MicroRNA-140 is elevated and mitofusin-1 is downregulated in the right ventricle of the Sugen5416/hypoxia/normoxia model of pulmonary arterial hypertension.

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MicroRNA-140 is elevated and mitofusin-1 is downregulated in the right ventricle of the Sugen5416/hypoxia/normoxia model of pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol 311: H689–H698, 2016. First published July 15, 2016; doi:10.1152/ajpheart.00264.2016.—Heart failure, a major cause of morbidity and mortality in patients with pulmonary arterial hypertension (PAH), is an outcome of complex biochemical processes. In this study, we determined changes in microRNAs (miRs) in the right and left ventricles of normal and PAH rats. Using an unbiased quantitative miR microarray analysis, we found 1) miR-21-5p, miR-31-5p and 3p, miR-140-5p and 3p, miR-208b-3p, miR-221-3p, miR-222-3p, miR-702-3p, and miR-1298 were upregulated (>2-fold; P < 0.05) in the right ventricle (RV) of PAH compared with normal rats; 2) miR-31-5p and 3p, and miR-208b-3p were upregulated (>2-fold; P < 0.05) in the left ventricle plus septum (LV+S) of PAH compared with normal rats; 3) miR-187-5p, miR-208a-3p, and miR-877 were downregulated (>2-fold; P < 0.05) in the RV of PAH compared with normal rats; and 4) no miRs were up- or downregulated with >2-fold in LV+S compared with RV of PAH and normal. Upregulation of miR-140 and miR-31 in the hypertrophic RV was further confirmed by quantitative PCR. Interestingly, compared with control rats, expression of mitofusin-1 (MFN1), a mitochondrial fusion protein that regulates apoptosis, and which is a direct target of miR-140, was reduced in the RV relative to LV+S of PAH rats. We found a correlation between increased miR-140 and decreased MFN1 expression in the hypertrophic RV. Our results also demonstrate that upregulation of miR-140 and downregulation of MFN1 correlated with increased RV systolic pressure and hypertrophy. These results suggest that miR-140 and MFN1 play a role in the pathogenesis of PAH-associated RV dysfunction.

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miR; heart; hypertrophy; heart failure; pulmonary; hypertension; lungs; rats

NEW & NOTEWORTHY

We show that in an experimental model of severe pulmonary arterial hypertension, miR-140 is significantly upregulated in the hypertrophic right ventricle, and its target protein mitofusin-1 is significantly downregulated. The increase in miR-140 is significantly correlated with increase in right ventricular systolic pressure and right ventricle hypertrophy.

THE PATHOPHYSIOLOGY of pulmonary arterial hypertension (PAH) is heterogeneous. In PAH, pulmonary vascular resistance and arterial pressure are increased, and the right ventricle (RV) is hypertrophied in response to increased afterload-pressure overload. Increased afterload in PAH leads to myocardial dysfunction, tricuspid regurgitation with progressive annular dilatation, and ultimately RV failure (67).

Heart failure, a major cause of mortality in PAH patients (4, 5), is a complex syndrome. In the failing hearts, oxidative stress is increased and the rate of cardiomycocyte apoptotic and autophagic death is augmented (6, 49, 51, 60, 62). Activation of these processes causes myocardial stiffening, dilatation, and loss of contractility (49, 51). However, the mechanisms that regulate apoptosis and autophagy in this complex pathology are unclear and are an area under investigation.

MicroRNAs (miRs) are a class of small noncoding RNAs that regulate gene expression by either inhibiting mRNA translation or promoting mRNA degradation (8, 29). The miR profile is altered in various left heart cardiomyopathies compared with nonfailing hearts (8, 14, 66). Several studies have shown that miRs are involved in the pathogenesis of left heart failure (7, 9, 24, 66), and that manipulation of miR expression is a promising therapeutic target (8, 24). In this regard, very little work has been done to evaluate the profile of miRs in the right and left ventricles, and the roles of various miRs in the pathogenesis of PAH-associated heart failure remains elusive. Also, whether increased afterload or unknown factors released from diseased lungs mediate right and left heart failure in PAH is still unclear. We postulated that the miR profile in the hypertrophic RV, induced by increased afterload, would be different from that in the left ventricle (LV) in Sugen5416/hypoxia/normoxia (Su/Hx/Nx)-induced PAH rats, as well as that in the RV of normotensive rats. Therefore, the objective of this study was to determine miR profiles and validate the expression of their targets in the RV vs. LV of control vs. PAH rats.

METHODS

Animal model of pulmonary arterial hypertension. All animal procedures were approved by the Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (200–250 g) were injected with 20 mg/kg Sugen-5416 (Cayman Chemical, Ann Arbor, MI) subcutaneously and exposed to normobaric hypoxia (10% O2) for 3 wk followed by exposure to normoxia (21% O2) for an additional 5 and 10 wk to establish 8-wk and 13-wk Su/Hx/Nx PAH as described previously (1, 63). At 8 and 13 wk, hemodynamic measurements were performed. After hemodynamic measurements, the heart was harvested and RV free wall was carefully separated from LV plus septal wall (LV+S). The ratio of RV to LV+S mass (Fulton’s index) was...
measured to assess RV hypertrophy. Immediately after this measurement, protocols were snap-frozen in liquid nitrogen.

**Hemodynamic.** Hemodynamic measurements were performed as described previously (41). Briefly, at the end of the protocol, rats were anesthetized by 2% isoflurane, and 1–2% isoflurane was used to maintain anesthesia for the entire duration of the surgery and data acquisition. Body temperature of the animal during surgery was maintained with a heating pad. The right jugular vein was then carefully exposed, and a liquid-filled catheter was inserted and pushed into the RV to measure right ventricular systolic pressure (RVSP) as described previously (41).

**MicroRNA microarrays.** Snap-frozen RV and LV tissue samples were homogenized in 700 μl QIAzol (Qiagen, No. 79306) as described previously (41). MiRs were extracted from homogenized tissues by using the miRNeasy Mini Kit (Qiagen, No. 27994). Total RNA concentration and quality were determined by absorbance at 260/280 nm. Total RNA quality was also determined by intact 28S and 18S rRNA and their ratios (28S/18S) of ~2:1, and miR expression profiling was then performed by using 0.5–2 μg total RNA on miRCURY LNA microRNA Arrays (v.11.0) by Exiqon. The array contained >1,700 locked nucleic acid modified oligonucleotide capture probes covering most of the known human, mouse, and rat miR sequences at the time of the assay. Samples of RNA from each animal were labeled with Hy5 dye and used as a common reference standard for interslide normalization. Intraslide normalization of expression data was performed by locally weighted scatterplot smoothing.

**MicroRNA expression by quantitative PCR.** MiR analysis was performed by quantitative PCR (qPCR) as previously described (11). Briefly, total RNA was extracted from the RV and LV + S with a Qiagen miRNeasy kit (No. 217004). Input RNA quality and concentration were measured on NanoDrop and cDNA was prepared by miR-specific TaqMan miR assays (Applied Biosystems, Foster City, CA). qPCR was performed in triplicates with TaqMan Universal PCR Master mix (No. 4324018). The primers for the miR-140 (Assay ID No. 001117), miR-31 (Assay ID No. 000185), and U6 (Assay ID No. 001973) were purchased from Thermofisher Scientific/TaqMan. Results were normalized to U6, and relative expression of microRNA was determined by ΔΔCt method as described previously (38).

**Cell culture.** H9c2 cells were maintained under 5% CO2 at 37°C in Dulbecco’s modified Eagle’s medium supplemented with l-glutamine, 4.5 g/l glucose (GIBCO, No. 11995-065), and 10% fetal bovine serum (GIBCO, No. 10082-147). Cells at ~70% confluence were subcultured weekly with 0.05% 100 trypsin-EDTA (GIBCO, No. 25300-054, Thermo Fischer Scientific, Grand Island, NY). About 3 × 10⁵ cells per well were plated in a 6-well plate. Next day, it was transfected with miRIDIAN microRNA Mimics, no-miR-140-5p mimic (50nM) (Dharmacon, No. C-320700-00) or Mimic Negative (Scramble) Control (50nM) (Dharmacon, No. CN-001000-01). Forty-eight hours posttransfection, cells were washed three times with 1X PBS and harvested by using NP-40 lysis buffer (50 mmol/l Tris HCl pH 7.4, 150 mmol/l NaCl, 0.5% NP-40) containing protease and phosphatase inhibitors (1X) (Roche Life Sciences). GAPDH was used as loading control, and protein levels were analyzed by densitometric analysis with ImageJ software.

**Mitochondrial DNA quantification.** Total DNA was extracted from H9c2 cells after 48 h of transfection with miRIDIAN microRNA Mimics, no-miR-140-5p mimic (Dharmacon, No. C-320700-00), or Mimic Negative (Scramble) Control (Dharmacon, No. CN-001000-01) with DNasey Blood & Tissue Kit (Qiagen, No. 69504) following manufacturer’s protocol. Mitochondrial DNA (mtDNA) copy number relative to nuclear DNA (nucDNA) copy number was quantified by qPCR using the equation: relative mtDNA content = 2^ΔΔCt, where Ct = (nucDNA CT – mtDNA CT) as described previously (53). To quantify the amount of mtDNA, the primer set located within the mitochondrial 16s RNA region was used, which are 5’-CCATTTATAGCTAGGATCCCTATGCTG-3’ Tm=59 and 5’-AGGGTAAAGCATATCCGTTG-3’ Tm = 62. To quantify the amount of nuclear DNA, the primer set located within the actin region was used, which are 5’-GGGATTGATAGCCGTAGCCCTTTT-3’ Tm=61 and 5’-GCGGTGCCCTAGAGATATCC-3’ Tm = 62.

**Mitochondrial membrane potential measurement.** Mitochondrial membrane potential (Δψm) was measured by Fluorescence-activated cell sorting (FACS) by using JC-1 (5,5’,6,6’-tetrachloro-1,1’,3,3’- tetracythylbenzimidazolocarbo-cyanine iodide) dye. It exhibits potential-dependent accumulation in mitochondria, indicated by formation of J-aggregates in energized mitochondria emitting red fluorescence (~590 nm). This is spectrally distinguishable from dye monomers emitting green (~488–529 nm) fluorescence. Mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio (55). For FACS study, H9c2 cells were transfected with miR-140 mimic or scramble control in the presence and absence of etodethlin-1 (ET1) (10 nM) for 48 h, followed by incubating them with 1 mM JC-1 for 30 min at 37°C. Cells were washed with 1X PBS, trypsinized, and transferred to a centrifuge tube. Cells were then washed twice in 1X PBS, and analyzed with a GuavaEasyCyte Mini (Millipore, Billerica, MA) flow cytometer. The relative Δψm was expressed as the ratio of the red-to-green fluorescence.

**Protein extraction and Western blotting.** Tissue samples were pulverized in liquid nitrogen and added to NP-40 lysis buffer (50 mmol/l Tris HCl pH 7.4, 150 mmol/l NaCl, 0.5% NP-40) containing protease and phosphatase inhibitors (1X) protease and phosphatase inhibitors (Roche Life Sciences). This was followed by centrifugation at 12,000 rpm for 15 min at 4°C, and then the supernatants were collected, discarding the pellets. These supernatants were used to obtain protein (20 μg) for Western blot analysis and to measure protein content. After mixing with 5X loading buffer, the protein samples were boiled for 5 min and then subjected to 10% SDS-PAGE, transferred to nitrocellulose membranes which were blocked by incubation in blocking buffer (5% Bovine Serum Antigen); followed by incubation with specific primary antibodies, mitofusin-1 (MFN1) (1:1,000, No. ab57602, Abcam), tubulin-1α (TUBA1A) (1:1,000, No. sc5286, Santa Cruz), c-kit (1:1,000, No. sc168, Santa Cruz), and GAPDH (1:1,000, No. 2118S, Cell Signaling), washed; and incubated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz). Signals were visualized by chemiluminescence detection (SuperSignal West Pico Chemiluminescent Substrate, Thermo scientific). GAPDH was used as loading control, and protein levels were analyzed by densitometric analysis with ImageJ software.

**Statistical analysis.** Values are means ± SE of the number of samples (n) from different animals. Statistical analysis was performed with a paired Student’s t-test, and a one-way ANOVA with Bonferroni correction was used for comparing multiple groups. P < 0.05 was used to establish statistical significance.

**RESULTS**

**RVSP and RV/LV+S are increased in PAH rats.** Right heart catheterization results indicate that RVSP was elevated in 8- and 13-wk Su/Hx/Nx PAH rats compared with normal rats (Fig. 1A). RV hypertrophy, measured as RV/LV+S, was similarly increased in the PAH rats (Fig. 1B). However, the RV hypertrophy was slightly lower in the 13-wk than in the 8-wk PAH rats (Fig. 1B), as observed previously by Tobia et al. (64). RVSP in the control and PAH rats was positively correlated with the respective RV/LV+S. Consistent with our previous studies (2, 49), the PAH rats were not in dilated cardiomyopathy stage at either 8 or 13 wk.

**miR-140 and miR-31 are elevated in the hypertrophic RV of PAH rats.** The degree of RV hypertrophy was greater at 8-wk than at 13-wk in PAH rats (Fig. 1B). Therefore, to investigate the miR signature of the hypertrophic RV, a microRNA microarray was performed in the RV of 8-wk PAH and normal

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rats. From an unbiased quantitative analysis, we found that a total of 304 miRs were either up- or downregulated in the hypertrophic RV. Out of these miRs, miR-21-5p, miR-31-5p, miR-31a-5p, miR-31a-3p, miR-140-3p, miR-208b-3p, miR-221-3p, miR-702-3p, and miR-1298 were upregulated (>1 logFC; P < 0.05), and miR-187-5p, miR-208a-3p, and miR-877 were downregulated (>1 logFC; P < 0.05) (Fig. 2A).

miR-140, which was upregulated in the hypertrophic RV (Fig. 2A), directly targets mitofusin-1 (MFN1) protein, one of the important mitochondrial fusion proteins that modifies cell function and plays a role in cardiomyocyte death (35). Although a recent study indicated a role for miR-140 in the pathogenesis of PAH (54), very little is known about the role of miR-140 in the PAH-associated hypertrophic RV. Furthermore, in the microRNA microarray analysis, miR-140 was the second highly upregulated microRNA (Fig. 2A). Therefore, we confirmed that the levels of miR-140 were elevated in hypertrophic RV by qPCR (Fig. 2B). However, the level of miR-140 was significantly lower in 13-wk PAH RV than in 8-wk PAH RV (Fig. 2B). Interestingly, increased miR-140 levels in the RV positively correlated with the elevated RVSP (Fig. 2, C and E) and the increased RV hypertrophy (Fig. 2, D and F).

Since miR-31 regulates the migration of endothelial progenitor cells to form microvascular tubes (68), we also confirmed elevated miR-31 levels in the hypertrophic RV of PAH rats by qPCR (Fig. 3A). A positive correlation (P < 0.05) was found between the increased miR-31 levels and elevated RVSP (Fig. 3, B and D) and RV hypertrophy (Fig. 3, C and E).

Expression of MFN1 is downregulated in the hypertrophic RV of PAH rats. Since Mfn1 gene is a direct target of miR-140 (35) (Fig. 4A), we investigated the expression levels of MFN1 in RV and LV of normal and PAH rats. We first tested the effects of a miR-140 mimic on MFN1 expression in cultured cells. Transfection of 50 nM miR-140 mimic in H9c2 cells decreased 1) expression of MFN1 by ~30% (Fig. 4, C and D) and 2) mitochondrial DNA content by ~32% (Fig. 4E). However, transfection of miR-140 mimic did not change mitochondrial potential either in the presence or absence of ET1 (10 nM; Fig. 4F). In the hypertrophic RV of 8-wk PAH rats, the expression of MFN1 was significantly deceased (Fig. 5, A and B). However, the expression of MFN1 in the RV of 13-wk PAH rats was not different from that in the normal RV (Fig. 5, A and B). Importantly, at 8 wk of PAH the elevated RVSP, RV hypertrophy, and increased miR-140 positively correlated with the decreased MFN1 expression (Fig. 5, C–E). However, at 13 wk of PAH, the elevated RVSP, RV hypertrophy, and increase in miR-140 levels did not correlate with the change in MFN1 expression (Fig. 5, F–H).

Expression of c-kit and TUBA1A are upregulated in the hypertrophic RV of PAH rats. Because miR-31 is associated with migration of c-kit+ endothelial progenitor cells (27), we investigated the expression levels of c-kit in RV and LV of normal and PAH rats. The c-kit expression was significantly higher in hypertrophic RV of 8-wk PAH rats than in normal RV (Fig. 6, A and B). Interestingly, at 13 wk, the expression level of c-kit in the RV was significantly lower than that in 8-wk PAH RV (Fig. 6, A and B). Importantly, the elevated RVSP, RV hypertrophy, and increased miR-140 positively correlated with increased c-kit expression at 8 wk (Fig. 6, C–E) but not at 13 wk (Fig. 6, F–H).

TUBA1A was also increased in the RV of both 8- and 13-wk PAH vs. control rats (Fig. 7, A and B). Notably, a correlation (P < 0.05) was found between the increased TUBA1A and elevated RVSP and RV hypertrophy (Fig. 7, C–F). The internal control GAPDH was not different between the RV free wall of the PAH vs. the control rats (Fig. 7, G and H).

MiR-208b is elevated in LV+S of PAH rats. We also estimated the microRNA expression levels in LV+S of PAH rats. From an unbiased quantitative analysis, we found that miR-208b-3p, miR-31a-5p, and miR-31a-3p were upregulated (Fig. 8) in the LV+S of 8-wk PAH compared with that of the control rats.

Expression of miRs in RV vs. LV+S of PAH rats. Although 304 miRs were differentially regulated in RV, no miRs were up- or downregulated with logFC>1 compared with LV+S of normal and PAH rats.
**DISCUSSION**

RV remodeling in response to increased afterload is a complex process that involves 1) changes in expression of cytoskeletal proteins in cardiac myocytes, 2) increase in cardiomyocyte size, and 3) proliferation of noncardiomyocytes in the myocardium (12, 13, 58). Emerging studies suggest that miRs play a critical role in regulating expression of proteins that are involved in gene regulation, cell proliferation, and cell apoptosis (8, 29). Accordingly, using an unbiased quantitative miR microarray analysis, we found that the miRs that increase cardiac fibrosis and hypertrophy

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**Fig. 2.** MiroRNA-140 is elevated in RV Su/Hx/Nx rats compared with RV normoxic rats. A: a subset of microRNA determined by unbiased microRNA microarrays that are differentially regulated more than 1 logFC between normal right ventricle (Nx RV) and hypertrophied RV at 8-wk Su/Hx/Nx PAH rats. B: qPCR validation of increase in miR-140 in the RV of the Su/Hx/Nx PAH rat. C: Pearson correlation between RVSP and miR-140 in the RV of 8-wk Su/Hx/Nx PAH rats. D: Pearson correlation between RVSP and miR-140 in the RV of normoxic rats. *P < 0.05 vs. normoxic control. #P < 0.05 vs. 8-wk Su/Hx/Nx. Data are means ± SE (n = 3–4).

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**Fig. 3.** MiroRNA-31 is elevated in RV Su/Hx/Nx rats compared with RV normoxic rats. A: qPCR validation of increase in miR-31 in the RV of the Su/Hx/Nx PAH rat. B: Pearson correlation between RVSP and miR-31 in the RV of normoxic rats. C: Pearson correlation between RVSP and miR-31 in the RV of 8-wk Su/Hx/Nx PAH rats. D: Pearson correlation between RVSP and miR-31 in the RV of 8-wk Su/Hx/Nx PAH rats. E: Pearson correlation between RVSP and miR-31 in the RV of 13-wk Su/Hx/Nx PAH rats. *P < 0.05 vs. normoxic control. #P < 0.05 vs. 8-wk Su/Hx/Nx. Data are means ± SE (n = 4).
(miR-21) (10, 15), increase cardiomyocyte apoptosis (miR-140) (35) and myocardial remodeling (miR-208b) (39), attenuate vascular smooth muscle cell proliferation and migration (miR-1298) (26), regulate mesenchymal/embryonic stem cell and endothelial progenitor cell proliferation or migration (miR-31, miR-221/222, and miR-702) (31, 68, 71, 72), and decrease cancer and stem cell apoptosis (miR-31 and miR-702) (17, 73) were augmented in the hypertrophic compared with control RV. In contrast, miRs associated with tumor growth (miR-877) (70), as well as suppression (miR-187) (74), and with cardiac arrhythmia and fibrosis (miR-208a) (45) were decreased in the hypertrophic RV. On the other hand, we

Fig. 4. MiR-140 targets 3'UTR of MFN1 and decreases the expression of Mfn1 in cardiomyocytes. A: a predicted consequential pairing of target region (top) position 476–483 of MFN-1 3’UTR and miR-140 (bottom) obtained from targetscan.org. B: conserved target sequence (red box) for miR-140 in position 476–483 of MFN1 3’UTR among different species. C: representative Western blot showing the decreased levels of mitofusin-1 (Mfn1) proteins in H9c2 cells after transfection of meridian mimic for miR-140-5p. D: quantitative determination of relative expression of mitofusin-1 (Mfn1) in H9c2 cells after transfection of meridian mimic for miR-140-5p. E: relative mitochondrial DNA copy number in H9c2 cells after transfection of meridian mimic for miR-140-5p. F: relative mitochondrial membrane potential (Δψm) expressed as a ratio of J-aggregates (Red) to monomers (Green), in H9c2 cells after transfection of meridian mimic for miR-140-5p or scramble control with and without endothelin-1 (ET1) treatment. *P < 0.05 vs. control, #P < 0.05 vs. scramble control. Data are means ± SE (n = 4).
found only three miRs (miR-208b, miR-31a-3p, and miR-31a-5p) that were elevated in the LV of PAH rats. miR-140 directly targets MFN1 and negatively regulates its expression (35). Consistently, we found high miR-140-5p and low MFN1 expression in hypertrophic RV of PAH rats. Furthermore, miR-140-5p knock-in downregulated MFN1 and mtDNA content in H9C2 cells. In these cells, mitochondrial membrane potential was unaffected by miR-140-5p knock-in in the absence and presence of ET1, which is a well-known mediator of pulmonary hypertension. These results suggest that upregulation of miR-140 potentially decreases mitochondrial biogenesis or damages the mitochondria, but it is unlikely to affect mitochondrial membrane potential. Consistently, studies show that miR-140 plays a role in promoting cardiomyocyte apoptosis by suppressing the expression of MFN1, and knockdown of miR-140 reduces myocardial infarct size (35). Previously, others and we have shown that apoptosis and autophagy/mitophagy are increased in the RV of PAH rats (2, 49) and of mice with pulmonary artery banding (48), suggesting a decline of myocardial function. Mitochondria constantly undergo fusion and fission to maintain organelle homeostasis (28, 30), and abnormality in this phenomenon triggers apoptotic cell death (47). Mitochondrial fission proteins initiate apoptosis, while mitochondrial fusion proteins inhibit apoptosis (59). Downregulation of MFN1 and 2 increases sensitivity to apoptotic stimuli (59). Conversely, MFN1 inhibits mitochondrial fission and apoptotic death of cardiomyocytes (36). It is accepted that apoptosis of cardiac myocytes plays a substantial role in the pathogenesis of dilated cardiomyopathy. In a late-stage dilated cardiomyopathy, ~80–250 cardiac myocytes per 10^5 cardiac nuclei undergo apoptosis (23, 46, 56). The use of transgenic mice conditionally expressing active caspase exclusively in the myocardium has shown that very low levels of cardiac myocyte apoptosis (23 cardiac myocytes per 10^5 cardiac nuclei) are sufficient to cause a lethal, dilated cardiomyopathy (69). Conversely, inhibition of
cardiac myocyte apoptosis has been shown to prevent the development of cardiac dilation and contractile dysfunction (69). Therefore, these studies and our current findings taken together suggest that MFN1 downregulated by miR-140 in RV of PAH rats could be a contributor to PAH-associated RV failure.

MiR-31 and c-kit expression were increased in the hypertrophic RV of PAH rats compared with control rats. These findings indirectly suggest that the number of c-kit+ cells was presumably increased in the hypertrophic RV. MiR-31 inhibits apoptosis, stimulates growth-maturation, and promotes migration of c-kit+ endothelial progenitor cells (17, 68). Furthermore, there is an association between high levels of miR-31 and c-kit activation (27). Therefore, it can be suggested that miR-31 is increased in the RV of PAH rats as a compensatory mechanism to reduce the decrease in capillary density, which is associated with the failing hearts (2). Alternatively, c-kit+ cells in the mammalian adult heart are endothelial cells (61), telocytes (3), or mast cells (75). Telocytes are involved in regeneration and repair of an organ and have been implicated in atrial remodeling (40) and mast cells in cardiac inflammation and myocardial remodeling (21, 34). Thus the accumulation of c-kit+ cells was likely a consequence of the RV hypertrophy in PAH rats, but a contribution to the progressive deterioration of heart function and finally failure cannot be ruled out.

Expression of miR-208b and miR-208a, correlates with the expression of MYH7 and MYH6, respectively (50). A switch from MYH6 to MYH7 in myocardium is an established marker of cardiomyopathy (16, 25, 32, 44, 65). Increased MYH7 expression in the myocardium also contributes to left heart failure (25, 33, 50). Notably, in the PAH rats, miR-208b was upregulated in RV and LV+S, while miR-208a was downregulated in the RV. This differential expression of miR-208a and miR-208b indicates that a switch in MYH6 to MYH7 occurred in both RV and LV in 8-wk PAH rats. While a recent study did not find miR-208b in the failing RV...
of the Sugen/hypoxia model of PAH (18), miR-208b and MYH7 have been reported to be upregulated in the mouse model of PA banding-induced RV failure (50). Clearly, the miR-208 that is involved in the normal functioning of myocardium was dysregulated in the PAH RV and LV. Therefore, elevated miR-208b and decreased miR-208a, signature miRs of heart failure, suggest right and left hearts were failing in the experimental model of Su/Hx/Nx-induced PAH. We also found a twofold increase of TUBA1A expression in the hypertrophic RV. Expression of TUBA1A and TUBA1B is increased in the hypertrophic and failing hearts (19, 57, 58), augmented in metabolic cardiomyopathies (22), and implicated in contributing to contractile dysfunction (19, 57).

Cardiac fibrosis, hypertrophy, and abnormal heart rhythm contribute to heart failure (52). Loss of vascular smooth muscle cell function or growth and apoptosis of endothelial cells also...
contribute to heart failure (20, 37, 43). A recent study showed that miR-1998 inhibits connexin-43 expression and vascular smooth muscle cell proliferation and migration (26). Connexin-43, a gap junction protein, is required for maintenance of the normal cardiac rhythm, regulation of vascular tone, and endothelial function (42). Since miR-1998 increased in the hypertrophic RV but not in the LV, we suggest that miR-1998-mediated decrease of connexin-43 could be one of the causes of arrhythmia and impaired coronary artery function in the right heart of PAH rats.

In summary, we have described the dynamic changes in the miR signatures in the RV and LV + S of the angioproliferative model of severe PAH. Of importance, we have shown that miR-140 is upregulated and that its target protein MFN1 is downregulated in the hypertrophic RV of PAH rats. Moreover, the increase in miR-140 is positively correlated with the increases in RVSP and RV hypertrophy, and the decrease in the increase in miR-140 is positively correlated with the decrease in RVSP. These changes in miR-140 and MFN1 clearly suggest that apoptosis of cardiac myocytes plays a potential role in the pathogenesis of PAH-induced RV failure.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

miR140 AND RIGHT HEART HYPERTROPHY


