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REVIEW

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Non-coding RNAs affecting NLRP3 inflammasome pathway in diabetic cardiomyopathy: a comprehensive review of potential therapeutic options

Elahe Radmehr[1,3], Niloufar Yazdanpanah[2,3,4] and Nima Rezaei[2,3,5*]

Abstract

Cardiomyopathies are a heterogeneous group of disorders that can lead to fulminant heart failure and sudden cardiac death. In recent years, the prevalence of all types of cardiomyopathies has shown an upward trend globally. Up to 40% of patients with cardiomyopathy-related heart failure have diabetes mellitus (DM). With the fast global spread of DM, the prevalence of DCM is increasing accordingly and it remains the leading cause of morbidity and mortality in chronic diabetic patients. NLRP3 inflammasome significantly contributes to the development and pathological progression of DCM. Targeting the inflammasome or any of the mediators along its activation pathway provides new potential therapeutic targets for developing specialized drugs to treat DCM.

In this comprehensive review, we sought to introduce and summarize the non-coding RNAs with potential therapeutic effects targeting NLRP3 inflammasome signaling in DCM. We hope this general overview can aid future research in developing new therapies for DCM.

Keywords Diabetic cardiomyopathies, Inflammasomes, NLRP3, Pyroptosis, Non-coding RNAs

Background

Cardiomyopathies are a heterogeneous group of disorders with various etiologies causing functional and structural abnormalities of the myocardium in the absence of valvular disease, hypertension, coronary artery disease (CAD), and congenital heart disease [1, 2]. Presenting signs and symptoms often fall into a spectrum from subtle clinical manifestations to fulminant heart failure [1]. Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) and hypertrophic cardiomyopathy (HCM) are known as important common causes of sudden cardiac death in young patients and athletes, respectively [1, 3]. Nearly 50% of children and adolescents with sudden cardiac death or waiting for heart transplantation, are affected by cardiomyopathies [4]. In 2020, about 60% of heart transplant recipients in the United States were

*Correspondence:
Nima Rezaei
rezaei_nima@tums.ac.ir; rezaei_nima@yahoo.com
[1]Colorectal Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences (TUMS), Tehran, Iran
[2]Research Center for Immunodeficiencies, Children's Medical Center Hospital, Tehran University of Medical Sciences, Dr. Qarib St, Keshavarz Blvd, Tehran 14194, Iran
[3]Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran
[4]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[5]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

primarily diagnosed with cardiomyopathy [5]. A study by Brownrigg et al. showed that the prevalence of all types of cardiomyopathies increased from 2010 to 2018 [6]. Globally, six million people were affected by cardiomyopathy and myocarditis in 2020 [5, 7].

To date, many classification systems have been proposed for cardiomyopathies. In 2006, the American Heart Association (AHA) classified cardiomyopathies as either primary or secondary based on the predominant organ involved, with primary cardiomyopathies further subdivided into genetic, mixed, and acquired cardiomyopathies [1]. In 2008, the European Society of Cardiology (ESC) categorized cardiomyopathies into specific functional and morphological phenotypes, with each phenotype subcategorized into familial and non-familial forms [2].

The most recent classification system endorsed by the World Heart Federation (WHF), the MOGE(S) nosology of cardiomyopathies, was adopted from the tumor, node, metastases (TNM) staging in oncology; this classification embodies and integrates five attributes: the morphofunctional phenotype (M), organ(s) involvement (O), genetic or familial inheritance pattern (G), etiological annotation (E), and the functional status (S) [8]. In recent years, the number of recognized cardiomyopathies has increased due to the advances in genetic attributes of cardiomyopathies [8–10].

Inflammasome-mediated inflammation plays a central role in the development and pathological progression of several cardiovascular diseases (CVDs), including cardiomyopathies [11–20]. Inflammasome is a high molecular weight multimeric protein complex [20–22] (generally composed of 3 proteins [17, 23–25]) in the cytosol of immune cells as well as the cardiovascular system cells. It plays a key role in response to cellular stress [23]. Inflammasome functions by triggering the activation of the proinflammatory cytokines interleukin (IL)-1β and IL-18 [20], resulting in a series of inflammatory responses, ultimately leading to the pathophysiology of CVDs [13, 26–28].

Many inflammasomes have been identified to date, but the nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome is the most widely studied inflammasome. Growing evidence highlights the critical role of NLRP3 inflammasome activation in the pathogenesis of ischemic and non-ischemic cardiomyopathies [11, 13, 23]. NLRP3 inflammasome induces pyroptosis, a form of inflammatory programmed cell death [23], which contributes to the development of dilated cardiomyopathy [15, 20]. Understanding the role of inflammasomes in cardiomyopathies and identifying the underlying mechanisms, has led to the emergence of novel therapies targeting the inflammasome [20, 21, 23].

In this review, we aim to provide a comprehensive summary of the non-coding RNAs targeting NLRP3 inflammasome signaling in diabetic cardiomyopathy (DCM).

NLRP3 inflammasome

Inflammasomes are an important component of innate immunity [22, 29], generally composed of three parts, sensor receptors that are triggered and activated by pathogen-associated molecular patterns (PAMPs) and host-derived danger-related molecular patterns (DAMPs) [30, 31], adaptors that facilitate an inflammatory reaction, and effectors that are able to initiate the inflammatory cascade [17, 25] (Fig. 1). The assembly of the NLRP3 inflammasome includes the receptor protein NLRP3, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), and the effector protein pro-caspase-1 [12, 29, 30, 32, 33]. The NLRP3 component belongs to the nucleotide-binding domain and leucine-rich repeat (NLR) protein family [12, 13], a group of pattern recognition receptors (PRRs) [17, 34, 35]. The NLR proteins share a mutual organization [12, 35]: a C-terminal leucine-rich repeats (LRR) domain [34, 36], a central nucleotide binding and oligomerization domain (NOD, also known as NACHT) [13, 37], and an N-terminal effector domain [17, 30, 35]. NLRP3 has the common structure of NLRs employing pyrin as its N-terminal effector (PYD) [12, 29, 33], which connects downstream bridging proteins to effector molecules [34, 36]. ASC is a speck-like protein consisting of two domains (a C-terminal CARD and an N-terminal PYD) that serves as the connection between NLRP3 and pro-caspase-1 [12, 38]. Pro-caspase-1, the effector protein of the inflammasome, is cleaved into caspase-1 after autocatalytic maturation on ASC filaments [12, 39]. Caspase-1 is a vital proteolytic enzyme in human homeostasis, resulting in the cleavage of pro-IL-1β and pro-IL-18 into mature cytokines [12, 40].

NLRP3 pathways in cardiomyopathy

NLRP3 inflammasome contributes to the pathophysiology of cardiomyopathy by inducing IL-1β and IL-18 release [12, 17]. In the cardiac tissue, IL-1β triggers calcium leak from the sarcoplasmic reticulum, promotes cell death and tissue remodeling, and ultimately impairs contractility [18, 41].

NLRP3 also plays a critical role in cardiomyocyte pyroptosis (a newly defined programmed cell death) that contributes to dilated cardiomyopathy [1, 12]. Increased mRNA levels of NLRP3 inflammasome components and over-activation of related pathways were observed in the serum and ventricular tissues of mice models of cardiomyopathies [13–16, 42, 43]. During physiological conditions, NLRP3 is inactive [44, 45]. Complete activation of the NLRP3 inflammasome occurs in two stages.

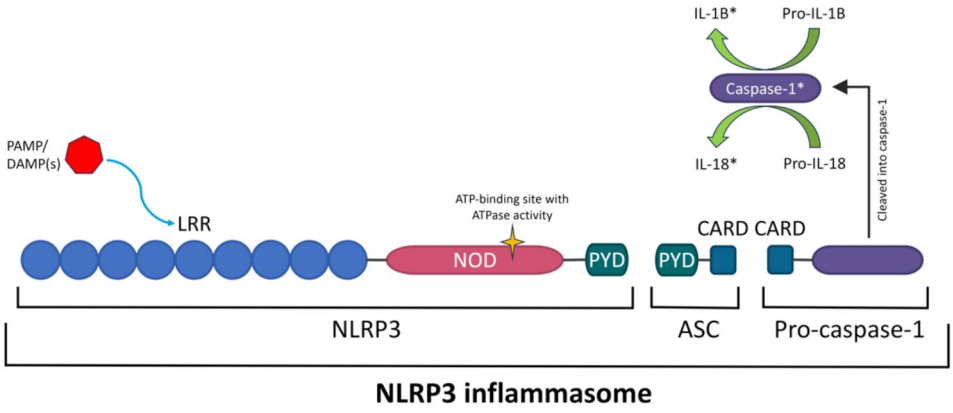

Fig. 1 Structure of the NLRP3 inflammasome. The receptor protein NLRP3 belongs to the NLR family, a group of pattern recognition receptors (PRRs) that function as cytoplasmic receptors. The common structure of NLR proteins is as follows: [1] a C-terminal leucine-rich repeats (LRR) domain that recognizes and binds PAMP or DAMP stimuli [2], a central nucleotide binding and oligomerization domain (NOD, also known as NACHT) that upon ligand binding, undergoes conformational changes, triggering oligomerization and activating adenosine triphosphate (ATP)ase activity through its ATP-binding site. This process is essential for NLRP3 self-association and activation [3]. and an N-terminal effector domain, which is pyrin (PYD) in the NLRP3 inflammasome. The adaptor apoptosis-associated speck-like protein containing a CARD (ASC) connects NLRP3 and pro-caspase-1. Its N-terminal PYD interacts with the PYD of the NLRP3, providing a tight connection, and its C-terminal CARD domain subsequently connects with pro-caspase-1 via the homotypic CARD–CARD reaction. The oligomerization of pro-caspase-1 on ASC filaments facilitates its proximity-driven autocatalytic cleavage into mature caspase-1, which then forms an active heterotetramer, resulting in the cleavage of pro-IL-1β and pro-IL-18 into mature cytokines and inducing their release

It is strictly regulated at the level of priming and activation [12, 30, 44, 45]. The priming step, also known as 'the first signal', is initiated by the recognition of PAMPs and DAMPs by Toll-like receptors (TLRs), which activate TLR-adaptor molecule myeloid differentiation primary response 88 (MyD88) signaling and/or cytokine receptors (TNFRs). This leads to stimulation of cytokines such as TNF-α which induce the release and nuclear translocation of active transcription factor nuclear factor kappa B (NF-κB), which, then, primes the transcription of inflammasome components such as pro-caspase-1, pro-IL-1β, pro-IL-18, and NLRP3 resulting in their increased intracellular transcript levels [11–13, 30, 33, 34, 44–53]. After recognition of the stimuli, TLRs also promote the transcription of inflammasome-related components through post-translational modification of the NLRP3 inflammasome [29, 54].

In the non-canonical (alternative) activation pathway of NLRP3, the transcription step is mediated by caspase-8 and depends on Gram-negative bacteria [13, 29]. Lipopolysaccharide (LPS) promotes the transcription of the inflammasome components through the TLR4-MyD88-TRIF-RIPK1-FADD-CASP8 signaling pathway [13, 29, 55]. The activation of this pathway also induces the oligomerization of the canonical NLRP3 inflammasome and results in the transcription of the caspase-11 gene [13, 55, 56].

When the inflammasome components are not adequately expressed, activation of NLRP3 alone will be insufficient to induce caspase-1 activation and IL-1B or IL-18 production, causing priming to act as the limiting step [44].

Once primed, subsequent NLRP3 inflammasome activation termed 'the second signal' is triggered via multiple inter-related pathways downstream of various unrelated stimuli leading to final inflammasome assembly, which is crucial for signal transduction from upstream molecules to the downstream effector molecules [11, 30, 34, 36, 44]. These complex activation pathways are classified according to the source of the activation signal [33].

Stimuli

Stimuli are divided into three main groups: ionic flux, mitochondrial dysfunction (via reactive oxygen species (ROS)), and lysosomal damage [11, 12, 57]. In addition, the Golgi apparatus, endoplasmic reticulum (ER) stress, and noncoding RNAs are gradually being discovered to contribute to NLRP3 inflammasome activation [11, 33].

Ionic flux

Alterations in the intracellular concentrations of K^+, Ca^{2+}, and Cl^- are different cellular signals regulating the activation of the NLRP3 inflammasome [29].

K+ efflux The most common mechanism of NLRP3 inflammasome activation is K^+ efflux [11, 29, 33] (Fig. 2A). Recognition of bacterial toxins, particulate matter, and ATP by PAMPs/DAMPs can converge on K^+ efflux [29, 34, 58, 59]. ATP is a common trigger of NLRP3 inflammasome activation via the purinergic receptor (P2 × 7R), a characteristic non-selective ATP-gated cationic channel that partially regulates K^+ currents [12, 29, 30, 60]. Cell membrane destruction and some microbial toxins are

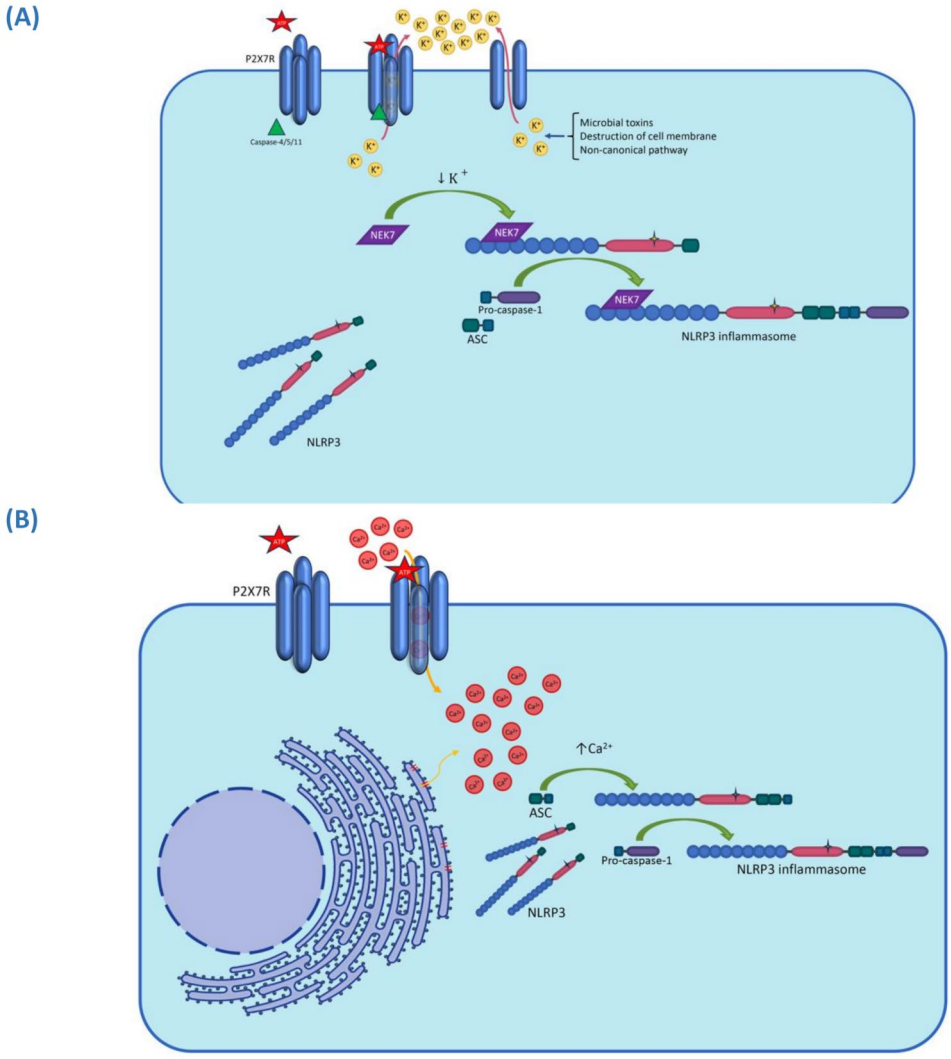

Fig. 2 Ionic flux. (**A**) K^+ efflux: Detection of the elevated levels of ATP in the extracellular medium by P2×7R and activation of this receptor by caspase-4/5/11 results in the opening of the channel which induces K^+ efflux. The decrease in intracellular potassium concentration activates the NLRP3 inflammasome. The binding of never-in-mitosis A-related kinase 7 (NEK7, a well-conserved serine/threonine kinase also known as NIMA-related kinase 7), to NLRP3 downstream to K^+ efflux plays a key role in this process resulting in the molecular complex formation and further activating the NLRP3 inflammasome. Cell membrane destruction, some microbial toxins, and the non-canonical pathway also contribute to the NLRP3 inflammasome activation via K^+ efflux. (**B**) Ca^{2+} mobilization: Calcium ion mobilization from the ER through Ca^{2+} channels or extracellular Ca^{2+} influx through the P2×7 receptor triggered by ATP results in increased intracellular Ca^{2+} levels. Ca^{2+} overload prompts the assembly of the NLRP3 inflammasome complex and plays an important role in the activation of the NLRP3 inflammasome. Ca^{2+} may facilitate the interaction between NLRP3 and ASC by inducing conformational changes

other triggers of K^+ efflux, which leads to NLRP3 inflammasome activation [29, 61, 62].

Ca2+ mobilization Increased cytosolic Ca^{2+} concentration (Ca^{2+} overload) due to the mobilization of Ca^{2+} from ER or extracellular Ca^{2+} influx through the P2×7 receptor contributes to NLRP3 inflammasome activation [33, 34] (Fig. 2B). Also, it is proposed that Ca^{2+} may facilitate the interaction between NLRP3 and ASC by inducing conformational changes [12, 63].

Cl-efflux Elevated extracellular Cl^- concentrations promote the maturation of IL-1β induced by ATP [12, 29]. Comparably, Na^+ influx also takes part in NLRP3 activation [29].

Mitochondrial dysfunction

Mitochondrial dysfunction and ROS production contribute to NLRP3 inflammasome activation [11] (Fig. 3). Once the respiratory chain reaction in mitochondria is inhibited, oxygen accumulates in the form of mitochondrial ROS (mtROS), which is essential for the response of NLRP3 to LPS and ATP [12, 64]. In addition to interacting with NLRP3, the mtDNA is also crucial for the activation process [12, 65, 66].

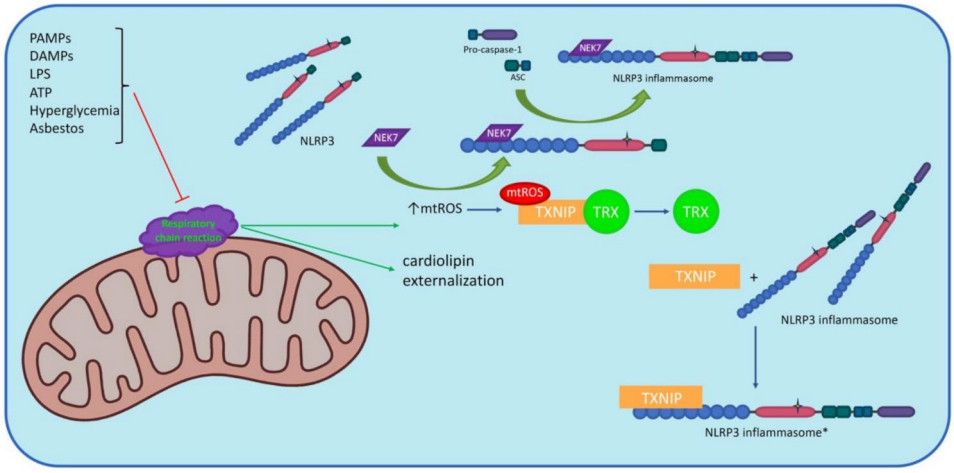

Fig. 3 Various stimuli, including PAMPs, DAMPs, LPS, ATP, Hyperglycemia, and asbestos, inhibit the respiratory chain reaction in mitochondria, leading to oxygen accumulation in the form of mtROS. Detection of increased ROS concentrations by thioredoxin interacting protein (TXNIP) results in its dissociation from thioredoxin (TRX). TXNIP then binds to NLRP3, activating the NLRP3 inflammasome complex. ROS can also promote the interaction between Nek7 and NLRP3, which recruits the ASC domain subsequently, inducing NLRP3 assembly and oligomerization

PAMPs, DAMPs, LPS, ATP, and asbestos induce mitochondrial dysfunction, ROS production, and cardiolipin externalization [12, 13, 33, 34, 57, 64]. Hyperglycemia leads to an increase in mtROS generation which contributes significantly to cardiac fibrosis in DCM [33, 67, 68]. Altered ROS concentrations are detected by thioredoxin interacting protein (TXNIP) causing it to dissociate from thioredoxin (TRX) and to bind to NLRP3, activating the NLRP3 inflammasome complex [11, 30, 34, 69–71]. Similar to potassium efflux, ROS can promote the interaction between Nek7 and NLRP3, which recruits the ASC domain subsequently, inducing NLRP3 assembly and oligomerization [13, 72].

Lysosomal damage

Formation of intracellular crystalline or granular structures following phagocytosis of foreign and self-derived particles, including monosodium urate, silica, amyloid-β (Aβ), cholesterol, and uric acid crystals causes lysosomal damage and rupture, leading to the release of its contents such as tissue protease B [11, 33, 34, 73–76]. Such lysosomal rupture also releases cathepsins that can activate the NLRP3 inflammasome [11, 34]. Cathepsin B, a lysosomal enzyme, is required for IL-1β release; it is an important mediator of NLRP3 activation promoting CVB3-induced myocarditis through pyroptosis [11, 12, 77].

Miscellaneous stimuli

Golgi apparatus and ER stress also contribute to NLRP3 inflammasome activation [11, 33]. ER-Golgi vesicle trafficking is suggested to be involved in NLRP3 inflammasome activation as the disruption of the ER-Golgi trafficking inhibited NLRP3 inflammasome assembly and caspase-1 activation. Furthermore, mitochondrial clustering around the Golgi was observed to accompany NLRP3 activation; so, the dispersed trans-Golgi network (dTGN) also provides a scaffold for NLRP3 inflammasome aggregation and activation [11, 78, 79].

Noncoding RNAs, mainly microRNAs (miRNAs), such as miR-223, miR-495-3p, miR-145a-5p, miR-30c-5p, and miR-9-5p, influence NLRP3 activation either by modulating the transcription process or targeting the upstream regulators of its activation [11, 80–84]. In addition, long noncoding RNAs (lncRNAs), such as lncRNA MEG3, can enhance NLRP3 expression by sponging miR-223 [11, 85].

Intracellular pH, metabolic alterations, and autophagy dysfunction have also been identified to be involved in NLRP3 activation [29, 33, 34, 36].

Pyroptosis

Pyroptosis is a kind of programmed cell death, characterized by pore formation in the plasma membrane resulting in persistent cell swelling and osmotic rupture, and the discharge of pro-inflammatory factors [12, 33, 86, 87].

In pyroptosis, the inflammasome serves as a mediator [12]. Concomitant with the cleavage of IL-1β and IL-18, the classical pyroptotic pathway also results in the cleavage of GSDMD into two parts (NT-GSDMD and CT-GSDMD) by the activated caspase-1. The cleaved lipophilic N-terminal fragment binds to membrane phospholipids and forms GSDMD micropores by oligomerizing into cell membranes, causing cell rupture and the release of cytokines, including IL-1β and IL-18, leading to an inflammatory cascade and cell death eventually [11, 12, 33, 88–90]. Cleaved GSDMD also facilitates

potassium efflux and activation of canonical NLRP3 signaling [13, 56].

In the non-canonical activation pathway of the NLRP3 inflammasome, at the oligomerization step, LPS released by Gram-negative bacteria triggers the activation of caspase-11 that induces the nonclassical pyroptotic pathway by cleaving GSDMD [13, 29, 33, 91–93].

Diabetic cardiomyopathy

Diabetes mellitus (DM) is a serious public health issue worldwide and one of the fastest-growing health emergencies in the 21st century [94, 95]. Diabetic cardiomyopathy (DCM), the most common diabetic complication, remains the leading cause of morbidity and mortality in chronic diabetic patients [94–98]. About 10–40% of patients with cardiomyopathy-related heart failure have diabetes [97, 99].

DCM is a chronic low-grade inflammatory cardiovascular disorder characterized by functional, structural, and metabolic changes of the heart resulting from the switch of cardiac energy substrates' supply to free fatty acids (FFAs) in the absence of CAD, hypertension, valvular and congenital heart disease, and other conventional risk factors for myocardial remodeling [94, 96, 100, 101].

DCM induces myocardial fibrosis and hypertrophy, followed by cardiac remodeling and early left ventricular diastolic dysfunction, leading to systolic dysfunction and ultimately to heart failure [94–96, 102]. With the fast global spread of DM, the prevalence of DCM is increasing accordingly; the incidence of DCM is about 1% and 17% in community-based populations and in diabetic patients, respectively [98, 103].

Myocardial inflammation is implicated as a key process in the multifactorial pathogenesis of DCM, with the cardiac inflammasome complexes being the key inducers of inflammation [94, 95, 104]. Insulin resistance in DM is closely associated with inflammation [95]. Major inducers of the pro-inflammatory state in the diabetic heart include hyperglycemia, hyperlipidemia (high levels of saturated FFAs), circulating cytokines, ROS, angiotensin II (AgII), endothelin-1, and insulin resistance [96, 104–107]. Several conserved mutual signal transduction pathways are abnormally regulated in cardiomyocytes due to dysregulation of glucose and lipid metabolism and DCM is closely associated with abnormal myocardial energy metabolism [94, 96, 108, 109].

NLRP3 inflammasome activation significantly contributes to the development of DCM [95, 98]. NLRP3 inflammasome-mediated pyroptosis of cardiomyocytes is a key participant in this process [98, 110]. Elevated expression of NLRP3 inflammasome components and upregulation of NLRP3 activation and cardiac pyroptosis have been shown in the heart tissues of diabetic patients compared with their nondiabetic counterparts [95, 98]. Through silencing the *NLRP3* gene, Luo et al. showed for the first time that NLRP3 contributes to the development of DCM [111]. The NLRP3 inflammasome is expressed across a wide range of cells, including macrophages, cardiofibroblasts (CFs), and cardiomyocytes [18, 104, 112]. Under a hyperglycemic environment, the myocardium induces NLRP3 inflammasome activation, ultimately leading to cardiomyocyte pyroptosis [95, 98, 101].

In addition, peroxisome proliferator-activated receptors (PPARs) and sirtuins (Sirts) also have a role in the myocardial substrate switch and can limit the inflammatory response by targeting different components of the NLRP3 inflammasome-dependent signal transduction pathways [104].

Targeting the inflammasome itself or any of the mediators along its activation pathway can be beneficial in slowing down the progression of DCM [97, 101]. The massive amount of research identifying the mechanisms involved in DCM development provides new potential therapeutic targets for developing specialized drugs to treat DCM [96, 97, 101] (Table 1). Although several substances have been introduced in different studies as potential drugs that can be helpful in treating DCM, none of them have been actively explored in the human population or in practice and thus, no such drug has been employed or developed, so far [95, 104].

Non-coding RNAs: possible treatments and therapeutic targets for DCM

MicroRNAs

miR-223

Xu et al. carried out in vitro and in vivo experiments investigating the role of miRNA-223 (miR-223) and the effects of miR-223 inhibition in DCM. They found that the level of miR-223 was significantly increased in high-glucose (HG)-induced H9c2 cells and also in the streptozotocin (STZ)-induced rat models of DCM. They discovered that after the introduction of the miR-223 inhibitor, the increase in the expression of the NLRP3 inflammasome-related gene (NLRP3), fibrosis-related genes (collagen-1 and collagen-3), and apoptosis-related genes (caspase-3 and bax) and the decrease in the expression of the apoptosis-related gene (bcl2) were partially or completely reversed and the NLRP3 inflammasome activation, myocardial fibrosis, and apoptosis were markedly attenuated in both HG-induced H9c2 cells and the DCM rat model. They also showed that inhibition of miR-223 prominently relieved cardiac dysfunction and significantly reduced blood glucose in DCM rat model. Evaluating and comparing the myocardial tissues of DCM rat model with those of the miR-223 inhibitor-treated group, they found that the miR-223 inhibitor significantly alleviated cardiac fibrosis and could improve

Table 1 Therapeutic targets for developing drugs for DCM

Category	Name of Agent		Effect and Pathway	Reference No.
MicroRNAs	miR-223		miR-223↓ → NLRP3↓, Col-I↓, Col-III↓, Casp-3↓, bax↓, bcl2↓ miR-223-3p↓ → SPI1↓ → Casp-1↓ → IL-1β and other pyroptosis-associated proteins↓ → pyroptosis↓	[113, 114]
	miR-21-3p		miR-21-3p↓ → AR↑, Casp-1↓	[115]
	miR-9		miR-9 → ELAVL1↓ → canonical pyroptosis↓	[116]
	miR-200a-3p		miR-200a-3p → FOXO3↓, Mst1↓, SIRT3↑, p-AMPK↑ → autophagy↑, apoptosis↓	[119]
	mIR-18a-3p	Berberin (BBR)	BBR → miR-18a-3p↑ → GSDMD↓, IL-1β↓ → ROS↓, Pyroptosis↓	[120]
LncRNAs	LncRNA-MALAT1	LncRNA-MALAT1	lncR-MALAT1↓ → Casp-1↓, IL-1β↓, IL-18↓ → pyroptosis↓	[121]
		Melatonin	Melatonin → lncR-MALAT1↓ → miR-141↑ → NLRP3 inflammasome activation↓ Melatonin → TGF-β1/Smads signaling↓	[122]
		Pomegranate peel extract (PPE)	PPE → NLRP3/Casp-1/IL-1β signaling↓ → pyroptosis↓ PPE → lncR-MALAT-1↓	[123]
	LncRNA GAS5		LncRNA GAS5 → miR-34b-3p↓ → AHR↑ → NLRP3 inflammasome-mediated pyroptosis↓	[124]
	TINCR LncRNA		METTL14 → TINCR lncRNA↓ → NLRP3↓ → pyroptosis↓	[126]
	LncRNA homeobox transcript antisense RNA (HOTAIR)		HOTAIR → FUS↑ → SIRT3↑ → pyroptosis↓	[127]
	LncRNA KCNQ1OT1		KCNQ1OT1↓ → miR-214-3p↑ → Casp-1↓ → pyroptosis↓	[134]
piRNAs	piR112710		piR112710 → pyroptosis and inflammation -associated proteins↓ (TXNIP, NLRP3, Casp-1, and GSDMD-N), ROS↓ → TXNIP/NLRP3 inflammasome↓ → pyroptosis↓	[135]

the morphological structure in myocardial tissues of the DCM model rats [113].

Zhao et al. discovered that along with the significantly increased expression level of miR-223–3p, SPI1 (a transcription factor) was significantly downregulated in HG-treated H9c2 cardiomyocytes. They found that miR-223–3p knockdown in HG-treated cardiomyocytes reversed the decrease in SPI1 levels. Also, the inflammasome/pyroptosis-associated proteins NLRP3, GSDMD-N, caspase-1, and IL-1β were downregulated following inhibition of miR-223–3p expression. Through further investigation, they confirmed that inhibiting the expression of miR-223–3p can relieve cardiomyocyte pyroptosis by up-regulating SPI1. Thus, they proposed a new signaling pathway based on their findings: miR-223–3p↑ → SPI1↓ → caspase-1↑ → IL-1β and other pyroptosis-associated proteins↑ → pyroptosis↑. These findings propose miR-223–3p inhibition as a potential therapeutic option for DCM [114].

miR-21-3p

In 2021, Shi et al. investigated the role of miR-21–3p and its target androgen receptor (AR) in diabetic cardiac fibrosis. They found that miR-21–3p was significantly upregulated in STZ-induced diabetic cardiac fibrotic tissue and HG-induced cardiac fibroblasts, while at the same time, the AR was downregulated. Overexpression of miR-21–3p resulted in reduced expression of AR in cardiac fibroblasts, whereas inhibition of miR-21–3p caused an elevation in the expression of AR in cardiac fibroblasts. Also, the levels of NLRP3 and caspase-1 and collagen I expression were reduced in cardiac fibroblasts transfected by miR-21–3p inhibitors. These findings demonstrated that AR is a direct target of miR-21–3p in modulating cardiac fibroblast pyroptosis in the diabetic heart. Furthermore, they discovered that AR up-regulation reduced fibrosis markers, collagen I expression, and NLRP3 and caspase-1 levels in HG-treated cardiac fibroblasts. Thus, considering that miR-21–3p aggravates diabetic cardiac pyroptosis and fibrosis by suppressing AR expression, they postulated that over-expression of AR or downregulation of miR-21–3p reduces pyroptosis via the AR/caspase-1 pathway [115].

miR-9

Through bioinformatics analysis and target validation assays, Jeyabal et al. discovered that miRNA-9 (miR-9) inhibits ELAVL1 expression in human cardiomyocytes. They found that ELAVL1 expression was augmented in human diabetic hearts and HG-treated human cardiomyocytes. Through further investigation, they showed that ELAVL1 knockdown nullified TNF-α induced canonical pyroptosis via NLRP3, caspase-1, and IL-1β suppression, while it did not affect LPS-induced noncanonical pyroptosis. Moreover, they found that inhibition of miR-9 upregulates ELAVL1 expression and activates caspase-1. Further evaluation showed that miR-9 expression was significantly downregulated in human diabetic heart tissues compared to their non-diabetic healthy counterparts. Also, blocking miR-9 expression in human cardiomyocytes resulted in an increase in the expression of caspase-1 and IL-1β, while transfecting cardiomyocytes with miR-9 mimics caused downregulation of these proteins and inhibited cardiac pyroptosis. These findings highlight targeting miR-9/ELAVL1 expression as a novel potential therapeutic option in preventing cardiac pyroptosis in DCM [116].

miR-200a-3p

In recent studies, a significant decrease in miR-200a-3p was observed in cardiomyocytes of myocardial injury models [117]. Additionally, lower expression of miR-200a-3p in the serum was associated with necrosis and inflammation in intestinal epithelial cells of necrotizing enterocolitis patients [118]. Based on these findings, You et al. investigated the role and mechanism of miR-200a-3p in the pathological process of DCM. They observed a significant decrease in miR-200a-3p expression in myocardial tissues of DCM-induced myocardial injury model mice. They also showed that overexpression of miR-200a-3p notably led to the alleviation of myocardial injury and cardiac dysfunction, reduced cardiac fibrosis, inflammation, and cardiomyocyte apoptosis, enhanced autophagy, and improved LV function in DCM mice. The researchers discovered that upregulation of miR-200a-3p ameliorates apoptosis by affecting various cellular processes. For instance, it downregulates FOXO3, inactivates Mst1 transcription, enhances SIRT3 expression and AMPK phosphorylation levels, and promotes autophagy. This ultimately slows down the progression of DCM. These findings suggest that overexpressing miR-200a-3p alleviates DCM in mice by regulating autophagy and inhibiting apoptosis through the FOXO3/Mst1/SIRT3/AMPK axis, which provides a new therapeutic approach for the prevention and treatment of DCM [119].

miR-18a-3p

Yang et al. studied the effects of Berberine (BBR), a natural compound extracted from a Chinese herb with application in treating inflammatory disorders and DM-induced cardiovascular injury, on DCM. They found that BBR treatment effectively improved cardiac dysfunction and fibrosis in the rat model of DCM. BBR markedly alleviated DCM by limiting GSDMD-driven pyroptosis via inhibiting IL-1β secretion and GSDMD expression at the post-transcriptional level in HG-stimulated H9c2 cells and diabetic rats. They discovered that the inhibitory

effects of BBR on GSDMD transcription are induced by the upregulation of miR-18a-3p expression in vivo and in vitro, confirming the direct negative regulatory effect of miR-18a-3p on GSDMD expression. They also indicated that miR-18a-3p reduced the ROS levels and rate of cell death by suppressing GSDMD production in vitro and in vivo. Moreover, it was confirmed that BBR regulates the expression levels of miR-18a-3p by enhancing the activation of the miR-18a-3p promoter. Collectively, these findings suggest that BBR alleviates DCM by inhibiting miR-18a-3p-mediated GSDMD activation and pore formation through the NLRP3 inflammasome pathway. Thus, BBR can be considered a potential novel therapy for treating DCM [120].

LncRNAs

LncRNA-MALAT1

Shi et al. carried out an experiment investigating the role of metastasis-associated lung adenocarcinoma transcript 1 lncRNA (lncRNA-MALAT1) in cardiomyocyte pyroptosis in DCM. They demonstrated that silencing MALAT1, which was overexpressed in myocardial tissue of diabetic mice and HG-induced cardiomyocytes, improves cardiac function and morphology in HG-induced cardiomyocytes and STZ-induced diabetic mice. They also showed that inhibition of MALAT1 attenuates cardiomyocyte pyroptosis through the NLRP3 pathway by inducing a significant decrease in caspase-1, IL 1β, and IL-18 protein levels. Hence, they suggested that MALAT1 could be a new therapeutic target for DCM [121].

Melatonin Che et al. found that along with the elevated levels of lncR-MALAT1, miRNA-141 (miR-141) was downregulated in the myocardium of DM mice and in HG-treated CFs. Investigating the role of melatonin in DCM, they reported that melatonin significantly restored cardiac hypertrophy, normalized the structural anomalies, ameliorated the damage to cellular organelles by improving sarcomere arrangement and mitochondria morphology, decreased interstitial collagen production, and finally alleviated cardiac dysfunction significantly in STZ-induced DM mice by suppressing the upregulation of lncR-MALAT1 and by upregulating miR-141 expression. These findings confirmed miR-141 as a downstream target of lncR-MALAT1, which is regulated by melatonin. Through further investigation, they signified lncR-MALAT1 as a competitive endogenous RNA (ceRNA) that can sponge miR-141 to limit its functional availability. Finally, they verified that melatonin exerts its antifibrotic effect by inhibiting lncR-MALAT1/miR-141-mediated NLRP3 inflammasome activation and TGF-β1/Smads signaling, making it a potentially effective agent for treating DCM [122].

Pomegranate Peel extract Abo-Saif et al. conducted a study on the effects of pomegranate peel extract (PPE) on DCM in rats. They showed that prophylactic administration of PPE to diabetic rats significantly increased their survival rate, ameliorated cardiac hypertrophy, and improved their lipid profile. Additionally, it decreased serum cardiac troponin-1 levels and reduced lipid peroxidation in the rats' myocardial tissue. They also found that the levels of lncRNA-MALAT1, IL-1β, and pyroptosis-related genes (NLRP3 and caspase-1) significantly decreased in the cardiac tissue of diabetic rats following treatment with PPE. In addition, histopathological examination of their cardiac tissues showed preservation of normal myocardial structures and a marked reduction in fibrosis. They concluded that PPE exhibits a cardioprotective effect against the development of DCM in diabetic rats. This effect is attributed to its exceptional antioxidant, anti-inflammatory, and anti-fibrotic properties, as well as its ability to improve the lipid profile. This protective effect is probably achieved via the inhibition of pyroptosis (the NLRP3/caspase-1/IL-1β signaling pathway) and downregulation of lncRNA-MALAT1. These data show that PPE could be a promising therapy to prevent the development of DCM [123].

LncRNA GAS5

Xu et al. observed a significant decrease in the lncRNA GAS5 levels in the heart tissues of mice with DCM. They also found that overexpression of GAS5 improved cardiac function and alleviated myocardial hypertrophy in DCM mice. Additionally, upregulation of GAS5 markedly repressed the increased expression of NLRP3, caspase- 1, pro-caspase-1, IL-1β, and IL-18 seen in cardiac tissues of DCM mice and HG-treated HL-1 cells (cardiac muscle cell line). Overexpressing GAS5 also suppressed caspase-1 activity and the release of lactate dehydrogenase (LDH) in the HG-treated HL-1 cells. Furthermore, they discovered that the expression of miR-34b-3p was increased in the heart tissues of DCM mice as well as in the HG-induced HL-1 cells, accompanied by a concomitant decrease in the expression of aryl hydrocarbon receptor (AHR). Through further investigation, they demonstrated that overexpression of GAS5 inhibits NLRP3 inflammasome activation-induced pyroptosis by regulating the miR-34b-3p/AHR axis via inhibition of miR-34b-3p expression. Collectively, these findings elucidate that lncRNA GAS5 acts as a ceRNA to enhance AHR expression by sponging miR-34b-3p, thus repressing NLRP3 inflammasome activation-mediated pyroptosis. This data provides lncRNA GAS5 as a novel valuable target for DCM treatment [124].

TINCR LncRNA

N6-methyladenosine (m6A) is an important epigenetic regulator of RNAs, including lncRNAs. Methyltransferase-like 14 (METTL14) is an m6A writer protein known to widely participate in the pathogenesis of major diseases, such as CVDs [125]. Since the underlying regulatory mechanism of m6A and METTL14 in DCM was not well established, Meng et al. aimed to uncover the role of METTL14-mediated m6A modification in pyroptosis and the progression of DCM throughout their study. They found that METTL14 was downregulated in HG-treated cardiomyocytes and myocardium tissues of STZ-induced DCM rat models. They revealed that METTL14 increased the m6A methylation level of the *TINCR* gene, suppressing its expression and reducing NLRP3 mRNA stability resulting in decreased expression of NLRP3. As a result, METTL14 prevented the occurrence of cardiomyocyte pyroptosis and remarkably suppressed DCM progression. This newly discovered regulatory mechanism not only enhances our understanding of the epigenetic regulation of pyroptosis in DCM progression but also holds promise for the development of novel therapeutic strategies to combat DCM [126].

LncRNA homeobox transcript antisense RNA (HOTAIR)

Since lncRNAs play important roles in the pathogenesis of DCM, Xiong et al. attempted to clarify the function of lncRNA homeobox transcript antisense RNA (HOTAIR) in the pyroptosis of cardiomyocytes and its underlying molecular mechanism in DCM. They reported that SIRT3 and HOTAIR were significantly downregulated in HG-induced H9C2 cells. Additionally, they found that HOTAIR overexpression alleviated pyroptosis and suppressed the inflammatory response in HG-treated cardiomyocytes. Upon further investigation, they unveiled that HOTAIR induced SIRT3 expression in HG-treated H9C2 cells by recruiting FUS (a multifunctional protein involved in multiple aspects of RNA metabolism, such as transcription regulation and alternative splicing), thereby attenuating pyroptosis. Moreover, they showed that SIRT3 knockdown notably reversed the suppressive effect of HOTAIR on HG-induced pyroptosis in cardiomyocytes, suggesting that SIRT3 acts as a downstream regulator of HOTAIR. Through these findings, researchers identified a vital and effective role for HOTAIR in DCM and strongly speculated that the HOTAIR/FUS/SIRT3 signaling pathway effectively influences the progression of DCM. They indicated that HOTAIR alleviates pyroptosis and the development of DCM, making it a potential marker for diagnosis and a novel therapeutic target for treating DCM [127].

LncRNA KCNQ1OT1

The lncRNA Kcnq1ot1, the full name of which is *KCNQ1* overlapping transcript 1 [128], has been reported to play a significant role in the heart and participate in many CVDs, such as long QT syndrome and myocardial ischemia/reperfusion injury [129–132]. A recent study found that Kcnq1ot1 might influence cataract formation by acting as a ceRNA to regulate caspase-1 expression via sponging miR-214-3p [133]. Considering the still unknown expression and mechanisms of Kcnq1ot1 in DCM, Yang et al. conducted a study to clarify the effects of Kcnq1ot1 on cardiac pyroptosis in DCM and define its mechanisms. They found that Kcnq1ot1 and caspase-1 were overexpressed in the serum of diabetic patients, the cardiac tissue of diabetic mice, and HG-induced cardiomyocytes. They demonstrated that silencing Kcnq1ot1 reduced cell death, DNA fractionation, cytoskeletal structure abnormalities, and calcium overload in HG-induced cardiomyocytes, improved cardiac function and morphology in STZ-induced diabetic mice, and significantly inhibited pyroptosis of cardiomyocytes in vitro and in vivo. They discovered that silencing Kcnq1ot1 attenuates pyroptosis by targeting caspase-1 via miR-214-3p in cardiomyocytes. Thus, they elaborated for the first time on the downregulation of miR-214-3p in HG-treated cardiomyocytes and fully illuminated the effect of Kcnq1ot1 on pyroptosis in DCM via miR-214-3p and caspase-1. Therefore, their research suggests Kcnq1ot1 as a novel therapeutic target for DCM [134].

PIWI-interacting RNAs (piRNAs)

piR112710

PIWI-interacting RNAs (piRNAs) are a group of small non-coding RNAs (ncRNAs) composed of 26–32 nucleotides. They are termed piRNAs due to their interaction with PIWI family proteins. piRNAs are abundantly expressed in the myocardial tissue and have been reported to regulate cardiomyocyte apoptosis and hypertrophic cardiomyopathy and to improve heart failure. In their attempt to identify the functions of piRNAs in regulating pyroptosis in DCM, Jiao et al. performed a combination of piRNA microarray expression analysis and transcriptome sequencing (RNA-seq). They identified piR112710 to be involved in DCM. The researchers then investigated the effects of piR112710 in DCM in db/db mice and HG + Palmitate-treated neonatal mice cardiomyocytes. They found that piR112710 was downregulated in HG + Palmitate-treated cardiomyocytes. Also, piR112710 significantly ameliorated cardiac dysfunction in db/db mice, characterized by downregulation of inflammatory factors and pyroptosis-associated proteins (NLRP3, ASC, TXNIP, caspase-1, and GSDMD-N), reduced fibrosis, enhanced mitochondrial respiratory functions of the myocardium, and improved

echocardiography. Moreover, supplementation with piR112710 significantly decreased oxidative stress and mitochondrial dysfunction and reduced the expression levels of pyroptosis-associated proteins and inflammatory factors in cultured HG + palmitate-induced neonatal mice cardiomyocytes. Through further investigation, the researchers discovered that piR112710 prevents pyroptosis by inhibiting the TXNIP/NLRP3 axis through the effective elimination of ROS and reducing the expression levels of the pyroptosis-associated proteins NLRP3, ASC, TXNIP, caspase-1, and GSDMD-N, which subsequently alleviate mitochondrial dysfunction and reduce IL-18 and IL-1β levels. Taken together, these findings revealed that the piR112710 protects against DCM by inhibiting the TXNIP/NLRP3-mediated pyroptosis, thereby providing a useful and novel therapeutic target for the treatment of DCM [135].

Conclusions

Cardiomyopathies are a heterogeneous group of disorders that can lead to fulminant heart failure and sudden cardiac death [1, 3]. From 2010 to 2018, the prevalence of all types of cardiomyopathies had an upward trend [6] and in 2020, six million people were affected by cardiomyopathy and myocarditis worldwide [5, 7]. It is noteworthy that up to 40% of patients with cardiomyopathy-related heart failure have diabetes [97, 99]. With the increasing global trend of DM, the prevalence of DCM is increasing accordingly [98, 103] and it remains the leading cause of morbidity and mortality in chronic diabetic patients [94–98]. NLRP3 inflammasome significantly contributes to the development and pathological progression of DCM [95, 98, 111].

Targeting the inflammasome itself or the mediators along its activation pathway provides new potential therapeutic targets for developing drugs for DCM treatment [96, 97, 101]. The massive amount of research identifying the underlying mechanisms of NLRP3 inflammasome in DCM has led to the emergence of novel therapies beneficial in slowing down the progression of DCM [20, 21, 23, 97, 101].

In this comprehensive review, we sought to provide a summary of the non-coding RNAs with potential therapeutic effects targeting NLRP3 inflammasome signaling in DCM. We hope that this general overview helps set a foundation for future research concerning the development of new therapies for DCM.

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