NETosis in myocardial ischemia-reperfusion injury: From mechanisms to therapies (Review)

ZIYANG ZHANG[1], YANXIN WANG[2], TIE LI[1] and HONGFENG WANG[1]

[1]College of Acupuncture and Massage, Changchun University of Chinese Medicine, Changchun, Jilin 130117, P.R. China; [2]Department of Cardiovascular Medicine, The Third Affiliated Hospital of Changchun University of Chinese Medicine, Changchun, Jilin 130117, P.R. China

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Abstract. The present review describes the mechanisms of NETosis and its role in myocardial ischemia-reperfusion injury (MIRI), focusing on the release of neutrophil extracellular traps (NETs) by activated neutrophils. NETs, composed of depolymerized chromatin and granule proteins, are crucial for pathogen entrapment, infection control and immune regulation. However, NET formation, linked to neutrophil death (NETosis), exacerbates MIRI by promoting inflammation and tissue damage. To address therapeutic strategies for NETosis in MIRI, several potential clinically significant approaches were explored, including peptidylarginine deaminase 4 inhibition, DNase therapy, antioxidants, inflammation modulation, and antithrombotic treatments, which not only provide novel diagnostic biomarkers and therapeutic targets in MIRI, but are also expected to improve patient prognosis and advance the development of personalised medicine.

Contents

Correspondence to: Dr Hongfeng Wang, College of Acupuncture and Massage, Changchun University of Chinese Medicine, 1035 Boshuo Road, Nanguan, Changchun, Jilin 130117, P.R. China
E-mail: ccwhf@126.com

Abbreviations: NE, neutrophil elastase; MPO, myeloperoxidase; NETs, neutrophil extracellular traps; MIRI, myocardial ischemia-reperfusion injury; ROS, reactive oxygen species; NOX, NADPH oxidase complex; PAD4, peptidylarginine deaminase 4; H3Cit, histone H3 citrullination; dsDNA, double-stranded DNA; NLRP3, NLR family, pyrin domain containing 3; DAMPs, damage-associated molecular patterns; IL-1β, interleukin-1β; mtDNA mitochondrial DNA; VWF, von Willebrand factor

Key words: MIRI, NETs, targeted therapy, NETosis, neutrophil activation, neutrophil death

1. Introduction

Neutrophils are the first immune cells to arrive at the site of inflammation, where they contribute to the destruction and containment of pathogenic microorganisms through phagocytosis, the release of bactericidal substances and the production of pro-inflammatory cytokines (1). Neutrophil activation is initiated by both microbial and endogenous stimuli, resulting in the release of chromatin and granule proteins, including neutrophil elastase (NE), myeloperoxidase (MPO) and other components such as histone G and defensins. These elements collectively form extracellular structures termed neutrophil extracellular traps (NETs) (2). The process, termed NETosis, is integral to the pathophysiology of myocardial ischemia-reperfusion injury (MIRI) by modulating mechanisms such as reactive oxygen species (ROS) production, inflammation and energy metabolism, thereby contributing to the advancement of myocardial injury (3,4). NETosis plays an important role in the physiopathology of aseptic inflammatory conditions, such as MIRI, by affecting ROS production, inflammation, energy metabolism and other such mechanisms, but the specific mechanisms and directions of its action are not yet fully understood.

Despite the development of more efficient and effective reperfusion techniques, including percutaneous coronary intervention and novel antiplatelet and antithrombotic therapies, effective treatments to prevent MIRI remain limited (5,6). Recent data show that cardiovascular disease is responsible for ~20% of all deaths worldwide each year (7). MIRI refers to myocardial injury that occurs after rapid restoration of blood flow in the ischemic area and accounts for 50% of the final infarct size (8). These injuries can be devastating, leading to fatal myocardial damage, myocardial dysfunction, vascular injury and tissue edema (9). The pathological mechanisms underlying these injuries include apoptosis, autophagy, endoplasmic reticulum stress, ferroptosis, pyroptosis, and ultimately, exacerbated oxidative stress, calcium imbalance, activation of inflammatory cascades, endothelial dysfunction

and microvascular injury. Recently, it has been shown that specific biomarkers (such as DNA, histones and NE) that are released during NETosis may provide novel biomarkers for the early diagnosis of MIRI (10). By regulating the NETosis process, the development of novel therapeutic strategies, such as inhibiting the formation of NETosis or promoting its clearance, may help to mitigate MIRI and improve the prognosis of patients.

The present review systematically compiles and analyzes the research progress on the role of NETosis in MIRI in recent years, focusing on the formation mechanism of NETosis, its dynamic changes during myocardial ischemia-reperfusion and the potential therapeutic mechanism of NETosis-targeted therapy for MIRI.

2. NETosis formation mechanisms

To date, the formation of NETosis has been classified into two forms: i) Suicidal NETosis; and ii) vital NETosis (11). Regardless of the stimulus, NETosis formation involves the cellular process of chromatin decondensation, which is essential for the release of NETs (12).

Suicidal NETosis. Suicidal NETosis denotes a mechanism of NET formation that is concomitant with neutrophil apoptosis. Upon activation by stimuli including phorbol ester, specific autoantibodies, immune complexes or conditions characterized by elevated calcium ion (Ca^{2+}) concentrations, neutrophils initiate the NADPH oxidase complex (NOX), leading to the production of ROS such as superoxide anion, hydroxyl radical and hydrogen peroxide (13,14). These ROS are highly reactive and play a crucial role in facilitating NOX-dependent NET formation (15). The release of ROS activates peptidylarginine deaminase 4 (PAD4), the only nuclear-localized isoenzyme in the PAD family, which is expressed in both the nucleus and cytoplasmic granules of immune cells (16). PAD4 facilitates the citrullination of core and linker histones, which diminishes their affinity for DNA, thereby promoting chromatin decondensation and the unfolding of densely packed chromatin structures (17,18).

Activation of PAD4 also triggers the translocation of NE and MPO from azurophilic granules to the nucleus (19), which further enhances histone H3 citrullination (H3Cit) and chromatin decondensation, a pivotal process in the formation of NETs (20,21). Consequently, H3Cit is considered a specific and well-established marker for NETs (22). With chromatin decondensation and nuclear membrane rupture, the decondensed chromatin mixes with granule proteins and is released into the cytoplasm (23). Eventually, the cytoplasmic membrane leaks, and the modified chromatin is expelled from the neutrophil, forming extracellular NETs in a meshwork structure. NETs are released into the extracellular space 3-8 h after neutrophil activation, leading to cell death due to membrane disintegration (24). Plasma levels of MPO have been shown to be positively correlated with the risk of coronary artery disease (25), and MPO-DNA complexes are associated with severe adverse cardiovascular events (26).

Moreover, the serum concentrations of critical markers of NETs, such as double-stranded DNA (dsDNA), MPO and NE, are markedly elevated in patients with coronary atherosclerotic disease (27). Additionally, extracellular H3Cit exhibits significant cytotoxicity, contributing to tissue damage and thereby elevating the risk of mortality among patients with cardiovascular disease (28). These observations indicate that MPO, NE and H3Cit are integral to NET formation, further implicating NETosis in the pathophysiological processes underlying MIRI.

Vital NETosis. Vital NET formation represents a type of NETosis that transpires shortly after neutrophil activation, distinguished by the generation of NETs without compromising the structural integrity of the neutrophil (29). In contrast to suicidal NETosis, this process involves the encapsulation of modified chromatin within vesicles that are secreted by the nucleus and subsequently expelled from the cell, thereby preserving neutrophil integrity (30). Consequently, neutrophils remain intact and retain the ability to perform additional functions (Fig. 1).

3. Pathogenesis of NETosis in MIRI

Myocardial ischemia is primarily attributed to atherosclerosis, thrombosis, insufficient coronary blood supply and elevated myocardial oxygen demand, with atherosclerosis and luminal thrombosis identified as the predominant mechanisms.

Pathogenesis of NETosis during ischemia. NETosis is a critical factor in the pathophysiology of myocardial ischemia, particularly within pro-inflammatory and pro-thrombotic contexts. NETs are observed to form in regions of elevated cholesterol within atherosclerotic lesions. Elevated blood cholesterol levels result in the deposition of cholesterol crystals within the vessel walls, which are subsequently recognized and phagocytosed by macrophages (31). This process induces lysosomal damage and activates the NLR family pyrin domain containing 3 (NLRP3) inflammasome within macrophages (32). PAD4 plays a critical role in the pathogenesis of atherosclerosis by regulating apoptosis-associated speck-like proteins containing a caspase recruitment domain (ASC proteins) and NLRP3 protein levels, thereby facilitating the assembly of NLRP3 inflammasomes (33). Upon activation, these multiprotein complexes lead to the production of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-18 (34,35).

These factors recruit more immune cells to the vascular endothelium, which exacerbates the inflammatory response and further activates neutrophils leading to NETosis (36). The release of NETs can exacerbate tissue damage and contribute to a pro-inflammatory milieu. Activated neutrophils secrete cytokines and chemokines, which further recruit additional immune cells and amplify the inflammatory response. This phenomenon is particularly evident in conditions such as acute myocardial infarction, where enhanced neutrophil-platelet interactions lead to increased thrombus formation (37). Thrombus formation plays a critical role in the pathogenesis of myocardial ischemia. For example, Zhou *et al* (38) identified elevated levels of NETs in coronary thrombosis among patients with ST-segment elevated myocardial infarction. Neutrophils retained within thrombi play a crucial role in initiating the endogenous coagulation cascade through the release of NETs, which facilitate

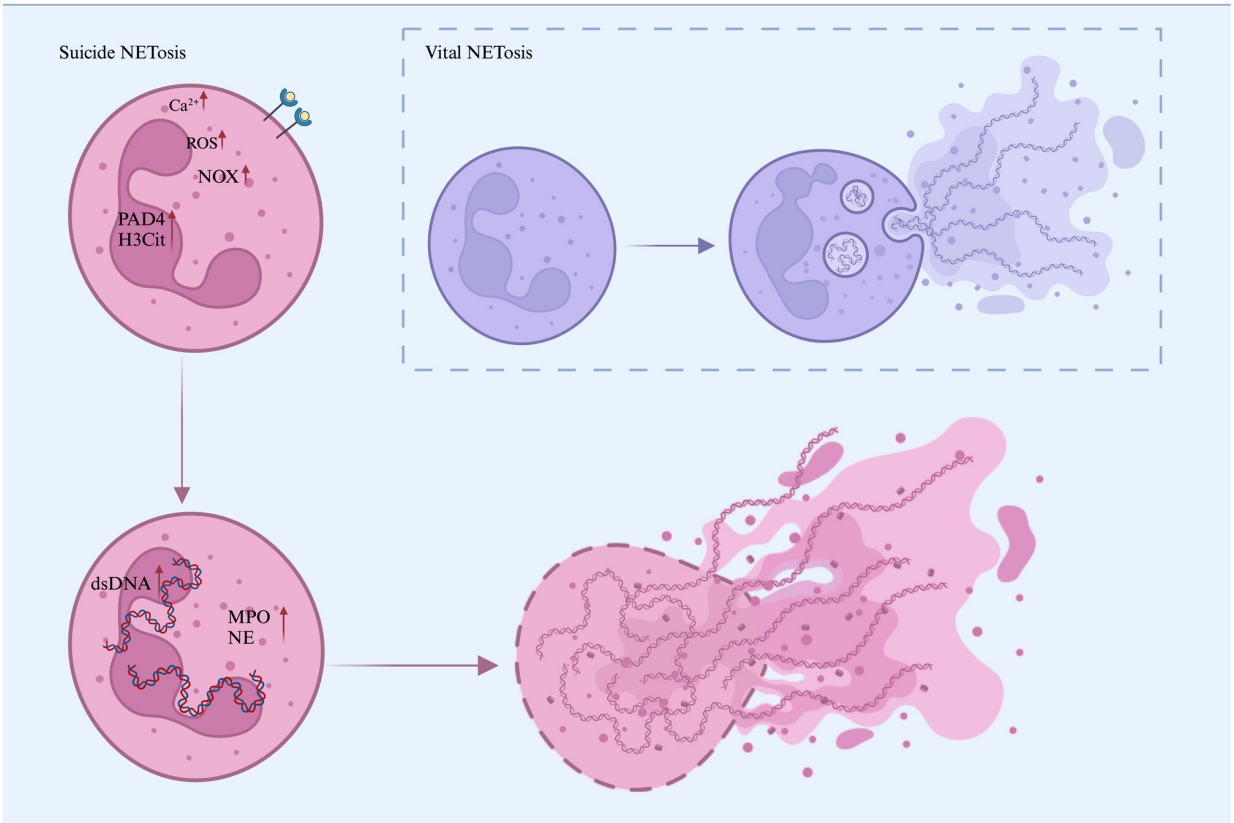

Figure 1. Mechanisms of NETosis. Suicidal NETosis is a process in which neutrophils die after releasing NETs whereas vital NETosis is a process in which neutrophils rapidly release NETs without destroying their own integrity. The red arrows represent elevated molecular levels. The graphic summary was generated with BioRender (https://BioRender.com). NE, neutrophil elastase; MPO, myeloperoxidase; ROS, reactive oxygen species; NOX, NADPH oxidase complex; PAD4, peptidylarginine deaminase 4; H3Cit, histone H3 citrullination; dsDNA, double-stranded DNA.

the activation of coagulation factor XII. This process can subsequently lead to thrombin generation and platelet activation (39). The structural network of NETs further supports the deposition of platelet adhesion molecules and promotes thrombin-mediated fibrin formation (40).

Pathogenesis of NETosis in MIRI

Oxidative stress. Myocardial energy supply is primarily derived from fatty acid and glucose metabolism (41), and neutrophils primarily rely on glycolysis, rather than oxidative phosphorylation, to meet their energy demands (42). During ischemia-reperfusion, due to a lack of synthetic substrates, mitochondrial dysfunction and calcium overload, cardiomyocytes rely on an attenuated glycolytic pathway for energy production for 0.5-2 h (43). When endogenous cellular free radical scavenging enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, are unable to neutralize ROS effectively, this leads to an imbalance between the oxidation system and antioxidant enzymes (44,45). This in turn disrupts cellular membrane integrity, altering its fluidity and permeability, leading to further membrane damage, cellular swelling and functional impairment (46). NETs play a significant role in the pathophysiological process of MIRI, particularly through NOX-dependent pathways. The restoration of blood flow following ischemia induces a severe inflammatory response, characterized by neutrophil activation. These activated neutrophils release NETs, which can trap pathogens, but also contribute to myocardial tissue damage and inflammation (47). Upon stimulation, neutrophils activate the NOX complex, initiating a pathway referred to as NOX-dependent NETosis. In this pathway, NOX catalyzes the production of ROS, which are crucial for the formation of NETs. This oxidative burst not only facilitates NET formation but also intensifies oxidative stress within myocardial tissues, thereby contributing to additional cellular damage and inflammation (48,49). The formation of NETs requires an increase in ATP availability, which further reduces the oxygen supply to cardiomyocytes, indirectly diminishing glycolytic efficiency (50). In addition, the excessive accumulation of NETs within the ischemic myocardium facilitates the formation of microthrombi, thereby obstructing blood flow and contributing to the 'no-reflow' phenomenon, wherein myocardial tissue remains insufficiently perfused despite the restoration of blood supply (51).

Calcium ions are crucial for the maintenance of normal cardiomyocyte function, with mitochondrial Ca^{2+} levels modulating the rate of oxidative metabolism to align with ATP depletion in the cytoplasm. This regulation ensures that cardiomyocytes receive adequate energy to sustain their normal systolic and diastolic functions (52). During reperfusion injury, cardiomyocytes exacerbate injury by reversing the Na^{+}/Ca^{2+} exchanger, leading to sodium efflux and subsequent intracellular calcium overload. Excessive calcium

efflux contributes to mitochondrial permeability transition pore opening and mitochondrial ROS production (53,54). In a high Ca^{2+} concentration environment, calcium ions can directly or indirectly activate NOX by altering its conformation or active state, triggering NOX activation and promoting NETosis (55). This pathway is referred to as NOX-independent NETosis. Research has shown that direct activation of small-conductance calcium-activated potassium (SK) channels can induce NETosis, with the involvement of SK3 (56,57).

Inflammation. During reperfusion, cells that have been compromised by ROS release endogenous damage-associated molecular patterns (DAMPs) and inflammatory cytokines, including tumor necrosis factor-α and IL-1β (58). The release of these cytokines further recruits and activates inflammatory cells, such as neutrophils and macrophages, thereby intensifying the inflammatory response and perpetuating a deleterious cycle of ROS production. Mitochondrial DNA (mtDNA) fragments are identified as DAMPs by pattern recognition receptors of the immune system, including Toll-like receptors (TLRs) and NOD-like receptors, thereby initiating inflammatory responses (59). mtDNA activates immune responses via CpG/TLR9 interactions, promoting mitogen-activated protein kinase (MAPK) signaling and thereby exacerbating MIRI (60,61). Additionally, the recognition of DNA within NETs by TLR9 activates plasmacytoid dendritic cells, which perpetuate vascular inflammation (62).

Extracellular histones, as critical DAMPs, contribute to myocardial injury during MIRI (63). Extracellular histones exacerbate the inflammatory response by activating TLR2 and TLR4, leading to the production of pro-inflammatory cytokines. Histone interactions with T cells promote Th17 cytokine production, further amplifying the inflammatory state (64-67). High mobility group protein B1, a critical DAMP released from necrotic cardiac tissue following prolonged ischemic injury, interacts with the receptor for advanced glycation end-products. This interaction facilitates the recruitment of a substantial number of neutrophils to the injured myocardium, thereby promoting the production of IL-6 through signaling pathways such as MAPK and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), along with the release of other inflammatory mediators, ultimately triggering NET formation (68). The inflammatory pathway factors involved in NETosis during reperfusion injury and potential treatments are described in Table I.

Endothelial dysfunction. During myocardial ischemia-reperfusion, endothelial cell damage occurs, accompanied by a reduction in antioxidant activity and a decrease in nitric oxide synthesis and release, culminating in impaired vasodilation (69,70). Myocardial injury is exacerbated by coronary artery constriction, neutrophil aggregation and abnormal platelet responses, resulting in microvascular spasm and restenosis (71,72).

Damaged endothelial cells release a variety of pro-inflammatory factors, such as IL-1β and IL-6, which further amplify the inflammatory response (73). NETs can induce NF-κB-dependent endothelial angiogenesis, altering the plaque microenvironment and promoting the development of unstable plaques (74,75). Angiotensin II has been demonstrated to induce NETosis through both the ROS/PAD4 pathway and autophagy-dependent mechanisms (76).

Involvement of NETosis in myocardial fibrosis after reperfusion injury. Subsequent to cardiomyocyte injury and necrosis, there is a pronounced accumulation of extracellular matrix components, accompanied by the differentiation of cardiac fibroblasts into myofibroblasts under stress conditions. These myofibroblasts exhibit enhanced proliferation and secretion activities, culminating in the development of cardiac fibrosis (77). NETs are intricately linked to inflammatory processes and may significantly influence the fibroblastic response during myocardial injury. Additionally, non-classical monocytes, integral components of the immune system, play a crucial role in myocardial healing. It has been observed that overproduction of NETs can reduce the expression of CX3C chemokine receptor 1 on non-classical monocytes at the site of injury. This reduction may impair the migration of non-classical monocytes to ischemic tissues, resulting in delayed or compromised myocardial healing (78).

The release of NETs also stimulates the proliferation and activation of cardiac fibroblasts, which secrete collagen and other fibrosis-associated proteins, thus contributing to the progression of myocardial fibrosis (79). NETs recognize and bind endocytosed dsDNA via TLR9, which activates the myeloid differentiation factor 88 and, in turn, triggers the NF-κB signaling pathway (80,81). Upon activation of the TLR-NF-κB signaling axis, myofibroblast differentiation is promoted by regulating the expression of genes such as transforming growth factor-β, collagen I (a major component of the cardiac extracellular matrix) and α-smooth muscle actin, a signature protein of myofibroblasts. These changes are accompanied by stress fiber formation and contribute to the progression of myocardial fibrosis (82).

4. NETosis predicts the prognosis in MIRI

During myocardial ischemia-reperfusion, excessive production of NETs can facilitate thrombosis and worsen microvascular obstruction, thereby elevating the risk of myocardial infarction. Chang *et al* (83) concluded that the combined use of circulating cell-free DNA (cfDNA) and creatine kinase-MB may enhance the diagnostic accuracy and sensitivity for acute myocardial ischemia, enabling a more comprehensive evaluation of the condition of patients. Recent research has demonstrated that in patients with acute myocardial ischemia, plasma cfDNA is predominantly released through NETosis, further substantiating its potential as a diagnostic marker for acute myocardial ischemia (84).

Additionally, markers such as platelet count, MPO-DNA complexes, NE-DNA complexes, H3Cit and soluble platelet selectin have been identified as independent predictors of major adverse cardiovascular events 1 year after myocardial infarction (85). A prospective clinical study demonstrated that plasma levels of bactericidal/permeability-increasing protein (BPI) in patients experiencing acute myocardial ischemia exhibited a positive correlation with IL-1β, high-sensitivity C-reactive protein, MPO-DNA and S100A8/A9. Consequently, BPI may serve as a novel biomarker for myocardial ischemia, with its primary mechanism potentially involving the

Table I. Inflammatory pathway agents involved in NETosis during reperfusion injury and potential therapeutic approaches.

First author/s, year	Drug/molecule	Type of experiment	Pathway of action	Outcome	(Refs.)
Xu *et al*, 2019	Adenosine	Clinical trial	Adenosine exerts anti-inflammatory effects by binding to its receptors and inhibiting neutrophil activation and NETosis.	HNE-DNA ELISA assay	(113)
Xie *et al*, 2018	Cpg-ODN	Animal	CpG-ODN treatment acts on the TLR9-p38 MAPK signaling pathway, leading to increased cardiomyocyte damage and inflammatory response.	Myocardial infarction	(61)
Clark *et al*, 2007	Histone	Clinical trial	Exogenous chromatin acts as a key driver to amplify the inflammatory response in aseptic diseases and infections by enhancing TLR4 binding.	Expression of IL-1β mRNA	(67)
Sayegh *et al*, 2023	Polyvinyl alcohol (PEG-4MAL) hydrogel	Animal	Delivery of CD39 and CD73 via hydrogel can modulate the innate immune response and thus protect cardiac function.	Echocardiography	(116)
Yu *et al*, 2022	S100A8/A9	Clinical trial	S100A8/A9 promotes granulopoiesis after myocardial infarction by inducing NETosis.	NETosis marker	(86)
Mangold *et al*, 2019	Monocyte subpopulation	Clinical trial	Exogenous chromatin may promote the differentiation response of monocytes and reduce their CX3CR1 expression at the site of the lesion, thus affecting the migration of non-classical monocytes to ischemic tissue and myocardial healing.	CX3CR1 expression level	(78)
Savchenko *et al*, 2014	DNase I and rhADAMTS13	Animal	Targeting extracellular DNA and VWF may be a novel cardioprotective therapeutic strategy.	Echocardiography	(98)
Ge *et al*, 2015	DNase and rt-PA	Animal	Combination therapy with DNase I and rt-PA effectively reduces NET density, improves coronary microcirculation patency and limits the extent of myocardial infarction.	Myocardial infarction	(99)

HNE, human neutrophil elastase; Cpg-ODN, CpG-oligodeoxynucleotide; TLR, Toll-like receptor; CX3CR1, CX3C motif chemokine receptor 1; ADAMTS13, recombinant ADAM metallopeptidase with thrombospondin type 1 motif 13; VWF, von Willebrand factor; rt-PA, recombinant tissue-type plasminogen activator; NET, neutrophil extracellular trap.

mediation of NET formation through interactions between platelets and neutrophils (86,87).

Animal studies have found that colchicine alleviates inflammation and cardiac remodeling after acute myocardial infarction by inhibiting the formation of NETs, thereby improving cardiac function (88). Additionally, colchicine reduces microvascular obstruction by suppressing the proliferation of neutrophils in the bone marrow and their migration to the ischemia-reperfusion injury area (89). Building on these findings from animal studies, clinical research has further validated the potential application of colchicine in cardiovascular diseases. Clinical studies have shown that low-dose colchicine provides significant clinical benefits in post-myocardial infarction patients, reducing the incidence of cardiovascular events with a favorable safety profile, suggesting its potential applicability in MIRI (90,91).

5. Targeting MIRI with NETosis

NETs may exert a dual role in the management of MIRI. Modulating NETs represents a potentially significant therapeutic strategy for the treatment of MIRI. By inhibiting the formation of NETs or facilitating their degradation, it may be possible to mitigate myocardial tissue damage and enhance tissue repair. There are no clinical or randomized controlled trials on the mechanism of NETosis in MIRI, and more basic research is needed to support this area of study. However, clinical studies on NETosis in other diseases, such as pneumonia and cancer, offer valuale insights (92-95).

PAD4. Considering that NETosis is primarily reliant on PAD4, targeting PAD4 represents a promising therapeutic strategy. The inhibitor GSK484 has demonstrated significant attenuation

of MIRI in an animal model (96). Additionally, BB-CLA, a potent PAD4 inhibitor, effectively prevented the formation and release of NETs induced by polyinosinic acid-polycytidylic acid, a synthetic analog of viral double-stranded RNA (97). Additionally, inhibition of von Willebrand factor (VWF) activity, which mediates leukocyte recruitment, also affects PAD4 activity and promotes NET formation, suggesting that drugs targeting both VWF and NETs may offer a novel therapeutic approach to reduce ischemia-related myocardial injury (98). Currently, studies on the combination therapy of NETosis in MIRI are limited and mainly focused on the animal experimental stage. Combination treatment of DNase I with rhADAMTS13 or rt-PA effectively improved myocardial infarct size and microcirculatory impairment in rats with MIRI (99). Combination of NETosis inhibitors with other anti-inflammatory or antithrombotic drugs may have a synergistic effect and significantly attenuate the pathological damage in MIRI (100). Based on the pathological mechanism of NETosis and related therapeutic targets, it is theorized that combination therapies, such as combining NETosis inhibitors with anti-inflammatory, antioxidant, or antithrombotic drugs may have greater therapeutic potential. The use of combination therapy in MIRI will be further explored in future studies.

The activity of DNase, an enzyme that degrades NETs, plays a crucial role in the clearance of NETs and subsequent modulation of inflammation and thrombosis. Alterations in DNase activity have been linked to ST-segment regression and the onset of myocardial infarction (101). DNase treatment can reduce the cytotoxicity of NETs by degrading their DNA backbone, thus offering potential for reducing thrombotic inflammation (102). However, DNase primarily targets DNA and has limited effects on other NET components, such as histones, which remain capable of causing vascular damage (103). Despite these limitations, dual-active DNase formulations, capable of targeting both DNA and histones, may offer further promise in modulating thrombotic inflammation (104). Recent studies have also suggested that DNase could serve as a prognostic biomarker in patients with atherosclerosis, offering new insights into recurrent ischemic events (105).

Antioxidants. The glutathione metabolic pathway plays a crucial antioxidant role in NETosis. Isocrystalline xanthophylls have been demonstrated to mitigate reperfusion-induced oxidative stress injury and ferroptosis in cardiomyocytes by upregulating glutathione peroxidase 4 (106). Studies indicate that the application of leukotriene C_4 (LTC_4) receptor antagonists can obstruct the LTC4 signaling pathway, thereby inhibiting NETosis and reducing MIRI (107,108). The expression of the transcription factor HIF-1α is upregulated under hypoxic conditions; notably, in myocardial infarction, the expression level of HIF-1α is significantly increased in neutrophils (109). HIF-1α induction may help promote neutrophil survival, mitigate oxidative stress and inhibit NET formation.

Krüppel-like factor 2 (KLF2) is a prominent member of the zinc finger protein transcription factor family. Under homeostatic conditions, KLF2 acts to inhibit HIF-1 signaling. The regulation of the neutrophil KLF2-NETosis-thrombosis pathway may represent a novel therapeutic target for the treatment of reperfusion injury (110). SkQ1, a mitochondria-targeted antioxidant, effectively scavenges ROS within mitochondria during NETosis (111). Furthermore, colchicine treatment has been shown to inhibit the formation of NETs and reduce inflammation by decreasing NOX2/ROS production, which significantly enhances survival rates and improves left ventricular ejection fraction in murine models (88).

Regulation of inflammation. Chemokines such as chemokine (C-X-C motif) ligand 4 (CXCL4) and chemokine (C-C motif) ligand 5 (CCL5) play a pivotal role in the inflammatory response. A synthetic peptide, MKEY, has been shown to block the CCL5-CXCL4 interaction, preventing NET formation by neutrophils *in vivo* (112). Adenosine, an immunosuppressive molecule, inhibits neutrophil activation and NET release (113). Inhibition of ectonucleotidases CD39 and CD73, which play a role in purinergic signaling, has garnered attention as a potential immunotherapeutic strategy in cancer treatment (114). Previous studies indicate that hydrogel-delivered CD39 and CD73 can reduce NETosis and immune infiltration, improving MIRI outcomes (115,116).

Antithrombotic. Recent research has demonstrated that in the context of coronary thrombosis, NETs primarily activate neutrophils and erythrocytes as the predominant constituents of most coronary thrombi, as opposed to the traditional emphasis on platelet aggregation (117). The inhibition of NET formation through VWF-mediated leukocyte recruitment presents a potentially novel therapeutic approach to mitigating ischemia-related myocardial injury. Research utilizing Lnk gene deletion mice (Lnk-/-) and LNK(TT) [neutrophils derived from human induced pluripotent stem cells with LNK(TT) mutations] indicates that the associated pathologies primarily manifest as abnormalities in platelets and neutrophils. These abnormalities include conditions such as coronary artery disease, thrombocytosis and neutropenia. The Lnk-/- and LNK(TT) models may exhibit heightened sensitivity of neutrophils to external stimuli, such as oxidized phospholipids (OxPL), which subsequently increases their susceptibility to NETosis (118). Consequently, therapeutic interventions targeting OxPL could be advantageous for genetically predisposed human populations. Recombinant tissue plasminogen activator (rt-PA) is a recombinant tissue-type plasminogen activator that restores blood flow in large vessels by dissolving thrombi. rt-PA combined with DNase I can simultaneously solve the problems of microcirculation and obstruction of large vessels, improve reperfusion and alleviate the phenomenon of 'no reflow'.

6. Conclusion

In the complex pathological process of MIRI, neutrophils exert multifaceted effects. They contribute to cardiomyocyte death, thereby exacerbating myocardial injury, and significantly impact normal vascular function. Additionally, neutrophils promote inflammation by releasing various cytokines and inflammatory mediators, which hinder the post-injury repair process, and their functions and phenotypes can vary depending on the inflammatory environment and stimuli present.

In detailed investigations of molecular mechanisms, PAD4 facilitates the formation of NETs through its interaction with granule proteins, thereby contributing to MIRI. Nonetheless, the excessive production and release of NETs in the context of MIRI present a dualistic effect. While they aid in the clearance of pathogens from the injury site, they also intensify cardiomyocyte apoptosis and the inflammatory response, potentially impairing cardiac function.

Recent therapeutic approaches for MIRI focus on targeting the NETosis process to attenuate or prevent the injury, including the use of PAD4 inhibitors, antioxidants, anti-inflammatory drugs and other related therapies. As research advances, the identification of different neutrophil subtypes with distinct functional properties in the inflammatory response has broadened the scope for potential treatments. Although these NETosis-based treatments show promise in laboratory and animal models, their clinical application requires further validation.

Therapeutic strategies targeting NETosis represent a promising novel approach for the treatment of MIRI. Although significant progress has been made in animal models, the translation of these strategies into clinical practice continues to face substantial challenges. Clinical trial outcomes have often been inconsistent, potentially due to individual variations in susceptibility to reperfusion injury and the limited therapeutic time window for intervention (119). Furthermore, there is a notable lack of high-quality clinical trials to rigorously validate the safety and efficacy of targeted therapies or to elucidate their precise mechanisms of action. Consequently, future research must focus on deepening the understanding of the specific mechanisms by which NETosis contributes to MIRI, as well as on developing more effective and clinically applicable interventions.

In conclusion, targeting NETosis in MIRI represents a promising therapeutic avenue that may significantly improve patient prognosis. By reducing myocardial damage and enhancing repair mechanisms, these therapies are expected to change the way MIRI is managed, providing patients with a better prognosis and quality of life. Future clinical trials should prioritize patient stratification, biomarker development, and evaluation of combination therapies to realise the full clinical potential of NETosis-targeted therapies.

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Authors' contributions

ZZ and YW conceived the review topic, conducted the literature search, and wrote the original draft. TL and HW reviewed and edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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