Molecular Cardiology

Conditional Dicer Gene Deletion in the Postnatal Myocardium Provokes Spontaneous Cardiac Remodeling

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Background—Dicer, an RNAse III endonuclease critical for processing of pre-microRNAs (miRNAs) into mature 22-nucleotide miRNAs, has proven a useful target to dissect the significance of miRNAs biogenesis in mammalian biology.

Methods and Results—To circumvent the embryonic lethality associated with germline null mutations for Dicer, we triggered conditional Dicer loss through the use of a tamoxifen-inducible Cre recombinase in the postnatal murine myocardium. Targeted Dicer deletion in 3-week-old mice provoked premature death within 1 week accompanied by mild ventricular remodeling and dramatic atrial enlargement. In the adult myocardium, loss of Dicer induced rapid and dramatic biventricular enlargement, accompanied by myocyte hypertrophy, myofiber disarray, ventricular fibrosis, and strong induction of fetal gene transcripts. Comparative miRNA profiling revealed a set of miRNAs that imply causality between miRNA depletion and spontaneous cardiac remodeling.

Conclusions—Overall, these results indicate that modifications in miRNA biogenesis affect both juvenile and adult myocardial morphology and function. (Circulation. 2008;118:1567-1576.)

Key Words: Dicer ■ genetics ■ heart failure ■ microRNA

MicroRNAs (miRNAs) are a class of endogenous, small, noncoding RNAs that regulate gene expression.1–3 Mature miRNAs (18 to 24 nucleotides long) are the result of sequential processing of primary transcripts (pri-miRNAs) mediated by 2 RNAse III enzymes, Drosha and Dicer.4 More than 400 miRNAs cloned and sequenced in humans negatively regulate protein expression by either mRNA degradation or translational inhibition.5–7 Each miRNA potentially targets multiple transcripts, and the latest estimates indicate that they may regulate up to one third of the human genome, suggesting that miRNAs have a fundamental role in regulating gene expression.8 Current research has focused on their role during development, and fewer studies have studied miRNA function during adulthood. With the discovery of miRNA function in adult organs, we are forced to adjust our understanding of their molecular regulatory mechanisms in normal and pathological cellular functions.

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Although miRNA studies predominate in the cancer field, miRNAs and their regulatory mechanisms have been highlighted recently in the cardiac field.9–15 Van Rooij and coworkers10 described a number of miRNAs modulated in hypertrophic or failing hearts from mice and humans. Additionally, overexpression of certain miRNAs in cardiomyocytes in vitro induced cardiac hypertrophy, whereas overexpression of the stress-inducible miR-195 in transgenic mice resulted in pathological cardiac remodeling and heart failure.10 The same group described miR-208 as an intrinsic, cardiac-specific miRNA encoded within the α-myosin heavy chain (αMHC) gene.11 Whereas in the adult heart, absence of miR-208 results in severe blunting of βMHC expression in response to pressure overload or activated calcineurin, expression of βMHC remains unaltered in hearts of newborn miR-208–null mice, demonstrating that this miRNA specifically participates in the mechanism of stress-dependent regulation of βMHC.11 With regard to embryonic heart development, mice lacking miR-1-2 or newborn hearts lacking Dicer have phenotypes ranging from cell cycle dysregulation, hyperproliferation of cardiac myocytes, electric conduction defects, and ventricular-septal malformation,15,16 reflecting the apparent role of single miRNA species to modulate multiple targets, miR-133 and miR-1, which are included in

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the same bicistronic unit, are specifically expressed in cardiac myocytes and skeletal muscle and play notable regulatory roles in cardiac hypertrophy and skeletal myoblast differentiation, respectively.\textsuperscript{13,17} Finally, miR-1 is overexpressed in patients with coronary artery disease and, when overexpressed in the rat heart, exacerbates arrhythmogenesis. Conversely, elimination of miR-1 by an antisense inhibitor in infarcted rat hearts relieved arrhythmogenesis.\textsuperscript{18}

Given the importance of the specific miRNAs in heart development and adult cardiac remodeling, it becomes crucial to assess the global requirement of miRNAs in the heart. Dicer, which is critical for processing of pre-miRNAs into their mature form, has been a popular target helping to dissect the significance of miRNAs in mammalian biology. Somatic Dicer loss results in embryonic lethality.\textsuperscript{19,20} Embryos or newborn mice lacking Dicer in developing heart succumb at embryonic day 12.5 or day 4 after birth with pericardial edema and a very poorly developed ventricular myocardium.\textsuperscript{15,16} In the present study, we triggered loss of Dicer in the postnatal myocardium through the use of a tamoxifen-inducible Cre recombinase expressed in murine heart cells. Targeted deletion of Dicer in 3-week-old mice resulted in spontaneous cardiac remodeling, impairment of cardiac function, and premature death within 1 week. In the adult myocardium, loss of Dicer induced rapid and dramatic biventricular enlargement, accompanied by hypertrophic growth of cardiomyocytes, myofiber disarray, ventricular fibrosis, strong induction of fetal gene transcripts, and functional defects. Overall, these results indicate that modification of miRNA biogenesis affects both juvenile and adult myocardial morphology and function.

\section*{Methods}

\subsection*{Transgenic Mice}

Mice homozygous for Dicer-floxed alleles\textsuperscript{21} (Dicer\textsuperscript{\textregistered}) and transgenic αMHC-MerCreMer (αMHC-MCM)\textsuperscript{22} mice were crossed to generate double-floxed (αMHC-MCM/Dicer\textsuperscript{\textregistered}) mice. Mice at 3 and 8 weeks of age (αMHC-MCM/Dicer\textsuperscript{\textregistered} and control Dicer\textsuperscript{\textregistered}) were treated with vehicle or tamoxifen (20 mg/kg per day) by daily intraperitoneal injections for consecutive 5 days. Tamoxifen was diluted in 10%/90% vol/vol ethanol/oil to a concentration of 1 mg/100 μL; for the vehicle group, only ethanol/oil solution was injected.

\subsection*{Western Blotting}

Mouse cardiac tissue samples were homogenized, proteins were extracted, and Western blotting was performed as described previously.\textsuperscript{23} Antibodies against Dicer (rabbit polyclonal), GAPDH (mouse monoclonal), and Irx5 (mouse monoclonal) were from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif), and the antibody against Kcnq2 (rabbit polyclonal) was from Chemicon (Temecula, Calif).

\subsection*{Histological Analysis and Immunofluorescence Microscopy}

For histological analysis, hearts were arrested in diastole, perfusion-fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm. Paraffin sections were stained with hematoxylin and eosin (H&E) for routine histological analysis, Sirius red for the detection of fibrillar collagen, FITC-labeled wheat-germ agglutinin (WGA) to visualize and quantify the cross-sectional area of the cardiomyocytes, and CD31 (marker of platelet/endothelial cell adhesion molecule-1) to visualize and quantify the number of capillary-ies per myofiber. Slides were visualized with a Nikon Eclipse E600 microscope or a Zeiss Axiovert 135 (immunofluorescence). Cell surface areas were determined with the use of SPOT imaging software (Diagnostic Instruments).

\subsection*{Transmural Echocardiography}

Echocardiographic measurements were performed on mice anesthetized with isoflurane as described before,\textsuperscript{21} 1 week and 4 weeks after the start of tamoxifen treatment.

\subsection*{miRNA Profiling}

Total RNA was isolated from mouse cardiac tissue samples with the use of TRIzol reagent (Invitrogen, Carlsbad, Calif). The quality of RNA was verified by an Agilent 2100 Bioanalyzer. The expression analysis of 483 miRNA was performed by a miRNA-profiling service (Exiqon, Denmark) with the use of miRCURY LNA arrays. In short, 1 μg of total RNA from tamoxifen-injected αMHC-MCM/Dicer\textsuperscript{\textregistered} mice was labeled with Hy3/Hy5, and the corresponding reference pool sample (vehicle-injected αMHC-MCM/Dicer\textsuperscript{\textregistered} mice) was labeled with Hy5/Hy3 in a dye-swap setup by using an LNA array labeling kit. The Hy3- and Hy5-labeled samples were mixed pairwise and hybridized to the miRCURY LNA array version 8.1, which contains capture probes targeting all miRNAs for all species registered in the miRBASE version 8.1 at the Sanger Institute. The hybridization was performed according to the miRCURY LNA array manual with the use of a Tecan HS4800 hybridization station (Tecan, Austria). After hybridization, the microarray slides were scanned and stored in an oxygen-free environment (ozone level <2.0 ppb) to prevent potential bleaching of the fluorescent dyes. The LNA array slides were scanned with the use of the Agilent G2505B Microarray Scanner System (Agilent Technologies, Inc) and image analysis was performed with the use of ImaGene 7.0 software (BioDiscovery, Inc). The raw signal for each probe was obtained by subtracting the maximum of the local background and negative control signals from the foreground signal. The data were preprocessed to remove poor-quality spots, and normalization was used to remove any systematic bias. Quantified signals were normalized with the use of the global Lowess (LOcally Weighted Scatterplot Smoothing) re- gression algorithm (Exiqon).

\subsection*{Northern Blotting}

Three micrograms of total RNA were fractionated on a denaturing 12% polyacrylamide gel containing 8 mol/L urea, transferred to Nytran N membrane (Schleicher & Schuell, Germany) by the capillary method, and fixed by UV cross-linking according to the manufacturer’s instructions. Membranes were hybridized with specific 5’-digoxigenin–labeled LNA probes for mir-1 or mir-21 (Exiqon). Detection was performed with an antibody to 5’-digoxigenin (Roche).

\subsection*{Quantitative Reverse Transcription Polymerase Chain Reaction}

One microgram of total RNA from mouse ventricular tissue was used as template for Superscript reverse transcriptase II (Promega). For real-time reverse transcription polymerase chain reaction (RT-PCR), a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, Calif) and SYBR Green were used in combination with specific primer sets designed to detect transcripts for nppa, nphb, acta1, myh7, lrr5, kcnq2, angpt1, angpt2, angpt3, vegla, vegfb, vegfc, vegfd, vegfr1, vegfr2, vegfr3, and hif1a (primer sequences available on request), as described previously in detail.\textsuperscript{24}

\subsection*{Statistical Analysis}

The results are presented as mean±SEM values. Statistical analyses were performed with the use of InStat 3.0 software (GraphPad Software Inc) and consisted of ANOVA followed by the Tukey posttest when group differences were detected at the 5% significance level or by the Student t test when 2 experimental groups were compared. Survival was compared by Kaplan–Meier analysis. Differences between parameters at 1 and 4 weeks after cardiac-restricted...
**Results**

**Targeted Deletion of Dicer in the Heart Induces Sudden Death and Mild Cardiac Remodeling in Juvenile Mice**

To assess the overall requirement of miRNAs in the mouse heart and to bypass the early embryonic lethality of Dicer-null mice, we first provoked deletion of a floxed Dicer (Dicer<sup>F/F</sup>) allele<sup>21</sup> using a tamoxifen-inducible Cre recombinase protein fused to 2 mutant estrogen-receptor ligand-binding domains (αMHC-MCM) under control of the cardiac-specific αMHC promoter. The first Dicer RNAse III domain encoded in exon 21 is flanked by loxP sites and removed by Cre recombinase activity. αMHC/Dicer<sup>F/F</sup> and Dicer<sup>F/F</sup> mice were treated with tamoxifen or vehicle at 3 or 8 weeks of age. Juvenile and adult mice were treated with vehicle or tamoxifen for 5 days (red arrow and line indicate start and duration of treatment). miRNA profiling and phenotypic characterization of Dicer-deleted hearts were performed 1 week (in juvenile and adult mice) and 4 weeks (in adult mice) after start of tamoxifen treatment. Cardiac function in the adult mice was determined by serial echocardiographic measurements at 1 week and 4 weeks after the start of tamoxifen treatment.

Dicer deletion were analyzed with SPSS software by applying a 2-way ANOVA for repeated measurements (with Greenhouse-Geisser epsilon correction) with time of measurement as the within factor and treatment as the grouping factor.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
in cardiac size accompanied by grossly enlarged atria filled with atrial thrombi (Figure 2C), which was reflected in a subtle increase in heart weight adjusted for body weight (Figure 2D). We also noted that within 5 days of the start of tamoxifen delivery, αMHC-MCM/DicerF/F mice displayed a weak condition and inactivity compared with tamoxifen-treated DicerF/F or vehicle-treated αMHC-MCM/DicerF/F control mice. Indeed, up to 25% of tamoxifen-treated αMHC-MCM/DicerF/F mice died within 1 week after the start of treatment, and this mortality rate increased up to 75% during the following 2 weeks (P=0.029; Figure 2E).

At 3 weeks of age, deletion of Dicer induced modest left ventricular histopathology. H&E-, Sirius red-, or WGA-stained histological sections displayed a mild degree of inflammatory cell infiltration but no additional myofiber disarray, myocyte hypertrophy, or interstitial fibrosis (Figure 3A). Quantitative real-time PCR on heart tissue from tamoxifen-treated αMHC-MCM/DicerF/F animals compared with tamoxifen- or vehicle-treated DicerF/F control mice revealed a mild but significant upregulation of the “fetal” cardiac genes encoding the natriuretic peptides atrial natriuretic factor (nppa) and brain natriuretic peptide (nppb) and those that encode for sarcomeric proteins α-skeletal actin (acta1) and βMHC (myh7). With a 700% increase, atrial natriuretic factor transcripts were most abundantly enriched in Dicer-deficient hearts, whereas no substantial change was observed in the expression of any of the analyzed marker genes in the control hearts (Figure 3B).

Abnormalities in cardiac conduction and repolarization often cause sudden death. Recently, targeted deletion of miR-1 resulted in upregulation of Irx5 and, as a consequence, downregulation of Kcnd2. Because we noted that miR-1 was among the most dramatically downregulated microRNAs in juvenile Dicer-depleted mice (Figure 2A and Table II in the online-only Data Supplement), we examined transcript levels for irx5 and kncd2. Consistent with a previous report, we observed a clear upregulation of irx5 transcripts and found that transcripts of the Irx5 target gene kncd2 were downregulated in the mutant hearts (Figure 3C). A clear increase in the transcriptional repressor Irx5 and a corresponding decrease in Kcnd2 at the protein level were also observed (Figure 3D). These data suggest that dysregulation of the cardiac electric system due to decreased Kcnd2 expression may, at least in part, provoke arrhythmogenesis and the observed sudden death in juvenile Dicer-depleted hearts. In conclusion, cardiac-restricted Dicer deletion in juvenile mice provokes selective reactivation of fetal cardiac genes and a high incidence of spontaneous sudden death, indicating the essential requirement for Dicer in the maturing heart.

**Targeted Deletion of Dicer in the Adult Heart Provokes Severe and Spontaneous Hypertrophy**

Because deletion of Dicer in juvenile mice resulted in high mortality, we next treated adult αMHC-MCM/DicerF/F and DicerF/F mice with vehicle or tamoxifen at the age of 8 weeks (Figure 1). Next, we analyzed the expression of miRNAs in adult hearts either 1 week or 4 weeks after tamoxifen-mediated Dicer deletion (Figure 1). miRNA profiling identi-
fied the same set of 161 miRNAs that displayed myocardial expression and identified 112 miRNAs that were 2-fold differentially expressed between tamoxifen- and vehicle-treated αMHC-MCM/DicerF/F mice at 4 weeks (Tables I and II in the online-only Data Supplement). At 4 weeks after tamoxifen treatment, 42 of 161 myocardial miRNAs remained unaffected, whereas 56 miRNAs were downregulated in expression (Figure 4A and Tables I and II in the online-only Data Supplement). To validate the array results, we confirmed the decrease of miR-1 and increase of miR-21...
Figure 4. Targeted deletion of Dicer in the adult heart provokes rapid and spontaneous pathological hypertrophy. A, miRNA profiling reveals a set of miRNAs that were differentially expressed on ablation of Dicer induced in 8-week-old αMHC-MCM/DicerF/F mice. B, Northern blot analysis of miR-1 and miR-21 expression levels in αMHC-MCM/DicerF/F mice treated with vehicle or tamoxifen. C, Representative image of DicerF/F and αMHC-MCM/DicerF/F murine hearts 4 weeks after tamoxifen or vehicle treatment and Western blot analysis demonstrating the level of Dicer expression in the different genotypes. Note the increase in heart size. Dicer protein levels were already depleted at 1 week in the tamoxifen-treated αMHC-MCM/DicerF/F hearts. Scale bar = 50 μm. D, Histological analysis of ventricular tissue sections from DicerF/F and αMHC-MCM/DicerF/F mice stained with H&E, Sirius red, WGA, and CD31. Note increased myocyte disarray and extracellular matrix (blue staining with H&E) and large fibrotic lesions (red color with Sirius red) in tamoxifen-treated αMHC-MCM/DicerF/F cardiac sections. Scale bar = 20 μm. E, Heart weight (HW)/body weight (BW) ratio between DicerF/F and αMHC-MCM/DicerF/F hearts, treated with vehicle (white bars) or tamoxifen (black bars), demonstrating increased cardiac mass in αMHC-MCM/DicerF/F after deletion of Dicer. Error bars are mean±SEM of n=5. F, Quantification of myofiber surface area of WGA-stained histological sections from vehicle- (white bars) or tamoxifen-treated (black bars) αMHC-MCM/DicerF/F and DicerF/F hearts, demonstrating a significant increase in myofiber size on deletion of Dicer. Error bars are mean±SEM of n = 150 individual myofibers. G, Capillary density determined by counting CD31-positive capillaries per myofiber. Error bars are mean±SEM of n ≥ 150 individual myofibers.
expression by Northern blot analysis in adult, tamoxifen-treated αMHC-MCM/DicerF/F mice (Figure B).

After 1 week, tamoxifen-treated αMHC-MCM/DicerF/F mice exhibited reduced activity in their cages but did not display premature sudden death. In agreement, no effects on survival were observed (data not shown). Low activity can be indicative of heart failure. One week after the tamoxifen regimen, cardiac Dicer protein levels were virtually absent, as confirmed by Western blot (Figure 4C). In addition, hearts from tamoxifen-treated αMHC-MCM/DicerF/F mice displayed doubling in heart weight compared with their control littermates (Figure 4C and 4E). A severe histopathology was evident after staining of histological sections with H&E, Sirius red, WGA, and CD31. Cardiac tissue revealed an intricate phenotype with hypertrophied myofibers, myocyte disarray, strong inflammatory infiltration, and interstitial fibrosis (Figure 4D), hallmark features observed in experimental and clinical heart failure biopsies. CD31 staining demonstrated an aberrant vascularity evidenced by strong vasodilation of preexisting vasculature in tamoxifen-treated αMHC-MCM/DicerF/F mice (Figure 4D), a tendency toward an increase in the relative capillary/myocyte ratio (P=0.077; Figure 4G), and increased expression of several proangiogenic factors and hypoxia-inducible factor-1α (Figure in the online-only Data Supplement). Combined, these results indicate that Dicer deletion in the adult myocardium affects processing of crucial cardiac miRNAs, leading to rapid and spontaneous pathological remodeling.

Targeted Deletion of Dicer in the Adult Heart Decreases Cardiac Function and Induces a Fetal Gene Profile

Cardiac geometry and function were assessed noninvasively by serial echocardiography at 1 and 4 weeks after tamoxifen treatment (Figure 5A, Table). After 1 or 4 weeks, vehicle-treated DicerF/F and MCM-DicerF/F mice demonstrated normal cardiac geometry and function as indicated by fractional shortening (1 week, 40±4% and 49±3%; 4 weeks, 45±3% and 51±3%, respectively; Table, Figure 5B), left ventricular internal dimensions, and left ventricular mass (Figure 5B, 5C, and 5D and Table). As expected, tamoxifen-treated DicerF/F mice showed no alterations in fractional shortening or other parameters Figure 5A through 5D). In contrast, at 1 and 4 weeks, MCM-DicerF/F animals demonstrated a rapid and significant decline in cardiac function, indicated by a 50% decrease in fractional shortening (P<0.05; Table, Figure 5A and 5B). At both time points after tamoxifen treatment, MCM-DicerF/F mice also showed a more pronounced deterioration in cardiac geometry than vehicle-treated MCM-DicerF/F mice, as demonstrated by an increase of 80% to 90% in left ventricular internal diameters (1 week, 1.5±0.3 and 2.7±0.1 mm; 4 weeks, 1.4±0.1 and 2.7±0.1 mm; P<0.05; Table, Figure 5C), indicating a rapid dilatation of the left ventricle. Cardiac mass continued to increase progressively by 45% to 60% compared with vehicle-treated MCM-DicerF/F mice (1 week, 48±6 and 71±13 mg; 4 weeks, 60±2 and 95±19 mg, respectively, P<0.05; Table, Figure 5D). These data indicate that Dicer depletion provokes progressive functional and geometric deterioration consistent with a heart failure phenotype.

Deletion of Dicer in adult mice also induced potent reactivation of embryonic genes such as acta1, npph, myh7, and nppa by 1300% to 1800% in 8-week-old αMHC-MCM/DicerF/F hearts (Figure 5E). There was no substantial change in the expression of any of these genes in hearts of tamoxifen- or vehicle-treated control genotypes. Conclusively, deletion of Dicer in the adult heart induces rapid and spontaneous cardiac dysfunction with strong induction of fetal hypertrophic marker genes.

Discussion

The production and maturation of miRNAs result from a 2-step processing pathway mediated by 2 major RNase III endonucleases, Dicer and Drosha. Whereas Drosha is involved in mRNA maturation in the nucleus, Dicer localizes in the cytoplasm, where it cleaves the pre-miRNA into the mature miRNA, leading to either translational repression or degradation of target mRNAs. Mice that lack Dicer succumb at embryonic day 7.5, which has posed a barrier to analyze Dicer and miRNA biogenesis in later development and adult animals. To assess the global requirement of miRNAs in the mouse heart and to overcome the embryonic lethality associated with a Dicer germine null allele, we have deleted a floxed Dicer allele using a myocardium-restricted, temporally regulated Cre deleter strain. In this report, we show the involvement and relevance of Dicer in ensuring accurate cardiac integrity and present definitive evidence that inactivation of Dicer in the postnatal heart results in severe cardiac malfunction and failure regardless of the timing of gene deletion.

Although several studies have described involvement of specific miRNAs in controlling embryonic development, myogenesis, cardiogenesis, and onset of cardiac disease, the overall requirement of miRNAs in the adult heart is less clear. Theoretically, 1 experimental approach to study the contribution of individual miRNA species in the myocardium would be to deplete the myocardium and restore expression of specific miRNAs or miRNA clusters in a myocardial Dicer-null background, as recently described for Dicer mutant zebrafish and miRNA-430. We found that deletion of Dicer in the adult mammalian heart resulted in severe myocardial histopathology, suggesting a crucial role for this enzyme in ensuring integrity of the postnatal heart. At early ages (3 weeks), Dicer deletion also provoked sudden death and atrial remodeling, which has previously also been reported for mice lacking miR-1-2 and neonates lacking cardiac Dicer. Mice lacking miR-1-2 presented evidence of atrial thrombi and sudden death by 2 to 3 months of age caused by cardiac repolarization and conduction defects. Indeed, our comparative miRNA profiling analysis at an early age demonstrated strong downregulation of miR-1, upregulation of Irx5 expression, and downregulation of Kcnd2 in Dicer-depleted hearts, suggesting that the phenotypes we observe may be attributable to miR-1 depletion, and implicates Dicer in developmental cardiac maturation and conduction.
To assess the role of Dicer and global requirement for miRNAs in the adult heart, we also deleted Dicer in 8-week-old mice, which provoked a remarkably rapid and spontaneous myocardial growth, accompanied by a severe histopathology. A surprising histological feature was the dramatic change in capillary organization, with strong vasodilation of preexisting vasculature and increased expression of proangiogenic factors. Myocardial vascularization may be affected from adaptive responses to cardiac oxidative stress, which regulates the transcription of an extensive repertoire of genes, including many involved in angiogenesis and vascular remodeling. Spontaneous adverse cardiac remodeling was further confirmed by functional deficits and acute upregulation of fetal cardiac genes, including those encoded by nppa, nppb, acta1, and myh7. In this respect, it is interesting to note that Thum et al recently established that cardiac microRNAs strongly contribute to induction of a fetal gene program in heart failure. The apparent phenotypic difference between

Figure 5. Targeted deletion of Dicer in the adult heart provokes heart failure and fetal gene activation. A, Representative M-mode images of adult DicerF/F and αMHC-MCM/DicerF/F mice 4 weeks after vehicle or tamoxifen treatment. The data demonstrate decreased contractility and increased left ventricular internal dimensions in αMHC-MCM/DicerF/F treated with tamoxifen. Quantification of fractional shortening (FS) (B), measurements of the left ventricular internal diameters in systole (LVIDs) (C), and quantification of the left ventricular mass (LV mass) (D) in the experimental groups, 1 week and 4 weeks after vehicle or tamoxifen treatment. Error bars are mean±SEM of n=7 to 10. E, Analysis of α-skeletal actin (acta1), brain natriuretic peptide (nppb), βMHC (myh7), and atrial natriuretic peptide (nppa) by quantitative RT-PCR in vehicle- (white bars) or tamoxifen-treated (black bars) αMHC-MCM/DicerF/F and DicerF/F ventricular tissue. Error bars are mean±SEM of n=3.
Table. Echocardiographic Characteristics of Adult DicerF/F or MCM-DicerF/F Mice 1 or 4 Weeks After the Start of Vehicle or Tamoxifen Treatment

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<th>DicerF/F</th>
<th>MCM-DicerF/F</th>
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<tr>
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<td>1 Week</td>
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<td>Anterior wall thickness in diastole, mm</td>
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<tr>
<td>LV mass, mg</td>
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<td>Fractional shortening, %</td>
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<td>47.7±1.3</td>
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Data are expressed as mean±SEM. LV indicates left ventricular.

*P<0.05 vs corresponding vehicle-treated group.

postnatal Dicer ablation in juvenile and adult myocardium likely reflects the different maturing states of the postnatal myocardium and could point toward relative differences between overall miRNA biogenesis and biological contribution in the juvenile versus adult myocardium. Clearly, Dicer depletion in the juvenile heart provoked an overall tendency toward arrhythmogenesis and less marked myocyte hypertrophy, whereas in the adult myocardium pronounced myocyte hypertrophy and angiogenic defects were observed. Future studies should seek to provide in-depth understanding of individual, differentially expressed miRNAs in a cardiac disease framework.

Despite efficient, genetic Dicer deletion in the adult heart, we did not observe a preponderance of miRNA repression, which may reflect, in part, the long half-life of certain endogenous miRNAs,33 given that the conditional allele used in this study was recently shown to efficiently reduce miRNA processing in the limb mesoderm.21 In addition, we observed a subset of miRNAs upregulated after tamoxifen treatment. It seems counterintuitive that miRNAs are upregulated in response to Dicer depletion, and we suspect that this is due to the particulars of the genetic models chosen. Indeed, here we used a Cre deleter strain that expresses the transgene only in heart muscle (MHC-MerCreMer), which will only allow for Dicer depletion in the heart muscle when crossbred with DicerF/F mice after tamoxifen treatment. Accordingly, the nonmyocyte fraction remains untouched with our strategy of Dicer depletion. It is likely that miRNAs expressed in the nonmyocyte fraction may respond in a secondary fashion to the observed remodeling processes, resulting in the upregulation of miRNAs in our expression profiling. Finally, we also noted that absolute miRNA expression levels can differ considerably between juvenile and adult hearts, such that for certain miRNAs, absolute expression before Dicer manipulation either increased (eg, mmu-let-7b, mmu-miR-21) or substantially decreased (eg, mmu-miR-145, mmu-miR-451) between the juvenile and adult heart. These 3 factors likely influenced in a combinatorial fashion the observed differential expression of individual miRNAs in our array results (Table I in the online-only Data Supplement). Taken together, our data provide, for the first time, clear evidence for a decisive role for Dicer and miRNA biogenesis in postnatal myocardial integrity. Our profiling lists yielded miRNAs that have not yet been linked to myocardial pathology.

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Disclosures

None.

References

The adult heart is exquisitely sensitive to slight alterations in gene expression to maintain normal morphological and hemodynamic homeostasis. A newly discovered class of small ribonucleotide-based regulators of gene expression, microRNAs, is being progressively implicated in an increasing number of biological processes, and the role of microRNAs in cardiovascular homeostasis and disease is only recently being uncovered. MicroRNAs are small RNAs that do not encode proteins but rather pair with their target mRNAs to negatively regulate protein expression. In this study, the global involvement of microRNAs in heart development and function was assessed by deleting Dicer, an endonuclease required for the proper biogenesis of all microRNA species, specifically in the postnatal myocardium. Targeted deletion of Dicer in the mouse heart and concomitant repression of proper microRNA processing and target protein expression resulted in spontaneous adverse ventricular remodeling, severe histopathology in juvenile and adult myocardium, and premature death of juvenile animals. Signs of heart failure including hemodynamic defects and acute upregulation of “fetal” cardiac genes accompanied the remarkably fast myocardial remodeling process of the Dicer-depleted adult myocardium. Altogether, these data provide clear evidence for a crucial role for microRNAs in controlling postnatal myocardial integrity and suggest a therapeutic potential of subsets of microRNAs in the treatment of cardiac disorders such as chronic heart failure.
Conditional *Dicer* Gene Deletion in the Postnatal Myocardium Provokes Spontaneous Cardiac Remodeling

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