

Heart Failure in Chronic Myocarditis: A Role for microRNAs?

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Abstract: Myocarditis is an inflammatory disease of the heart, which can persist over a long time. During this time, known as the chronic phase of myocarditis, ongoing inflammation damages the cardiomyocytes. The loss of cardiac cells culminates in the development of dilated cardiomyopathy, often followed by non-ischemic heart failure due to diminished cardiac function. During the course of the disease, expression levels of non-coding small RNAs, called microRNAs (miRNAs), change. Although mainly studied in the acute setting, some of these changes in expression level appear to persist in the chronic phase. In addition to being a much-needed diagnostic tool, these miRNA could provide new treatment options. miRNA-based intervention strategies already showed promising results in the treatment of ischemic cardiovascular diseases in preclinical animal models. By implementing more knowledge on the role of miRNAs in the progression towards heart failure, this can potentially be used in the development of miRNA-based therapeutic interventions in the treatment of myocarditis and thereby preventing the progression towards heart failure. The first part of this review will focus on the natural course of myocarditis and the progression towards heart failure. Secondly, we will discuss the current knowledge on alterations of miRNA expression patterns, and suggest some possible future interventions.

Keywords: Dilated cardiomyopathy, Heart failure, Inflammation, miRNA, Myocarditis, Therapy.

1. MYOCARDITIS

Myocarditis is an inflammatory disease of the heart, which often results in heart failure or sudden cardiac death [1]. The disease mostly occurs in young, healthy people in the age of 20-51 years [2]. Estimations of the incidence of myocarditis are variable due to the non-specific symptoms, leading to underestimation of the disease. The incidence of myocarditis in cases with unexplained heart failure is estimated at 9,6% [2]. Bacterial infections, viruses, autoimmune diseases and other factors are able to induce myocarditis, with viruses being the most common cause [3, 4]. Structural and functional damage of the myocardium, caused by these factors, activates the innate and adaptive immune response, which can lead to severe inflammation [5]. The immune response is eventually downregulated, however, myocardial inflammation can also persist. Persistent inflammation is characterized by an ongoing damage to the cardiomyocytes and ultimately results in non-ischemic heart failure [6]. In 30% of the cases, dilated cardiomyopathy (DCM) occurs, which is a major cause of heart failure and an important indication for cardiac transplantation [7]. Hence, the treatment of myocarditis is difficult due to late diagnosis and irreversible damage that has occurred [8]. Final clinical outcome of the disease depends on the host response, the amount of irreversible damage, and the use of therapeutic interventions [9, 10]. Patients with severe acute myocarditis

have a better prognosis, while patients with moderate chronic myocarditis are more prone to develop heart failure [1, 2].

Phases

Myocarditis is a three-phase process, consisting of (1) an acute phase, (2) a sub-acute phase and (3) a chronic phase.

In the (1) acute phase (first 3-4 days), infection induces cardiomyocyte damage via the induction of apoptotic signaling pathways and the release of proteolytic enzymes [3, 11]. This leads to the activation of the immune system and the production of pro-inflammatory cytokines, such as interferon- γ (IFN- γ), interleukins-1,-6 and -12 (IL-1, IL-6, IL-12) and tumor necrosis factor- α (TNF- α) [1, 12]. Classical activated macrophages type 1 (M1) become activated, which have pro-inflammatory properties and further enhance the immune response [1]. In addition, up-regulation of adhesion molecules on endothelial cells leads to the recruitment of more immune cells, thereby activating the innate immune response [11, 12].

In the (2) sub-acute phase (day 4-5), the innate immune response remains activated and immune cells infiltrate into the heart [13]. Phagocytosis of dead cells and debris is initiated by monocytes, which augment the expression of pro-inflammatory cytokines [5]. Immune cells of the adaptive immune system, such as T-cells and B-cells, also accumulate in the infected heart [14]. Licensed cytotoxic T-cells (CD8+) recognize virus-infected cardiomyocytes via the interaction and presentation of antigens loaded on major histocompatibility complex type 1 (MHC-I) [15]. The cytotoxic T-cell directly kills the infected cardiomyocyte by releasing per-

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forin and granzymes, triggering the caspase cascade and inducing apoptosis. Antigen presenting cells (APCs), like dendritic cells (DCs), take up the debris of dead cardiomyocytes and present the ingested antigens on MHC-II [16]. T-helper cells (CD4+) are able to recognize these presented antigens via the interaction of the T-cell receptor and the presented antigen loaded on MHC-II. This subsequently leads to the activation of T-helper cells, which can license cytotoxic T-cells to kill infected cells or activate B-cells, which produce neutralizing antibodies [16]. During the sub-acute phase, the immune response not only eliminates infected and dead cells, but also significantly contributes to irreversible damage of the myocardium by damaging of healthy cardiomyocytes [1].

In the (3) chronic phase (day 14), repair and remodeling of the myocardium is initiated [1, 12]. Regulatory T-cells (CD4+CD25+FoxP3+) respond to the production of IL-2 by T-helper cells and start proliferating rapidly [1]. Anti-inflammatory cytokines are being produced, such as transforming growth factor-beta (TGF- β) and IL-10, to down-regulate the immune response and to reduce cardiac damage [11, 12]. Secondly, an alternative type of macrophages becomes activated, macrophage type 2 (M2). This type of macrophage produces more TGF- β and IL-10 to reduce inflammation and to stimulate repair mechanisms [17]. Fibroblasts start to proliferate and differentiate into myofibroblasts, which contain contractile properties like smooth muscle cells, to replace the lost cardiomyocytes [18]. Fibroblasts and M2-macrophages produce collagen to restore the extracellular matrix and form a permanent scar [1, 12]. In some cases, the immune system fails to completely clear all the infected cells or auto-reactive immune cells persist in the myocardium, resulting in chronic inflammation [2, 9]. The chronically activated immune system produces cytokines, which activates matrix metalloproteases (MMPs) that can digest interstitial collagen and elastin. Additionally, profibrotic factors are produced and thereby facilitate the dilatation of the heart [11], which can lead to dilated cardiomyopathy (DCM), an irreversible disease with systolic and diastolic dysfunction [7, 19].

Diagnosis

Clinical manifestation of myocarditis includes a broad spectrum of non-specific flu-like symptoms and signs of myocardial infarction [20]. Clinical presentation is usually not sufficient for the diagnosis of myocarditis. Myocarditis is considered in young patients with rapidly progressive cardiomyopathy, arrhythmias and acute myocardial-infarction-like symptoms in combination with normal healthy coronary arteries [20]. In these cases, symptoms often mimic myocardial infarction due to segmental wall abnormalities and elevated levels of troponin and creatine kinases found in blood samples [8]. In patients with acute myocarditis, concentrations of troponin I and T are more elevated than creatine kinase [21]. In addition, inflammatory markers such as C-reactive protein and the number of circulating leukocytes can be increased [5]. In sub-acute and chronic myocarditis, anti-cardiac auto-antibodies and immunoglobulins can be detected in blood samples [19]. High levels of IgM are an indication for the presence of an active virus and can be used to determine the possible cause of myocarditis [11].

Electrocardiogram (ECG) is widely used as one of the diagnostic tools for the diagnosis of myocarditis, although it has a low sensitivity of only 47% [22]. Most of the patients with (acute) myocarditis show abnormalities in their ECG [6, 23], which often mimics the ECG of a patient with a myocardial infarction [20]. Although ECGs can vary between patients, they often show non-specific T-waves, ventricular arrhythmias, PQ-segment depressions, and ST-segment changes by which high levels of Q waves are associated with higher rates of deaths and transplantations [5].

When myocarditis is suspected, an endomyocardial biopsy (EMB) is taken and analyzed to detect presence of a virus via immunohistological techniques. Diagnosis of these histopathological analysis is based on the Dallas-criteria, which indicates myocarditis if inflammatory cellular infiltrates with or without associated cardiomyocyte necrosis is present [24]. However, the interpretation of different clinicians and the lack of prognostic values make these criteria not very sensitive or conclusive [19].

The most effective tool in the diagnosis of myocarditis is cardiac magnetic resonance imaging (CMR), which is able to distinguish ischemic and non-ischemic cardiomyopathy [21]. It makes use of different parameters such as gadolinium late enhancement (LGE) and transmural enhancement (TE). High levels of LGE are an indication of myocardial injury and can be used to distinguish between myocardial infarction and myocarditis [21]. In myocarditis, the elevated LGE levels are more diffuse and nodular, whereas myocardial infarction shows a smaller distribution [3]. T1-weighted-CMR marks capillary leakage and T2-weighted-CMR images are able to mark interstitial and extracellular edema [9, 21]. Interstitial edema occurs during the inflammatory response and can be predictive for myocarditis. A combined approach, using T2-weighted-CMR images and LGE parameters, increases the accuracy of the diagnosis and assessment of myocarditis patients [25].

2. NON-ISCHEMIC HEART FAILURE

Heart failure is a complex clinical syndrome that can result from an abnormal cardiac structure or function leading to failure of the heart to deliver oxygen and nutrients to metabolizing tissues [26]. According to the etiology and pathophysiology, heart failure can be categorized in ischemic or non-ischemic and in anatomical designations as hypertrophic and dilated. Of these, dilated cardiomyopathy (DCM) is the most prevalent form of non-ischemic cardiomyopathy with a relative poor overall prognosis of 25% mortality in 5-years [27]. DCM is predominantly defined by left ventricular dilatation and reduced cardiac contraction in the absence of coronary artery disease and abnormal loading conditions. Amongst other secondary causes, e.g. chemotherapy, neuromuscular disease and alcohol abuse, myocarditis can result in DCM and eventually heart failure [7, 28].

Early diagnosis of DCM is hindered by the fact that the majority of patients are asymptomatic in early stages of the disease, therefore patients often present themselves in the late stage of the disease with symptoms of exercise intolerance, breathlessness and edema [26]. Additionally, in some of these patients the conduction system is affected that lead to arrhythmias and an increased risk for sudden cardiac

death. For diagnosis of DCM, laboratory test and imaging techniques are important to define disease severity. Imaging often show DCM, with general wall motion abnormalities and MRI using LGE could show diffuse damage throughout the heart [21]. Since fast initiation of treatment is beneficial for the prognosis of heart failure, a clear diagnosis of DCM and also the contributing etiology should be known to give the best available therapy [27]. Accordingly, taking cardiac biopsies is indicated in patients with DCM from unknown etiology, and surprisingly, in 9-16% of patients presenting with DCM, traces of prior myocarditis are observed [7].

After diagnosis of heart failure, all patient with DCM are treated with standard heart failure therapy such as ACE-inhibitors, mineralocorticoid receptor antagonist, diuretics and β -blockers [10, 26, 29]. The primary aim of this treatment is to relieve symptoms of heart failure, to improve survival and to prevent ongoing remodeling and worsening of heart function [26, 29]. For myocarditis induced DCM, additional immunosuppressive drugs are being used [3], however this is not beneficial for viral myocarditis and can even cause harm when used for this purpose [12].

Despite poor overall prognosis and lack of specific treatments, 25% of DCM patients with recent onset heart failure will undergo reverse remodeling and demonstrate a (partially) improved cardiac function [30]. The ability to recover is observed in the majority of DCM etiologies, including myocarditis. The fact that there is a certain degree of recovery and reversibility in cardiac function, attention should be focused on inducing this process to guide improvement.

Since prominent roles have been uncovered for miRNAs in the treatment of cardiovascular disorders [31], miRNAs might also have a potential role in treatment of myocarditis and reversibility of myocarditis induced-DCM.

3. THE ROLE OF miRNAs

microRNAs (miRNAs, miR) are small non-coding RNAs with a length of approximately 20-24 nucleotides, which are involved in the post-transcriptional regulation of protein expression by binding messenger RNAs (mRNAs) [32]. miRNAs are able to use two silencing mechanisms to down-regulate specific target genes: degradation or translational repression of the mRNA, depending on the complementarity with the target gene. If the miRNA and their target gene are complementary, the mRNA will be cleaved and degraded. If the miRNA and the target gene are not perfectly complementary, miRNAs suppress translation of the target gene, however without affecting the stability of the mRNA. miRNAs can be directly produced by cells themselves or secreted via e.g. microvesicles, such as exosomes [33]. miRNAs are involved in many processes, such as cell proliferation and apoptosis but also in the regulation of the immune response. Emerging evidence is found for the contribution of miRNAs in myocardial pathological processes by regulating angiogenesis, apoptosis and differentiation of cardiomyocytes [34, 35]. The expression of certain miRNAs changes during cardiac disease and heart failure, which makes them interesting targets in the potential treatment of cardiovascular diseases, such as myocarditis [32, 36].

4. ROLE OF miRNAs IN MYOCARDITIS

The role of miRNAs in human myocarditis is not fully elucidated. Currently, only a couple of miRNAs have been identified, which might be correlated with viral myocarditis, especially during the acute phase of myocarditis. Two identified microRNAs, miR-208b and miR-499, are elevated in viral myocarditis and can be detected in plasma of myocarditis patients [37]. miR-208b is expressed by cardiomyocytes and is involved in pathological processes, such as cardiac growth, fibrosis and inflammation by increasing MHC-expression [35]. Upregulation of miR-208b induces adverse cardiac remodeling. miR-499 is also expressed by cardiomyocytes and upregulated during cardiac disease [38]. Both of these microRNAs are released upon myocardial damage and can potentially be used in the diagnosis of myocarditis to determine the severity of the disease [37]. Elevated levels of miR-499 can be detected in all patients, whereas the expression of both miR-208b and miR-499 was only found in fulminant virus-induced myocarditis [37]. These miRNAs, however, are not specific for myocarditis but probably reflect myocardial injury in general, as was observed for acute coronary syndrome (ACS) patients [39].

miRNAs Involved in Acute Myocarditis

Current research is focusing on miRNA screenings in the different stages of myocarditis to identify additional miRNAs involved in the pathogenesis of the disease. In myocarditis patients, a cardiac miRNA profile was identified involved in the acute viral phase and the inflammatory phase of acute myocarditis. The study of Corsten *et al.* showed that, for example, miRNA-155 is highly expressed in cardiac tissue of myocarditis patients [40]. miR-155 was found to be up-regulated in cardiac tissue in both human and mice with viral-induced myocarditis [40]. miR-155 is known to be pro-inflammatory and involved in multiple processes, such as immune cell functioning, and is expressed by inflammatory cells especially in the acute inflammatory phase of myocarditis [41]. In viral-induced myocarditis mice models, blockage of miR-155 by antagonists showed attenuated cardiac inflammation and less necrosis [41]. These results indicate that miR-155 plays an important role in the inflammatory response of viral-induced myocarditis. Next to miR-155, miR-21 and miR-146b were found to be upregulated in myocarditis patients, which also have a central role in immune activation and inflammation [40]. Silencing of these miRNAs by specific inhibitors showed a strong attenuation of myocarditis in viral-induced myocarditis mice [42]. The exact role of these miRNAs in the pathology of myocarditis is not elucidated, however it is shown that the expression of miR-21 and miR-146b is correlated with IL-17 expression [42]. These findings suggest that miR-21 and miR-146b are involved in the regulation of TH17 differentiation and thereby control autoimmunity. The function of ROR γ t, a transcription factor of TH17 differentiation, is enhanced by increased expression of these miRNAs, which leads to the differentiation of mature T-cells towards TH17 cells [42]. Furthermore, miR-21 is also involved in interstitial fibrosis and cardiac hypertrophy [43]. Interestingly, other studies showed contradictory results as significantly decreased levels of miR-21 expression were found. The expression of miR-21 in the myocardium in coxsackievirus B3 (CVB3)-

myocarditis mice was significantly reduced and a negative correlation was found between the severity of myocarditis and the expression of miR-21 [44]. When mice were treated with miR-21 precursors, the myocarditis was alleviated and apoptosis was reduced, indicating that miR-21 also has a protective role by regulating programmed cell death 4 (PDC4)-mediated apoptosis [44]. Based on the different outcomes of these studies, it might be that the role of miR-21 in myocarditis depends on the temporal and spatial expression of its targets.

In one study, additional elevated miRNAs, including miR-511 and miR-212 [40], were found in patients with acute viral myocarditis. miR-511 functions as a positive regulator of Toll Like Receptor-4 (TLR) signaling, whereas miR-212 is involved in the hypertrophic responses of cardiomyocytes [40, 45].

Next to upregulated miRNAs there is also evidence that miRNAs are involved by their down-regulation. Anti-inflammatory miR-106a and miR-93 were downregulated in the acute phase of human myocarditis [40]; both are involved in anti-inflammatory responses by inhibiting the production of inflammatory cytokines [43, 46].

miRNAs Involved in Chronic Myocarditis

Most research thus far was focused on miRNAs involved in the acute phase of myocarditis, however, less is known about miRNAs involved in the chronic phase of the disease. Inflammatory miRNAs and miRNAs associated with cardiac damage, involved in the acute phase of myocarditis, might remain affected and involved in the chronic phase, stimulating the progression of the disease [7]. Recently, miR-21 was

found to be involved in the progression of viral myocarditis towards DCM [47]. These findings indicate that changes in miR-21 expression might contribute to the progression of myocarditis to DCM. In addition, elevated levels of miR-208b and miR-499 are also found in later phases after viral-induced myocarditis in patients and in patients with DCM [48]. Besides miR-21, miR-208b and miR-499 no specific miRNAs involved in the chronic phase of myocarditis are identified, however, it might be that inflammatory and damage-associated miRNAs in the acute phase are also persistently expressed in the chronic phase of myocarditis.

Overall, miRNAs involved in inflammatory responses are dysregulated and muscle specific miRNAs (myomiRs) are mostly upregulated during human myocarditis (Table 1). Since the chronic phase of myocarditis can progress into heart failure, it is also hypothesized that miRNAs involved in the development of (non-ischemic) heart failure might also be involved.

5. ROLE OF miRNAs IN (NON-ISCHEMIC) HEART FAILURE

microRNAs are involved in the pathogenesis and progression of heart failure [49]. It is known that the heart responds to cardiac injury by activating signaling pathways, which leads to remodeling and hypertrophy of cardiomyocytes [50]. Eventually, myocardial fibrosis and dilation of the left ventricle can result in heart failure. Multiple patterns of miRNAs, which are consistently aberrantly expressed, are identified in ischemic heart failure patients [51]–[54]. For example miR-21, -22, -23, -146, -195, -199 and 499 are found to be upregulated in heart failure patients, whereas

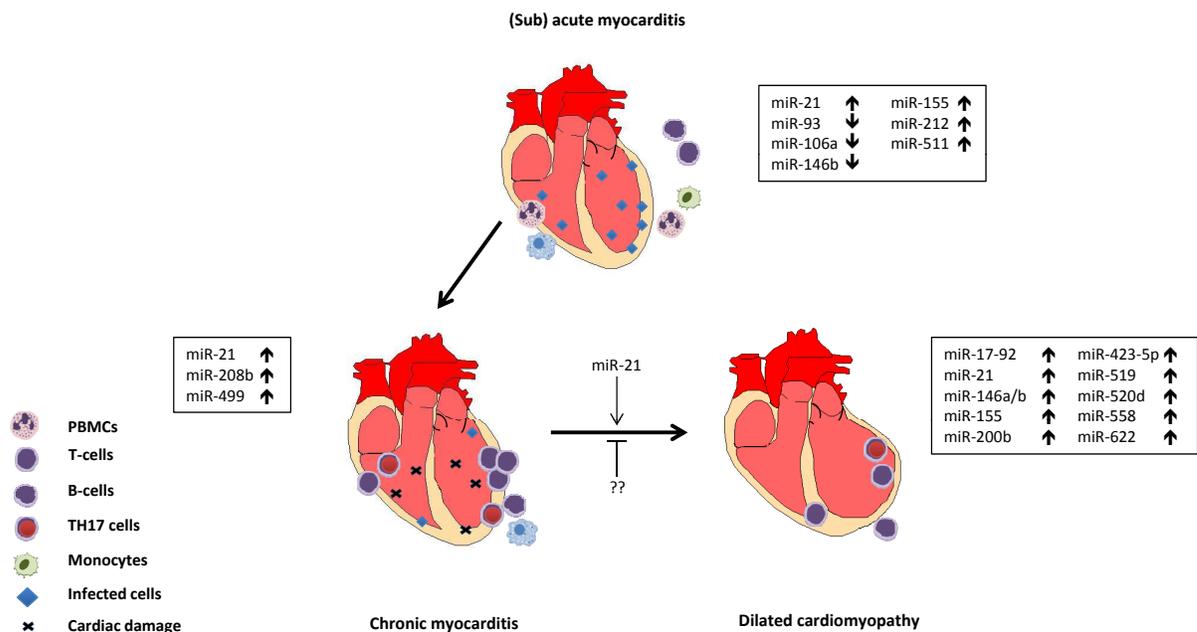


Fig. (1). Different stages of myocarditis and the progression towards dilated cardiomyopathy. In the (sub)-acute phase of myocarditis, infected cells are recognized by immune cells and inflammation is initiated. The immune response can either be downregulated or infected cells persist, which leads to chronic myocarditis. Chronic inflammatory reactions are causing damage to the myocardium, which is further enhanced by autoimmune responses. Ultimately, chronic myocarditis can progress towards dilated cardiomyopathy (DCM). Multiple miRNAs have shown to be involved in the different phases of myocarditis and DCM, however miRNAs involved in the progression of myocarditis towards heart failure are not identified yet. These miRNAs can be of great importance for developing potential therapeutic interventions.

miR-1, -29, -133 and -150 are found to be downregulated [55, 56]. Clinical studies showed that reactivation of fetal gene expression patterns are induced in failing hearts, which results in an altered contractile function of the heart [51].

miRNAs involved in inflammatory responses, leading to non-ischemic heart failure, are still subject of investigation. Currently, some potential miRNAs have been identified in patients with non-ischemic heart failure, such as miR-21, miR-146a/b, -155, -423-5p, and the miR-17-92 cluster [57]–[59]. Furthermore, miR-200b, -519, -520d, -558, and -622 are identified as biomarkers for non-ischemic heart failure

with reduced ejection fraction (HF-REF), however, these miRNAs still have to be validated in other studies [58]. Some of the miRNAs that have been identified in heart failure patients are also aberrantly expressed in myocarditis patients (Table 1), thereby pointing to a potential role of these miRNAs in the progression of myocarditis towards heart failure. However, additional research has to be performed to verify these miRNAs and to identify whether additional myocarditis-specific miRNAs can be identified and are specifically involved in the progression towards heart failure (Fig. 1).

Table 1. microRNAs involved in myocarditis and (non-ischemic) heart failure.

	microRNA	Expression	Function	Reference
Acute myocarditis	miR-21	↑	Interstitial fibrosis, cardiac hypertrophy, immune activation	[40]–[43]
	miR-21	↓	PDC4-mediated apoptosis	[43, 44]
	miR-93	↓	Inhibition pro-inflammatory cytokine production	[40, 43, 46]
	miR-106a	↓	Inhibition pro-inflammatory cytokine production	[40, 43, 46]
	miR146b	↑	Immune activation TH-17 differentiation	[40]–[42]
	miR-155	↑	Pro-inflammatory immune cell functioning	[40, 41]
	miR-212	↑	Cardiomyocyte hypertrophy	[38, 40]
	miR-511	↑	TLR signaling	[38, 40]
Chronic myocarditis	miR-21	↑	Interstitial fibrosis, cardiac hypertrophy, immune activation, progression DCM	[47]
	miR-208b	↑	Myocardial damage, fibrosis and dilatation	[35, 37]
	miR-499	↑	Myocardial damage	[37, 38]
(Non-ischemic) heart failure	miR-21	↑	Interstitial fibrosis, cardiac hypertrophy, immune activation, progression DCM	[50]
	miR-17-92	↑	Immune cell proliferation, cardiac development	[43, 52]
	miR-146a/b	↑	Immune activation TH-17 differentiation	[52]
	miR-155	↑	Pro-inflammatory immune cell functioning	[41, 52]
	miR-423-5p	↑	Biomarker of heart failure	[37, 58, 59]
	miR-200b,-519,-520d,-558,-622	↑	Biomarkers non-ischemic heart failure	[58]

6. THERAPEUTIC OPTIONS USING miRNA-BASED INTERVENTIONS

In addition to current used therapeutics, miRNA-based interventions can potentially be used for the treatment of cardiovascular diseases, thereby including myocarditis. miRNA expression can therapeutically be manipulated via different mechanisms, having promising results in animal models [31] but also already in phase I clinical trials for hepatitis C virus (HCV) infection [60]. One approach that can be used is the inhibition of specific miRNAs involved in disease progression by using modified antisense oligonucleotides (antimiRs) [61, 62]. antimiRs are able to inhibit

miRNA function through complementary base pairing with their corresponding miRNA [31] and, as a result, the miRNA is inhibited and the target mRNA expression is restored.

In addition, miRNA mimics (pre-miRs) can be used to elevate the expression of certain miRNAs [50]. miRNA mimics consist of a synthetic double stranded structure of oligonucleotides, which is complementary to the miRNA sequence [63]. In situations in which decreased levels of miRNAs are causing a disease, miRNA mimics can be used to restore miRNA expression. Although these approaches are promising, their organ selectivity is still limited and improved targeting or local delivery is essential [64].

CONCLUSION

Many studies demonstrated the role of miRNAs in the development and progression of cardiac diseases, thereby recently also including myocarditis. Aberrantly expressed miRNAs are mostly examined in the acute phase of myocarditis, whereas only a few miRNAs are studied in the chronic phase of myocarditis. Since the chronic phase often progress into heart failure, the question remains whether myocarditis specific miRNAs are involved. By studying miRNAs involved in the progression of chronic myocarditis towards heart failure, potential miRNA-based therapeutic approaches can be developed. Modulation of miRNA expression can be a promising strategy in the treatment of chronic myocarditis by limiting inflammation and cardiac damage and thereby preventing non-ischemic heart failure.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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