic cardiomyopathy, and latent coronary artery disease. Angina can be classified into three types: Stable angina is typically triggered by physical exertion, emotional stress, or cold exposure. It manifests as transient (3–5 min) retrosternal pressure/tightness, with pain radiating to the left arm, neck, or jaw, and is relieved by rest or nitrates [16]. The underlying mechanism involves fixed coronary stenosis, which prevents adequate blood flow to meet increased myocardial oxygen demand [16]. Unstable angina occurs unpredictably, even at rest, and is characterized by prolonged (>30 min) severe chest pain that responds poorly to nitrates [17]. It primarily results from plaque rupture accompanied by thrombus formation, vasospasm, or inflammatory responses. Acute myocardial infarction (AMI) is caused by sudden coronary occlusion (usually thrombotic). It presents with persistent (>30 min) crushing substernal pain unrelieved by nitrates, often accompanied by autonomic symptoms (e.g., sweating, nausea) and potential complications such as heart failure [18] (Fig. 1).

2.2 Incidence Status and Hazards

Myocardial ischemia-related diseases, including coronary heart disease and myocardial infarction, remain a leading global health threat, accounting for high morbidity and mortality rates [19]. Cardiovascular diseases—the world's top cause of death—claimed 17.9 million lives in 2016, with myocardial ischemia playing a major role. In China, such conditions account for ~40% of annual deaths [20]. Rising risk factors (e.g., aging populations, high-salt/fat diets, physical inactivity, and smoking) have driven increasing incidence

rates, now affecting younger demographics. Acute events like AMI carry high fatality risks, while survivors often face severe complications (e.g., heart failure, arrhythmias), significantly impairing cardiac function and independence. Chronic ischemia can progress to irreversible ischemic cardiomyopathy [21]. Even non-fatal angina episodes reduce quality of life through recurrent chest pain and functional limitations. These outcomes impose substantial medical and socioeconomic burdens. Thus, advancing research into molecular mechanisms and therapies is critical to mitigate disease impact (Fig. 1). Through the above content, we have clearly grasped the clinical scope, classification characteristics, and global disease burden of myocardial ischemia, which lays a clinical foundation for further analyzing its pathological essence. In the pathological cascade of myocardial ischemia, energy metabolism disorder is the earliest activated core link. The next section will focus on this pathway, explaining the transformation mechanism of myocardial cells from aerobic oxidation to anaerobic glycolysis under ischemic conditions and the metabolic abnormal characteristics during the reperfusion phase.

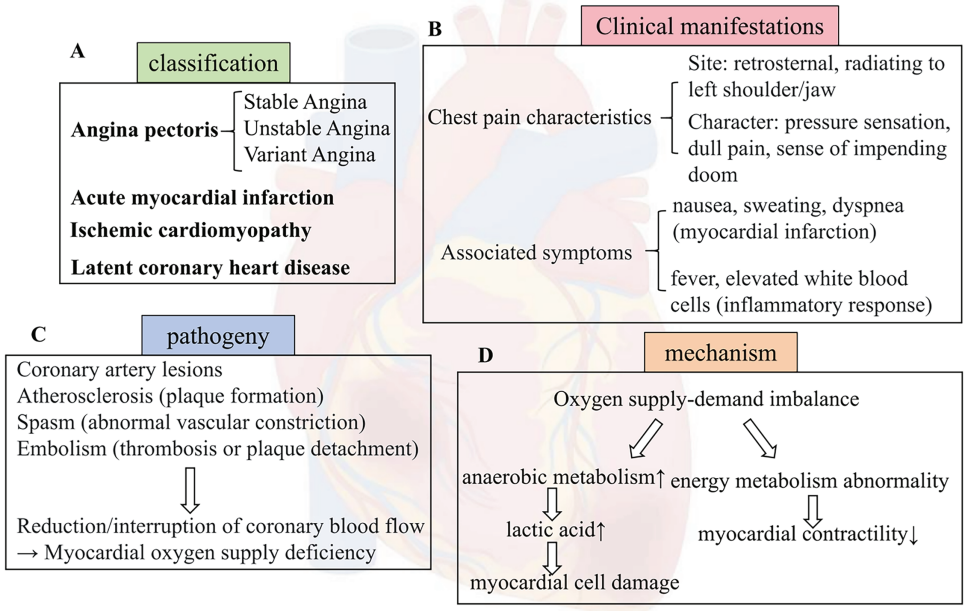

Figure 1: Classification, clinical manifestations, pathogenesis, and mechanism of myocardial ischemia. (**A**) Classifies myocardial ischemia into four types: angina pectoris (stable, unstable, variant), acute myocardial infarction (AMI), ischemic cardiomyopathy, and latent coronary heart disease. (**B**) Highlights retrosternal pain (radiating to left shoulder/jaw, described as pressure/dull ache) as the core symptom, with associated manifestations like nausea/sweating (in AMI), fever (in inflammation), and dyspnea. (**C**) Details coronary artery lesions (atherosclerosis, spasm, embolism) that reduce/interrupt blood flow, causing myocardial oxygen deficit. (**D**) Explains the oxygen supply-demand imbalance: coronary stenosis/occlusion or increased oxygen demand (exercise/emotion) forces anaerobic metabolism, leading to lactic acid accumulation, myocardial cell damage, and impaired cardiac function

3 Energy Metabolism Disorder Signaling Pathway

3.1 Energy Metabolism Changes during Ischemia

During early myocardial ischemia (within seconds), cardiomyocytes rapidly shift from aerobic mitochondrial oxidation to anaerobic glycolysis due to oxygen deprivation [22]. While glycolysis provides immediate ATP, its yield is insufficient for cellular demands, forcing reliance on stored high-energy phosphates (creatine phosphate and ATP), which degrade rapidly [23]. This process increases inorganic phosphate and lactate accumulation, causing intracellular acidosis. The resulting pH drop inhibits phosphofructokinase

(PFK), a key glycolytic enzyme, further impairing ATP regeneration. Concurrently, ADP converts to AMP and eventually adenosine, which leaks from cells, depleting the adenine nucleotide pool and worsening the energy crisis.

3.2 Abnormal Energy Metabolism during Reperfusion

Following coronary reperfusion, myocardial energy metabolism remains impaired for an extended period despite restored blood flow [24]. Early reperfusion is characterized by persistent mitochondrial dysfunction, limiting aerobic oxidation capacity, while washout of lactate and inorganic phosphate reduces glycolysis inhibition, maintaining glycolytic dominance [24]. Studies in rat ischemia-reperfusion models demonstrate that even 24 h post-reperfusion-when contractile function has recovered-regional blood flow remains reduced with persistent glycolytic activation [25]. Paradoxically, fatty acid oxidation rapidly rebounds beyond pre-ischemic levels during reperfusion. Ischemia-induced AMP accumulation activates adenosine 5′-monophosphate-activated protein kinase (AMPK), which phosphorylates acetyl-CoA carboxylase (p-ACC), relieving inhibition of carnitine palmitoyltransferase 1 (CPT-1) and enhancing mitochondrial fatty acid uptake [26]. Upon reoxygenation, this leads to excessive β-oxidation. Both experimental and clinical evidence show this metabolic shift impairs functional recovery: while β-oxidation increases ATP production, its higher oxygen demand (compared to glucose metabolism) is detrimental in the oxygen-limited reperfusion environment [27]. Additionally, enhanced fatty acid oxidation suppresses glucose oxidative phosphorylation, exacerbating cellular injury.

3.3 Mechanisms of Damage Caused by Energy Metabolism Disorder

Energy metabolism disorder is closely linked to calcium overload. Normally, myocardial cells maintain stable intracellular ion concentrations through membrane pumps like Na^+-K^+-ATPase and Ca^{2+}-ATPase, ensuring proper Ca^{2+} transport [28]. However, when energy metabolism is disrupted, ATP production declines, impairing these pumps. Reduced Na^+-K^+-ATPase activity raises intracellular Na^+ levels, prompting excessive Ca^{2+} influx via the Na^+-Ca^{2+} exchanger, causing calcium overload [29]. This overload activates calcium-dependent enzymes like proteases and phospholipases, degrading cellular proteins and membranes, damaging myocardial fibers, increasing membrane permeability, and disrupting mitochondrial function [30]. Additionally, calcium overload exacerbates oxidative stress by generating oxygen-free radicals. Mitochondrial dysfunction is another consequence of an energy metabolism disorder. As the primary site of energy production, mitochondria are impaired during myocardial ischemia due to oxygen deficiency, which disrupts the respiratory chain, reduces ATP synthesis, and increases reactive oxygen species (ROS) [31]. ROS oxidizes mitochondrial lipids, proteins, and DNA, lowering membrane potential, causing swelling, rupture, and release of apoptotic factors like cytochrome C (cyt C), triggering cell death [32]. Oxidative stress further amplifies damage from energy metabolism disorder. Ischemia-reperfusion generates excessive ROS, while antioxidant defenses (e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)) are weakened, failing to neutralize ROS effectively. This oxidative attack damages cell membranes, proteins, and DNA, disrupting cellular integrity [33]. Oxidative stress also activates inflammatory pathways, releasing mediators that worsen myocardial injury (Fig. 2). In summary, myocardial energy metabolism imbalance during ischemia and reperfusion not only leads to ATP depletion and lactic acid accumulation but also disrupts ion homeostasis by inhibiting Na^+/K^+-ATPase activity, creating prerequisites for the occurrence of calcium overload. The next section will focus on the calcium overload signaling pathway, elaborating on how abnormal elevation of intracellular Ca^{2+} exacerbates the structural and functional damage of myocardial cells by activating effector molecules such as calmodulin (CaM)-dependent kinases and phospholipases.

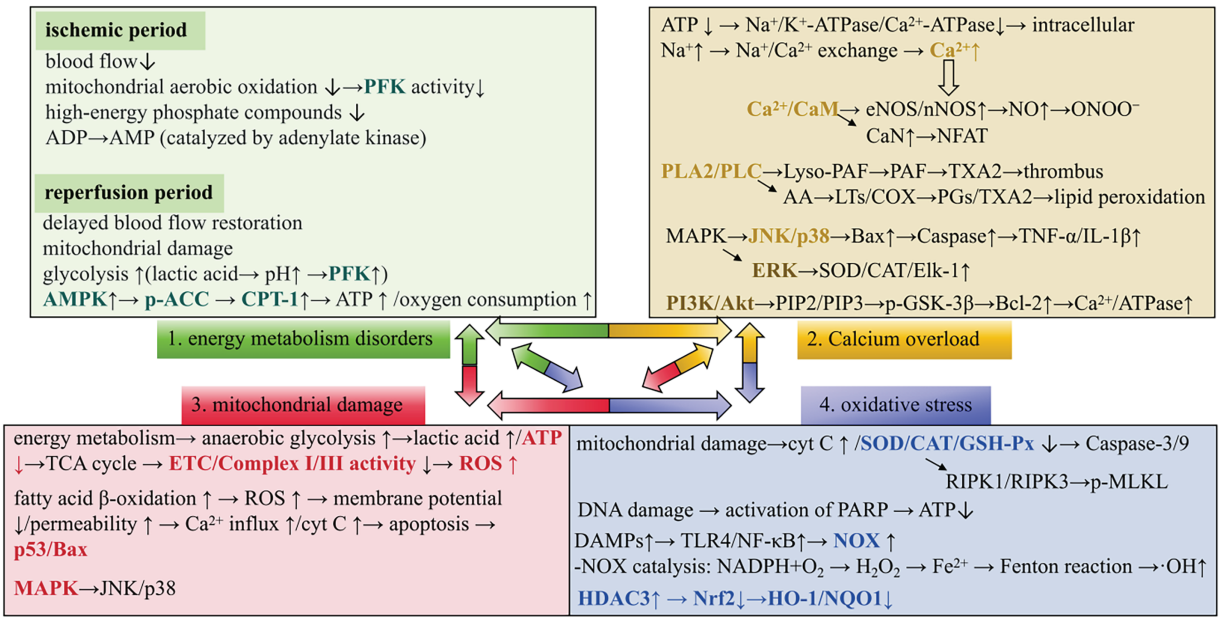

Figure 2: Core pathological mechanisms of myocardial ischemia and their crosstalk. 1. Energy metabolism disorder: Ischemia reduces blood flow and myocardial oxygen supply, suppressing mitochondrial aerobic oxidation while enhancing glycolysis (causing lactic acid accumulation and ATP depletion). Reperfusion increases fatty acid β-oxidation, partially restoring ATP but promoting ROS overproduction. 2. Calcium Overload: ATP depletion impairs Na^+/K^+-ATPase and Ca^{2+} pump activity, leading to intracellular Na^+ accumulation and subsequent Na^+/Ca^{2+} reverse exchange, resulting in abnormal intracellular Ca^{2+} elevation. This activates calcium-dependent enzymes (e.g., CaN, eNOS) and apoptotic pathways. 3. Mitochondrial Damage: Ischemia-reperfusion disrupts mitochondrial ETC function, causing excessive ROS generation, mitochondrial membrane potential decline, and cyt C efflux, which activates the Caspase cascade and promotes apoptosis. 4. Oxidative Stress: Excessive ROS (e.g., ·OH, H_2O_2) and antioxidant system (SOD, CAT, GSH-Px) imbalance induce lipid peroxidation, DNA damage, and release of inflammatory cytokines (TNF-α, IL-1β), exacerbating tissue injury. These mechanisms interact reciprocally (indicated by double-headed arrows), forming a vicious cycle that ultimately leads to myocardial cell dysfunction or death. Abbreviation: Phosphofructokinase (PFK), Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), Phosphorylated acetyl-CoA carboxylase (p-ACC), Mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), Bcl-2-associated X protein (Bax), Extracellular signal-regulated kinase (ERK), Superoxide dismutase (SOD), Catalase (CAT), Phosphatidylinositol 4,5-bisphosphate (PIP2), Phosphatidylinositol 3,4,5-trisphosphate (PIP3), Phosphorylated glycogen synthase kinase-3β (p-GSK-3β), Calmodulin (CaM), Endothelial nitric oxide synthase (eNOS), Neuronal nitric oxide synthase (nNOS), Nitric oxide (NO), Peroxynitrite (ONOO–), Nuclear factor of activated T-cells (NFAT), Phospholipase A2 (PLA2), Phospholipase C (PLC), Lysophosphatidic acid platelet-activating factor (Lyso-PAF), Platelet-activating factor (PAF), Thromboxane A2 (TXA2), Arachidonic acid (AA), Leukotrienes (LTs), Cyclooxygenase (COX), proteoglycan (PGs), Tricarboxylic acid cycle (TCA), Electron transport chain (ETC), Glutathione peroxidase (GSH-Px), Receptor-interacting serine/threonine-protein kinase 1/3 (RIPK1/3), Phosphorylated mixed lineage kinase domain-like protein (p-MLKL), Poly(ADP-ribose) polymerase (PARP), Damage-associated molecular patterns (DAMPs), Toll-like receptor 4 (TLR4), Nicotinamide adenine dinucleotide phosphate oxidase (NOX), Histone deacetylase 3 (HDAC3), Nuclear factor erythroid 2-related factor 2 (Nrf2), Heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1)

4 Calcium Overload Signaling Pathway

4.1 Mechanisms of Calcium Overload Formation

Myocardial ischemia induces calcium overload through three key mechanisms. First, ATP depletion disrupts Ca^{2+} homeostasis, reducing mitochondrial ATP production impairs the ATP-Ca^{2+} exchanger's

ability to pump Ca^{2+} out of cells, allowing extracellular Ca^{2+} influx to raise intracellular concentrations [34]. Second, ischemic acidosis activates ion exchange cascades. Anaerobic metabolism generates lactic acid, increasing intracellular H^+ and triggering H^+-Na^+ exchange. The resulting Na^+ accumulation then stimulates Na^+-Ca^{2+} exchange, further elevating Ca^{2+} levels [35]. Third, pathological conditions reverse Na^+/Ca^{2+} exchanger activity. Normally expelling Ca^{2+} (forward mode), this exchanger switches direction during ischemia-reperfusion due to membrane potential changes and Na^+ overload, actively importing Ca^{2+} and exacerbating cellular damage [36]. These interconnected pathways collectively drive calcium overload in ischemic myocardium.

4.2 Pathways of Myocardial Damage Induced by Calcium Overload

Calcium overload triggers enzymatic cascades that severely damage cardiomyocytes. The Ca^{2+}/CaM system plays a central role-elevated Ca^{2+} binds CaM to form complexes that activate Ca^{2+}/CaM enzymes, stimulating excessive nitric oxide synthase endothelial nitric oxide synthase (eNOS)/neuronal nitric oxide synthase (nNOS) expression [37]. While nitric oxide (NO) normally has protective effects, calcium overload leads to harmful peroxynitrite (ONOO–) formation through reaction with superoxide, causing oxidative damage to cellular components [38]. Concurrently, calcium overload activates phospholipases A2 and C (PLA2/PLC). PLA2 generates lysophosphatidic acid (Lyso)-platelet-activating factor (PAF) and arachidonic acid (AA), with Lyso-PAF converting to pro-inflammatory PAF [21]. PAF promotes thrombus formation through thromboxane A2 (TXA2) while suppressing protective prostacyclin I2 (PGI2), disrupting vascular homeostasis [39,40]. PLC-mediated AA metabolism produces inflammatory leukotrienes and lipid peroxidation products that damage cell membranes through oxidative stress, ultimately impairing cellular function and viability [41]. These pathways collectively exacerbate myocardial injury during calcium overload.

4.3 Signaling Pathways and Regulatory Mechanisms for Calcium Overload Homeostasis

The phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt) pathway plays a critical role in regulating calcium overload during myocardial ischemia. Activated by extracellular signals, PI3K generates phosphatidylinositol 3,4,5-trisphosphate (PIP3) to recruit and phosphorylate Akt [42]. Akt then modulates calcium homeostasis through multiple mechanisms: (1) phosphorylating glycogen synthase kinase-3β (GSK-3β) to enhance anti-apoptotic Bcl-2 expression [43]; (2) activating Ca^{2+}-ATPase to promote calcium efflux; and (3) inhibiting the reverse Na^+/Ca^{2+} exchanger to reduce calcium influx [44]. These actions collectively alleviate calcium overload and protect cardiomyocytes. The mitogen-activated protein kinase (MAPK) pathway exhibits dual roles in calcium regulation. Early ischemia activates extracellular signal-regulated kinase (ERK), which upregulates antioxidant enzymes (SOD, CAT) and survival genes via Ets-like protein 1 (Elk-1), providing temporary protection [45]. However, prolonged ischemia excessively activates c-Jun N-terminal kinase (JNK)/p38 MAPK, promoting apoptosis through Bcl-2-associated X protein (Bax)/cyt C and inflammation via tumor necrosis factor-α (TNF-α)/interleukin-1β (IL-1β), ultimately worsening calcium overload damage [46]. The calcineurin (CaN) pathway directly responds to calcium overload. Ca^{2+}/calmodulin activates CaN, which dephosphorylates nuclear factor of activated T-cells (NFAT) to drive pathological gene expression, leading to hypertrophy, fibrosis, and apoptosis [47]. CaN inhibitors like cyclosporine A show protective effects, while crosstalk with MAPK pathways further influences cell fate under calcium overload conditions. These interconnected pathways collectively determine the severity of calcium overload injury in ischemic myocardium (Fig. 2). Calcium overload serves as a key link connecting energy metabolism disorder and mitochondrial damage through mechanisms such as activating the CaN-NFAT pathway and promoting mitochondrial Ca^{2+} overload. As the "powerhouse" of myocardial cells, mitochondrial dysfunction plays a connecting role in the pathological process of myocardial ischemia.

The next section will delve into the molecular mechanisms of mitochondrial respiratory chain damage, membrane potential collapse, and mitochondrial permeability transition pores (MPTP) opening under ischemic stress.

5 Mitochondrial Dysfunction

5.1 Physiological Functions of Mitochondria

Mitochondria serve as vital cellular organelles with several essential functions. First, they act as the cell's powerhouse, generating the majority of cellular ATP through oxidative metabolism. In addition to glucose, fatty acids represent a major substrate for mitochondrial energy production, especially in tissues such as adult cardiomyocytes, where fatty-acid oxidation predominates. These substrates are oxidized via the tricarboxylic acid (TCA) cycle and the electron transport chain to drive ATP synthesis, supporting critical cellular processes including biosynthesis and contraction [48]. Second, mitochondria play a central role in regulating programmed cell death. Under stress conditions, they release cyt C, which combines with apoptotic protease-activating factor-1 (Apaf-1) to form the apoptosome, leading to caspase-9 activation and initiation of apoptosis. Mitochondria also release proteins such as second mitochondria-derived activator of caspases (SMAC/DIABLO), which counteract inhibitor of apoptosis proteins (IAPs), further promoting cell death [49]. Third, mitochondria contribute to calcium homeostasis. Through uptake mechanisms mediated by the mitochondrial calcium uniporter (MCU) and efflux via the sodium-calcium exchanger (NCLX), mitochondria help buffer cytoplasmic Ca^{2+} levels and coordinate with the endoplasmic reticulum to modulate calcium-dependent signaling [50]. Together, these functions underscore the role of mitochondria as integrative hubs governing cellular energy balance, apoptosis, and calcium dynamics.

5.2 Ischemia-Induced Mitochondrial Damage

Myocardial ischemia causes significant mitochondrial damage through structural and functional alterations. Structurally, ischemia induces mitochondrial swelling due to increased membrane permeability, along with cristae fragmentation and membrane damage from lipid oxidation. These changes, observable via electron microscopy, disrupt respiratory chain assembly and correlate with cellular injury severity [51]. Functionally, ischemia impairs respiratory chain complexes (particularly I and III), reducing ATP production and causing electron leakage that generates excessive ROS [52,53]. This oxidative stress further damages mitochondria. Additionally, the proton gradient collapses, lowering membrane potential [54]. This impairs energy production and triggers MPTP opening, leading to matrix content leakage and cell death pathways [55]. Together, these ischemic changes severely compromise mitochondrial and cellular function.

5.3 Mechanisms and Signaling Pathways Mediating Mitochondrial Dysfunction

Myocardial ischemia triggers mitochondrial dysfunction through multiple interconnected mechanisms. Energy metabolism impairment is central, as ischemia shifts ATP production from aerobic oxidation to inefficient glycolysis, causing ATP depletion and acidosis [56]. This energy deficit reduces respiratory chain activity and membrane potential, creating a vicious cycle of worsening mitochondrial function [57]. Oxidative stress and mitochondrial damage reinforce each other. Ischemia-reperfusion generates excessive ROS that attack mitochondrial components, while dysfunctional mitochondria produce more ROS due to electron leakage [58]. Similarly, calcium overload exacerbates mitochondrial damage through multiple pathways: Ca^{2+} precipitates cause swelling, MPTP opening triggers apoptosis, and respiratory chain inhibition worsens energy deficits. Key signaling pathways regulate these processes. The p53 pathway promotes dysfunction by increasing Bax (pro-apoptotic) while decreasing Bcl-2 (anti-apoptotic), facilitating Cyt C release [59]. MAPK pathways show dual roles-while ERK provides early protection, sustained JNK/p38

activation worsens damage through mitochondrial apoptosis pathways [60]. Therapeutic interventions targeting these mechanisms (energy substrates, antioxidants, calcium blockers, or pathway inhibitors) show protective potential in experimental models [61] (Fig. 2). Mitochondrial dysfunction not only causes energy production disorders but also generates a large amount of ROS through respiratory chain electron leakage, becoming the main source of oxidative stress. As an important pathological mediator of myocardial ischemia, oxidative stress can amplify cell damage through lipid peroxidation, protein oxidative modification, and other ways. The next section will systematically analyze the generation mechanism of oxidative stress and its interaction with inflammatory response and apoptosis.

6 Oxidative Stress Signaling Pathway

6.1 Inducing Factors of Oxidative Stress

Oxidative stress occurs when harmful stimuli disrupt the balance between ROS production and antioxidant defenses, causing cellular damage [62]. In myocardial ischemia, oxidative stress mainly stems from two sources: mitochondrial dysfunction and neutrophil activity. Mitochondria become major ROS producers during ischemia. Impaired oxygen supply disrupts the respiratory chain's electron flow, leading to electron leakage and superoxide anion (O_2^-) formation [63]. These radicals further generate more damaging ROS like H_2O_2 and ·OH, which oxidize mitochondrial components, causing structural damage and worsening energy metabolism in a destructive cycle. Neutrophils amplify oxidative stress during reperfusion. Activated neutrophils employ NADPH oxidase to produce superoxide anions, which transform into highly reactive hydroxyl radicals via the Fenton reaction [64]. These ROS severely damage cardiac cells and tissues, exacerbating ischemia-reperfusion injury.

6.2 Mechanisms of Myocardial Damage Caused by Oxidative Stress

Oxidative stress generates highly reactive free radicals (superoxide anions, hydroxyl radicals, hydrogen peroxide) that severely damage cardiomyocytes through multiple mechanisms. These radicals primarily attack: Cell membranes: Initiating lipid peroxidation of unsaturated fatty acids, forming toxic aldehydes like malondialdehyde (MDA) that disrupt membrane integrity, increasing permeability, and causing calcium imbalance. Proteins: Oxidizing critical amino acids in ion channels, mitochondrial enzymes, and metabolic proteins, impairing their function [65]. DNA: Causing strand breaks, base modifications, and crosslinks that disrupt genetic integrity and cellular function [66]. Such damage triggers cell death pathways—either programmed apoptosis (via mitochondrial Cyt C release and caspase activation) or unregulated necrosis with inflammatory consequences [67]. Additionally, free radicals activate inflammatory cells to release cytokines (TNF-α, IL-1β, IL-6), creating a vicious cycle of inflammation that worsens ischemia through vascular dysfunction and thrombosis [68]. These interconnected pathways collectively contribute to progressive myocardial damage under oxidative stress conditions.

6.3 Core Signaling Pathways and Key Regulatory Molecules of Oxidative Stress

The nuclear factor E2-related factor 2 (Nrf2) signaling pathway serves as a crucial cellular defense against oxidative stress. Normally bound to kelch-like ECH-associated protein 1 (Keap1) and targeted for degradation, Nrf2 is released during oxidative stress and translocates to the nucleus. There, it activates antioxidant genes (heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), and glutamate cysteine ligase (GCL)) through antioxidant response elements (AREs) binding, enhancing ROS clearance [69]. Experimental studies demonstrate Nrf2 activation (via sulforaphane or curcumin) reduces ischemia-reperfusion injury by decreasing cell death and improving cardiac function. Histone deacetylase 3 (HDAC3) negatively regulates this protective mechanism by suppressing Nrf2 activity. During ischemia, elevated HDAC3 inhibits

Nrf2 acetylation, reducing antioxidant gene expression [70]. Pharmacological inhibition of HDAC3 (e.g., with RGFP966 or kaempferol) restores Nrf2 function, demonstrating therapeutic potential in both animal models (isoproterenol-induced) and cell studies ($CoCl_2$-stimulated) by mitigating oxidative damage and preserving cardiac function [70]. These findings highlight the interplay between Nrf2 and HDAC3 as a key regulatory axis in myocardial oxidative stress responses (Fig. 2). The above content indicates that oxidative stress forms a positive feedback loop with inflammatory response by activating pathways such as the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome and promoting nuclear factor kappa-B (NF-κB) nuclear translocation, jointly exacerbating myocardial ischemia-reperfusion injury. The next section will focus on the inflammatory response signaling pathway, analyzing the regulatory mechanisms of neutrophil infiltration, macrophage M1 polarization, and the release of pro-inflammatory cytokines (such as TNF-α and IL-1β).

7 Inflammatory Response Signaling Pathway

7.1 Roles of Inflammatory Cells and Cytokines in Myocardial Ischemia

Myocardial ischemia triggers a rapid inflammatory response involving multiple immune cells and cytokines that collectively damage cardiac tissue. Neutrophils are the first responders, infiltrating ischemic areas to clear debris but also releasing harmful ROS and lysosomal enzymes that oxidize cellular components and degrade structural proteins [71]. Macrophages play dual roles in this process. Early-stage pro-inflammatory M1 macrophages secrete damaging cytokines (TNF-α, IL-1β, IL-6) that promote apoptosis, reduce contractility, and amplify inflammation [72]. While later-stage anti-inflammatory M2 macrophages attempt tissue repair through IL-10 secretion, their effects are often insufficient against established damage [73]. Key cytokines like TNF-α and IL-6 activate destructive signaling pathways. TNF-α stimulates NF-κB to upregulate inflammatory genes (inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)), while IL-6 activates janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) signaling, further amplifying immune cell activation [74]. These interconnected inflammatory processes create a vicious cycle of myocardial damage, exacerbating cell dysfunction and death while impairing cardiac function.

7.2 Core Inflammatory Signaling Pathways in Myocardial Ischemia

The JAK2-STAT3 pathway is crucial in myocardial ischemia-induced inflammation. When IL-6 binds its receptor, it activates JAK2, which phosphorylates STAT3. Activated STAT3 dimers then regulate pro-inflammatory genes (IL-6, IL-8, intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1)), amplifying inflammation [75]. Inhibiting this pathway reduces cytokine release and improves cardiac function in ischemia-reperfusion models. The NLRP3 inflammasome pathway responds to ischemic damage signals (ATP, ROS) by assembling with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and pro-caspase-1. Active caspase-1 processes pro-IL-1β/IL-18, triggering inflammatory cell recruitment and tissue damage [76]. NLRP3 inhibition has shown protective effects in myocardial ischemia. The lysyl oxidase (LOX)-NF-κB pathway contributes through arachidonic acid metabolites. LOX-derived leukotrienes activate IκB kinase (IKK), leading to IκB degradation and NF-κB nuclear translocation. This upregulates inflammatory mediators (TNF-α, IL-1β, IL-6) that worsen tissue injury [77]. Both LOX and NF-κB inhibition demonstrate therapeutic potential for ischemia-reperfusion injury.

7.3 Interactions between Inflammatory Response and Other Pathological Mechanisms

Inflammation and oxidative stress form a vicious cycle that worsens myocardial ischemic injury. Oxidative stress generates ROS that not only directly damage cellular components but also activate inflammatory pathways like NLRP3 inflammasome and NF-κB, triggering cytokine release (IL-1β, IL-18) and amplifying inflammation [78]. Conversely, inflammatory cells (macrophages, neutrophils) produce additional ROS through respiratory bursts, while cytokines like TNF-α further disrupt redox balance by both increasing ROS production and suppressing antioxidant defenses [79]. This pathological interplay extends to calcium homeostasis. Inflammation promotes calcium overload by impairing ion pump function (Na^+-K^+-ATPase, Ca^{2+}-ATPase) and activating CaN [80]. Conversely, excess calcium activates proteases and NF-κB, releasing inflammatory mediators that further exacerbate inflammation. Therapeutic studies show that targeting either calcium overload or inflammation can break this cycle, with calcium suppression reducing inflammation and anti-inflammatory treatments mitigating calcium dysregulation in ischemia-reperfusion models (Fig. 3). Inflammatory response damages the vascular endothelial barrier and aggravates myocardial interstitial edema by releasing pro-inflammatory mediators, and at the same time cooperates with oxidative stress to promote myocardial cells to transform into apoptosis or necrosis. The next section will focus on the cell death pathway, comparatively analyzing the functional differences and regulatory networks of mitochondrial pathway apoptosis (Cyt C-Apaf-1-caspase-9 pathway) and necroptosis (Receptor-interacting serine/threonine-protein kinase 1 (RIPK1)-RIPK3-mixed lineage kinase domain-like protein (MLKL) pathway) in myocardial ischemia.

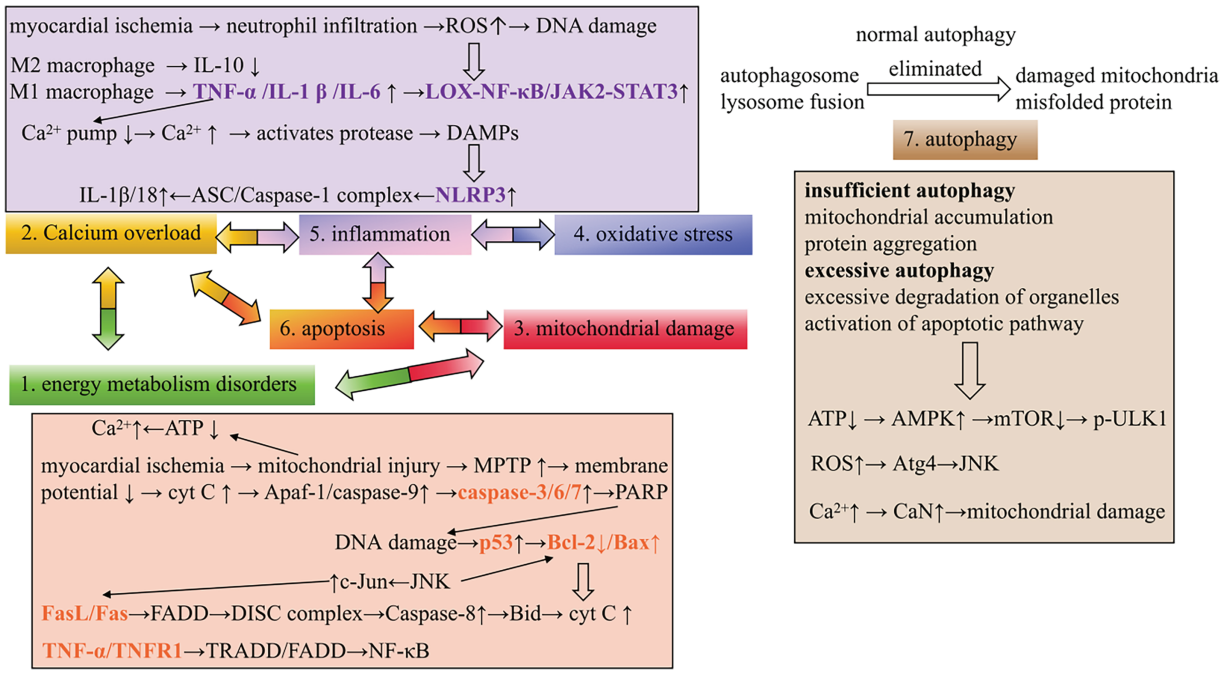

Figure 3: Intertwined pathological mechanisms driving inflammation, apoptosis, and autophagic dysregulation in myocardial ischemia. Inflammation: Myocardial ischemia induces neutrophil infiltration (elevating ROS and DNA damage) and macrophage polarization imbalance (reduced anti-inflammatory IL-10 from M2 macrophages; pro-inflammatory TNF-α/IL-1β/IL-6 from M1 macrophages activating LOX-NF-κB and JAK2-STAT3 pathways). Calcium overload (due to impaired Ca^{2+} pump and ATP depletion) releases DAMPs, while NLRP3 inflammasome activation promotes ASC/Caspase-1 complex formation to amplify IL-1β/IL-18 production. Apoptosis: Mitochondrial injury (MPTP opening, membrane potential collapse, cyt C release) activates the caspase

(Continued)

Figure 3: (continued) cascade (caspase-9/-3/-6/-7), leading to PARP-mediated DNA repair failure. DNA damage upregulates p53 (tilting Bcl-2/Bax toward apoptosis), JNK activates c-Jun, and Fas/FasL/TNF-α/TNFR1 pathways recruit FADD/TRADD to amplify caspase-8 and NF-κB activity. Autophagy: Normal autophagy clears damaged mitochondria/misfolded proteins via autophagosome-lysosome fusion; insufficient autophagy causes organelle/protein accumulation, while excessive autophagy induces apoptosis. Energy stress activates AMPK/mTOR/ULK1 signaling, and ROS/Ca^{2+} overload impairs mitochondria via Atg4/JNK and calcineurin pathways. These mechanisms interact to drive ischemic myocardial injury. Abbreviation: Interleukin-10 (IL-10), Interleukin-6 (IL-6), Lysyl oxidase (LOX), Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), Interleukin-18 (IL-18), Mitochondrial permeability transition pore (MPTP), Apoptotic protease activating factor 1 (Apaf-1), Fas ligand (FasL), Fas-associated death domain (FADD), Death-inducing signaling complex (DISC), BH3-interacting domain death agonist (Bid), TNF receptor 1 (TNFR1), TNF receptor-associated death domain (TRADD), Phosphorylated UNC-51-like kinase 1 (p-ULK1), Autophagy-related gene 4 (Atg4)

8 Apoptosis and Necrosis

8.1 Mechanisms of Apoptosis and Necrosis in Myocardial Ischemia

Myocardial ischemia triggers apoptosis through two main pathways: mitochondrial and death receptor-mediated. The mitochondrial pathway initiates when ischemia damages mitochondria, causing MPTP opening, membrane potential collapse, and Cyt C release [81]. Cyt C binds Apaf-1 to form apoptosomes that activate caspase-9 and downstream executioner caspases (caspase-3/-6/-7), leading to cellular breakdown [82]. The death receptor pathway activates when Fas ligand (FasL) or TNF-α binds their respective receptors (Fas/tumor necrosis factor receptor (TNFR)), recruiting Fas-associated death domain protein (FADD)/TNFR1-associated death domain protein (TRADD) and forming death-inducing signaling complex (DISC) to activate caspase-8 [83]. Caspase-8 both directly triggers apoptosis and amplifies mitochondrial signaling via BH3-interacting domain death agonist (Bid) cleavage. TNF-α additionally activates NF-κB, linking apoptosis to inflammation [84]. Necrosis occurs when severe ischemia causes ATP depletion, disrupting ion homeostasis and activating destructive enzymes (proteases, phospholipases, nucleases) [85]. Membrane rupture releases cellular contents, inducing inflammation that further damages surrounding tissue and impairs cardiac function [86]. These cell death pathways collectively contribute to ischemic myocardial injury.

8.2 Regulatory Factors and Signaling Pathways of Apoptosis and Necrosis

The Bcl-2 protein family serves as a central regulator of apoptosis, comprising both anti-apoptotic (Bcl-2, Bcl-xL) and pro-apoptotic (Bax, Bak) members. Anti-apoptotic proteins maintain mitochondrial integrity by preventing cyt C release and neutralizing pro-apoptotic proteins [87], while pro-apoptotic members like Bax translocate to mitochondria during stress to form pores that facilitate apoptotic factor release. Their dynamic interactions through dimerization determine cell fate [88]. In myocardial ischemia, decreased Bcl-2/Bcl-xL expression shifts this balance toward apoptosis. The caspase cascade executes apoptosis through initiator (caspase-8/-9) and executioner (caspase-3/-6/-7) members [89]. Activated through distinct pathways (DISC formation in death receptor signaling or apoptosome assembly in mitochondrial pathways), these proteases cleave critical substrates like poly(ADP-ribose) polymerase (PARP) and cytoskeletal proteins to dismantle the cell [90]. p53, which responds to ischemia by upregulating pro-apoptotic genes (Bax) while suppressing anti-apoptotic Bcl-2, directly promotes mitochondrial cyt C release [91]; JNK signaling, which phosphorylates and inactivates Bcl-2/Bcl-xL while activating pro-apoptotic transcription via c-Jun/AP-1 [92]. Experimental inhibition of these pathways (particularly JNK) demonstrates therapeutic potential by reducing apoptosis in ischemia-reperfusion models. This multi-layered regulation highlights the complex interplay between survival and death signals in ischemic cardiomyocytes.

8.3 Impacts of Apoptosis and Necrosis on Myocardial Function

Cardiomyocyte death through apoptosis and necrosis significantly impairs cardiac function by reducing the number of functional heart muscle cells [93]. During myocardial ischemia, apoptosis predominates initially, with necrosis increasing as ischemia persists. This cellular loss disrupts myocardial tissue structure, diminishing both contractile and relaxation capabilities. In myocardial infarction, widespread necrosis in the affected area leads to complete loss of local contraction, compromising overall cardiac output [94]. The mechanisms involve: Systolic dysfunction-energy depletion from ATP deficiency and calcium imbalance disrupts excitation-contraction coupling, causing abnormal contractions and reduced cardiac output [95]. Diastolic impairmen-cellular damage, and subsequent fibrosis decrease myocardial elasticity, limiting ventricular filling capacity [96]. These pathological changes manifest clinically as reduced ejection fraction and symptoms like dyspnea, with the extent of dysfunction correlating with the degree of cardiomyocyte loss. The transition from apoptotic to necrotic cell death patterns reflects worsening ischemic injury over time (Fig. 3). As irreversible forms of cell damage in myocardial ischemia, apoptosis and necrosis are closely related to upstream pathways such as energy metabolism disorder and oxidative stress. Autophagy, as the "self-repair mechanism" of cells, presents an imbalanced state of excessive activation or insufficient activation under ischemic conditions. The next section will explore how autophagic imbalance participates in the pathological process of myocardial ischemia by affecting the clearance of damaged mitochondria and the degradation of misfolded proteins.

8.4 Emerging Regulated Cell Death Pathways: Pyroptosis and Ferroptosis in Myocardial Ischemia

In addition to classical apoptosis and necrosis, emerging regulated cell death pathways—pyroptosis and ferroptosis—have been identified as critical contributors to myocardial ischemic injury (Fig. 4), enriching the understanding of cell death mechanisms in this pathological process [97,98]. Pyroptosis is a pro-inflammatory programmed cell death pathway mediated by gasdermin (GSDM) family proteins [99]. Myocardial ischemia triggers pyroptosis through two main mechanisms: the canonical pathway activated by NLRP3 inflammasomes and the non-canonical pathway mediated by caspase-4/5/11. During ischemia, mitochondrial dysfunction and oxidative stress induce the release of DAMPs (e.g., ATP, mtDNA) and ROS, which activate the NLRP3 inflammasome [100,101]. This leads to cleavage of pro-caspase-1, which in turn processes pro-IL-1β/IL-18 into mature inflammatory cytokines and cleaves gasdermin D (GSDMD) into N-terminal fragments. These fragments form pores on the cell membrane, causing cell swelling and lysis, and releasing inflammatory factors to amplify myocardial inflammation. The non-canonical pathway is activated by lipopolysaccharide (LPS) or other pathogenic signals, which directly activate caspase-4/5/11 to cleave GSDMD, triggering pyroptosis independently of NLRP3 [102]. Studies have shown that inhibition of pyroptosis (e.g., targeting NLRP3, caspase-1, or GSDMD) reduces myocardial infarct size and improves cardiac function in ischemia-reperfusion models, highlighting its therapeutic potential [103,104].

Ferroptosis is an iron-dependent regulated cell death characterized by excessive accumulation of lipid peroxides. Myocardial ischemia promotes ferroptosis through multiple interconnected mechanisms [105]. Ischemia-induced oxidative stress disrupts the balance of iron metabolism: increased expression of transferrin receptor 1 (TfR1) enhances iron uptake, while decreased expression of ferroportin 1 (FPN1) inhibits iron export, leading to intracellular iron overload [106]. Iron-catalyzed Fenton reactions generate massive lipid peroxides, which damage cell membranes and induce ferroptosis. Ischemia impairs the antioxidant defense system against ferroptosis: reduced glutathione (GSH) levels (due to ATP depletion and oxidative stress) weaken the activity of glutathione peroxidase 4 (GPX4), a key enzyme that degrades lipid peroxides [107]. Additionally, activation of the Nrf2-HO-1 pathway (a major antioxidant signaling axis) is suppressed during ischemia, further exacerbating ferroptosis [108]. Preclinical studies demonstrate that ferroptosis inhibitors

(e.g., ferrostatin-1, liproxstatin-1) or regulators of iron metabolism (e.g., deferoxamine) alleviate myocardial ischemic injury by reducing lipid peroxidation and preserving cardiomyocyte viability [109].

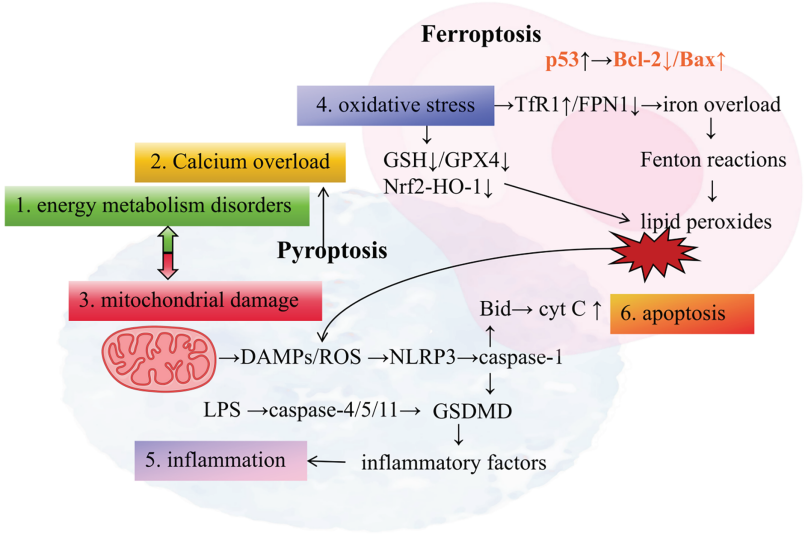

Figure 4: In myocardial ischemic injury, the interaction network between ferroptosis and pyroptosis cell death pathways. Mitochondrial damage releases DAMPs and ROS, which activate the NLRP3 inflammasome. This further triggers pyroptosis through caspase-1-mediated cleavage of GSDMD. Meanwhile, LPS can induce pyroptosis via the non-canonical caspase-4/5/11 pathway, and the inflammatory factors released by pyroptosis exacerbate the inflammatory response. Oxidative stress, on one hand, leads to iron overload by upregulating TfR1 and downregulating FPN1. Combined with the reduction of GSH and GPX4, as well as the inhibition of the Nrf2-HO-1 pathway, this causes the accumulation of lipid peroxides associated with ferroptosis. On the other hand, caspase-1, activated during pyroptosis, can cleave the Bid protein to promote the release of cyt C, and the upregulation of p53 related to ferroptosis also regulates the Bcl-2/Bax balance, ultimately jointly promoting apoptosis. These pathways crosstalk with each other, forming a complex molecular regulatory network in myocardial ischemia. Abbreviation: Transferrin receptor 1 (TfR1), Ferroportin 1 (FPN1), Glutathione (GSH), Glutathione peroxidase 4 (GPX4), Lipopolysaccharide (LPS), Gasdermin D (GSDMD)

These emerging cell death pathways interact closely with classical mechanisms. Pyroptosis amplifies inflammatory responses by releasing pro-inflammatory cytokines, which in turn promote oxidative stress and calcium overload. Ferroptosis is tightly linked to mitochondrial dysfunction and oxidative stress—mitochondrial ROS production accelerates iron-dependent lipid peroxidation, while ferroptosis-induced mitochondrial damage further impairs energy metabolism. Moreover, cross-talk exists between pyroptosis, ferroptosis, and apoptosis: for example, activated caspase-1 in pyroptosis can cleave Bid to trigger mitochondrial apoptosis, and iron overload promotes both ferroptosis and apoptosis via p53 activation [110,111]. Incorporating these new pathways into the existing regulatory network enhances the comprehensiveness of myocardial ischemia mechanism research and provides novel targets for multi-dimensional therapeutic strategies.

9 Autophagic Imbalance

9.1 Physiological Roles of Autophagy

Autophagy is a highly conserved self-degradation process in cells, playing a vital role in maintaining intracellular homeostasis and metabolic balance. Under normal physiological conditions, cells eliminate damaged, senescent, or dysfunctional organelles such as mitochondria and endoplasmic reticulum through autophagy [112]. If not cleared in time, damaged mitochondria produce large amounts of ROS, causing

oxidative damage to cells. Autophagy can identify and encapsulate these damaged mitochondria to form autophagosomes, which then fuse with lysosomes to degrade the mitochondria, thereby reducing ROS production and protecting cells from oxidative stress. Autophagy also removes misfolded or aggregated proteins accumulated in cells [113]. The accumulation of these abnormal proteins disrupts normal cellular physiology and even induces cytotoxicity. Autophagy degrades them into small molecules such as amino acids, achieving protein quality control and providing raw materials for cellular metabolism. In this way, autophagy not only maintains intracellular homeostasis but also supplies essential nutrients and energy to ensure normal cellular functions. It also participates in processes such as cell development, differentiation, and immune regulation, playing an indispensable role in maintaining normal physiological states of the body.

9.2 Inducing Factors of Autophagic Imbalance in Myocardial Ischemia

In myocardial ischemia, multiple factors lead to autophagic imbalance and disrupt cellular homeostasis. Energy deficiency is one of the primary causes—reduced coronary blood flow during ischemia leads to myocardial cell hypoxia, inhibiting mitochondrial aerobic oxidation and significantly decreasing ATP production. The decline in ATP levels activates the energy sensor AMPK, which in turn activates autophagy by inhibiting mammalian target of rapamycin (mTOR) [114]. However, prolonged energy deprivation may overactivate autophagy, causing excessive degradation of cellular components and impairing normal functions. Oxidative stress represents another critical factor [115]. Ischemia-induced mitochondrial respiratory chain dysfunction and neutrophil infiltration lead to massive production of ROS. ROS can oxidatively modify autophagy-related proteins (autophagy-related 4 (Atg4)), disrupt autophagosome formation, and block autophagic flux [116]. Additionally, ROS indirectly regulates autophagy by activating pathways like JNK. Furthermore, calcium overload is closely associated with autophagic imbalance. Ischemia-induced dysfunction of cell membrane ion pumps causes calcium overload, activating the CaN-NFAT signaling pathway to regulate autophagy-related gene expression and induce abnormal autophagy [117]. Calcium overload also exacerbates mitochondrial dysfunction, further intensifying energy metabolic disorders and oxidative stress, thereby indirectly disrupting autophagic balance.

9.3 Pathological Effects and Regulatory Signaling Pathways of Autophagic Imbalance

Autophagic dysregulation, whether excessive or insufficient, significantly contributes to cardiomyocyte dysfunction during myocardial ischemia. Excessive autophagy exerts harmful effects by degrading essential cellular components (such as contractile proteins and mitochondria), impairing cardiac contractility and energy metabolism, and initiating apoptosis through degradation of Bcl-2 and activation of Bax, thereby leading to mitochondrial dysfunction and caspase activation [118]. In contrast, autophagic deficiency causes toxic accumulation of damaged organelles (leading to increased reactive oxygen species) and misfolded proteins [119], while impairing cellular immunity and stress resistance. Key regulatory pathways include the mTOR and AMPK pathways: the mTOR pathway normally suppresses autophagy under nutrient-rich conditions [120], but ischemia-induced energy depletion inhibits mTOR and triggers autophagy [121]; the AMPK pathway is activated by energy stress (high AMP/ATP ratio), promoting autophagy via Unc-51 like autophagy activating kinase 1 (ULK1) activation and mTOR inhibition [122], though sustained AMPK activation, while initially protective, may lead to excessive autophagy. These pathways highlight the delicate balance required for autophagic regulation, as both hyperactivity and insufficiency of autophagy can detrimentally affect cardiomyocyte survival and function under ischemic stress (Fig. 3). In summary, energy metabolism disorder, calcium overload, mitochondrial dysfunction, oxidative stress, inflammatory response, apoptosis, and autophagic imbalance do not exist independently but form a complex regulatory network through cross-signaling pathways such as AMPK-mTOR, CaN-NFAT, and Nrf2-HO-1, jointly driving the

progression of myocardial ischemia. The next section will analyze the interaction mechanisms between these pathways and construct an overall regulatory map of the pathological process of myocardial ischemia.

10 Interconnections between Mechanisms

Myocardial ischemia triggers an interconnected cascade of pathological mechanisms. The initial energy metabolism disorder arises from impaired aerobic oxidation, forcing reliance on inefficient glycolysis that fails to meet ATP demands. This ATP depletion disrupts ion pumps, causing calcium overload, which further worsens metabolic dysfunction through enzyme activation and cellular damage. Oxidative stress emerges through mitochondrial dysfunction and neutrophil activity, with ROS damaging cellular components and perpetuating calcium overload. These reactive species simultaneously activate inflammatory pathways like NLRP3 and NF-κB, creating a vicious cycle as inflammatory cells generate additional ROS [123]. The resulting inflammation promotes cell death through TNF-α and IL-1β, which trigger both apoptotic (via death receptors) and necrotic pathways while releasing DAMPs that amplify inflammation [124]. Autophagy plays a dual role-its dysregulation (either excessive or insufficient) exacerbates cellular damage through organelle degradation or toxic accumulation. Central to this network is mitochondrial dysfunction, manifesting as structural damage and impaired respiration. This not only worsens energy deficits but also promotes apoptosis through cyt C release while aggravating oxidative stress and calcium dysregulation [125]. These intertwined mechanisms collectively drive ischemic myocardial injury through metabolic, oxidative, inflammatory, and cell death pathways (Table 2).

Table 2: The regulatory role and interaction relationship of core signaling pathways in myocardial ischemia

Signaling pathway	Core functions (Myocardial ischemia scenario)	Upstream activating factors	Downstream regulatory targets	Interaction with other pathways
PI3K-Akt [42–44]	Inhibits apoptosis, alleviates calcium overload	Growth factors, cellular stress	GSK-3β, Ca^{2+}-ATPase	Antagonizes the pro-damaging effects of the MAPK pathway
MAPK (ERK/JNK/p38) [60,61]	Early protection, late pro-damage	ROS, inflammatory factors	Elk-1, Bax	ERK activates Nrf2 for antioxidant defense; JNK/p38 exacerbates inflammation
NF-κB [74,77,78]	Promotes an inflammatory response	TNF-α, ROS, DAMPs	TNF-α, IL-1β, iNOS	Synergizes with JAK2-STAT3 to amplify inflammation
JAK2-STAT3 [83,84]	Inflammation regulation, cell survival	IL-6, IL-10	Inflammatory factors, anti-apoptotic proteins	Cooperates with NF-κB to promote the release of pro-inflammatory cytokines

(Continued)

Table 2 (continued)

Signaling pathway	Core functions (Myocardial ischemia scenario)	Upstream activating factors	Downstream regulatory targets	Interaction with other pathways
Nrf2-HO-1 [42,79,80]	Resists oxidative stress	ROS, HDAC3 inhibition	SOD, CAT, GSH-Px	Inhibits the NF-κB pathway to reduce inflammation
AMPK-mTOR [114,120]	Regulates autophagy and energy metabolism	Decreased ATP, increased AMP	ULK1, CPT-1	Activates autophagy during ischemia; activity needs to be balanced during reperfusion

Note: Abbreviation: Ets-like protein 1 (Elk-1).

11 Research Status and Prospects

11.1 Summary of Current Research Progress

Research on myocardial ischemia has significantly advanced our understanding of its molecular mechanisms and potential treatments. Key pathways, including energy metabolism dysfunction, oxidative stress, calcium overload, inflammation, cell death, autophagy imbalance, and mitochondrial damage, have been extensively studied. During ischemia, the shift from aerobic to anaerobic metabolism triggers calcium overload and mitochondrial damage, while oxidative stress from mitochondrial dysfunction and neutrophil activation causes cellular damage through molecules like Nrf2 and HDAC3. Calcium overload mechanisms involving ATP depletion and ion exchange pathways (H^+-Na^+/Na^+-Ca^{2+}) have been clarified, along with their damaging effects through PI3K-Akt and MAPK signaling. Inflammatory pathways involving TNF-α, IL-1β, and signaling cascades like JAK2-STAT3 and NF-κB have been mapped, revealing their interactions with oxidative stress and calcium overload. Cell death mechanisms through mitochondrial/apoptotic pathways (Bcl-2, caspases, p53) and necrosis have been characterized, while autophagy dysregulation via mTOR/AMPK pathways and mitochondrial dysfunction through p53/MAPK signaling have been elucidated.

In recent years, emerging regulated cell death pathways such as pyroptosis and ferroptosis have become research hotspots. Studies have confirmed that pyroptosis mediated by NLRP3 inflammasome/caspase-1/GSDMD and ferroptosis driven by iron overload/GPX4/lipid peroxidation play important roles in myocardial ischemic injury. These pathways interact with classical mechanisms to form a more complex regulatory network, providing new perspectives for understanding disease progression.

These findings provide multiple therapeutic targets: metabolic modulators to improve energy supply, Nrf2 agonists/HDAC3 inhibitors to combat oxidative stress, PI3K-Akt activators/CaN inhibitors for calcium overload, JAK2-STAT3/NLRP3/NF-κB blockers for inflammation, Bcl-2/caspase/p53 modulators to prevent cell death, mTOR/AMPK regulators for autophagy, mitochondrial protectants to maintain function, and pyroptosis/ferroptosis inhibitors (e.g., GSDMD antagonists, GPX4 activators) to target emerging cell death pathways. This comprehensive understanding offers promising avenues for developing targeted therapies against myocardial ischemia.

11.2 Existing Challenges and Unsolved Issues

Despite progress in understanding myocardial ischemia, significant knowledge gaps remain regarding its molecular mechanisms and treatment. While key pathways like energy metabolism dysfunction, oxidative stress, calcium overload, inflammation, and cell death have been identified, many details remain unclear. For instance, the regulatory mechanisms of glycolytic enzymes, specific free radical interactions, spatial calcium signaling, inflammatory cascade dynamics, and differential susceptibility of cardiomyocytes to death signals all require further study. Additionally, the complex crosstalk between pathways during different ischemia stages complicates the development of effective multi-target therapies. For emerging pathways such as pyroptosis and ferroptosis, several critical questions remain unresolved. The specific molecular triggers of pyroptosis in myocardial ischemia (e.g., the role of different DAMPs in NLRP3 activation) and the cell-type specificity of pyroptosis (cardiomyocytes vs. immune cells) need clarification. For ferroptosis, the regulatory mechanisms of iron metabolism in ischemic myocardium (e.g., the balance between iron uptake, storage, and export) and the interaction between ferroptosis and other cell death pathways (e.g., how ferroptosis regulates apoptosis or autophagy) are not fully understood.

Current therapeutic targets face translational challenges. Some pathway modulators may improve ischemia but cause unintended effects like arrhythmias or organ dysfunction. Promising preclinical results often fail in human trials due to physiological complexity, individual variability, and drug metabolism differences. Developing safe, synergistic multi-target therapies remains particularly difficult, especially for combining inhibitors of classical pathways with emerging cell death modulators. Research models also have limitations. Animal and cell models cannot fully replicate human pathophysiology, with species differences and lack of tissue complexity affecting result reliability. Existing systems also poorly simulate chronic ischemia progression and complications, hindering long-term therapeutic studies. The lack of clinically relevant models for studying pyroptosis and ferroptosis in human myocardial tissue further limits translational progress. Overcoming these challenges is crucial for advancing both mechanistic understanding and clinical translation.

11.3 Future Research Directions and Perspectives

Future research on myocardial ischemia should focus on three key areas to advance understanding and treatment. First, a deeper exploration of signaling pathways using advanced techniques like CRISPR-Cas9, proteomics, and single-cell sequencing can clarify molecular interactions and regulatory networks. These methods enable precise gene manipulation, protein profiling, and single-cell analysis, helping identify new therapeutic targets. For emerging pathways, single-cell sequencing can reveal the cell-type-specific roles of pyroptosis and ferroptosis in ischemic myocardium, while spatial transcriptomics can map the spatial distribution of key molecules in these pathways.

Developing targeted therapies is crucial. Computer-aided drug design and high-throughput screening can optimize small molecules, biologics, or gene therapies that modulate key pathways. For pyroptosis, developing selective GSDMD inhibitors or NLRP3 inflammasome modulators with good tissue penetration and low toxicity is a priority. For ferroptosis, optimizing GPX4 activators or iron chelators to specifically target ischemic myocardium can improve therapeutic efficacy. Gene and cell therapies, such as stem cell transplantation or gene correction (e.g., overexpressing FPN1 to reduce iron overload), may also promote myocardial repair. Additionally, exploring combination therapies (e.g., combining ferroptosis inhibitors with anti-inflammatory drugs) to target multiple interconnected pathways holds promise for enhancing therapeutic effects.

Personalized approaches should consider genetic and environmental influences. Genome-wide association studies (GWAS) and epidemiological research can identify risk factors, guiding tailored treatments

for better outcomes. For example, identifying genetic variants associated with pyroptosis/ferroptosis susceptibility can help select patients who may benefit most from targeted inhibitors. Integrating multi-omics data (genomics, transcriptomics, metabolomics) to construct patient-specific molecular profiles can further optimize personalized treatment strategies. Combining these strategies may improve both mechanistic understanding and clinical management of myocardial ischemia.

12 Conclusions

Myocardial ischemia, a cardiovascular disease posing significant threats to human health, demands critical exploration of its molecular mechanisms. Through in-depth investigation of key pathways—including energy metabolism disorders, oxidative stress, calcium overload, inflammatory responses, apoptosis and necrosis, autophagy imbalance, and mitochondrial dysfunction—we have gradually unraveled the intricate molecular regulatory networks underlying the pathogenesis of myocardial ischemia. These mechanisms are not isolated but interconnected, synergistically driving disease progression. Current research has provided substantial insights into the pathological processes of myocardial ischemia and identified potential therapeutic targets for novel strategies and drug development. However, several limitations remain: incomplete elucidation of signaling pathway details, insufficient validation of therapeutic target efficacy and safety, and constraints in research models. Moving forward, integrating advanced technologies to dissect regulatory mechanisms of signaling pathways, developing more effective therapies and drugs, and addressing genetic and environmental influences will be pivotal for achieving precision therapy and effective prevention of myocardial ischemia. With continued research and technological advancements, our understanding of its molecular basis will deepen, paving the way for breakthroughs in clinical treatment and prevention. These efforts hold promise for reducing the incidence and mortality of myocardial ischemia, improving patient quality of life, and making profound contributions to global health.

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