MicroRNAs in right ventricular remodelling

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Abstract

Right ventricular (RV) remodelling is a lesser understood process of the chronic, progressive transformation of the RV structure leading to reduced functional capacity and subsequent failure. Besides conditions concerning whole hearts, some pathology selectively affects the RV, leading to a distinct RV-specific clinical phenotype. MicroRNAs have been identified as key regulators of biological processes that drive the progression of chronic diseases. The role of microRNAs in diseases affecting the left ventricle has been studied for many years, however there is still limited information on microRNAs specific to diseases in the right ventricle. Here, we review recently described details on the expression, regulation, and function of microRNAs in the pathological remodelling of the right heart. Recently identified strategies using microRNAs as pharmacological targets or biomarkers will be highlighted. Increasing knowledge of pathogenic microRNAs will finally help improve our understanding of underlying distinct mechanisms and help utilize novel targets or biomarkers to develop treatments for patients suffering from right heart diseases.

Keywords

MicroRNAs • Right ventricular remodelling • Pulmonary hypertension • Noncoding RNAs

1. Right ventricular remodelling and pulmonary hypertension

Myocardial remodelling is a biological process reactive to physiologic or pathologic stimuli involving the transformation of cardiac wall structure and function. The long-term prognosis for patients depends on the heart’s ability to cope with altered hemodynamic status. The concept was coined by Pfeffer et al.¹ as the progressive transformation of the left ventricle following myocardial infarction. Since then, the myocardial remodelling process has been recognized as a common pathway for heart failure progression. Ventricular remodelling manifests as an adaptive transformation of the heart’s wall structure and the subsequent change in chamber geometry and wall thickness, increases in end-diastolic and end-systolic volumes. These parameters become inadequate as the heart undergoes a continuous decline in contractile function, reducing ejection fraction which ultimately leads to heart failure. The clinical importance of remodelling is highlighted by the broad consensus of clinical trial experts to recommend using the change in ventricular volumes as mechanistic endpoints in early heart failure trials.²

Assumptions of function and disease biology based on studies of the left ventricular remodelling can be generalized and applied to the right ventricle to some extent. However, the two ventricles differ in many fundamental ways, thus remodelling the right ventricle has distinct, unique elements unobserved in the left ventricle. The difference between the right and left ventricle is already devised at the developmental level; the RV originates from the secondary embryonic heart field and the LV from the primary heart field.³ The two chambers also differ in morphology and metabolism. The RV is a low-pressure chamber; it normally deals with a low impedance pulmonary circulation. As a result, the walls are thinner than in the LV, which is exposed to higher pressure conditions. Thus, it is not surprising that under pathologic conditions the two chambers display different responses.

The RV remodelling process is commonly triggered by pressure or volume overload, causing initial hypertrophy and subsequent maladaptive remodelling. The primary cause of altered hemodynamic conditions is pulmonary hypertension (PH) or pulmonary valve dysfunction, such as stenosis or regurgitation. In addition, any disease that affects the right ventricle may also initiate the remodelling process, such as reduction of myocardial contractility owing to ischemia (e.g. RV myocardial infarction), arrhythmias or various forms of cardiomyopathies.⁴ PH, defined as a resting mean pulmonary artery pressure (mPAP) at or above 25 mmHg⁵ is the leading cause of RV remodelling. PH can be of
idiopathic origin or secondary to lung diseases such as chronic obstructive pulmonary disease (COPD) or various forms of pulmonary fibrosis. As a result of increased afterload, initial adaptive transformation of the right ventricle occurs to maintain sufficient output. Over time, the transformation of the RV becomes maladaptive, leading to adverse morphological changes and functional decline. If not reversed, the clinical symptoms of right heart failure (cor pulmonale) will manifest leading to the death of the patient.

Our current understanding of the complex molecular and cellular mechanisms of ventricular remodelling is derived from various small and large animal models. There are RV-specific models that mimic many of the key underlying pathological features of RV remodelling, hypertrophy, and failure (for review, see 11). The rodent models of chronic hypoxia exposure and monocrotaline (MCT) induced lung injury have been central to the investigation of the effect of PH on RV remodelling.7 The partial ligation of the pulmonary artery (pulmonary artery banding) has been a frequently used procedure to induce pressure overload in the right ventricle, inducing rapid progression to failure. In addition to these ‘classical’ models, new, refined models have also been established to study some of the specific features of human pathology. The combination of a vascular endothelial growth factor receptor antagonist,8 Sugen 5416 (SUS416), and chronic hypoxia followed by normoxia (SU/Hx/Nx) induces pronounced PH accompanied by inflammation and angiobliteration and subsequent RV remodelling and failure in both rats and mice. The underlying mechanism is thought to involve primarily pulmonary artery endothelial cell death followed by the emergence of an apoptosis-resistant cell type. The return to normoxia induces the development of neointimal lesions closely resembling human plexogenic arteriopathy.8 Although the cellular and molecular mechanism and the interplay between various cellular elements of progressive RV remodelling and the development of cor pulmonale have been extensively studied, there are still many aspects that are less understood. In general, the hallmark of maladaptive transformation is the alteration of the cardiomyocyte phenotype in both the right and the left ventricle. Early adaptation due to increased workload manifests as reactive hypertrophy of functional cardiomyocytes. The increased burden on cardiomyocytes over time is accompanied by maladaptive, stress-related activation of their foetal gene program, which results in altered protein synthesis, defective excitation–contraction coupling, disturbed intracellular Ca2+ handling, loss of cardiac energy reserve, and apoptosis. Additional features of adverse remodelling include activation and proliferation of fibroblasts which result in the transformation and stiffening of the myocardial extracellular matrix, as well as the rearrangement of the coronary microvascular structure.7

2. MicroRNA biology

The recognition of non-coding genomic transcripts as regulators of biological and pathological processes was a fundamental discovery with wide ranging implications in cardiovascular research. Several classes of non-coding RNA transcripts have been identified and characterized. One particular class is the microRNAs that are short, up to 22 nucleotides long. They are essential regulators of RNA translation by binding to specific, complementary sequences, mainly in the 3’-untranslated region (UTR) of the target messenger RNA transcripts which leads to an inhibition of protein synthesis.10 MicroRNAs are abundantly expressed in all human cells, regulating the expression of thousands of protein-coding genes.11 Biogenesis and maturation of microRNAs has been extensively studied and reviewed elsewhere.12 The mature microRNA is incorporated into the RNA-induced silencing complex (RISC) where the microRNA interacts with its messengerRNA target leading to suppression of protein synthesis due to for example the degradation of the messenger RNA or blockade of ribosomal translation.10 The most important aspect of microRNA-mediated transcriptional regulation is the fact that an individual microRNA can affect the transcription of a specific set of genes, thereby having fundamental importance in regulating cellular pathways and biological functions in health and disease.13 During pathologic processes, specific and distinct microRNAs become dysregulated with dramatic consequences in cell function due to changes in protein synthesis. It is therefore unsurprising that many microRNA-related therapeutic strategies arose with the idea to modulate a single microRNA for the purpose of normalizing derailed gene expression in diseased tissues (reviewed in 14–16). Approaches to evaluate the distinct role of microRNAs in diseases associated with the right heart are essential to uncover mechanisms and therapeutic targets, specific to the right ventricle. A key clinical importance of such approaches has been highlighted by the results of several clinical trials suggesting that medications for left ventricular heart failure lack clear benefit when used for patients with cor pulmonale (Figure 1).17,18

The role of microRNAs in the context of PH induced RV dysfunction has been reviewed elsewhere.19 In this review, we summarize the latest developments in identifying specific microRNAs that have been associated with various stages of the RV remodelling process.

3. RV-specific expression profile of microRNAs

Evaluating the spatiotemporal microRNA expression profile in stressed and remodelled hearts is a key to better understand RV-specific mechanistic details. Fundamental differences in gene expressions between the left and right ventricle have been comprehensively studied in healthy and diseased hearts from animal models as well as in human samples.20 Expression of microRNAs in both healthy and diseased left ventricles has also been well documented.21,22 In contrast, data involving microRNA expression in the right ventricle is still scarce. MicroRNA expression profile of various cardiac regions including right ventricle and right atrium has been reported in a few animal studies,23 revealing that different microRNA cluster show region-specific expression patterns. For instance, miR-208 b levels were high in ventricles, but low in atria in three different species investigated (rat, dog, and monkey). The report did not identify major differences in microRNA expression under baseline conditions between the left and the right ventricle.24 However, it is highly likely that in response to various stressors and during disease conditions microRNA expression is differently affected in the two sides of the heart. Small but significant difference in the expression of miR-27 b and miR-34c were found between the normal left and right ventricle in rats.24 A recent report demonstrated that miR-223 expression levels in samples of murine lung and right ventricle are higher than in the left ventricle.25 This data underscore the side-specific difference of microRNA regulation in the heart. These differences may be attributable to the separate embryological development of the two chambers or to the hemodynamic differences, i.e. mechanical load affecting the chambers.
4. MicroRNAs in the pathogenesis of RV remodelling

Reddy et al. investigated microRNA expression patterns over a time period of 10 days in a murine model of pulmonary artery constriction. The increase in RV mass has been observed as early as 2 days after banding with further increase up to 10 days post banding. Expression analysis revealed that a distinct set of microRNA and their target gene expression were altered at early stage of RV hypertrophy. Those genes are associated with cardiomyocyte survival and growth (miR-199a). During the late phase of remodelling, different genes were altered, associated with the reactivation of the foetal gene program (miR-208b).

The transition from adaptive to maladaptive hypertrophy and heart failure was characterized by alteration of microRNAs associated with apoptosis and fibrosis (miRs-34, -21, -1). Not surprisingly, key differences between microRNA expressions in right and left ventricular remodelling have also been identified. MicroRNAs miR-34a, -28, -148a, and -93 were upregulated in the right ventricle and were at the same time downregulated during LV remodelling. In particular, the increase of miR-34a expression is of great interest as this microRNA has been found to be key in cardiac apoptosis and aging. The transition to maladaptive RV hypertrophy is associated with a decrease in RV- Hypoxia-inducible factor-1α (Hif1α) which is a target of miR-199a. Moreover, miR-199a also targets Smad3, thus potentially regulating Ca2+ concentration and NO release. Interestingly, miR-199a was increased in lung tissue of patients with COPD.

The progression of RV hypertrophy, from early remodelling to right ventricular failure was studied by Drake et al. using the models of pulmonary artery banding and chronic hypoxia. Early remodelling was associated with decreased expression of miR-21 and miR-34c*, whereas miR-133a was normal in the RV. In the later stage of RV failing, besides miR-21 and miR-34c*, the expression of miR-133a and miR-139-3p was also decreased, pointing out the different role of each individual microRNAs during the chronic remodelling process.

Researchers also explored the link between miR-193 and oxidized lipids. Mir-193 expression was significantly downregulated in the lungs of two experimental animal models of PH. Treatment with 4F, an Apolipoprotein A-I mimetic peptide, fully restored expression of miR-193 in pulmonary arterial hypertension PAH. Furthermore, restoration of miR-193 levels in the lungs reversed the decline of RV function due to PH in both the monocrotaline (MCT) and hypoxia model of PH. MiR-193 is also known to control SMCs proliferation, further supporting the role of miR-193 as a target in the pathology of PH and RV remodelling.

In a recent study, the beneficial effect of exercise training was demonstrated in a rodent model of RV hypertrophy and remodelling. Functional improvements due to training were accompanied by miR-1 downregulation in the right ventricle. However, it should be emphasized, these observations were only of associative nature.

The expression of 26 microRNAs was altered in the right heart under 21-day hypoxic conditions; among those, miR-223 was downregulated. In a different model of pulmonary artery banding, miR-223 was also downregulated in the lung and the right ventricle. Downregulation of
miR-223 was coupled with increased expression of the miR-223 target insulin-like growth factor-1 receptor (IGF-IR) and increased IGF-I downstream signalling. Conversely, miR-223 overexpression attenuated right-ventricular hypertrophy and improved right heart function. In human pulmonary hypertension tissue samples, miR-223 also decreased with concomitant IGF-IR increase.

Right and left cardiac tissue samples from rats with Sugen/Hx/Nx-induced PAH were analysed in a recent study using an unbiased quantitative microRNA microarray. In hypertrophic RV samples, miR-21-5p, -31-5, and 3p, -140-5 and 3p, -208b-3p, -221-3p, -222-3p, -702-3p, and -1298 were upregulated and miR-187-5p, -208a-3p, and -877 were downregulated compared to control samples. Interestingly, in the same group of animals with RV remodelling, only miR-31-5 and 3p and -208b-3p were upregulated in the left ventricle, revealing a distinct chamber specific pattern. The authors further validated the upregulation of miR-140 and miR-31 in the hypertrophic right ventricle with qPCR. Interestingly, mitofusin-1 (MFN1), a direct target of miR-140 and regulator of apoptosis, was downregulated in the RV relative to the left ventricle. The extent of miR-140 expression changes correlated with the degree of hypertrophic RV remodelling. Thus, the miR-140—MFN1 axis may also be further explored as a therapeutic target.

The potential role for the miR-214 stem loop microRNA and the closely linked miR-199a microRNAs were also investigated in the Sugen/Hx/Nx model. Expression levels of both strands of miR-214/199 were upregulated in the lung and right ventricle in both mice and rats exposed to Sugen/Hx/Nx induced PAH. Furthermore, miR-214 deficient mice exposed to SU/Hx/Nx induced PAH, showed a significant increase in RV hypertrophy. A direct target of miR-214, phosphatase and tensin homolog (PTEN) was upregulated in these mice. Thus, miR-214 is likely contributing to the PAH induced RV remodelling and may be exploited for therapeutic purposes.

In the MCT-induced PAH model of RV remodelling, the expression level of a specific set of microRNAs were significantly altered (Up: miRs17, 21, and 223; down: miRs126, 145, 150, 204, 424, and 503) compared to vehicle controls. Amongst those miR-223 was constantly upregulated in various organs such as the lung, RV, wall of pulmonary arteries, and blood.

The pathogenic role of miR-126 dysregulation in RV remodelling has been recently further confirmed. MiR-126 expression was downregulated in RV tissues from patients with decompensated cor pulmonale vs. patients with compensated hypertrophy, whereas left ventricular tissues were not affected. Lower levels of miR-126 de-repressed Sprouty-related EVH1 domain-containing protein 1 (SPRED-1), which in turn led to decreased activation of RAF (phosphorylated RAF/RAF) and mitogen-activated protein kinase (MAPK), thus inhibiting the vascular endothelial growth factor pathway. In contrast, therapeutic activation using miR-126 mimics resulted in better cardiac vascular density and function.

Typical characteristic of severe PH in humans is the presence of pulmonary plexiform and concentric obliterative vascular lesions. Using laser-assisted microdissection with microRNA expression analysis, miR-143/miR-142 and let-7e-miR-140—MFN1 axis may also be further explored to reduce susceptibility to fatal arrhythmias.

In another study, microRNA expression was assessed in cardiac tissue samples of ARVC patients. The tissue levels of 1078 human microRNAs were assessed and compared to the control group. Statistical analysis found 21 validated microRNAs to be signatures of ARVC. 11 and 10 microRNAs were significantly increased or decreased in ARVC heart tissues, respectively, compared to healthy controls. Of those, miR-21-5p and miR-135b were correlated with Wnt and Hippo pathways by functional enrichment analysis. Analysis of the latter showed significant changes in miR-21-5p and miR-135b expression, which are potential disease pathways for arrhythmogenic right ventricular cardiomyopathy and, therefore, might be evaluated as therapeutic targets in the future (Table 1).

### 6. MicroRNAs in arrhythmogenic RV remodelling

A highly relevant aspect of heart failure characteristics is the increased propensity for arrhythmias due to arrhythmogenic remodelling. Arrhythmic right ventricular cardiomyopathy (ARVC) is a type of primary cardiomyopathy characterized by fibro-fatty replacement of right ventricular myocardium, a main cause for sudden death in young people and athletes.

Desmosome proteins have been implicated in this process and, interestingly Connexin43 and Desmocollin2 (DSC2) were found as targets of miR-130. Mice overexpressing miR-130a had a right ventricular dilation and arrhythmic phenotype. Thus, the miR-130—desmosome protein axis may be further exploited to reduce susceptibility to fatal arrhythmias.

In another study, microRNA expression was assessed in cardiac tissue samples of ARVC patients. The tissue levels of 1078 human microRNAs were assessed and compared to the control group. Statistical analysis found 21 validated microRNAs to be signatures of ARVC. 11 and 10 microRNAs were significantly increased or decreased in ARVC heart tissues, respectively, compared to healthy controls. Of those, miR-21-5p and miR-135b were correlated with Wnt and Hippo pathways by functional enrichment analysis. Analysis of the latter showed significant changes in miR-21-5p and miR-135b expression, which are potential disease pathways for arrhythmogenic right ventricular cardiomyopathy and, therefore, might be evaluated as therapeutic targets in the future (Table 1).

### 5. MicroRNAs in congenital heart diseases

Most congenital heart defects affect the pulmonary circulation to a varying degree and the ensuing pressure and volume overload can lead to the development of PH, RV remodelling and heart failure, unless repairs take place in early childhood. There are a few reports exploring microRNA expression changes in congenital heart disorders. Tetralogy of Fallot (TOF) is a congenital heart defect characterized by a large ventricular septal defect and stenosis of the right ventricular outflow tract, leading to right ventricular hypertrophy and failure. One of the first studies in this area characterized microRNA expression in RV tissue samples from infants with nonsyndromic TOFs compared to tissues from foetal myocardium and myocardial tissues from normal infants. In total, 61 microRNAs were identified to be significantly altered in TOF samples. Interestingly, the microRNA expression profile bore a striking resemblance to the pattern of foetal heart tissue, suggesting a foetal microRNA program reactivation during TOF-related development of hypertrophy and adverse remodelling. A similar reactivation of foetal gene program is also evident during the development of left ventricular heart failure.

In a similar study, 18 microRNAs were identified as being deregulated in RV outflow tract tissues samples from infants with nonsyndromic TOF (miR-146-5p, -155, -19a, -222, -424, -337-5p, -363, -130b, -154, -708, -181c, -424a, -181d, -192, -660, -29c, -720, -181a). Target gene network analysis showed that 16 of the 18 microRNAs targeted 97 genes that are involved in heart development. Further analysis indicated that miR-424/424a and miR-222 are involved in hypertrophy and remodelling processes.

Ventricular septal defect (VSD) is another common type of congenital heart disease. Differential regulation of eight microRNAs was found in VSD and gene ontology analysis indicated that their top target genes were mainly related to cardiac right ventricle morphogenesis (e.g. let-7e-5p, miR-222-3p, -433, and NOTCH1, HAND1, ZFPM2, and GATA3 as predicted as targets).
In summary, dysregulation of microRs has been associated with structural abnormalities in the heart. Our understanding of the microRNA signalling pathways will help to uncover new targets and biomarker patterns in the development of congenital heart diseases.

7. Cell-type specific MicroRNA changes during remodelling

One of the major shortcomings of most studies is that microRNA changes were investigated in healthy or diseased whole heart tissue but lack cell-type specific data (e.g., fibroblasts, cardiomyocytes, and endothelial cells). Given the fundamental structural changes during the development of heart failure some of the reported expression changes of microRNAs (e.g., left vs. right ventricle, failing vs. non-failing) may only reflect changes in the heart’s cell composition. For instance, a fibroblast-specific microRNA may be overrepresented in the fibrotic heart due to a higher fibroblast:cardiomyocyte ratio but not because of a higher fibroblast-specific expression in comparison to healthy heart. In contrast, true cell-type specific microRNA differences during remodelling may be masked by the accompanying cell-types when analyzing whole tissue. Indeed, one of the very limited studies characterizing tissue- and cell-type specific microRNA changes demonstrated that in canine model of heart failure by ventricular tachypacing no changes were detected when analyzing LV tissue, whereas cell-selective analysis revealed changes in fibroblasts and cardiomyocytes. Strikingly, some cardiomyocyte specific microRNAs (miR-1, miR-208b, and miR133a/b) changed almost exclusively in fibroblasts in this model. This report therefore highlights that future studies should address both tissue- and cell-type specific expression changes.

8. MicroRNA therapeutic approaches in RV remodelling

The expression level of microRNAs may be modified in vivo by the application of specific microRNA inhibitors or mimics. Specific synthetic antisense oligonucleotides against microRNAs (AntimiR ASO) are a promising therapeutic approach to pharmacologically modulate pathogenic microRNAs. Several chemical classes of ASO have been proposed with distinct features. Many of the AntimiR ASO can be systematically applied and there are several reports about the successful use of microRNA modulators as a potential therapy for PH and RV diseases. For instance, the miR-145/143 cluster is well known to play a significant role in smooth muscle cell biology and disease pathology. This cluster also plays a dominant role in the lung and pulmonary circulation, for example during the development of increased pulmonary resistance and subsequent PH. In a hypoxia model of PAH in mice, both genetic and pharmacologic loss of miR-145 were protective. Indeed, RV systolic pressure and sign of RV hypertrophy were reduced by miR-145 inhibition in comparison to the placebo. Therefore, pharmacological inhibition of miR-145, acting on pulmonary vasculature, represents a therapeutic
opportunity to treat PAH thereby reducing pressure overload and thus indirectly preventing RV remodelling leading to cor pulmonale. 47

Along these lines, in a mouse model of PAH, due to chronic hypoxia, pharmacological treatment with AntimiR ASO against miR-17 reduced right ventricular pressure and RV remodelling, representing an additional potential therapeutic approach. 48

As previously mentioned, miR-223 is a potential target in RV remodelling. Tissue levels of increased miR-223 levels in lungs and in the wall of pulmonary vessels were effectively silenced in rats with MCT-induced PAH using systemic application of AntimiR ASO against miR-223. The inhibitor, however, did not attenuate the clinical picture of MCT-induced PAH, neither RV systolic pressure nor RV mass. In contrast, analysis of lung specimens of MCT-induced PAH rats overexpressing human prostacyclin synthase (hPGIS) demonstrated reversal of MCT-induced microRNA alterations, among others upregulation of miR-223. The expression of bone morphogenetic receptor 2 (BMPR2) was not altered by miR-223 inhibition. Expression of hPGIS, on the other hand, restored BMPR2 mRNA to levels in MCT-induced PAH to control lev-

els. 34 Taken together, this study failed to confirm the efficacy of a specific AntimiR ASO, but the target’s therapeutic utility may be further explored using different chemistries.

In some cases, the therapeutic approach requires enhancing tissue level-
s of microRNA in vivo. For that, microRNA ‘mimics’ have been developed. MicroRNA mimics are small, synthetic, chemically modified double-stranded oligonucleotides, analogues to the endogenous microRNA. Mimics are taken up by the RISC complex and function like endogenous microRNAs.

Such an approach has been successfully tested for miR-204 which is suppressed in lung tissue from patients and from animal with pulmonary hypertension. 49 Delivery of a synthetic mimic to restore miR-204 levels significantly reduced disease severity in the lung and reversed the concomitant symptomatic of RV hypertrophy and failure in animals with MCT-induced PAH. Moreover, miR-126 is a key player in developmental angiogenesis and essential for vascular integrity. Its role has been established in the pro-
gression of RV remodelling where RV tissue levels of miR-126 negatively correlate with disease severity. The use of a specific miR-126 mimic to restore tissue levels, improved overall RV function by increasing cardiac vascular density and reducing fibrosis, even if it was applied in the late stage of RV remodelling in an MCT-induced rodent PAH model. 35 Thus, restoration of miR-126 levels in PAH is a likely promising treatment approach for patients with PAH induced cor pulmonale.

In summary, there is compelling pre-clinical data on microRNA-based therapeutics in RV heart disease with regards to both microRNA inhibi-
tory and activating approaches. These studies may provide a foundation for further clinical development of such approaches in the future. However, major concerns such as pharmacokinetics/dynamics, targeted delivery and potential adverse long-term effects need to be addressed before microRNA-based therapeutics will become clinical reality.

9. Circulating microRNAs as potential biomarkers for RV remodelling

Circulating microRNAs are attractive potential biomarkers and have been explored in a variety of cardiovascular diseases. 50 Several microRNAs have been suggested to be a potential diagnostic biomarker of left ventricular function. 51 On the other hand, studies focusing on microRNA biomarkers in diseases associated with the right heart are still uncommon.

For instance, miR-423_5p has been associated with left ventricular heart failure. In contrast, patients with systemic right ventricle, reduced ejection fraction and heart failure after atrial repair for the congenital condition of transposition of the great arteries did not show elevated levels of circulating miR-423_5p. 52 These results reinforce the notion that distinct microRNA regulatory pathways or secretory mechanisms exist for the right ventricle.

In patients with RV pressure overload due to PH, the circulating levels of miR-1, miR26a, miR-29c, miR-34b, miR-451, and miR-1246 were lower and the levels of miR-21, miR-130a, miR-133b, miR-191, miR-204, and miR-208b were elevated in comparison to matching controls. 53 From those microRNAs, the altered level of miR-21 and miR-29c have been shown to be associated with myocardial fibrosis, indicating a me-

chanistic link to the pathogenesis of pulmonary hypertension and conse-
quent RV remodelling. 21,22 Alteration of cardiomyocyte specific microRNAs such as the elevation of circulating miR-133b and miR-208b may reflect cardiac stress and adverse remodelling for both ven-

tricle. Indeed, a recent biomarker study confirmed the correlation between the severity of PH and circulating levels of miR-133b and -208b. 53

Another study found strongly reduced circulating miR-150 levels in patients with chronic PAH and cor pulmonale. Plasma miR-150 levels were an independent predictor of survival in PAH, showing potential prognostic power of miR-150 in this disease. 54

Moreover, the expression profile of circulating microRNAs in congeni-
tal heart malformations with a systemic right ventricle was assessed in patients late after atrial switch operation for complete transposition of the great arteries. The relationship between alteration of circulating microRNAs and systemic ventricular contractility were evaluated. Out of 23 identified microRNAs, 11 were validated to be upregulated in patients compared with controls: miR-16, -106a, -144*, -18a, -25, -451, -486-3p, -486-5p, -505*, -93, and let-7e. Among those, miR-18a and -486-

5p negatively correlated with ventricular function. 56

Additional studies are warranted to explore novel or re-evaluate cir-
culating microRNAs in a systematic way that may be useful to assess right ventricular function and predict outcome in patients with RV remodelling and cor pulmonale.

10. MicroRNA approaches in clinical development

As discussed in this review, several microRNAs are dysregulated in right heart pathologies. Studies in animal models have demonstrated that their manipulation is sufficient to halt or ameliorate specific disease processes. This was often achieved by genetic manipulation, but more relevant to the clinical setting, through pharmacological modulation of microRNAs. The development of antisense oligonucleotide tools, designed for steric blocking of microRNAs, and the development of novel delivery tools for microRNA mimics, has spurred interest in developing treatments of cardiac diseases. The implementation of the concept of pharmacological inhibition of microRNAs into clinical development of antisense oligonucleotide microRNA inhibitors underwent an astonishing progress in the recent years. The first example is Miravirsen, a specific miR-122 inhibitor for the treatment of patients with chronic hepatitis C virus infection. Importantly, a phase 2a study of Miravirsen revealed a prolonged dose-
dependent reduction of viral RNA in the absence of dose-limiting adverse events or the occurrence of viral resistance.\textsuperscript{56} Drawing on the success, several companies are currently developing various antisense oligo inhibitors against miR-122. A more recent example is miR-21 inhibition in Alport syndrome. Alport syndrome is an inherited form of kidney disease caused by mutations in collagen genes. AntimiR-21 treatment in patients with cutaneous T-cell lymphoma (CTCL, mycosis fungoides subtype).\textsuperscript{57} MI-155 is found at high levels in malignant T-cells of many mycosis fungoides patients and miR-155 is a promoter of growth and survival of these cancer cells.\textsuperscript{58} The inhibitor is currently tested by local administration directly into CTCL lesions in the skin. Both trials are currently ongoing, reports are expected at the earliest by late 2017. Another treatment approach is to enhance microRNA activity using mimics. A miR-29 mimic decreases the expression of collagen and other proteins that are involved in scar formation in preclinical models.\textsuperscript{59} A miR-29 mimics is currently clinically evaluated for safety, tolerability, and pharmacokinetic properties.

11. Conclusion and outlook

The discovery of microRNA and other noncoding RNAs that are involved in transcriptional regulation has fundamentally transformed our understanding of biological processes and disease development. Many general, microRNA-mediated mechanisms have been described in the left ventricle, which were also confirmed in the RV. More importantly, many RV-specific pathways have been identified. These efforts lead to the identification of novel targets that may be exploited as biomarkers or therapeutic targets. First therapeutic approaches have shown indirect or direct efficacy of microRNA modulating strategies in several animal models of RV remodelling and related diseases. Studies with novel microRNA therapeutics need to evaluate their efficacy in preventing or slowing down the progression of RV remodelling and development of cor pulmonale. Beyond the discovery process, the diagnostic and prognostic potential of individual circulating microRNAs and microRNA patterns need further validation in large patient cohorts before they can be implemented in therapeutic guidelines. Despite the hurdles for translation that still need to be overcome, when considering the potential for miRNA it is likely that microRNA based tools will help patients with RV remodelling and related diseases in the future. Ongoing and anticipated studies will also access the role of other classes of noncoding RNAs in RV biology, such as long noncoding RNAs and circular RNAs.\textsuperscript{60}

Disclosures

T.T. filed and licensed patents about microRNAs. T.T. and S.B. are co-founders of Cardior Pharmaceuticals GmbH.

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