FUNCTIONAL CHARACTERIZATION OF THE MICRO-RNA DEFICIENT ADULT MURINE HEART

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BACKGROUND AND SIGNIFICANCE

MicroRNAs (MiRNAs) are small endogenous RNA molecules about 22 nucleotides in length. MiRNAs are capable of post-transcriptional gene regulation by binding to their target messenger RNAs (mRNAs), leading to mRNA degradation or suppression of translation. MiRNAs have recently been shown to play pivotal roles in cardiovascular biology. Dicer, a RNAse III endonuclease, plays a key role in processing of miRNA into their functional mature form. A number of recent reports point towards a central role of miRNA in cardiac development and function. We have developed cardiomyocyte-specific conditional dicer knockout mice. Dicer knockout of adult mice resulted in a overt phenotype featuring ventricular enlargement, myocyte hypertrophy, and heart failure. In the current study we sought to functionally characterize the dicer deficient adult murine heart.

MR image acquisition.
MR images were acquired as follows: mice were anesthetized with isoflurane followed by placement of ECG leads and respiratory sensors. Core body temperature, respiration and ECG were followed using monitoring and gating systems Model 1025 (Small Animal Instruments Inc., Stony Brook, NY, USA). The MRI protocol included: *1)* localizer FLASH scans, and *2)* short-axis cine loops (12-16 slices. MR imaging was performed on a rodent-dedicated Bruker 11.7 T MR scanner with maximum gradient strength of 1000 mT/m). A 30 mm birdcage RF coil was used. The full imaging protocol was completed within 1h after anesthetizing the mouse.

Image processing and determination of cardiac functional parameters.
MR images were converted to DICOM format using Paravision 4.0 and processed with Segment. After the end-diastolic and end-systolic phases were identified on a slice-by-slice basis, end-diastolic volumes (EDV), and end-systolic volumes (ESV) were computed from the traced boarders. The areas were summed from all short axis slices and multiplied by slice thickness to get chamber volume. The difference between end-diastolic and end-systolic volumes provided the Left Ventricular (LV) stroke volume (SV) which was used to calculate cardiac output (SV*HR) and LV ejection fraction (SV*100/EDV). LV mass was calculated by multiplying LV myocardial volume with density of the myocardium {Gnyawali, 2009}.

Results
Dicer knockout resulted in decreased LV fractional shortening and ejection fraction within one week of tamoxifen injection. During the same time point cardiac hypertrophy was evident as increased LV myocardial mass in DKO (pmDicer-/-; pair matched Dicer knockout) mice. Compromised ejection fraction and cardiac output as well as increased stroke volume were noted in *pmDicer* $^{-/-}$ compared to *Dicer*$^{+/+}$ mice. Interestingly, we observed that *pmDicer* $^{-/-}$ mice suffered from oxidative stress as evident by increased tissue lipid peroxidation.

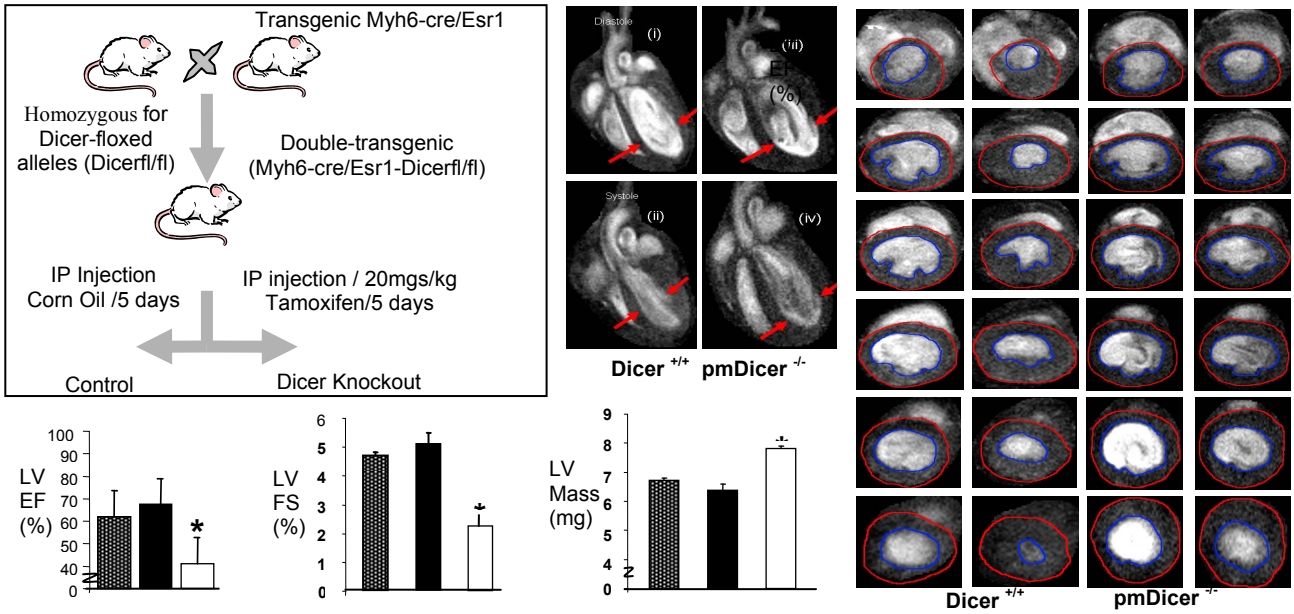

Figure 1. Conditional disruption of the mouse gene *Dicer* and measurement of cardiac function: Mouse homozygous for a floxed *Dicer* allele (Dicer$^{fl/fl}$) was bred with another mouse having a tamoxifen-inducible Cre-recombinase protein fused to mutant estrogen-receptor ligand-binding domains under the control of the cardiac-specific MYH6 promoter. Eight week old double transgenic mouse was injected with tamoxifen (DKO set) or corn oil (control set) for 5 days. MRI (11.7T) images of long axis at diastole (i & iii), systole (ii & iv) cardiac 11.7T MRI images in DKO and control mice are shown. The diastole of KO mice was enlarged because of insufficient contraction of the heart (shown by arrows). The panel on the right displays 11.7T images of short axis at diastole, systole of 1.0 mm slices stacked along columns from basal to apical of the LV of both control and DKO mice. Sixteen time frames per slice of such images were collected per cardiac cycle of the murine heart. Left ventricular volumes (blue contour lines) were determined from short axial frames by planimetry and applying Simpson's rule. Representative M-mode images of control and DKO mice are shown.

Conclusion: Arrest of microRNA biogenesis caused by conditional knockout of Dicer results in rapid (within one week) and overt changes in cardiac phenotype. Cardiac hypertrophy is associated with marked compromise of cardiac function. Such changes are associated with oxidative stress in the heart.