

Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure

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Cardiovascular disease is the leading cause of human morbidity and mortality. Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy associated with heart failure. Here, we report that cardiac-specific knockout of Dicer, a gene encoding a RNase III endonuclease essential for microRNA (miRNA) processing, leads to rapidly progressive DCM, heart failure, and postnatal lethality. Dicer mutant mice show misexpression of cardiac contractile proteins and profound sarcomere disarray. Functional analyses indicate significantly reduced heart rates and decreased fractional shortening of Dicer mutant hearts. Consistent with the role of Dicer in animal hearts, Dicer expression was decreased in end-stage human DCM and failing hearts and, most importantly, a significant increase of Dicer expression was observed in those hearts after left ventricle assist devices were inserted to improve cardiac function. Together, our studies demonstrate essential roles for Dicer in cardiac contraction and indicate that miRNAs play critical roles in normal cardiac function and under pathological conditions.

cardiac function | microRNA

The heart is the first organ to form and to function during vertebrate embryogenesis (1). Perturbations in normal cardiac development and function result in a variety of cardiovascular diseases, the overall leading cause of death in developed countries (2, 3). Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy, in which the heart becomes weakened and affects the ability of the cardiovascular system to meet the metabolic demands of the body. DCM, characterized by cardiac chamber dilation and systolic impairment, has been associated with mutation of specific contractile proteins and components of stress sensor machinery (2, 4). However, the regulatory events required for appropriate coordination of contractile function are still elusive.

MicroRNAs (miRNAs) are a class of recently discovered ≈22-nt regulatory RNAs that posttranscriptionally regulate gene expression (5). Despite the large number of miRNAs identified thus far, the biological roles of most miRNAs and the molecular mechanisms underlying their function remain largely unknown. Emerging evidence suggests that miRNAs play important roles in a variety of biological processes, including cancer and stem cell biology (6, 7). Recent studies uncovered the involvement of several muscle-specific miRNAs, miR-1, -133, and -208 in particular, in the regulation of cardiac and skeletal muscle gene expression and muscle proliferation and differentiation (8–11). Specifically, gene targeting studies demonstrate that miR-1 and -208 are required for proper cardiac development and/or function (9, 10).

In this study, we took a global approach to study cardiac miRNAs by deleting Dicer, an endonuclease required for the processing of all miRNAs, in the heart. Here we report that loss of Dicer results in a dramatic decrease in the level of mature miRNAs. All Dicer mutant mice die postnatally due to severe DCM and heart failure.

Furthermore, we have found that Dicer protein level was significantly decreased in human patients with DCM and failing hearts. Our data therefore reveal an essential role of Dicer and miRNAs in normal heart formation and function.

Results

Cardiac-Specific Deletion of Dicer Allele. To understand the global involvement of miRNAs in heart development and function, we applied the Cre-loxP system to disrupt the Dicer tissue-specifically in the heart. Mice homozygous for the floxed Dicer alleles (Dicer^{flox/flox}), which are viable and fertile without any apparent abnormalities (12), were crossed to a transgenic mouse line in which the Cre recombinase is expressed under the control of alpha myosin heavy chain (α -MHC) promoter (MHC^{Cre/+}), which directs cardiac-specific expression (13) (Fig. 1*a*). Double-heterozygous progenies (MHC^{Cre/+}; Dicer^{flox/+}) were identified and mated with Dicer^{flox/flox} to obtain cardiac-specific Dicer mutant animals (Fig. 1*b*). Genotyping of offspring from the latter mating did not identify any Dicer mutant mice (MHC^{Cre/+}; Dicer^{flox/flox}) at weaning age (data not shown), indicating that cardiac-specific deletion of Dicer caused premature lethality. We then genotyped postnatal day 0 (P0) mice from the above intercrossing and found all possible genotypes in expected Mendelian frequency (Fig. 1*c*), suggesting that cardiac-specific Dicer deletion did not cause embryonic lethality. We confirmed, using Western blot analysis, that Dicer protein was indeed removed from the hearts of knockout mice (Fig. 1*b*).

To confirm that cardiac-specific disruption of Dicer indeed results in the loss of mature miRNAs in the hearts, we performed miRNA microarray analysis using total RNA samples isolated from P0 wild-type Dicer heterozygous and homozygous hearts. There is a dramatic reduction in mature miRNA expression in Dicer mutant hearts compared with wild-type control hearts. The very low level of mature miRNA expression detected in the Dicer knockout hearts is likely due to the accumulation of miRNAs processed before the functional Dicer was effectively deleted. A substantial reduction of mature miRNA expression was also observed in heterozygous hearts [Fig. 1*d* and [supporting information \(SI\) Table 2](#)]. In contrast, no difference in mature miRNA expression was observed in the livers of wild-type and

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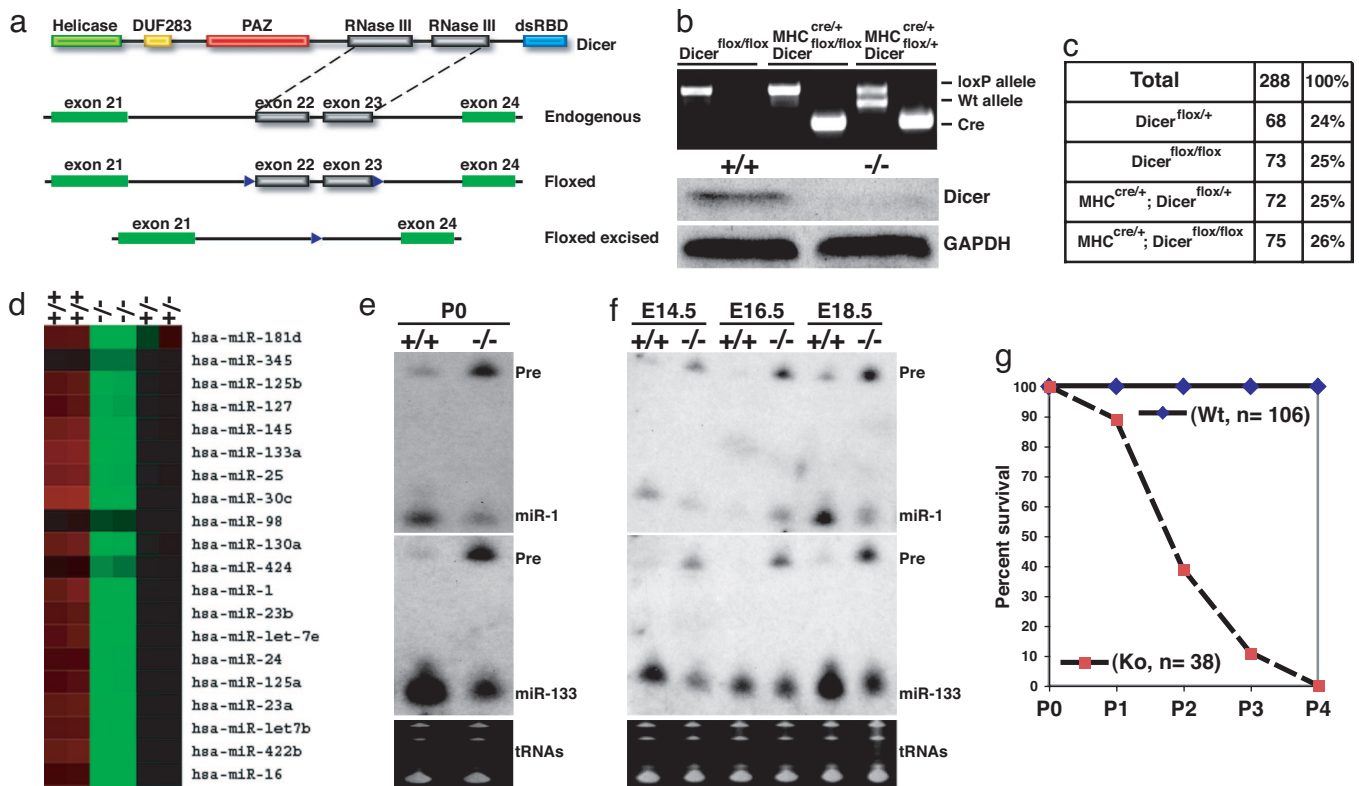


Fig. 1. Generation of cardiac-specific Dicer knockout mice. (a) The gene targeting strategy for cardiac-specific deletion of Dicer. (b) PCR genotyping analysis of P0 mice. Wild-type loxP alleles and Cre products are indicated (Upper). Western blot analysis of Dicer protein from wild-type (+/+) and Dicer mutant (-/-) hearts. GAPDH serves as a loading control (Lower). (c) Genotyping results of P0 offspring from MHC^{Cre/+}; Dicer^{loxP/+} mice and Dicer^{loxP/loxP} mice intercrossing. (d) miRNA microarray analysis of miRNA expression in P0 wild-type (+/+), Dicer heterozygous (+/-), and homozygous (-/-) mutant hearts. Red denotes high expression and green denotes low expression. (e and f) miRNA Northern blot analysis to detect the expression of precursor (Pre-) and mature miR-1 and -133 in P0 or embryonic wild-type (+/+) and Dicer mutant (-/-) hearts. tRNAs were used as a loading control. (g) Kaplan-Meier survival curves for wild-type (Wt) and Dicer knockout (Ko) mice. All Dicer mutant mice die before P4.

Dicer mutant mice (data not shown), confirming cardiac-specific depletion of mature miRNAs. Northern blot analysis of Dicer mutant hearts further demonstrated a marked decrease in the expression of mature forms of miR-1 and -133, two of the most abundantly expressed muscle miRNAs (Fig. 1e) (8). Similarly, the expression of cardiac-specific miR-208 was significantly lower in Dicer mutant hearts (SI Fig. 6). Notably, miRNA precursors accumulated in the Dicer mutant hearts, consistent with the view that Dicer is required for processing of mature miRNAs from their precursors. We have examined miRNA expression in the embryonic hearts and found decreased expression of mature miRNAs in Dicer mutant hearts as early as embryonic day 14.5 (E14.5) (Fig. 1f).

Cardiac-Specific Dicer Knockout Mice Die Shortly After Birth. Newborn Dicer mutant mice are externally indistinguishable from their control littermates. However, all mutant mice die within 4 days after birth (Fig. 1g). Preceding death, mutant animals become very fragile and exhibit decreased spontaneous activity. The hearts of mutant mice are substantially larger than that of their littermates (Fig. 2a), even though there is no difference in body size. Histological analyses of mutant hearts indicate dramatic left ventricle dilation (Fig. 2b). We always observe accumulation of blood clots and/or thrombin in the left ventricle and left atrium of mutant hearts (Fig. 2b), indicating that Dicer mutant hearts suffer from impaired cardiac function. The myocardium is less organized, and the integrity of cardiomyocytes is impaired in Dicer mutant hearts (Fig. 2c). However, no substantial increase in fibrosis was observed in the mutant heart

(data not shown). Consistent with the observed cardiomyocyte cyto-architectural defect, a profound decrease in the expression of contractile proteins was observed in Dicer mutant ventricles (Fig. 2d and e). Isolated neonatal cardiomyocytes from Dicer mutant hearts also show disarrayed myofibrils (Fig. 2f).

Ultrastructural analyses using transmission electronic microscopy show striking alterations in the myocardial structure of Dicer mutant hearts. Foremost, there are substantially less sarcomeres in the mutant ventricles (Fig. 2g). Second, the sarcomeres that are present are dramatically disarrayed and substantially shorter in the mutant hearts (Fig. 2h). These observations were confirmed by quantitative measurement of sarcomere length of Dicer mutant cardiomyocytes from E18.5 and P0 Dicer mutant hearts versus the controls (Fig. 2i). However, we did not detect a significant difference in the structure of intercalated discs between Dicer mutant and control hearts (data not shown). These defects in the cardiac muscle contractile apparatus likely account for the abnormalities in heart morphogenesis and the dysfunction in cardiac contraction found in the Dicer mutant hearts (see below).

There is no significant difference in the proliferation of cardiomyocytes of control compared with Dicer mutant hearts, as determined by phosphorylated histone H3 and Ki-67 staining, and bromodeoxyuridine (BrdU) labeling (SI Figs. 7-9). A substantial increase in apoptosis, detected by TUNEL staining, was found in the left atrium of P2 mutant hearts (Fig. 2j and l). In addition, restricted areas of the ventricular apex and left ventricle wall of P2 Dicer mutant hearts also show an increase in apoptosis (Fig. 2k and l). However, we did not observe increased apoptosis in other regions of the Dicer mutant hearts examined.

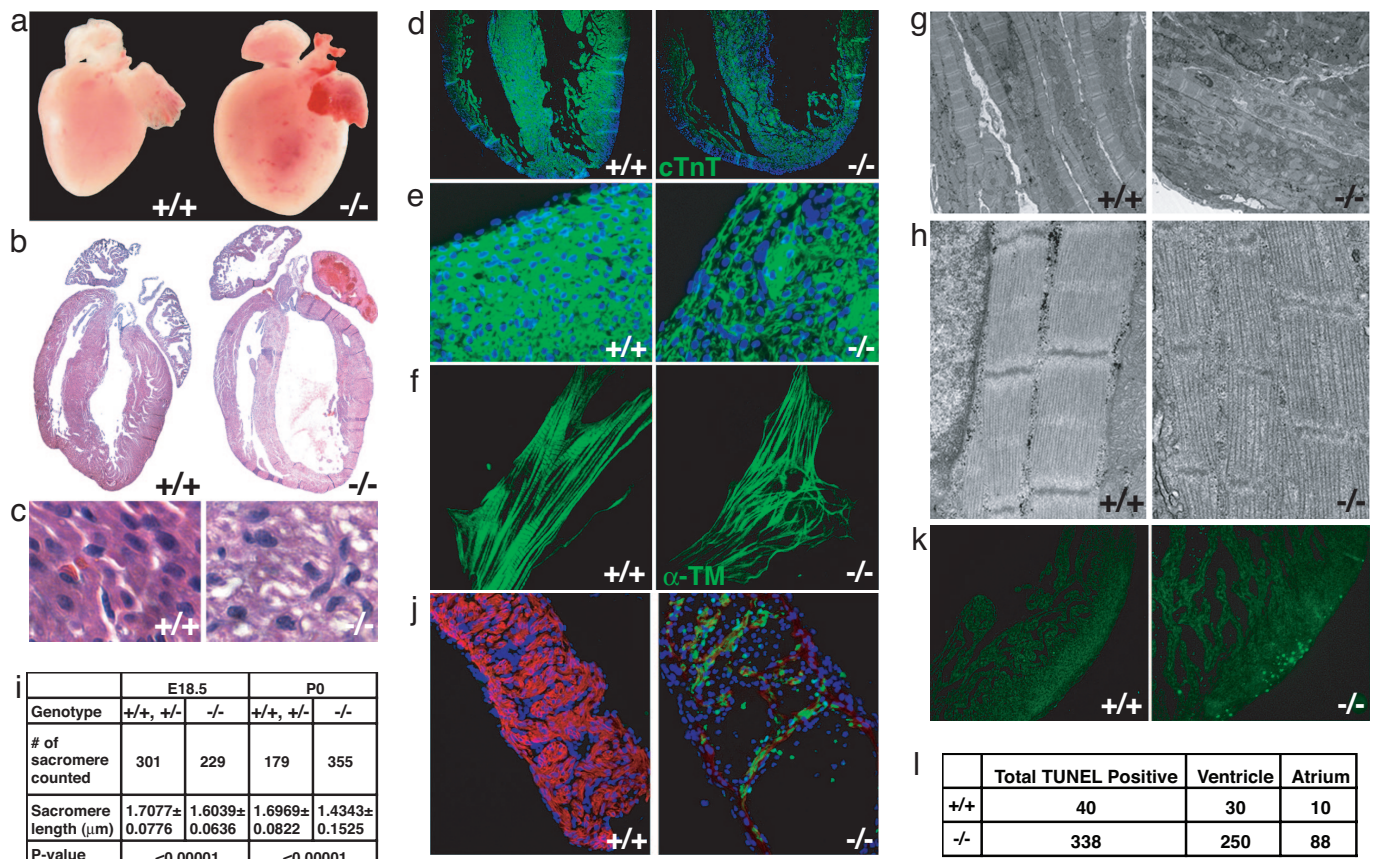


Fig. 2. Cardiac-specific Dicer deletion leads to DCM. (a) Gross morphology of P0 wild-type (+/+) and Dicer mutant (-/-) hearts. (b) H&E staining of sagittal sections of P0 wild-type (+/+) and Dicer mutant (-/-) hearts. (c) Higher-magnification images from H&E stained left ventricular myocardium of P0 wild-type (+/+) and Dicer mutant (-/-) hearts. (d) Immunohistology of P0 wild-type (+/+) and Dicer mutant (-/-) hearts for cTnT (green). DAPI stains nuclei. (e) Higher-magnification images from cTnT immunohistology-stained left ventricular myocardium of P0 wild-type (+/+) and Dicer mutant (-/-) hearts. (f) Confocal microscopic images of cultured neonatal cardiomyocytes from wild-type (+/+) and Dicer mutant (-/-) hearts stained for cardiac α -TM. (g) Electron microscopic images of cultured neonatal cardiomyocytes from wild-type (+/+) and Dicer mutant (-/-) hearts stained for cardiac α -TM. (h) Higher magnification of electron micrographs of wild-type (+/+) and Dicer mutant (-/-) myocardium to show disarrayed sarcomere in mutant hearts. (i) Quantitative measurement of sarcomere length in wild-type (+/+) and Dicer mutant (-/-) hearts. (j) TUNEL staining (green) detected increased apoptosis in the left atrium and ventricle of P2 Dicer mutant (-/-) heart. Antibody against cTnT was used to label cardiomyocytes (red). DAPI stains nuclei (blue). (k) Increased apoptosis in the left ventricular wall of P2 Dicer mutant (-/-) hearts as revealed by TUNEL staining. (l) Quantitative measurement of TUNEL positive cells in P2 wild-type (+/+) and Dicer mutant (-/-) hearts.

Neither did we detect any significant increase in apoptosis in the Dicer mutant hearts before P0, before obvious signs of cardiac dysfunction (data not shown). We measured the cell number and size of cardiomyocytes of Dicer mutant heart. Overall, no substantial change in the cell size or cell number in P0 Dicer knockout hearts was observed (SI Fig. 10 and data not shown).

Dicer Knockout Mice Display Features of DCM and Heart Failure.

Cardiac function of Dicer mutant mice was analyzed by noninvasive transthoracic echocardiography and compared with littermate controls (Fig. 3a and Table 1). Dicer mutant mice exhibit severe left ventricle dilation coupled with a dramatic decrease in fractional shortening. Notably, the left ventricle posterior wall thickness at end diastole was significantly increased, without significant change at end systole, indicating a significant loss of ventricle force generation and cardiac malfunction in Dicer mutant hearts. Interestingly, Dicer mutant mice display remarkable decrease in heart rate (Table 1). Although wild-type and heterozygous hearts contract at \approx 550 beats per minute (bpm), the mutant hearts only beat about half of the rate (278 ± 64 bpm), suggesting that Dicer mutant mice may also suffer from conduction defects (Table 1). Together,

these data demonstrate that cardiac-restricted Dicer knockout mice exhibit several features of DCM and heart failure.

Many DCMs are also associated with defects in cardiac conduction (2). We examined the expression of connexin (Cx) 40 (Cx40), Cx43, and Cx45, three major gap junction proteins expressed in the heart and are responsible for the proper function of the cardiac conduction system (14). The expression of Cx40 was dramatically decreased in the cardiac conduction system of the Dicer mutant hearts (Fig. 3b-d, f, and g). However, the expression of Cx40 in the coronary vessels was not disturbed in Dicer knockout hearts (Fig. 3b, e, and h), which is not surprising because Dicer was specifically removed in cardiomyocytes, not coronary vessels. Western blot analyses quantitatively demonstrated a significant decrease of Cx40 protein in Dicer mutant hearts (Fig. 3i). Remarkably, the expression of Cx45 was significantly increased in Dicer mutant hearts (Fig. 3i-k), whereas there was no significant change in the expression of Cx43 (Fig. 3i and data not shown). The change of connexin protein levels in Dicer mutant hearts could be detected as early as E16.5 and is evident at E18.5 (SI Fig. 11).

Mishandling of calcium (Ca^{2+}) has been associated with animal models of heart failure and human failing hearts. In particular, several sarcoplasmic reticulum (SR) Ca^{2+} regulatory proteins are

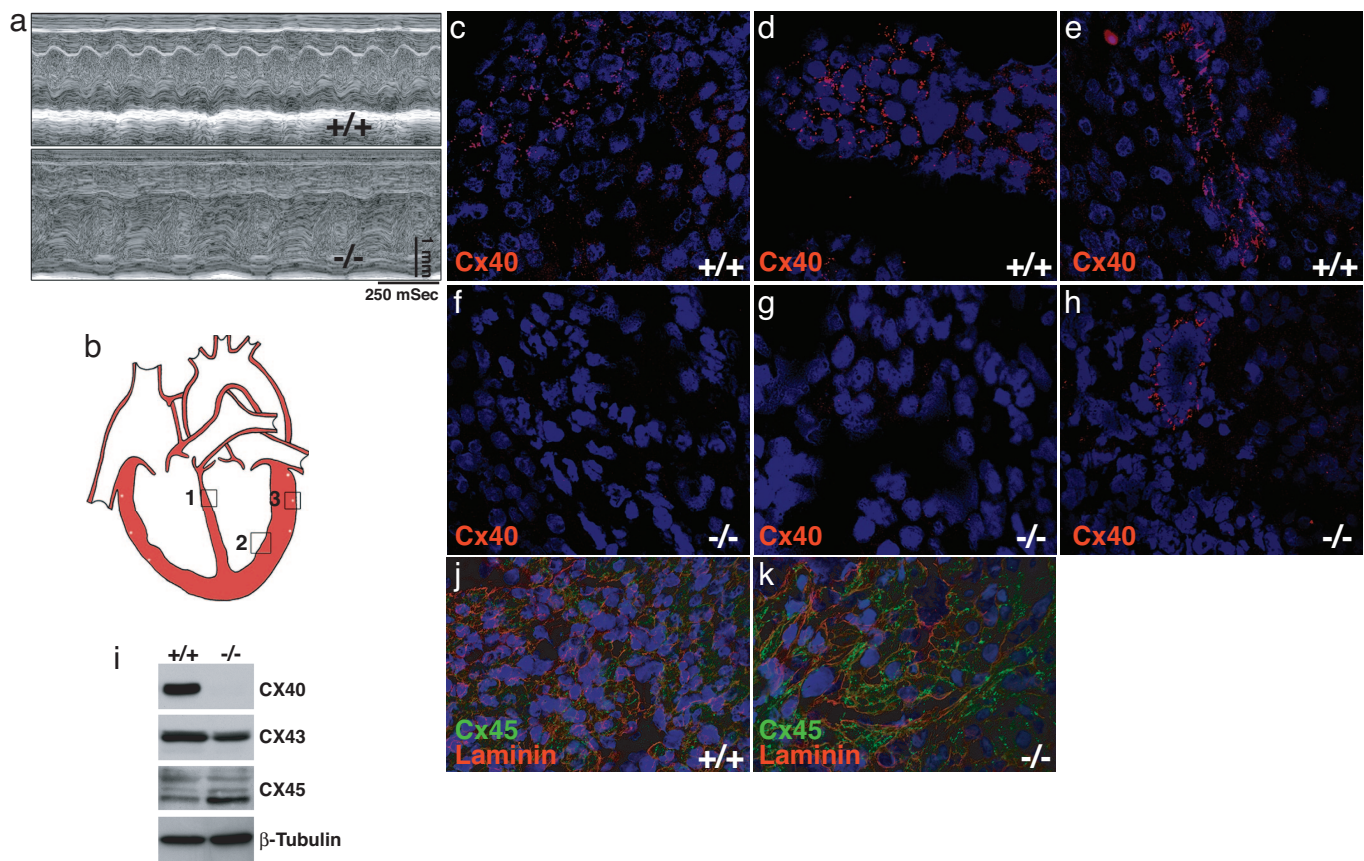


Fig. 3. Impaired cardiac function in Dicer mutant hearts. (a) M-mode echocardiograms of P0 wild-type (+/+) and Dicer mutant (-/-) mice. (b) Diagram to show the areas of ventricles examined in confocal immunohistochemistry images: area 1 for c and f; area 2 for d and g; area 3 for e and h. (c–h) Confocal microscopic images for Cx40 protein expression (red) in the ventricles of P0 wild-type (+/+) and Dicer mutant (-/-) mice. DAPI stains nuclei. (i) Western blot analyses of connexin protein expression in P0 wild-type (+/+) and Dicer mutant (-/-) mice. β -Tubulin serves as a loading control. (j and k) Confocal microscopic images of Cx45 in P0 wild-type (+/+) and Dicer mutant (-/-) hearts. Laminin marks the cell surface and DAPI stains nuclei.

suggested to be involved in the progression of heart failure (15). We determined the B_{max} values of [3H]ryanodine binding [as a measurement of SR Ca^{2+} release channel (RyR2) expression levels] and SR $^{45}Ca^{2+}$ uptake rates [as a measurement of SR Ca^{2+} pump (SERCA2a) activity] (16). B_{max} values (\pm SE) of [3H]ryanodine binding were 0.15 ± 0.01 and 0.13 ± 0.02 pmol/mg protein of whole membrane fractions isolated from control and Dicer mutant hearts, respectively ($n = 5$). SR $^{45}Ca^{2+}$ uptake rates were 0.99 ± 0.14 and 0.88 ± 0.05 nmol/mg protein/min for control and Dicer mutant membrane fractions, respectively. Neither difference was significant, which suggests there are no pronounced differences in the expression levels of the two key Ca^{2+} handling proteins of SR.

Dysregulation of Cardiac Contractile Protein Expression in Dicer Mutant Hearts. Our observations suggest that Dicer mutant hearts suffer from a deficiency in contractile force generation, as well as

a likely defect in conduction. Failure to provide sufficient contraction is the hallmark of human heart failure and is often associated with defects in the assembly of the contractile apparatus, the sarcomere, and/or the expression of contractile proteins (17). Therefore, we examined the expression of cardiac contractile proteins in control and Dicer mutant hearts. Indeed, decreased expression of cardiac troponin T (cTnT), an actin-binding protein linked to DCM in human patients (18), was evident in the Dicer mutant hearts (Fig. 2 d and e), which was further confirmed by Western blot analyses (Fig. 4g).

Human DCM is associated with mutations in genes important for myocyte contraction and cell integrity, including MHC and α -tropomyosin (α -TM) (4, 19, 20). We examine the expression of those contractile proteins and desmin, an intermediate filament found near the sarcomeric Z line, and vinculin, a cytoskeletal

Table 1. Dicer mutant mice are defective in cardiac function

Genotypes	Heart rate, bpm	IVSd, mm	IVSs, mm	LVPWd, mm	LVPWs, mm	LVIDd, mm	LVIDs, mm	FS, %
+/+ ($n = 10$)	555.9 \pm 42.33	0.348 \pm 0.06	0.604 \pm 0.12	0.355 \pm 0.07	0.624 \pm 0.13	1.248 \pm 0.13	0.696 \pm 0.15	44.63 \pm 8.86
-/- ($n = 7$)	278.6 \pm 64.38	0.40 \pm 0.12	0.57 \pm 0.15	0.571 \pm 0.10	0.713 \pm 0.10	1.517 \pm 0.25	1.287 \pm 0.16	19.77 \pm 5.31
+/- ($n = 8$)	537.4 \pm 48.28	0.335 \pm 0.05	0.573 \pm 0.09	0.295 \pm 0.04	0.567 \pm 0.17	1.306 \pm 0.16	0.756 \pm 0.16	42.34 \pm 8.52
P value	<0.0001	0.296	0.629	0.0001	0.170	0.01	<0.0001	<0.0001

Echocardiographic analyses of P0 wild-type (+/+), Dicer heterozygous (+/-), and homozygous (-/-) mutant mice. Values were quantified from five independent M-mode measurements. IVSd, interventricular septal thickness at end diastole; IVSs, interventricular septal thickness at end systole; LVPWd, left ventricular posterior wall thickness at end diastole; LVPWs, left ventricular posterior wall thickness at end systole; LVIDd, left ventricular internal diameter at end diastole; LVIDs, left ventricular internal diameter at end systole; and FS, fractional shortening.

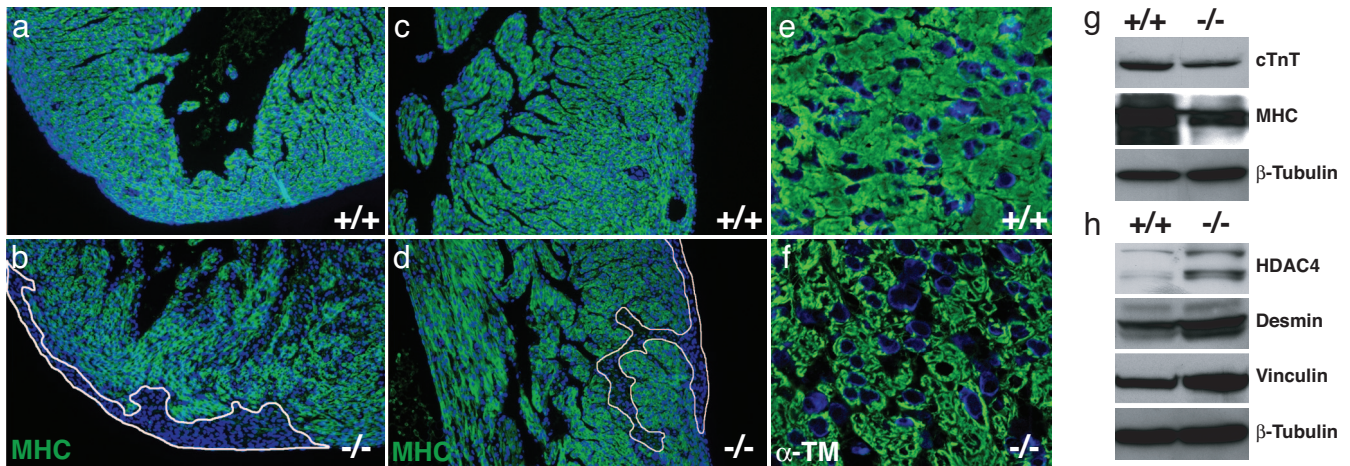


Fig. 4. Expression of cardiac contractile proteins in Dicer mutant hearts. (a–d) Confocal microscopic images of P0 wild-type (+/+) and Dicer mutant (–/–) hearts stained with MHC in apexes (a and b) or left ventricle (c and d). Note patches of myocardium that lack expression of MHC in Dicer mutant (–/–) hearts. DAPI stains nuclei. (e and f) Confocal microscopic images of P0 wild-type (+/+) and Dicer mutant (–/–) hearts stained with antibody against α -TM. Note dramatic decrease in the expression of α -TM in Dicer mutant (–/–) hearts. DAPI stains nuclei. (g and h) Western blot analyses of indicated proteins using protein extracts from P0 wild-type (+/+) and Dicer mutant (–/–) hearts. β -tubulin serves as a loading control.

protein involved in cell adhesion, in Dicer mutant hearts (Fig. 4). Mutations in both desmin and vinculin proteins have also been linked to DCM (21, 22). Strikingly, the expression of MHC proteins was altered in Dicer mutant hearts in a pattern where patches of ventricular myocytes lost the expression of those proteins (Fig. 4 a–d). Decreased MHC protein level in Dicer mutant hearts was further confirmed by Western blot analysis (Fig. 4g). Interestingly, whereas the expression of total MHC proteins decreased in Dicer mutant hearts, we noticed that the expression of β -MHC protein appears unchanged (data not shown). We also examined the expression of MHC in embryonic Dicer mutant hearts and found that its protein level decreased as early as E18.5 (SI Fig. 12). Confocal microscopy images of ventricular myocytes demonstrate a decrease in α -TM protein level in Dicer mutant hearts (Fig. 4 e and f). However, we did not detect any decrease in the protein level from E16.5 or E18.5 Dicer mutant hearts (SI Fig. 13). Interestingly, the expression of vinculin and desmin was increased in Dicer mutant hearts (Fig. 4h). In addition, we examined protein levels of HDAC4 and SRF, two proteins previously identified as regulatory targets for miR-1 and -133, respectively, in Dicer mutant hearts (8). Although the expression of HDAC4 was increased in the Dicer mutant hearts, the protein expression level of SRF was unchanged (Fig. 4h and data not shown). Notably, the expression of those proteins was unaltered in E16.5 or E18.5 Dicer mutant hearts (SI Fig. 13).

Decreased Dicer Expression in Human Patients with End-Stage DCM and Heart Failure. Having demonstrated that Dicer is essential for normal heart function in animals, by which loss of Dicer led to acute DCM and heart failure, we sought to examine the expression of Dicer protein in human patients with DCM and/or heart failure. Indeed, Dicer protein level is very low in human failing hearts (Fig. 5, lanes 1–4). Remarkably, the expression of Dicer protein is significantly elevated in recovering hearts after installation of a left ventricular assist device (LVAD) in those patients (Fig. 5, lanes 5–8). Clinically, LVADs are used in patients with end-stage failing hearts to mechanically support medically refractory hearts. Recent evidence indicates that reverse remodeling can occur even in the most advanced DCM after the application of LVAD (23). In contrast, we found that Dicer was expressed at a modest level in nonfailure patient hearts (Fig. 5, lanes 9–11). These data suggest that Dicer and therefore miR-

NAs are likely involved the progression and/or regression of DCM and heart failure in human patients.

Discussion

In this study, we assessed the global role of miRNAs in the heart, using a cardiac-specific conditional Dicer knockout mouse model. We found that Dicer and miRNAs play a critical role in normal cardiac function and animal survival. Dicer knockout leads to a severe decrease in cardiac contractility and loss of contractile proteins in the hearts. Most importantly, we report that heart failure patients lose normal Dicer protein expression.

DCM patients are clinically diagnosed by chamber dilation, decreased contractility, and impaired relaxation with no evidence of hypertrophic cardiomyopathy. Most previously reported DCM cases, particular those associated with sarcomeric protein mutations, have shown a progressive phenotype (2, 4). However, we found that deletion of Dicer in mouse hearts leads to a severe neonatal DCM phenotype, indicating that miRNAs are required for proper formation and function of cardiac contractile proteins. Whereas we found that in Dicer mutant heart, the expression level of some DCM-associated proteins (cTnT and MHC) was decreased, others such as desmin and vinculin was increased. The immediate downstream regulatory targets affected by the loss of potentially hundreds of miRNAs by Dicer deletion remain to be determined, which will likely require genetic studies of specific miRNAs.

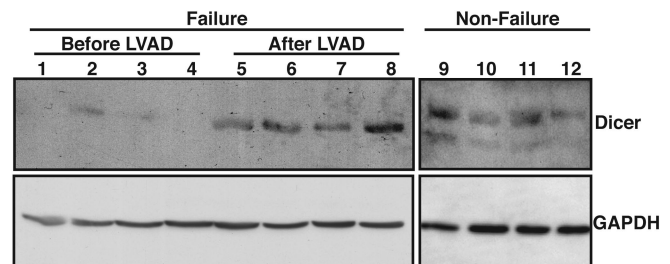


Fig. 5. Dicer protein expression in patients with failure or nonfailure hearts. Western blot analyses for Dicer from total protein extracts of ventricles of human subjects with end-stage heart failure before (lanes 1–4) or after (lanes 5–8) the application of LVAD, or nonfailure heart samples (lanes 9–11). GAPDH serves as a loading control.

Dicer is the only known enzyme responsible for processing miRNA precursors into mature products, making Dicer an ideal target to study and understand the global role of miRNAs. Gene targeting studies in mice revealed that Dicer is required for early embryogenesis and embryonic mesoderm formation (24). Surprisingly, embryonic removal of Dicer in zebrafish led to a much later and less severe phenotype during development (25). Most recently, it has been reported that cardiac-specific deletion of Dicer, using *Nkx2.5-Cre*, led to defects in heart development and embryonic lethality (10). In the present study, deletion of Dicer from the heart at a later developmental stage results in postnatal lethality with complete penetration, further supporting the view that Dicer (and miRNAs) are essential for cardiac development and function. The cardiac phenotype associated with the Dicer mutant mice resembles the human clinical features of DCM and heart failure. Indeed, we find decreased Dicer expression is associated with human patients with DCM and heart failure.

The heart is very sensitive to many stimuli and stresses; therefore, it is not surprising that even slight perturbations during cardiogenesis or in the adult heart have catastrophic consequences. Several reports have found the global miRNA expression profile regulated in models of physiological and/or pathological cardiac hypertrophy and in human patients with failing hearts (26–29). Interestingly, many of those studies documented a correlation of increased miRNA expression and cardiac abnormality (27–29). Dysregulated miRNA expression likely contributes to heart disease by mediating pathological changes in gene expression. Given the complexity of cardiac development and function, it is therefore very exciting to speculate that miRNAs are among the critical regulators of this process. The identification of specific regulatory mRNA targets for those miRNAs involved in cardiovascular system will provide more insight into the molecular mechanisms underlying this disease process.

It is intriguing that a single miRNA (miR-1) appears to be involved in the regulation of diverse cardiac and skeletal muscle function, including cellular proliferation, differentiation, cardi-

omyocyte hypertrophy, cardiac conduction, and arrhythmias (8, 10, 11, 26, 30). Those studies suggest that miRNAs may perform distinct functions in divergent biological settings by targeting different sets of mRNA targets. Furthermore, cardiac-specifically expressed miR-208 has been shown to play a key role in stress-dependent cardiac function (9). Together with what we have reported in this study, it becomes ever more evident that miRNAs are an important class of molecules that control cardiovascular development and function.

Methods

Details of materials and methods are found in *SI Text*.

Generation of Dicer Conditional Null Mice. Generation and genotyping of Dicer floxed (*Dicer^{fllox/fllox}*) mice (31) and α -MHC-Cre (*MHC^{Cre/+}*) mice (13) have been described.

Immunohistochemistry, TUNEL Staining, and Western Blot Analysis. Immunohistochemistry and Western blotting analyses were performed as described (8) using protein extracts from frozen mouse hearts. TUNEL staining was as described (8).

Transthoracic Echocardiography. Echocardiography was performed on control and Dicer knockout neonatal mice as described in detail in *SI Text*.

MicroRNA Microarray, Northern Blot Analyses, and RT-PCR. MicroRNA microarray, RT-PCR, and Northern blot analyses are essentially as described (8).

Human Patient's Samples. Under a University of North Carolina Institutional Review Board (UNC-CH IRB)-approved protocol, left ventricular tissue was taken from patients with end-stage DCM requiring mechanical circulatory support as a bridge to transplantation. More detailed analyses are described in *SI Text*.

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