REVIEW ARTICLE

Emerging role of microRNAs in dilated cardiomyopathy: evidence regarding etiology

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Dilated cardiomyopathy (DCM) is a heart muscle disease characterized by ventricular dilation and systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease. This cardiac disorder is a major health problem due to its high prevalence, morbidity, and mortality. DCM is a complex disease with a common phenotype but heterogeneous pathological mechanisms. Early etiological diagnosis and prognosis stratification is crucial for the clinical management of the patient. Advances in imaging technology and genetic tests have provided useful tools for clinical practice. Nevertheless, the assessment of the disease remains challenging. Novel noninvasive indicators are still needed to assist in decision-making. microRNAs (miRNAs), a group of small noncoding RNAs, have been identified as key mediators of cell biology. They are found in a stable form in body fluids and their concentration is altered in response to stress. Previous research has suggested that the miRNA signature constitutes a novel source of noninvasive biomarkers for a wide array of cardiovascular diseases. Specifically, several studies have reported the potential role of miRNAs as clinical indicators among the etiologies of DCM. However, this field has not been reviewed in detail. Here, we summarize the evidence of intracellular and circulating miRNAs in DCM and their usefulness in the development of novel diagnostic, prognostic and therapeutic approaches, with a focus on DCM etiology. Although the findings are still preliminary, due to methodological and technical limitations and the lack of robust

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population-based studies, miRNAs constitute a promising tool to assist in the clinical management of DCM. (Translational Research 2019; 000:1–16)

Abbreviations: AGO2 = Argonaute 2; ALDH 2 = Aldehyde dehydrogenase 2; AUC = Area under the curve; *BAG3* = BCL-associated athanogene 3gene; CAD = Coronary artery disease; circRNA = Circular RNA; DCM = Dilated cardiomyopathy; HCM = Hypertrophic cardiomyopathy; HF = Heart failure; *LMNA* = Lamin A/C gene; LV = Left ventricle; LVEF = LV ejection fraction; MI = Myocardial infarction; miRNA = microRNA; mRNA = messenger RNA; ncRNA = non-coding RNA; PBMC = Peripheral blood mononuclear cells; PPCM = Peripartum cardiomyopathy; RISC = RNA-induced silencing complex; *TTN* = Titin gene

DILATED CARDIOMYOPATHY: A HETEROGENEOUS ENTITY ENCOMPASSING A COMMON PHENOTYPE

Dilated cardiomyopathy (DCM) is a heart muscle disease defined as left ventricular (LV) or biventricular dilatation and systolic dysfunction that are not explained by abnormal loading conditions (hypertension or valve disease) or coronary artery disease (CAD) sufficient to cause global systolic impairment.[1] DCM is a common form of cardiomyopathy with an estimated prevalence range from 1/2500 up to 1/250.[2] It is a significant health concern in adult and pediatric populations. DCM constitutes the third most common cause of heart failure (HF) and the leading cause of heart transplantation and is associated with high rates of morbidity and mortality.[3] More than a single disease entity, DCM constitutes a cardiac phenotype that arises from multiple etiologies.[4] Indeed, DCM has been considered the outcome of several pathological pathways (Fig 1). Therefore, after identifying morphologically enlarged LV and systolic dysfunction, recognition of the underlying disease mechanism is crucial to properly manage the DCM patient, for example, specific treatment or the need for family screening or prognosis prediction.[2]

To date, the influence of underlying molecular etiology has not been sufficiently considered in the management of DCM patient.[5] Indeed, a lack of knowledge about the pathophysiology means there is no unique classification of the disease. Various efforts have been made to classify cardiomyopathies, including DCM,[1] but there is no clear consensus. The European Society of Cardiology proposed a genetic and nongenetic classification, including etiologies such as peripartum, antineoplastic therapies, drug or alcohol abuse or myocarditis, among others.[6] In this context, a multidisciplinary approach is required to reach the diagnosis. Many health professionals should be involved, including cardiologists, radiologists and geneticists.[7] Nonetheless, overlapping phenotypes often make differential diagnosis unclear. This situation represents a major challenge in the cardiology arena.

In recent years, management of DCM patients has improved due to pharmacological and nonpharmacological advances.[8] Consequently, the identification of presymptomatic DCM patients and patients with established and latent DCM could reduce the clinical burden of the disease.[4] However, diagnosis and risk stratification are still a challenge. Myocardial biopsies are a potential and powerful diagnostic tool. However, their application is limited due to invasiveness and poor accuracy in certain scenarios.[8] Transthoracic echocardiography has been traditionally used for DCM diagnosis due to its noninvasiveness and widespread availability, in spite of its inter- and intraobserver variability and the lack of etiological patterns defined for DCM.[9] Indeed, transthoracic echocardiography can reach a DCM diagnosis but cannot determine a morphological or functional pattern for each underlying pathogenic mechanism, and it fails to detect preclinical stages without clinical expression of the disease.[9] Cardiac magnetic resonance has emerged as the gold-standard tool for structural and functional evaluation of cardiomyopathies.[10] Its advantages include high spatial resolution, unrestricted imaging planes, and lack of ionizing radiation. Conversely, the potential of cardiac magnetic resonance to be optimally integrated into clinical practice has been limited in part by the long total scan time, the contrast agents used, and the presence of claustrophobia or metal devices.[11] A blood-borne test could provide relevant insight into pathological processes: necrosis, inflammation, and remodeling, among others. However, there are still several hurdles that need to be overcome. For example, troponins have been used to determine myocyte death, mostly in ischemic cardiomyopathy.[12] Although "organ-specific," this established clinical test is not "disease-specific."[13] Therefore, it does not provide information on DCM pathophysiology. In relation to the family of natriuretic peptides, the N-terminal pro-B-type natriuretic peptide (NT proBNP) seems to be a very efficient biomarker to assist clinical decision-making in HF.[14] However, although it is highly sensitive, the specificity is low.[13] C-reactive protein is a biochemical marker of inflammation with clinical utility in DCM.[15] Studies have shown a link between C-reactive protein concentrations and several cardiovascular disorders such as CAD and stroke.[16,17] Similar to troponins and NT-proBNP, its usefulness in identification of underlying DCM etiology is limited.

The heterogeneity of DCM pathophysiology, the limitations of imaging cardiovascular tests, the complications of myocardial biopsy, and the lack of specific biomarkers to confirm the etiology create a need for novel approaches to

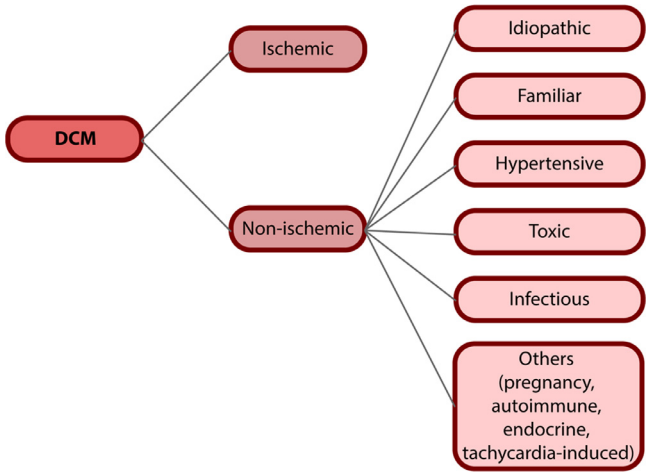

Fig 1. Classification of dilated cardiomyopathy, modified from Merlo et al.[8]

identify pathological mechanisms and support clinical decision-making. In this paper, we aim to discuss state-of-the-art research into microRNAs (miRNAs) in DCM, from their role in the disease to their potential usefulness as indicators and therapeutic tools, with special emphasis on the etiologies of DCM. It should be noted that the term DCM has been used to describe the disease independently of the etiology. The specific etiologies have been clearly defined in the text.

MICRORNAS

miRNAs are short noncoding RNAs (ncRNAs) with around 19−25 nucleotides that induce silencing of gene expression at the post-transcriptional level (Fig 2). miRNAs may target over 60% of protein-coding genes.[18] The mechanisms of action are based on protein synthesis inhibition or messenger RNA (mRNA) degradation by binding to complementary sequences, mainly in the 3-untranslated region (3'-UTR), but also the 5'-untranslated region (5-UTR) and the amino acid coding sequence region. A direct interaction with other ncRNAs and viral RNA has also been described.[19,20] To date, over 2500 human mature miRNAs have been annotated in the miRBase database (Release 22.1, October 2018, http://www.mirbase.org/).[21] miRNAs are usually expressed in complex networks that enable subtle control of cellular phenotype. An individual miRNA can target hundreds of distinct mRNAs, and a single mRNA can be regulated by multiple miRNAs. These small ncRNAs are involved in almost all biological processes, including proliferation, development, differentiation, metabolism, immunity, inflammation, apoptosis, autophagy, stress response, and cell cycle control.[22-26] As such, studies from independent laboratories have suggested the crucial role of deregulation of miRNA expression in the pathogenesis of numerous diseases, including cardiovascular disorders.[27-30] The disruption of the Dicer allele, an endonuclease essential in miRNA biogenesis, in cardiac progenitors is associated

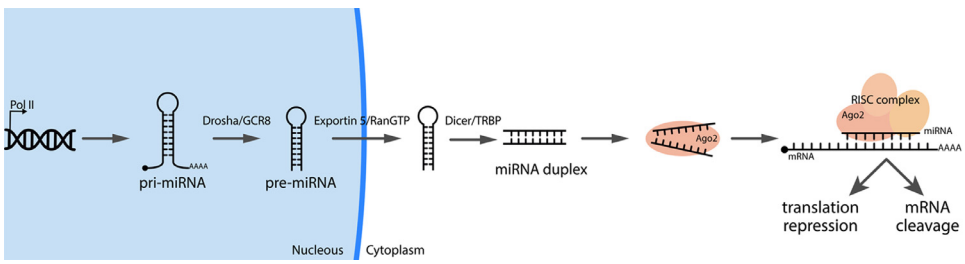

Fig 2. Biogenesis and function of microRNAs. The microRNAs are transcribed by Pol II resulting in the pri-microRNA that has a distinctive hairpin structure. The pri-microRNA is processed by Drosha/GCR8 to produce the pre-microRNA. By Exportin 5/RanGTP action, the pre-mRNA is exported to the cytoplasm where it is cleaved by the action of Dicer/TRBP producing a microRNA duplex of about 20 bp. Finally, the microRNA duplex is loaded into protein argonaute-2 (AGO2). One chain is degraded, and the other chain constitutes the mature microRNA. The microRNA with AGO and other proteins constitute the RNA-induced silencing complex (RISC) complex. Together, they act as regulators of gene expression at posttranscriptional level.

with a poorly developed ventricular myocardium and embryonic lethality.[31] Interesting, Chen et al[32] demonstrated that cardiac-specific knockout of Dicer not only leads to HF and postnatal lethality, but also to DCM. Dicer expression was decreased in end-stage DCM and failing hearts in humans and therapeutic interventions such as LV assist devices increase the expression of Dicer and improved cardiac function. For further information about miRNAs as novel regulators in cardiac physiology and pathophysiology, see other excellent reviews.[33-35]

In addition to their intracellular localization, miRNAs have been detected in the extracellular space and circulation.[36-39] Extracellular miRNAs are transported in protein or lipoprotein complexes[40,41] or packed into extracellular vesicles (exosomes, microvesicles, and apoptotic bodies).[36,42] Similar to other soluble factors, various pieces of evidence have suggested a potential role of extracellular miRNAs in cell-to-cell communication, mainly at paracrine level[43] and probably at endocrine level.[44] Extracellular miRNAs seem to be implicated in physiological responses and in the onset of cardiovascular disease.[45,46]

From a clinical perspective, extracellular miRNAs have interesting biochemical properties.[47] They have a long half-life within the sample, can be obtained by minimally invasive techniques in samples used in clinical laboratories (eg, serum and plasma) and can be quantified through standard techniques such as quantitative reverse transcription PCR (qPCR). Global profiles can be obtained in a single experiment using next-generation sequencing or microarrays. Furthermore, miRNA-based tests have been described as a cost-effective alternative for risk assessment and disease monitoring.[48] Importantly, extracellular ncRNAs have been proposed as useful tools for clinical practice. In 2012, the FDA approved the prostate cancer antigen 3 (Progensa PCA3 assay) to diagnose prostate cancer. Indeed, circulating miRNAs constitute potential noninvasive biomarkers with a clinical application.[49,50] Alterations in the circulating miRNA profile have been well-correlated with a number of cardiovascular conditions, including CAD, myocardial infarction (MI), HF and cardiomyopathies.[51-53] Therefore, the circulating levels of these ncRNAs emerged as a promising source of clinical indicators for the diagnosis and prognosis of DCM.

MICRORNAS AND DILATED CARDIOMYOPATHY

Several publications have explored the relationship between miRNA profiles and DCM without a detailed definition of the etiological mechanism (Table 1). In general, this condition has been associated with altered expression of the miRNA profile in the myocardium. For instance, genome-wide profiling of miRNAs in human LV identified miR-17-5p, miR-28, and miR-106a as differentially expressed in DCM samples compared to control samples.[54] These miRNAs were not deregulated in ischemic cardiomyopathy or aortic stenosis samples. The expression of miRNAs seems to be differentially regulated depending on the location. Using human cardiac tissues from patients, Wang et al[55] observed that miR-21 was upregulated in the apex, left, and right ventricles of patients with DCM compared with normal cardiac tissues. Conversely, miR-29a, miR-29b, miR-29c, miR-133a, and miR-133b were significantly downregulated in these locations. Intracellular miRNAs may not only be indicative of the mechanisms affected in the disease but could also play a role in diagnosis and prognosis. A correlation between miR-133a expression levels in endomyocardial biopsies with fibrosis, myocyte necrosis, LV function, and clinical outcome has been observed in patients with inflammatory DCM.[56] In the same type of samples, expression levels of let-7i, miR-126, and miR-155 were downregulated, and miR-21 was upregulated, in patients with DCM compared to subjects without LV dysfunction as control subjects.[57] The authors demonstrated that low let-7i levels were a strong independent predictor of cardiac death and HF (relative risk = 3.76, 95% confidence interval = 1.04−13.63). Recently, miRNA expression in LV has been proposed as a useful tool to distinguish between types of cardiomyopathies: hypertrophic cardiomyopathy (HCM) and DCM.[58] miR-1-3p and miR-27a were differentially expressed between both types of samples and showed an optimal discrimination performance: area under the receiver operating characteristic Curve (AUC) = 0.850 for miR-1-3p and AUC = 0.860 for miR-27a. The use of miRNAs as therapeutic agents has also been proposed. The long−term delivery of adeno-associated viral−mediated intraventricular miR−669a reduced adverse remodeling and enhanced systolic fractional shortening of the LV in a transgenic model of chronic muscular dystrophy-associated DCM.[59]

Some studies have evaluated the potential of circulating miRNAs as easily accessible biomarkers of DCM in clinical contexts. In 2010, Voellenkle et al[60] compared the miRNA expression pattern of peripheral blood mononuclear cells (PBMC) isolated from healthy individuals and chronic HF patients affected by ischemic cardiomyopathy and nonischemic DCM. The authors reported a specific miRNA signature in nonischemic DCM patients. The bioinformatic analysis predicted miRNA targets in molecular pathways that are intimately associated with chronic HF, which suggests that miRNAs participate in the pathophysiology of the

Table 1. Selected microRNAs associated with DCM and its etiologies

Etiology	Patients/Model	Source	Main findings	Reference
DCM	67 subjects (25 DCM, 19 ICM, 13 AS and 10 nonfailing controls)	LV	miR-17-5p, miR-28 and miR-106a were differentially expressed in DCM compared to control No deregulation in ICM or AS samples	54
	3 DCM patients and 3 control subjects	Cardiac tissue	miR-21 was upregulated and miR-29a, miR-29b, miR-29c, miR-133a and miR-133b were downregulated in the apex, LV and RV of DCM patients	55
	76 iCMP patients and 22 noninflammatory DCM patients	Endomyocardial biopsy	miR-133a and miR-155 were upregulated in iCMP compared with DCM miR-133a correlated with fibrosis, myocyte necrosis, LV function and clinical outcome	56
	103 DCM patients and 37 control subjects without LV dysfunction	Endomyocardial biopsy	Let-7i, miR-126 and miR-155 were downregulated and miR-21 was upregulated in DCM Low let-7i levels were associated with poor clinical outcomes	57
	27 HCM patients, 10 DCM patients and 10 control subjects	LV	miR-1-3p and miR-27a were differentially expressed in HCM and DCM	58
	Mouse model of chronic muscular dystrophy-associated DCM	Cardiac tissue	Intraventricular delivery of miR-669a reduces adverse remodeling and improves LV function	59
	26 NIDCM patients, 23 ICM patients and 28 control subjects	PBMC	Specific miRNA signatures were reported in NIDCM and ICM	60
	8 patients with isolated diastolic dysfunction and preserved systolic function, 10 patients with stable compensated DCM, 13 patients with decompensated congestive HF secondary to DCM and 8 healthy individuals	Buffy coats	miR-142-3p was downregulated in stable compensated DCM and decompensated congestive HF secondary to DCM miR-124-5p was upregulated in stable compensated DCM	61
	70 DCM patients	Plasma	Expression levels of miR-21, miR-26, miR-29, miR-30 and miR-133a were similar in new-onset and chronic DCM patients with and without fibrosis Specific associations with markers of collagen metabolism and extracellular matrix fibrosis	62
	43 DCM-AHF patients and 34 control subjects	Serum	Exosomal miR-92b-5p was increased in DCM-AHF and was correlated with functional and morphological parameters	63
	23 DCM patients and 23 control subjects	Plasma	miR-3135b, miR-3908 and miR-5571-5p were upregulated in DCM	64
	50 DCM patients and 41 control subjects	Plasma	miR-185 was upregulated in DCM Higher miR-185 levels associated with better prognosis	65
	46 CDCM patients and 28 control subjects	Serum	A signature including miR-27b-3p, miR-126-3p, miR-142-5p and miR-143-3p differentiated with CDCM	66

(*continued on next page*)

Table 1. (*Continued*)

Etiology	Patients/Model	Source	Main findings	Reference
	55 children <18 years old with DCM	Serum	miR-155 and miR-636 were upregulated and miR-639 and miR-646 were downregulated in DCM patients who were transplanted or died compared to DCM patients that recovered their ventricular function	67
Ischemic	6 nonfailing, 5 idiopathic DCM, and 5 ischemic DCM patients	Cardiac tissue	miR-100 and miR-195 were upregulated and miR-92 and miR-133b were downregulated in idiopathic and ischemic cardiomyopathy	69
	10 diabetic HF and 19 non-diabetic HF patients affected by non-end stage ischemic DCM and 16 matched non-diabetic and non-HF control subjects	LV	miR-34b, miR-34c, miR-199b, miR-210, miR-223 and miR-650 were differently expressed between diabetic HF and 19 non-diabetic HF patients miR-216a was inversely correlated with LVEF	70
	Mouse model of MI. Young (3 month) and old (22 month) mice	Cardiac tissue	miR-1, miR-34a and miR-133a were upregulated in aged mice miR-1 showed a further increase in old mouse following MI	71
	25 ischemic DCM patients, 25 idiopathic DCM patients and 10 healthy controls	Plasma	let-7b-5p, let-7c-5p, miR-24-3p, miR-28-5p, miR-100-5p, miR-103a-3p, miR-125b-5p, and miR-214-3p were overexpressed in ischemic and idiopathic DCM groups compared to the control group miR-15b-5p and miR-106a-5p discriminated between ischemic and idiopathic DCM	72
Idiopathic	14 idiopathic DCM patients	Endomyocardial biopsy	miR-422 was upregulated and miR-10, miR-300, miR-302 and miR-323 were downregulated in reduced patients with reduced catecholamine sensitivity	75
	43 idiopathic DCM patients treated with β-blockers	Endomyocardial biopsy	miR-1-3p, miR-21-5p, miR-199a-5p, miR-208a-3p, miR-208b-3p and miR-591 were deregulated at 3 and 12 months of treatment	76
	48 nonfailing controls and 44 patients with relatively stable chronic HF associated with idiopathic DCM	PBMC	miR-548 family members were downregulated in patients with stable chronic HF	77
	23 children with isolated idiopathic DCM and 26 control children	Plasma	miR-454 and miR-518f were upregulated and miR-99b, miR-147, miR-155, miR-194, miR-205, miR-218, miR-302a, miR-544, miR-618, miR-875-3p were downregulated in idiopathic DCM patients	78
Familial	Inducible DCM mouse model carrying a human TTN mutation 30 patients undergoing LV assist device implantation: 10 idiopathic DCM, 10 ischemic heart disease and 10 myocarditis	Cardiac tissue	miR-208b regulated cardiac remodeling and pathogenesis of DCM miR-208b was upregulated in patients with DCM	85
	25 patients with pathogenic mutations in the LMNA gene responsible for DCM, 25	Plasma	miRNA signature (let-7a-5p, miR-142-3p, miR-145-5p and miR-454-3p) that could assist in the diagnosis of	86

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Table 1. (*Continued*)

Etiology	Patients/Model	Source	Main findings	Reference
	idiopathic DCM patients and 25 control subjects		patients with DCM caused by pathogenic mutations in LMNA	
	21 patients with pathogenic mutations in the BAG3 gene responsible for DCM and 44 control subjects	Whole blood	miR-154-5p, miR-182-5p, miR-3191-3p, miR-6769b-3p and miR-6855-5p were deregulated patients with pathogenic mutations in BAG3	87
Infectious	60 patients with latent and reactivated Parvovirus B19 infection	Endomyocardial biopsy	Signature of 29 miRNAs differentially regulated in patients with latent and reactivated Parvovirus B19 infection	91
	Mouse model of VMC 6 DCM patients and 8 control subjects	Cardiac tissue LV	miR-21 was upregulated in the mouse model of VMC and in VMC and DCM patients	92
	Coxsackievirus B3-infected mouse model	Whole blood	miR-191, miR-216a, miR-337, miR-710 and miR-713 were deregulated after infection	93
Hypertensive	15 spontaneously hypertensive rats and normotensive control Wistar Kyoto rats	Neonatal rat ventricular myocyte	miR-18 protected against DCM during hypertension-induced HF	95
	Mouse model of hypertension	Endomyocardial biopsy	Downregulation of miR-221/222 led to fibrosis and LV dilation and dysfunction	96
	79 DCM patients and 28 patients with severe isolated aortic valve stenosis		miR-221 and miR-222 were downregulated in patients with severe fibrosis and DCM	
Toxic	Mouse model of alcoholic cardiomyopathy	LV	19 miRNAs were differential expressed in alcohol-treated group	98
	Adult rats	Rat cardiomyocytes	Increased expression of miR-378a-5p in acute ethanol exposure	99
Peripartum	38 patients with acute PPCM, 18healthy postpartum controls, 5 healthy age-matched nonpregnant women, 30 patients with HF due to DCM, and 12 patients with PPCM receiving bromocriptine treatment	Plasma/LV	miR-146a was upregulated in the plasma and hearts of PPCM patients miR-146a plasma levels were within normal range after standard therapy	102

AHF, acute heart failure; AS, aortic stenosis; BAG3, BCL-associated athanogene 3; CDCM, childhood dilated cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HF, heart failure; ICM, ischemic cardiomyopathy; iCMP, inflammatory cardiomyopathy; LMNA, Lamin A/C; LV, left ventricle; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NIDCM, nonischemic dilated cardiomyopathy; PBMC, peripheral blood mononuclear cells; PPMC, peripartum cardiomyopathy; RV, right ventricle; TTN, Titin; VMC, viral myocarditis.
Intracellular and circulating microRNAs associated with dilated cardiomyopathy and its etiologies are displayed. See the text for detailed information.

disease. In particular, miR-107, miR-139, and miR-142-5p displayed 21, 16, and 29 potential mRNA targets in patients with nonischemic DCM. The SON DNA binding protein, a protein involved in cell proliferation and apoptosis, was targeted by more than one miRNA. The specific downregulation in the expression levels of circulating miR-142-3p was observed in patients with stable compensated DCM and with decompensated congestive HF secondary to DCM compared to patients with isolated diastolic dysfunction with preserved systolic function.[61] In the same study, miR-124-5p was highly upregulated in patients with stable compensated DCM but not in patients with isolated diastolic dysfunction or decompensated congestive HF. Rubiś et al[62] suggested that the circulating levels of miRNAs linked to fibrosis (miR-21, miR-26, miR-29, miR-30, and miR-133a) are similar between DCM patients with different durations of the condition: new-onset DCM with and without fibrosis, and chronic DCM with and without fibrosis. Nonetheless, some of these candidates could offer information about fibrotic mechanisms. In acute HF due to DCM, expression levels of serum exosomal miR-92b-5p were upregulated in patients compared to controls.[63] The levels of this miRNA were correlated with different functional parameters and provided an interesting discriminative value (AUC = 0.808), which suggests its role as a diagnostic biomarker. Plasma miR-3135b and miR-5571-5p also provided optimal discriminative potential to distinguish between healthy controls and DCM patients (AUC = 1.00 and 0.91, respectively).[64] In the field of prognostication, plasma levels of miR-185 have been proposed as a biomarker in newly diagnosed patients with DCM.[65] Patients with high miR-185 levels showed improvements in LV ejection fraction (LVEF) and LV end diastolic diameter, and a significant decline in both cardiovascular mortality and total admissions for HF rehospitalizations during a 1-year follow-up period.

Profound deregulation in the circulating miRNA profile has been observed in childhood DCM. The circulating profile of miR-27b-3p, miR-126-3p, miR-142-5p, and miR-143-3p seems to discriminate between patients with DCM from healthy subjects.[66] Interestingly, Miyamoto et al[67] identified specific serum miRNAs that can be used for risk stratification in children with DCM. Using an array for 754 miRNAs and RT-qPCR validation, the authors found a differential expression profile of miR-155, miR-636, miR-639, and miR-646 in patients who were transplanted or died compared with patients who recovered their ventricular function. All 4 miRNAs were highly predictive of transplant need (AUC for the presence of any 1 of the 4 miRNAs = 0.875).

In summary, DCM is intimately associated with an altered intracellular and circulating miRNA profile compared to healthy controls and other cardiac conditions or cardiomyopathies such as HCM. Findings from different investigations suggest miRNAs as biomarkers and potential therapeutic tools, and simultaneously, as a source of knowledge to explore pathological mechanisms linked to the disease.

MICRORNAS AND DILATED CARDIOMYOPATHY ETIOLOGY

In the current section, we summarize the results obtained by several publications that explored the association between intracellular and circulating miRNAs within DCM etiologies (Table 1). Only studies that clearly define the DCM etiology have been included.

microRNAs and ischemic dilated cardiomyopathy. Although the inclusion of ischemic DCM is contradictory with the definition of DCM,[1] in this review we have also considered this etiology due to its clinical relevance. Ischemic DCM is one of the most common types of DCM. Approximately half of DCM patients may have an ischemic cause.[2] Most patients with ischemic DCM have known CAD, which is associated with the development of LV remodeling and dysfunction. The main difference between ischemic and nonischemic DCM is the presence of atherosclerotic lesions in the coronary arteries of patients with ischemic DCM and the absence of this lesion in patients with nonischemic DCM. Unlike idiopathic genetic DCM and depending on dysfunctional tissue viability, LV function can be improved in ischemic DCM.[68]

Some research has described intracellular expression patterns of miRNAs in patient-based studies and animal models of ischemic DCM. In 2008, Sucharov et al[69] analyzed the miRNA expression profile in samples obtained from nonfailing, idiopathic DCM and ischemic DCM patients. Using a microarray approach, the authors identified a profile of miRNAs deregulated in both idiopathic DCM and ischemic DCM patients or specifically in each disorder. After subsequent RT-qPCR validation, they reported the upregulation of miR-100 and miR-195 and the downregulation of miR-92 and miR-133b in tissue samples from both idiopathic and ischemic hearts. Greco et al[70] explored the miRNA expression profiles in LV biopsies from diabetic and nondiabetic patients affected by non-end-stage ischemic DCM. Both groups were compared with control subjects. The authors reported 17 miRNAs differentially expressed in diabetic and/or nondiabetic samples compared with control subjects. Six miRNAs, miR-34b, miR-34c, miR-199b, miR-210, miR-223, and

miR-650, were differentially expressed between both patient groups. One of the miRNAs upregulated in both patient classes, miR-216a, was inversely correlated with LVEF. Using in vitro and bioinformatics approaches, the miRNA intracellular profile was linked to several pathological mechanisms related to HF, some of them specific for diabetic patients: free radical scavenging, oxidative stress, cardiac damage, cardiac dysfunction, cardiac fibrosis, and HF. Recently, the possible role of miR-1, miR-133a and miR-34a in LV dilation and subsequent LV dysfunction was explored in an in vivo model of MI in young and old mice.[71] An age-dependent effect was observed. Indeed, the 3 miRNAs were increased in aged mice. MI-injury in aged mice caused a further increase of miR-1 levels, a finding that was not observed for young mice.

A recent study by Onrat et al[72] tested the potential of plasma miRNAs to distinguish between ischemic DCM and idiopathic DCM. Using RT-qPCR-based technologies, the authors sought to investigate the expression profiles of 30 miRNAs in patient groups and healthy controls. They reported that the expression levels of let-7b-5p, let-7c-5p, miR-24-3p, miR-28-5p, miR-100-5p, miR-103-3p, miR-125b-5p, and miR-214-3p were upregulated in DCM groups compared to controls and suggested that circulating miR-15b-5p and miR-106a-5p allow the discrimination of patients with ischemic and idiopathic DCM.

MICRORNAS AND NONISCHEMIC DILATED CARDIOMYOPATHY

Nonischemic DCM comprises various etiologies that are not related to CAD. This condition includes pathogenic mechanisms concluding in a common phenotype. Nonischemic DCM could be inherited. Various environmental factors and pathological insults are implicated in the pathogenesis: inflammatory processes, viral and nonviral infections, toxins, mechanical load or nutritional deficiencies, among others.[73] The identification of the precise etiological mechanisms could allow for specific treatment targeted to the underlying cause.[2]

microRNAs and idiopathic dilated cardiomyopathy. A clinical diagnosis of idiopathic DCM is assigned when after etiological assessment the identifiable cause of DCM is not found, including the genetic origin. Unfortunately, no robust epidemiological data are available on the prevalence of idiopathic DCM. Nonetheless, it has been estimated that idiopathic DCM may constitute more than 70% of all diagnoses of nonischemic cardiomyopathies.[2] The prognosis of this condition has been improved due to novel pharmacological therapies and device-based therapeutic strategies developed in recent decades.[74] Accurate diagnosis of idiopathic DCM is therefore fundamental.

Reduced catecholamine sensitivity is considered an early HF characteristic, which is related to deregulation of the expression of several contractile proteins in DCM patients. In 2011, using genome-wide miRNA expression profiling in endomyocardial biopsy specimens, Funahashi et al[75] demonstrated that 32 intracellular miRNAs were deregulated in idiopathic DCM patients with reduced catecholamine sensitivity compared to preserved catecholamine sensitivity patients. In particular, the expression of miR-422 was upregulated and expression levels of miR-10, miR-300, miR-302, and miR-323 were downregulated in reduced catecholamine sensitivity patients. The authors suggested the potential of miRNAs as therapeutic targets for chronic HF. Sucharov et al[76] reported miRNA profile changes dependent on the response to β-blocker treatments in endomyocardial biopsies of idiopathic DCM patients. The miRNA differential expression was related to the presence or absence of a reverse-remodeling response to the therapy. In the presence of a reverse-remodeling response, miR-1-3p, miR-21-5p, miR-199a-5p, miR-208a-3p, miR-208b-3p, and miR-591 were deregulated at 3 and 12 months of treatment. Bioinformatic analyses predicted alteration in molecular pathways related to apoptosis, cardiac myocyte cell death, hypertrophy, and HF.

As shown above and similar to results from Onrat et al,[72] publications have suggested a specific pattern of circulating miRNAs in patients with idiopathic DCM. Using a genome-wide miRNA microarray on PBMC samples, Gupta et al[77] identified several members of the miR-548 family that were downregulated in ambulatory patients with chronic stable HF with idiopathic DCM compared to healthy controls. The authors concluded that alteration in the miR-548 family signature in PBMC could help to detect early HF and proposed that miR-548 targets are enriched in molecular pathways associated with cardiovascular disease, including cardiac fibrosis hypertrophy, fibrosis, and necrosis/cell death. In children between 2 months and 192 months old with isolated idiopathic DCM the expression levels of plasma miR-454 and miR-518f were significantly higher than in control subjects.[78] Conversely, the expression levels of 10 miRNAs including miR-99b, miR-147, miR-155, miR-194, miR-205, miR-218 miR-302a, miR-544, miR-618, and miR-875-3p were significantly lower in DCM patients than in the control subjects.

microRNAs and familial dilated cardiomyopathy. Recent studies have proposed that nearly 60% of DCM cases have a genetic cause.[79] Nevertheless, the clinical presentation and phenotypic variation of familial DCM make its diagnosis complex.[79-82] Indeed, approximately 25% of patients with idiopathic DCM are likely to have a genetic basis for disease.[82] Over 400 pathogenic variants have been identified in over 60 genes. Titin (*TTN*) and Lamin A/C (*LMNA*) are the main genes responsible for familial DCM.[79]

Early recognition of carriers with pathogenic mutations could allow therapeutic interventions to prevent adverse events. Furthermore, a more rigorous assessment of DCM etiology is of clinical interest in terms of family or genetic screening, counseling and prognosis.[4,83] However, current diagnosis of familial DCM has limitations.[6] The clinical phenotype does not provide any real clue to detect the underlying genotype. A clinical screening of first-degree relatives is required during the screening protocol of familial DCM, but a detailed family history may not be conclusive in certain circumstances (ie, small families, low number of living family members, not all relatives are available, incomplete low and/or age-related penetrance or variable phenotypes, among others). Molecular genetic testing is becoming more widely available, although the diagnosis of familial DCM rarely begins with the identification of genetic mutations. Additionally, the cost, relatively low yield of screening known disease genes and the inability to predict clinical outcomes are limitations for genetic testing in clinical practice. Thus, the development of novel biomarkers for familial DCM, and particularly transcriptome-based approaches,[84] could provide a new scenario for patient management. Despite the high epidemiological relevance of familial DCM, studies analyzing the relationship between intracellular and circulating miRNAs in patients with the disease are scarce.

In 2017, Zhou et al[85] performed a miRNA profiling approach in myocardium samples from an inducible DCM mouse model carrying a human *TTN* mutation. The authors described the upregulation in the expression levels of cardiomyocyte-enriched miR-208b. Loss-of-function and gain-of-function studies using locked nucleic acid-modified miR-208b mimics and antimiR-208b demonstrated a key role of this miRNA in remodeling and pathogenesis of DCM. Based on their findings, miR-208b may constitute a novel therapeutic target for DCM. Interestingly, results were validated in heart tissue of the LV apex from patients with severe DCM.

Recently, we explored the potential of circulating miRNAs to identify patients with pathogenic *LMNA* mutations that are responsible for familial DCM.[86] After comprehensive screening of plasma miRNAs, we reported the signature of four miRNAs, let-7a-5p, miR-142-3p, miR-145-5p, and miR-454-3p, that was upregulated in carriers of pathogenic *LMNA* mutations compared to control subjects with wild-type variants (Fig 3). These circulating miRNAs, and their combination in a score, allowed discrimination between carriers of pathogenic *LMNA* mutations and subjects with wild-type variants, including patients with idiopathic DCM (AUC from 0.713 to 0.836). The discriminative potential was higher than that observed for the established clinical test NT-proBNP (AUC = 0.578). In addition, we demonstrated

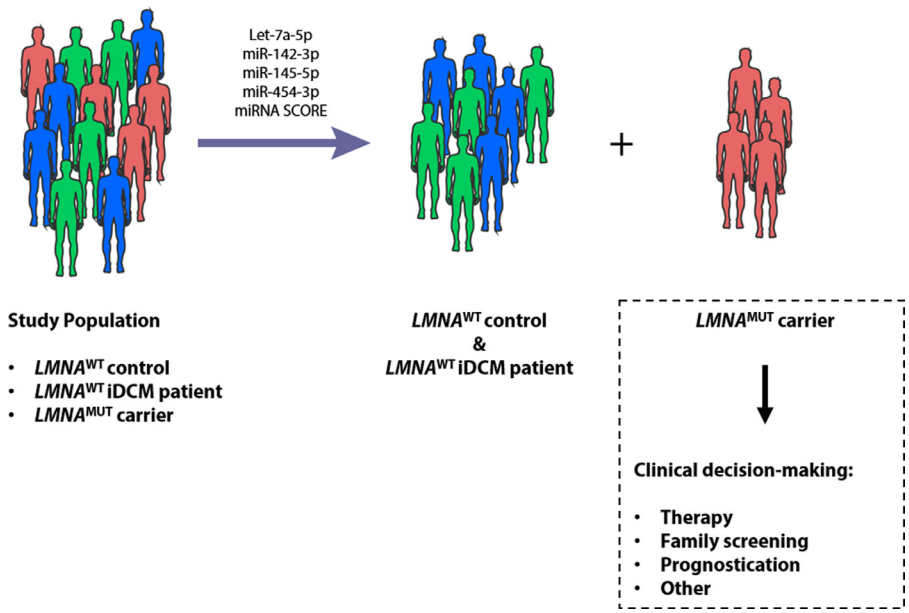

Fig 3. Potential clinical application of circulating microRNAs in the identification of patients with pathogenic *LMNA* mutations responsible for dilated cardiomyopathy (DCM). Proposed model for the clinical application of circulating miRNAs as a tool to assist in the diagnosis of patients with dilated cardiomyopathy (DCM) caused by *LMNA* pathogenic mutations. *LMNA*^{MUT} carrier: patients with pathogenic *LMNA* mutations responsible for DCM, *LMNA*^{WT} control: *LMNA*-wild-type healthy controls, *LMNA*^{WT} iDCM patient: *LMNA* wild-type idiopathic DCM patients.

that the 4 miRNAs and their subsequent score allowed discrimination between healthy subjects and carriers who were phenotypically negative for DCM and between idiopathic DCM patients and carriers who were phenotypically positive for DCM, which could have a direct impact on the clinical management of the disease. Eleven molecular pathways were enriched with the predicted targets of our miRNA signature, including pathways intimately associated with cardiac pathophysiology: TGF-beta, FoxO, and Wnt signaling pathways.

The use of circulating miRNAs as biomarkers has also been recently proposed in *BAG3*-related familial DCM.[87] Using sequencing in whole blood samples, the authors identified a profile of miRNAs composed by miR-154-5p, miR-182-5p, miR-3191-3p, miR-6769b-3p, and miR-6855-5p that showed different expression levels in carriers of pathogenic *BAG3* mutations and subjects with wild-type variants. In addition, miR-154-5p, miR-182-5p, and miR-6855-5p were deregulated in *BAG3* carriers that were phenotypically positive for DCM when compared to carriers that have not developed the condition. These miRNAs were also correlated with echocardiographic variables including A wave, left atrium length, and left atrium area.

microRNAs and infectious dilated cardiomyopathy. The persistence of viral infection, mainly coxsackievirus B, adenovirus, parvovirus B19 and human herpes virus 6,[88] within cardiomyocytes has been associated with the development and progression of DCM.[89] Viral infections of the heart have been proposed as one of the underlying causes of inflammatory cardiomyopathies in developed countries. Up to 40% of the DCM has been associated with inflammation or viral infection.[90]

In 2012, Kühl et al[91] tested whether miRNAs are differentially expressed in endomyocardial tissue specimens from patients with latent and reactivated erythrovirus infection, specifically parvovirus B19. After a profiling phase including 906 human miRNAs, the authors identified 29 miRNAs that are extremely deregulated in patients with latent and reactivated viral infection. Using a bioinformatic approach, they proposed that these miRNAs regulate the expression of 1330 mRNAs that are mainly associated with cardiomyopathies and inflammatory response related pathways. More recently, Xu et al[92] evaluated the potential role of miR-21 in viral myocarditis and DCM. The expression levels of the miRNA were increased in cardiac myocytes from viral myocarditis and DCM patients and from a mouse model infected with Coxsackievirus B3 in comparison with control samples. The levels of its target gene sprouty homolog 1 (*SPRY1*), a negative feedback regulator of the MAPK signaling pathway, were decreased in viral myocarditis and DCM samples. After an in vitro approach, they proposed that the expression of miR-21 may contribute to the pathogenesis of both conditions and suggested a potential therapeutic approach by modulating the levels of the miRNA.

Sun et al[93] have evaluated the profile of miRNAs in peripheral blood of Coxsackievirus B3-infected mice. Using a microarray approach, the authors demonstrated that specific miRNAs were differentially expressed in the peripheral blood of infected mice, and that the expression pattern varied with infection duration; 96 differentially expressed (33 upregulated and 63 downregulated) on day 3 after infection and 89 differentially expressed (37 upregulated and 52 downregulated) on day 6 after infection. Validation using RT-qPCR indicated that miR-216a and miR-710 were upregulated and miR-337 was downregulated on day 3 after infection. miR-216a and miR-713 were reported to be upregulated and miR-191 was downregulated on day 6 after infection. Bioinformatic analysis predicted several potential pathological roles of differentially expressed miRNAs including DCM, HCM, and arrhythmogenic right ventricular cardiomyopathy.

microRNAs and hypertensive dilated cardiomyopathy. Chronic high blood pressure can lead to DCM resulting in a hypertensive DCM. The prevalence of hypertensive DCM is estimated as 44% of all idiopathic DCM.[94] Strikingly, hypertensive DCM has a more rapid, favorable response to optimized therapy in terms of LV reverse remodeling than idiopathic DCM. Patients with hypertensive DCM have better long-term survival free from adverse outcomes, including cardiovascular death, heart transplant, arrhythmias, and ventricular assist devices.

Huang et al[95] have recently demonstrated that transgenic overexpression of miR-18 in cardiomyocytes protects against DCM during hypertension-induced HF. The p53/miR-18/heat shock factor 2/IGF-IIR axis was a critical regulator of cardiomyocyte hypertrophy in vitro and in vivo, and miR-18 was proposed as a therapeutic tool against hypertension-induced HF. The role of intracellular miRNAs in the regulation of the pathophysiological mechanism linked to hypertension-induced HF has been corroborated by an independent study.[96] Downregulation in the expression levels of members of the miR-221/222 family in mice led to increased fibrosis and LV dilation and dysfunction. Of note, the expression levels of miR-221 and miR-222 were downregulated in myocardial biopsies of patients with severe fibrosis and DCM compared to matched patients with nonsevere fibrosis.

microRNAs and toxic dilated cardiomyopathy. Alcohol and chemotherapeutic agents are the most common causes of toxic nonischemic DCM. In particular, alcoholism may represent one of the main factors responsible for nonischemic DCM in Western countries, accounting for 23%−37% of DCM cases.[97]

Deregulation in the expression levels of specific miRNA signatures, in addition to other ncRNAs such as circular RNAs (circRNAs), has been reported in a murine alcoholic cardiomyopathy model. In heart samples, Yang et al[98] demonstrated the differential expression of 19 miRNAs and 265 circRNAs in the alcohol-treated group compared with the control group. Bioinformatic analysis suggested that the molecular pathways that are affected are mainly associated with carbohydrate metabolism. Wang et al[99] explored in vitro the induction of cardiomyocyte apoptosis due to ethanol exposure in rat cardiomyocytes. Ethanol exposure caused apoptosis and reduced expression of alcohol detoxification enzyme aldehyde dehydrogenase 2 (ALDH 2). These effects were concomitant with higher intracellular miR-378a-5p levels. Moreover, the overexpression of miR-378a-5p in cardiomyocytes led to ethanol-induced apoptosis and ALDH 2 reduction, which suggests that miR-378a-5p plays a role in cardiomyocyte apoptosis in this condition.

microRNAs and peripartum dilated cardiomyopathy. Peripartum cardiomyopathy (PPCM), also known as postpartum cardiomyopathy, is an uncommon form of DCM that present with signs of HF during the last month of pregnancy or up to 5 months postpartum.[100] Related to the etiological factors, further studies are needed as the fundamental molecular processes are still uncertain. However, a critical role for late-gestational hormones on the maternal vasculature and a possible genetic basis has been proposed.[101]

Halkein et al[102] demonstrated that the cathepsin D-cleaved 16kDa form of nursing hormone prolactin (16K PRL), which may mediate PPCM,[103] induces the expression of miR-146a in endothelial cells and stimulates the release of miR-146a-loaded exosomes. This miRNA seems to participate in cell-to-cell communication. Indeed, exosomal miR-146a released by the endothelial cell was incorporated by cardiomyocytes in vitro and in vivo regulating their metabolism. In the same study, miR-146a levels in LV tissue were higher in patients with PPCM than in nonfailing organ donors or patients with end-stage HF due to DCM. Interestingly, plasma miR-146a expression was elevated in PPCM patients but not in patients with DCM, which suggest a potential diagnostic approach. Additionally, in patients who recovered after being treated with standard therapy for HF, miR-146a plasma levels were within the normal range. Therefore, miR-146a could be used as a novel tool to monitor disease recovery.

PERSPECTIVES

Over the last decade, an explosion of research on miRNAs has been observed. A considerable number of miRNAs are being identified as pathological mediators of DCM. Interestingly, specific alterations in their intracellular and circulating profiles seem to correlate with DCM and its various etiologies. Considering the evidence shown here, miRNAs are emerging as a helpful tool to explore the underlying pathological mechanism and for clinical decision-making.[49] The incorporation of miRNAs into current clinical workflows may provide substantial benefits to the patient and healthcare systems. Once the DCM phenotype is identified using current diagnostic tools, an easily-accessible and cost-effective test based on miRNAs may be useful for the identification of the specific underlying etiology which ultimately could affect the clinical management of the patient in terms of prognostication and therapy. The use of miRNAs as a gatekeeper for the inclusion or exclusion of patients with suspected DCM in subsequent invasive studies should also be considered. In addition, the potential of miRNAs to identify patients with pathogenic mutations associated with DCM in large population screenings, even when these subjects have not developed the disease, constitutes an alternative that should not be discarded.

Although miRNAs have been extensively studied in a wide array of diseases, numerous limitations must be addressed before they are incorporated into clinical practice, especially in the field of biomarkers. For instance, standard, reproducible, comparable methodology is still required when extracellular miRNAs are analyzed. Circulating miRNA quantification is influenced by sample collection, RNA isolation methods and the procedures and techniques used for miRNAs quantification and data analysis. All these variables contribute to the discrepancy in published miRNA studies.[53] Most of the studies presented here were performed in a small number of subjects with limited power to obtain convincing results. In addition, in most cases the research compared healthy controls with diseased patients, which could overestimate the accuracy of miRNAs as indicators. It is fundamental to explore the potential of miRNAs as biomarkers in patients with different DCM etiologies, a context that is closer to the real clinical problem. Larger, multicenter, and multiethnic studies are needed to fully evaluate the potential clinical application of miRNAs in DCM. Furthermore, and like other biomarkers, it is crucial to analyze the role of miRNAs as indicators in conjunction with a full clinical assessment and comparison with established biochemical tests.[104] The confounding of nondisease factors, demographic and clinical characteristics, and pharmacological treatments on miRNA levels should also be considered when the studies are designed. The information provided by ongoing clinical trials is therefore crucial to clinically adopt circulating miRNAs as biomarkers of cardiac disease in the medium- and long-term (www.clinicaltrials.gov; NCT03090763,

NCT03635255, NCT03855891), and more specifically as indicators of DCM (www.clinicaltrials.gov; NCT03076580) and conditions related to DCM (www.clinicaltrials.gov; NCT02109692, NCT02672683).

Importantly, the biology of ncRNAs in the circulation is far from being completely understood.[105] Indeed, the mechanisms that mediate their secretion, transport, and function in the target cell remain an active research area. Although the findings presented here point to a key role of miRNAs in the molecular pathways implicated in each DCM etiology, additional efforts are needed to determine their precise function. Additionally, the evaluation of molecular mechanisms and signaling pathways regulated by intracellular miRNAs in DCM pathology requires the acquisition of cardiac biopsies. However, these are performed in the end stages of the disease when it is clinically manifest. Consequently, reliable in vitro and in vivo models are essential to describe the molecular and biological processes mediated by intracellular and extracellular miRNAs in the various etiologies.

CONCLUSIONS

miRNAs are an attractive tool to facilitate the understanding of the disease mechanism, the identification of novel biomarkers for clinical management and the development of therapeutic strategies. Additional research is still needed to further explore the clinical application of miRNAs in DCM and their respective etiologies.

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