

The role of non-coding RNAs in myocarditis: a narrative review

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Background and Objective: Myocarditis is a heterogeneous disease that can lead to acute heart failure, dilated cardiomyopathy (DCM), and sudden death. However, the knowledge of the precise molecular mechanisms of myocarditis is fairly limited. In recent years, non-coding RNAs (ncRNAs) have been demonstrated to be involved in many physiological and pathological processes in myocarditis and to have the potential to be used as novel diagnostic and therapeutic strategies for myocarditis. This review summarizes the role of ncRNAs in myocarditis and discusses their potential as noninvasive biomarkers and therapeutic targets for myocarditis.

Methods: Literature on ncRNAs and myocarditis published in PubMed was extensively reviewed for analysis and discussion.

Key Content and Findings: This review describes the roles of different ncRNAs in myocarditis and summarizes their potential in diagnosing and treating myocarditis. Multiple functions and mechanisms of ncRNAs in myocarditis have been uncovered.

Conclusions: Current studies show that ncRNAs are widely involved in the occurrence and development of myocarditis caused by infection, autoimmunity, and the use of immune checkpoint inhibitors (ICIs) through their regulation of cell apoptosis, immune response, viral replication, and other aspects. Small-sample clinical studies have assessed the diagnostic value of ncRNAs. These results provide a new theoretical basis for diagnosing and treating myocarditis.

Keywords: Non-coding RNAs (ncRNAs); viral myocarditis; experimental autoimmune myocarditis (EAM); Chagas disease; immune checkpoint inhibitors (ICIs)

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Introduction

Myocarditis is an inflammatory disease of the myocardium that exhibits vast clinical heterogeneity, ranging from mild chest discomfort to cardiogenic shock. Precise diagnosis of myocarditis is difficult to achieve because of the high heterogeneity of its clinical presentation and the scarcity of endomyocardial biopsy (EMB) (1). To date, no specific blood test has been established to reliably diagnose myocarditis. Patients with mild myocarditis often recover without any treatment (2). However, in some cases, myocarditis progresses to dilated cardiomyopathy (DCM) and heart failure requiring heart transplantation, even after the use of major treatment methods, such as

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Table 1 Search strategy summary

Items	Specification
Date of search	14/9/2021
Databases and other sources searched	PubMed database
Search terms used	"non-coding RNAs and myocarditis", "miRNAs and myocarditis", "IncRNAs and myocarditis", "circRNAs and myocarditis
Time frame	From December, 2010 to September, 2021
Inclusion and exclusion criteria	Published English-language literature on the role of non-coding RNAs in myocarditis. All study types were included
Selection process	Wenhu Liu and Jing Hu searched the database independently. The corresponding author mediated any cases of disagreement between the two researchers

immunosuppressant, antiviral, and circulatory support (3,4). Therefore, exploration of novel diagnostic and therapeutic methods for myocarditis is important.

Non-coding RNAs (ncRNAs) are ribonucleic acids transcribed from DNA that are not translated into proteins. They include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), transfer RNAs, ribosomal RNAs, small nuclear RNAs, and piwiinteracting RNAs (5). Mounting evidence suggests that ncRNAs play a pivotal role in cardiovascular and inflammatory diseases (6-9). Therefore, future therapies may focus on these ncRNAs to reduce the severity of myocarditis. Furthermore, considering that some ncRNAs in blood are stable to degradation, have long half-lives, and can be measured by classical laboratory methods, they also hold great potential for the diagnosis of myocarditis. In this review, we focus on the roles of miRNAs, lncRNAs, and circRNAs in the pathogenesis of myocarditis and discuss the possibility of using ncRNAs as novel diagnostic markers and therapeutic targets for myocarditis. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups. com/article/view/10.21037/atm-21-6116/rc).

Methods

Literature on ncRNAs and myocarditis published in PubMed up to September 2021 was extensively reviewed and analyzed using the following keywords: "non-coding RNAs and myocarditis", "miRNAs and myocarditis", "lncRNAs and myocarditis", and "circRNAs and myocarditis". The search strategy is summarized in *Table 1*.

Results

Animal models of myocarditis

Myocarditis is a cardiovascular disease caused by a variety of factors, most of which are associated with infections, immunity, drugs, and toxins (4,10,11). Myocarditis has been understood mainly through animal models, and several clinically relevant models have been developed over the years. Animal models of myocarditis with different etiologies are summarized in *Table 2* (12).

Coxsackievirus B3-induced myocarditis models

The most common animal model of viral myocarditis is the coxsackievirus B3 (CVB3)-induced myocarditis mouse model, which was first established in the 1950s (13,14). The CVB3-infected mouse model remains the animal model of choice for studying viral myocarditis because it closely resembles the heart injury observed in patients with myocarditis (15). The model is developed by intraperitoneal injection of mice with purified CVB3 viruses, which cause acute cardiac inflammation between days 7 and 14 after infection (16). Susceptible mouse strains, including A/J and BALB/c mice, develop chronic myocarditis; in contrast, C57 mice are resistant and develop only acute myocarditis (17). Due to the ability of the virus to induce multiorgan dysfunction, mice often also develop pancreatitis (18). Although pancreatitis may influence cardiotoxicity, this concept has been challenged by experiments in mice with pancreas-specific deletion of the coxsackievirus and adenovirus receptor, which showed that infection of the pancreas has very little effect on cardiac CVB3 infection and pathology (19). However, pancreatitis is rare in patients

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Model	Susceptible strains	Disease features	Advantages	Disadvantages
CVB3-induced myocarditis	A/J, BALB/c, ABY/SNJ	Acute myocarditis progressing to DCM	Recapitulates clinical pathogenesis of myocarditis caused by viruses	High-grade virus lab required; severe pancreatitis with clinical inconsistency; high mortality rate
	C57 BL/6	Acute myocarditis		
Experimental autoimmune myocarditis	A/J, BALB/c, SW	Acute myocarditis progressing to DCM	High biosecurity, recapitulates virus-negative myocarditis; study of the progression to DCM	Non-clinical natural causes of morbidity
Trypanosoma cruzi-induced myocarditis	BALB/c, C57BL/6, A/J, DBA-2, C3H/He	Chronic myocarditis	Recapitulates clinical pathogenesis of Chagas disease-induced myocarditis	Classical models for which there is no definitive model; a long time required to reach the chronic phase
ICI-induced myocarditis	MRL, BALB/C, PD1 ^{-/-} or PDL1 ^{-/-} mice	Fulminant myocarditis or DCM	r Study of ICI-induced myocarditis	High mortality rate; strict mouse strain restrictions
	BALB/c, C57BL/6, CTLA4 ^{-/-} mice	Fulminant myocarditis		High mortality rate; systemic non- specific lymphoid proliferative disease with non-specific myocarditis
	C57BL/6, PD1-lg treatment, and cardiac radiation	Fulminant myocarditis		High mortality rate; the influence of radiation factors

Table 2 Animal models of myocarditis caused by different etiologies

DCM, dilated cardiomyopathy; IPSCs, induced pluripotent stem cells; ICI, immune checkpoint inhibitor.

with myocarditis, and the high frequency of pancreatitis is a key limitation of this mouse model (20).

Experimental autoimmune myocarditis (EAM) models

Myocarditis has been linked to autoimmunity (21,22). Circulating heart-specific autoantibodies are often detected in patients with myocarditis or inflammatory cardiomyopathy (23). These cardiac-specific autoantibodies can recognize many cardiac antigens, particularly cardiac alpha-myosin heavy chain and beta-myosin heavy chain (24,25). EAM can be induced by subcutaneous injection of mice with cardiac myosin with adjuvant on days 0 and 7. In these mice, cardiac inflammation is evident at 21 days after injection, and DCM usually develops within 40 days after injection (26,27). The injection of cardiac myosin induces severe myocarditis and the production of high titers of antimyosin autoantibodies in A/J, Swiss Webster, and BALB/ c mice. In contrast, C57BL/6J and C57BL/10J mice are resistant to cardiac myosin-induced myocarditis and do not develop myosin-specific autoantibodies (28). A key advantage of this model is that it allows both autoimmunity and the transition from myocarditis to DCM to be studied. However, most transgenic and knockout mice have a C57 background, which limits the wide application of the EAM model (12).

Trypanosoma cruzi-induced myocarditis models

Trypanosoma cruzi (T. cruzi) infection, also known as Chagas disease, may cause myocarditis. Experimental Chagas heart disease has been successfully established in several mouse lines using various strains of T. cruzi, including the Colombian, Tulahuen, CL Brener, Y, and Sylvio X10 strains. However, no model has been generally accepted as the classical one because of the diversity of pathogenic Trypanosoma strains isolated from chagastic patients, insect vectors, and animal hosts (12,29,30).

Myocarditis models induced by immune checkpoint inhibitors (ICIs)

ICIs have become the treatment of choice for many cancers. Myocarditis induced by ICI therapy is rare but can have serious consequences (31,32). Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are the most common targets in animal models of ICI-induced myocarditis (33). In view of the extremely low incidence of myocarditis induced through the direct use of ICIs, the current animal models mostly adopt strategies targeting immune sites, in particular, the use of knockout mice for PD-1 and CTLA-4. Tivol *et al.* reported that CTLA-4^{-/-}

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mice rapidly develop lymphoproliferative disease with multiorgan lymphocyte infiltration and tissue destruction, as well as severe myocarditis and pancreatitis (34). Similarly, PD-1^{-/-}, PD-L1^{-/-}, BALB/c, and MRL mice develop myocarditis and DCM (35-37). Du *et al.* (38) developed a technique to deliver cardiac irradiation in a mouse model while using PD-1 blockade, and histological examination of their model revealed significant inflammation in the heart.

The role of ncRNAs in myocarditis

Classification of ncRNAs

At present, the mechanism of myocarditis is still unclear. Myocarditis is believed to be caused by the damaging effects of factors that activate the immune system (39), and the role immune-induced myocardial injury plays in inducing myocarditis is becoming increasingly evident (10,40). With the completion of the Human Genome Project and the development of next-generation sequencing technologies, thousands of ncRNAs have been identified, some of which appear to be important regulators of the initiation and progression of myocarditis (41,42).

miRNAs are the most abundant ncRNAs and have lengths ranging between 18 and 25 nucleotides (43). They are generated in the nucleus from primary miRNAs (pri-miRNAs), which are sheared by the intranuclear nucleic acid endonuclease Drosha into miRNA precursors (pre-miRNAs). Pre-miRNAs are translocated into the cytoplasm, where they are cleaved by Dicer to produce mature miRNAs (44,45). Often, a single pre-miRNA gives rise to multiple mature miRNAs, which can bind to the 3' untranslated region of one or more mRNA molecules. Upon miRNA-mRNA binding, the RNA-induced silencing complex suppresses the expression of the target gene (46,47). Many studies have shown that miRNAs can regulate myocarditis by modulating the expression of target genes.

Long ncRNAs comprise a class of ncRNAs with a length exceeding 200 nucleotides (48). Although not highly conserved in their sequence, most lncRNAs have a conserved secondary structure and subcellular localization, both of which are critical to their function (49). Unlike that of miRNAs, the mechanism of action of lncRNAs is poorly understood. Long ncRNAs can bind to other nucleic acids (e.g., DNA, mRNA, and miRNA) or certain proteins to participate in epigenetic, transcriptional, and posttranscriptional regulation of gene expression (50). A class of lncRNAs known as competing endogenous RNAs (ceRNAs) can act as molecular sponges for miRNAs, thereby

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antagonizing their molecular effects (51).

Covalently closed single-stranded circRNAs were first discovered as a viroid form of nucleic acid (52). Circular RNAs have no 5' cap or 3' poly(A) tail and are insensitive to nucleases, making them more stable than ordinary linear RNAs (53). Although many circRNAs have been identified, the mechanisms underlying their maturation and function, as well as their role in cardiovascular diseases, are poorly understood (54). The mechanisms of action of circRNAs are believed to fall into three main categories: ceRNA or miRNA sponges, translation into short polypeptides, and circRNA-protein interactions (55).

The role of ncRNAs in viral infection-induced myocarditis

Viral myocarditis is the most extensively studied form of myocarditis. The onset and progression of this disease are determined by the interaction between the virus and the host (56). The pathogenic mechanisms of viruses in myocarditis include the direct effects of the virus on the host cells and the immunopathological damage caused by the infection (57). Critically, many mechanisms underlying viral myocarditis have been described in animal models (58). Parvovirus B19 is commonly found in human myocarditis biopsies; however, an animal model of parvovirus B19-induced myocarditis has yet to be established. In contrast, animal models of CVB3-induced myocarditis are widely used (10,59). Multiple events in the pathogenesis of CVB3-induced myocarditis are regulated by ncRNAs (*Figure 1*).

Many ncRNAs can directly regulate the apoptotic process in infected cardiomyocytes. In the early stage of viral infection, cells initiate the process of apoptosis to prevent viral invasion. The death of infected cells eliminates the environment needed for pathogen replication, which interrupts the viral replication process (60). However, in the later stages of viral proliferation, the release of progeny viruses can be promoted by triggering apoptosis, thereby accelerating the infection of nearby living cells (61). Current research on ncRNA in viral myocarditis focuses on the negative aspects of apoptosis. For instance, miR-222 is upregulated in CVB3-infected murine hearts and H9c2 cells; it regulates cardiomyocyte apoptosis and viability by modulating the expression of phosphatase and tensin homolog (PTEN) (62). PTEN is a 3'-lipid phosphatase which exerts a wide range of cardiac regulatory effects, mainly through the PI3K/Akt pathway (63). It regulates cell survival, cardiac hypertrophy, myocardial contractility, coronary angiogenesis, metabolism,



Figure 1 The role of ncRNAs in CVB3-induced myocarditis. ncRNAs regulate multiple processes in the pathogenesis of CVB3-induced myocarditis. Upregulated and downregulated ncRNAs in CVB3 infection are shown in red and blue, respectively; ncRNAs whose expression levels are not stated in the original article are in black; ncRNAs that promote the process are followed by (+) and those that inhibit the process are followed by (-). Mø, macrophages; AK085865, LncRNA AK085865; MEG3, lncRNA maternally expressed 3; HF1A-AS1, lncRNA HIF1A-AS1; ncRNAs, non-coding RNAs.

and mechanotransduction (64). In cardiomyocytes, miR-34a promotes CVB3-induced apoptosis by regulating the expression of the silent information regulator T1 (SIRT1)-p53 pathway (65). Similar roles have been described for miR-15 with its targeting of nucleotidebinding oligomerization domain (NOD)-like receptor X1 (NLRX1) (66). Previous studies have showed that NLRX1 is an anti-apoptotic protein (67,68). The lncRNA hypoxiainducible factor 1 alpha-antisense RNA 1 (HIF1A-AS1) binds to miR-138, which acts as a bridge in various ceRNA networks to promote apoptosis (69,70), and represses its expression, thereby modulating the nuclear factor kappa B (NF-κB) pathway. Reduction of the expression of lncRNA HIF1A-AS1 by siRNA was found to attenuate CVB3induced oxidative stress and apoptosis in cardiomyocytes, and alleviate CVB3-induced myocardial inflammation (71). However, other ncRNAs can inhibit apoptosis. For example, upregulation of miR-21 expression followed by suppression of the MAP2K3/P38 signaling causes apoptosis evasion and attenuates cardiac injury after CVB3 infection (72,73). These observations show that ncRNAs can facilitate virus

release and spread by regulating host cell apoptosis.

ncRNAs also regulate immune responses after CVB3 infection. CVB3 infection promotes immune damage in various cells in the heart. Given that cytokines, including interleukin (IL)-1, IL-2, IL-6, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ , are elevated before inflammatory cell infiltration, cells intrinsic to the myocardium, such as cardiomyocytes and fibroblasts as well as innate immune cells, are thought to play a central role in their expression (74). Zhang et al. reported that overexpression of miR-133b in cardiomyocytes inhibited cardiomyocyte injury after viral infection, and suppressed the synthesis and release of the proinflammatory cytokines TNF- α and IL-6 (75). Another study found that miR-146a was upregulated in CVB3-infected fibroblasts and exerted anti-inflammatory effects through toll-like receptor 3 (TLR3) and TNF receptor-associated factor 6 (TRAF6) (76). In the subsequent stage, innate immune-related cells are recruited to the myocardium, where monocytes differentiate into macrophages after reaching the tissues. Of note, macrophages are central regulators of inflammatory

diseases through their ability to orchestrate immunological networks. Polarization of macrophages toward the M1 (proinflammatory) or M2 (anti-inflammatory) phenotype is also a key factor regulating cardiac inflammation (77). Upregulation of lncRNA AK085865 has been observed in CVB3-infected C57 mouse hearts and murine bone marrow-derived macrophages after M2 polarization. The lncRNA AK085865 binds to IL enhancer-binding factor 2 (ILF2), thereby regulating the ILF2/ILF3 complex and subsequently, miR-192/IL-1 receptor-associated kinase 1 (IRAK1) function (78,79). IRAK1 is the main mediator of toll-like receptor 4 (TLR4) activation and binds to TRAF6, ultimately leading to the induction of NF-KB, which is essential for lipopolysaccharide-mediated inflammatory response in macrophages (80-82). Xue et al. found that silencing of the lncRNA MEG3 inhibited M1 polarization and promoted M2 polarization in macrophages, alleviating myocarditis. MEG3 regulates the expression of TRAF6 by inhibiting miR-223 (83). TRAF6-first identified as a mediator of IL-1 receptor-mediated activation of NF-KB—is critical for activating various immune cells, including macrophages (84,85). miR-223 can also regulate macrophage polarization and function, consequently affecting the progression of viral myocarditis (86). The role of miR-155 in inflammatory diseases has been extensively studied, and this miRNA has been proposed as a marker of inflammation (87). In Corsten et al.'s study, silencing of miR-155 in mice with viral myocarditis attenuated the infiltration of monocytes and macrophages in the heart by repressing the expression of the direct miR-155 target PU.1 (88). With the infiltration and activation of innate immune cells, acquired immune-related cells (especially T cells) are activated to produce cytokines and inflammatory factors (11). Nevertheless, there is a limited number of studies on this aspect. To sum up, regulating ncRNA expression can promote an inflammatory response mediated by immune cells or non-immune cells in CVB3 infection.

Further, ncRNAs can regulate CVB3 replication. A miRNA microarray analysis of hearts from mice infected with CVB3 revealed that miR-203 was one of the most upregulated genes in the infected hearts. miR-203 enhances the replication of CVB3 and the survival of infected HeLa cells by regulating the expression of zinc finger protein-148 (ZFP-148) (89). Silencing of ZFP-148 by siRNA has shown similar effects on CVB3 replication (89). miR-126 promotes CVB3 replication by targeting sprouty-related EVH1 domain-containing protein 1 (SPRED1) (90), which can regulate Ras-MAPK signaling by effectively inhibiting

growth factor-mediated extracellular signal-regulated protein kinase (ERK) activation (91). Research has shown that the ERK1/2 signaling pathway is one of the important pillars of CVB3 infection (92). Also, in Ye et al.'s study, miR-126 sensitized cells to CVB3-induced cell death and increased viral progeny release to initiate new infections by targeting Wnt-responsive CDC42 homolog 1 (WRCH1) and low-density lipoprotein receptor-related protein 6 (LRP6), as well as by promoting β -catenin degradation (90). Corsten et al. reported that inhibition of miR-221/-222 increased cardiac injury and immune cell infiltration during acute viral myocarditis in vivo and increased viral replication on days 4 and 7 post infection in vitro and in vivo. Targets of miR-221/-222 include proinflammatory transcription factors (e.g., ETS1 and ETS2), IFN regulatory factor 2 (IRF2), CXC chemokines (e.g., CXCL12), anti-apoptotic proteins (e.g., BCL2L11), proapoptotic proteins (e.g., BMF), and T-cell thymocyte selection-associated HMG box (TOX) (93). All of these target genes have been implicated in playing roles in myocarditis (94-98). Interestingly, miRNAs can also directly regulate several viral functions. For instance, miR-10a* regulates the expression of the three dimensional-coding region of the CVB3 genome and enhances CVB3 replication (99). Further, high expression of miR-590-5p confirmed to be associated with viral replication was found in exosomes produced after viral infection of cells (100). However, the functional mechanism of this virus-mediated exosome release needs to be further explored. These findings confirmed that the expression of ncRNAs could promote viral replication and aggravate the severity of myocarditis in CVB3-induced myocarditis.

In conclusion, the above-discussed studies suggest that ncRNA can regulate the occurrence and development of viral myocarditis from the aspects of viral replication, immune response, and apoptosis, and provide promising data on ncRNAs as effective options for therapeutic strategies in myocarditis. However, considering the complexity of ncRNA involvement in the pathogenesis of viral myocarditis, as well as the limited number of researchers and funding for myocarditis, the number of studies in this area is few. Moreover, the research methods and experimental technologies lag behind those of many other diseases and are often unable to keep up with the speed of technological developments relating to ncRNAs. Therefore, the literature on myocarditis and ncRNAs is still lacking, and more studies need to be conducted to evaluate the role of ncRNAs in viral myocarditis.

The role of ncRNAs in EAM

As mentioned above, since the EAM model is mainly used

Name	Organ	Expression	Description	Mechanism	Reference
miR-590-3p	Heart	Downregulation	Overexpression of miR-590-3P improves cardiac function	Target genes: NF-KB	(101)
miR-141-3p	Heart	Downregulation	miR-141-3p inhibits the expression of inflammation-related factors in the heart	Target genes: STAT4	(102)
miR-21a-5p	Heart	Upregulation	Silencing miR-21a-5p reduces cardiac inflammation and fibrosis	Unknown	(103)
miR-155	Heart, splenic CD4 ⁺ cells	Upregulation	miR155 promotes Th17 cell development and function	Unknown	(27)
miR-142	Circulating exosomes	Upregulation	miR-142 regulates immunometabolism in CD4+ T cells	Target genes: MBD2, SOCS1	(104)
miR-98	B cells in the heart	Upregulation	Blocking miR-98 reduces myocarditis	Unknown	(105)
CircSnx5	DC stimulated with lipopolysaccharide	Upregulation	Transplantation of circSnx5-overexpressing DCs attenuates experimental autoimmune myocarditis	CeRNA: CircSnx5-miR- 544-SOCS1/PU.1	(106)
MALAT1	DC stimulated with lipopolysaccharide	Upregulation	In vivo transfer of DCs overexpressing MALAT1 delays the progression of myocarditis	CeRNA: MALAT1- miR155-5p-DC-SIGN	(107)

Table 3 The role of ncRNAs in experimental autoimmune myocarditis

NF-KB, nuclear factor kappa B; STAT4, signal transducer and activator of transcription 4; MBD2, methyl-binding domain protein 2; SOCS1, suppressor of cytokine signaling 1; CircSnx5, cirRNA circSnx5; Snx5, sorting nexin 5; ceRNA, competing endogenous RNA; PU.1, transcription factor PU.1; MALAT1, IncRNA metastasis-associated lung adenocarcinoma transcript 1; DC-SIGN, dendritic cell-specific ICAM (intercellular adhesion molecule)-grabbing non-integrin.

to study autoimmunity in the heart, its primary concern is the function and mechanism of immune cells (10). Several studies have shown that ncRNAs can influence the progression of autoimmune myocarditis by regulating the activity of immune cells (Table 3). In this model, dendritic cells (DCs) attenuate myocardial injury by blocking the antigen presentation process. DCs loaded with an immunogenic peptide derived from cardiac α-myosin heavy chain activate self-reactive CD4⁺ T cells, leading to the dysregulation of T helper cells and regulatory T cells, which are critical for the induction of EAM (108). In Wu et al.'s study, in vivo transfer of DCs overexpressing metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) delayed the progression of autoimmune myocarditis, and MALAT1 promoted DC-SIGN expression by acting as a miR-155-5p sponge in the cytoplasm of DCs (107). As a member of the C-type lectin receptor (CLR) family, DC-SIGN has been proved to play an important role in mediating DC adhesion, migration, inflammation, activating primary T cells, triggering immune response, and participating in the immune escape of pathogens and tumor cells (109). In another study, circSnx5 was found to play a role in DC-driven immunity and tolerance by inhibiting the activation and enhancing the tolerogenic functions of DCs.

Importantly, circSnx5 knockdown promoted DC activation and inflammation. Mechanistically, circSnx5 can act as a miR-544 sponge to attenuate miRNA-mediated inhibition of SOCS1 expression and inhibit nuclear translocation of PU.1 (106). Previous studies had demonstrated that SOCS1 and PU.1 are pivotal regulators in DC function (110,111).

The role of B cells in myocarditis is poorly understood except in one aspect. Autoantibodies are known to be a key factor in B-cell production and to contribute to the pathogenesis of myocarditis (112). In Chen *et al.*'s study, mice immunized with alpha-myosin heavy chain (MyHC- α) showed a reduced frequency of IL-10+ B cells in the heart. B cells isolated from the hearts of mice with myocarditis showed higher expression of miR-98 and lower levels of IL-10. Exposure to TNF- α upregulated miR-98, and miR-98 overexpression inhibited IL-10 expression in B cells. Blockade of miR-98 or transplantation of B10 cells prevented experimental myocarditis (105).

Evidence suggests that CD4⁺ cells play a central role in autoimmune diseases, although non-pathogenic IL-10⁺ Th17 cells have also been described (113,114). In mice with EAM, miR-155 was found to be upregulated and to regulate the balance of Th17/Treg cells. miR-155 also promoted the differentiation of CD4⁺ cells into Th17 cells and the production of Th17-stimulating cytokines in DCs (27). Additionally, in Sun *et al.*'s study, the injection of serum exosomes from mice with EAM induced global CD4⁺ T cell activation and cardiac inflammation. Mechanistically, circulating exosomes from mice with EAM were selectively loaded with abundant miR-142, which was confirmed by luciferase assays to regulate CD4⁺ T cell dysfunction and glycolytic reprogramming by targeting methyl-CpG binding domain protein 2 (MBD2) and suppressor of cytokine signaling 1 (SOCS1) (104). Further, other studies have reported that miR-590-3p and miR-141-3p can also play a role in EAM by reducing cardiac inflammation; however, more detailed mechanisms have yet to be elucidated (101,102).

ncRNA replacement therapy has been hampered for a long time due to the lack of robust nonviral delivery methods for in vivo administration (115). To overcome this challenge, several explorations have been undertaken. For example, systemic treatment consisting of antagomiR-21a-5p formulated with polymeric nanoparticles based on low molecular weight polyethyleneimine was found to significantly reduce myocardial inflammation and fibrosis (103). This study suggests that ncRNAs may provide new targets for treating autoimmune myocarditis. However, most studies on ncRNAs in the EAM model have focused on the replacement therapy of ncRNAs, such as DC adoptive transfer or exosome therapy. There is a lack of research on the mechanism of ncRNAs in EAM, especially on Th17 cells, as the key cells leading to EAM, and further research is needed to understand the specific mechanisms of the therapeutic effects of ncRNAs.

The role of ncRNAs in myocarditis caused by T. cruzi infection

Although the function of ncRNAs in the hearts of trypanosome-infected individuals is not well understood, some studies have suggested that ncRNAs may play a role in T. cruzi-induced myocarditis. A genome-wide mRNA and miRNA analysis demonstrated that some differentially expressed miRNAs in the hearts of mice infected with T. cruzi type I were significantly associated with clinically relevant parameters of parasitemia and electrocardiographic changes (116,117). Bioinformatics analysis to integrate the overall miRNA and mRNA expression profiles of myocardial tissues from patients with chronic Chagas disease cardiomyopathy showed that miRNAs modulated the expression of multiple genes that regulate many pathophysiological processes of chronic Chagas disease cardiomyopathy. These miRNAs also regulated the expression of genes associated with cardiomyopathy-related processes, including fibrosis, hypertrophy, myocarditis, and arrhythmias (118). In a cohort of patients with chronic Chagas disease cardiomyopathy, the miRNAs miR-19a-3p, miR-21-5p, and miR-29b-3p were found to be upregulated. Receiver operating characteristic (ROC) analysis was performed to evaluate the sensitivity, specificity, and accuracy of these miRNAs; the areas under the ROC curves for miR-19a-3p, miR-21-5p, and miR-29b-3p were 0.77, 0.54 and 0.70, respectively. Moreover, these miRNAs were positively correlated with cardiac dysfunction and fibrosis, and negatively correlated with ejection fraction and left ventricular strain (119). These findings suggest that ncRNAs play an important role in trypanosome-induced myocarditis. Still, most studies are focused on the differential expression of these ncRNAs discovered by sequencing or correlation analysis with clinical practice. The roles and mechanisms of these ncRNAs in the T. cruzi-induced model are still unknown and need to be further studied.

The role of ncRNAs in myocarditis caused by ICIs

Immune checkpoint inhibitors have become the treatment of choice for many cancers. However, ICIs may cause adverse cardiovascular effects, including different forms of cardiotoxicity, such as myocarditis, acute coronary syndrome, and proximal membrane disease (120,121). However, the exact mechanisms by which ICIs cause myocarditis are still unclear, and studying ncRNAs may provide new insights. PD-1 inhibitors induce M1 macrophage polarization and myocardial inflammation by modulating the miR-34a/Krüppel-like factor 4 (KLF4) signaling pathway (122). Notably, miR-34a was found to be highly expressed in macrophage-derived exosomes treated with PD-1 inhibitors, with treatment of cardiomyocytes with these exosomes triggering cardiomyocyte senescence (123). Most studies have focused on the efficacy of ncRNAs in patients with ICItreated tumors (124-127), and the concomitant cardiac damage has been poorly studied. Further, although a few reports have suggested that modulation of ncRNAs in vivo has a beneficial effect in combating ICI-induced cardiotoxicity, none of them used clinically relevant models. This situation highlights the need for new studies to evaluate the therapeutic effect of ncRNAs in models of ICI-induced cardiotoxicity.

Name	Organ	Expression	Sample size	Biomarker type	Reference
miR-146b, miR-214	Heart	Upregulation	VM/CON: 8/8	Diagnostic	(134)
miR-155, miR-148a	Heart	Upregulation	VM/CON: 6/6	Diagnostic	(135)
miR-721	Peripheral blood	Upregulation	M/MI/CON: 42/90/80	Diagnostic	(136)
miR-30a, miR-181d, miR-125a	Circulating exosomes	Upregulation	VM/CON: 23/12	Diagnostic	(137)
miR-155, miR-21	Circulating exosomes	Downregulation	VM/CON: 23/12	Diagnostic	(137)
miR-155, miR-320a	Circulating exosomes	Upregulation	FM/NFM/CON: 99/32/105	Diagnostic	(138)
miR-4763-3p	Peripheral blood	Upregulation	FM/CON: 20/10	Diagnostic	(139)
miR-155, miR-133a	Heart	Upregulation	iCMP/non-inflammatory DCM: 76/22	Prognostic	(140)
miR-208a	Peripheral blood	Upregulation	M acute phase/M chronic phase: 8/8	Prognostic	(141)

Table 4 Non-coding RNAs as biomarkers in diagnosis and prognosis of viral myocarditis

VM, viral myocarditis; CON, control; iCMP, inflammatory cardiomyopathy; DCM, dilated cardiomyopathy; M, myocarditis; MI, myocardial infarction; FM, fulminant myocarditis; NFM, non-fulminant myocarditis.

The potential of ncRNAs in the diagnosis and treatment of myocarditis

The potential of ncRNAs in the treatment of myocarditis

As mentioned above, ncRNAs can be involved in regulating multiple links in the occurrence and development of myocarditis, such as apoptosis of cardiomyocytes, replication of viruses, and regulation of immune cells. Therefore, ncRNAs may serve as potential therapeutic targets for myocarditis. At present, miRNAs are the most studied ncRNAs, and mimics or inhibitors of miRNAs can be designed to reduce myocardial inflammation and damage. Clinical trials of miRNA-based therapies are already underway for other diseases, including mesothelioma, T-cell leukemia/lymphoma, and hepatitis C infection (128). Therefore, these therapies may become reliable treatments for myocarditis in the future.

Therapeutics based on ncRNAs may hold promise for the treatment of myocarditis, but there are currently some issues that must be addressed. First, one ncRNA may target multiple molecules simultaneously, which leads to the possibility that some ncRNAs may exert opposite or unpredictable effects. Second, a major barrier to implementing miRNA-based therapies in clinical practice is the lack of efficient delivery systems that can protect RNA molecules from nuclease degradation and deliver them to cardiac tissue without adverse effects. Encouragingly, delivery systems based on lipid and lipid-like materials, polymers, conjugates, and 3D scaffolds have been developed to address this problem (129). Moreover, exosomes containing ncRNAs may serve as natural medicines for myocarditis. Third, ncRNAs are poorly conserved, and there are considerable differences between mouse body structures and those of humans. Consequently, ncRNAs with functions in mice may not work in humans. Therefore, there is a long way to go before ncRNA-based therapy can become a clinical application.

The potential of ncRNAs in the diagnosis and prognosis of myocarditis

For patients with acute myocarditis, the typical symptoms and signs at presentation include chest pain, dyspnea, fatigue, palpitations, syncope, and cardiogenic shock, but these are still non-specific (130). Electrocardiograms, laboratory tests, echocardiography, and cardiac magnetic resonance have low predictive value in myocarditis, and EMB—the gold standard for myocarditis diagnosis—is not a routine practice due to its invasive nature and potential complications (131,132). Moreover, available biomarkers, such as troponins and N-terminal pro-brain natriuretic peptide (NT-proBNP), lack sufficient specificity and are widely changed during myocardial injury and cardiac dysfunction (133). Therefore, novel biomarkers with higher sensitivity and specificity in diagnosing myocarditis are urgently needed.

Several studies have been conducted to assess the value of ncRNAs in diagnosing viral myocarditis (*Table 4*). Studies

of cardiac tissues from patients with viral myocarditis found that miR-146b, miR-214, miR-155, and miR-148a were significantly upregulated, which suggests their potential as diagnostic markers, but corresponding clinical analysis is lacking (134,135). Noninvasive diagnostic methods for myocarditis are also lacking. Circulating ncRNAs hold great potential as diagnostic biomarkers of myocarditis due to their presence in the blood (142,143). For example, miR-721, a miRNA produced by TH17 cells, was found to be increased in the plasma of patients and mice with myocarditis but not in those with myocardial infarction, suggesting its potential use as a diagnostic marker for acute myocarditis (136). In another study of 23 patients with acute myocarditis, five miRNAs (miR-30a, miR-181d, miR-125a, miR-155, and miR-21) were found to be differentially expressed in serum exosomes, but insufficient clinical information limited their use as biomarkers for the diagnosis of viral myocarditis (137).

Fulminant myocarditis is a rare and serious form of myocarditis that presents as a severe and sudden attack, with symptoms typically lasting for less than 2 weeks. It can lead to cardiogenic shock and electrical instability requiring inotropic or mechanical circulatory support (144). Given the rapid progression of this potentially fatal condition, diagnostic indicators of fulminant myocarditis are of high clinical importance (145). The serum exosome levels of miR-155 and miR-320a have been suggested as noninvasive indicators of fulminant myocarditis. Notably, a multivariate logistic regression model combining the levels of miR-155 and miR-320a could accurately predict fulminant myocarditis, providing areas under the ROC curves that were significantly higher than those provided by individual miRNAs, C-reactive protein, or cardiac troponin (138). Transcriptomic analyses of circulating miRNAs revealed that miR-4763-3p was upregulated in patients with fulminant myocarditis and restored to a normal level after proper treatment. The miR-4763-3p level was negatively correlated with LVEF (R=-0.66, P=0.03) and was positively correlated with the concentration of interferon- β . Also, miR-4763-3p had an area under the ROC curve of 0.85 (95% confidence interval: 0.68 to 1.00), suggesting that miR-4763-3p may represent a highly specific diagnostic biomarker for fulminant myocarditis (139).

DCM may be the result of myocarditis or the first presentation of heart failure, and carries an increased risk of cardiac death and heart transplantation (10). Unfortunately, no established clinical markers are available to identify the risk of adverse outcomes in patients with myocarditis (131). Research has started to consider ncRNAs as a prognostic marker of myocarditis, and relevant exploration has been carried out. For example, a study by Besler et al. using EMB found that patients with inflammatory cardiomyopathy (iCMP) had increased levels of miR-155 and miR-133a compared to patients with non-inflammatory DCM. Additionally, the levels of miR-133a (R=0.73; P<0.01) and miR-155 (R=0.63; P<0.01) were correlated with inflammatory cell counts in patients with iCMP. More importantly, the Kaplan-Meier curve of adverse cardiovascular outcomes (cardiovascular death, ventricular tachycardia, and hospitalizations for heart failure) at a mean follow-up of 38 months showed that patients with low miR-133a levels had a worse cardiovascular outcome than those with high miR-133a levels, suggesting that miR-133a may be a potential prognostic biomarker for human iCMP (140). A prospective study of cardiac and inflammationassociated plasma miRNA levels in eight children with viral myocarditis showed that the circulating levels of miR-208a and miR-21 were significantly elevated in the acute phase of the disease compared to the subacute and chronic phases. Moreover, miR-208b levels during the subacute phase were correlated with systolic left ventricular function during the chronic/resolution phase, which could evidence prognostic significance for left ventricular functional recovery in patients with myocarditis (141).

Although the above findings suggest that ncRNAs may play an important role in the clinical diagnosis and prognosis of myocarditis, most studies involved limited sample sizes. Therefore, large prospective clinical trials are required to confirm the value of ncRNAs as minimally invasive biomarkers for the diagnosis and prognosis of myocarditis.

Conclusions

Although there has been great progress in research on myocarditis in recent years, great challenges remain in the diagnosis and treatment of the condition. Accumulating evidence suggests that ncRNAs play a pivotal role in myocarditis (*Figure 2*) and that targeting ncRNAs may represent a novel therapeutic approach for myocarditis. However, the clinical application of ncRNAs in myocarditis is hindered by an insufficient understanding of ncRNAs and the technical limitations of ncRNA oligonucleotide in targeting the design and *in vivo* delivery methods. The good news is that some therapeutic clinical trials of ncRNAs have begun and have achieved varying degrees of success.



Viral infection-induced myocarditis

Figure 2 The main findings about ncRNA mechanisms in myocarditis. "?" represents the unknown function of ncRNAs in trypanosomiasis.

Improvement of oligonucleotide chemistry and delivery methods is continuously being pursued to accelerate the clinical application of ncRNAs. Notably, circulating ncRNAs can be used as diagnostic biomarkers for myocarditis due to their unique biochemical properties, overcoming the need for invasive diagnostic methods. However, larger patient cohort studies are needed to validate these findings and assess the prognostic potential of circulating ncRNAs. Also, although most studies on the role of ncRNAs in myocarditis have focused on miRNAs, lncRNAs and circRNAs may also be a treasure trove worth mining, which requires more attention in future research. With the rapid development of bio-sequencing technologies, ncRNA research is expected to provide new directions for the diagnosis and treatment of myocarditis.

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