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Contribution of miR-199b to right ventricular remodelling due to pressure overload

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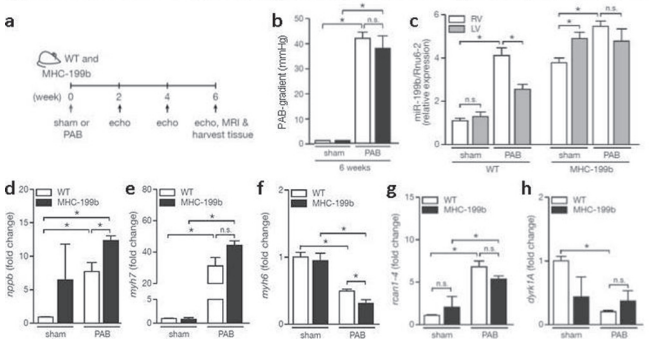
Background: MicroRNA-199b (miR-199b) has been identified as a direct target gene of calcineurin/NFAT signalling in both mouse and human heart. miR-199b expression levels are increased in left ventricular (LV) pressure overload and heart failure. Overexpression of miR-199b inhibits nuclear NFAT kinase dual-specificity tyrosine-(Y)-phosphorylation (Dyrk1a), contributing to adverse left ventricular remodelling. The role of miR-199b in right ventricular (RV) pressure load is currently unknown.
Purpose: We aimed to unravel the role of miR-199b in calcineurin/NFAT signalling in RV remodelling due to pressure overload.
Methods: In the present study, wild type (wt) and transgenic mice with cardiac-specific overexpression of miR-199b (Tg) were subjected to six weeks of RV pressure overload induced by pulmonary artery banding (PAB). Echocardiographic and MRI derived hemodynamic parameters, and molecular remodelling were assessed for experimental groups (PAB-Tg, PAB-wt) and compared to sham-operated controls (sham-Tg, sham-wt). (figure a)
Results: Increased RV pressure load was confirmed by increased PAB-gradient (figure b) and septal flattening RV. miR-199b levels in PAB-Tg were increased compared to both PAB-wt and sham-Tg, whereas miR-199b levels did not differ between PAB-wt and sham-Tg (figure c). PAB led to significant RV-adaptation, assessed by hemodynamic, histopathological and molecular measurements in both PAB groups when compared to both sham groups. RV hypertrophy and fibrosis tended to be increased in PAB-Tg compared to PAB-wt. Also RV end diastolic (76.4 vs. 72.9 μl, resp.) and systolic volumes (52.8 vs. 46.0 μl, resp.), RV ejection fraction (31.9 vs. 36.1%, resp.) and tricuspid annular plane systolic excursion (TAPSE) (0.72 vs. 0.91 mm, resp.) tended to be worsened in PAB-Tg. The slopes of TAPSE over time suggested progressive impairment of RV function in PAB-Tg compared to PAB-wt (-0.0125 vs. 0.045, resp. $p<0.001$). Markers of ventricular stress (NPPB) (figure 1d) and fetal gene program (MYH7/MYH6 ratio) (figure e-f) were increased in PAB-Tg compared to PAB-wt as well. Activation of calcineurin/NFAT signalling, however, did not differ between PAB-wt and PAB-Tg as reflected by levels of rcan1–4 (figure g) and dyrk1a (figure h). Remarkably, in the LV, levels of dyrk1a decreased in PAB-Tg compared to PAB-wt, inversely related to miR-199b expression. This suggests greater susceptibility of the LV to increased levels of miRNA-199b during RV stress.

Figure. Contribution of miR-199b to right ventricular remodelling due to pressure overload



Conclusion: Increased expression levels of miR-199b were associated with impaired RV function in RV pressure overload, whereas increased miR-199b expression without RV pressure overload was insufficient to worsen RV function. In the LV, in contrary to the RV, upregulation of miR-199b leading to inhibition of dyrk1a and activation of calcineurin/NFAT signalling, increases LV susceptibility to RV stress. Altogether, these data suggest a less prominent role for miR-199b in the RV compared to the LV.

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Cardiac fibroblast-enriched long non-coding RNA lnc-fibrogen promotes myocardial fibrosis by sponging miR-29a

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Introduction: Heart failure (HF) is one of leading causes of death worldwide. The loss of myocardium following cardiac injury is compensated by the excessive production of extracellular matrix (ECM) and the formation of a collagen-rich fibrotic scar. Scar formation and interstitial fibrosis lead to progressive myocardial dysfunction and ultimately HF. There is, however, no effective therapy to prevent or reverse cardiac fibrosis. There is a clear need to identify novel mediators/pathways underlying cardiac fibrosis to develop effective therapeutics. With the advances in genomic medicine, it is now known that up to 90% of mammalian genome is transcribed as long non-coding RNAs (lncRNAs). Studies have shown that lncRNAs are critically involved in cardiac development and diseases. Leveraging next-generation RNA sequencing on human failing heart, we have identified a cardiac fibroblast-enriched lncRNA, lnc-fibrogen, which is dysregulated in failing heart and its expression levels are highly correlated with that of fibrosis genes.
Purpose: To test the hypothesis that lnc-fibrogen contributes to the pathogenesis of cardiac fibrosis and to determine the molecular mechanisms
Methods and results: Lnc-fibrogen is specifically enriched in human ventricular fibroblasts (HuVF) and was significantly upregulated in response to pro-fibrotic stimuli such as TGFβ1 treatment. Knocking down lnc-fibrogen prevented TGFβ1-induced HuVF activation and ECM gene production. In addition, overexpression of lnc-fibrogen was sufficient to result in ECM gene, including COL1A1 and ACTA2, up-regulation, cell proliferation and myofibroblast transformation in HuVF. Lnc-fibrogen was mainly distributed in cytosol, where it modulates ECM gene transcript stability. Computational analyses predicts the interaction between lnc-fibrogen and miR-29a, a miRNA known to inhibit cardiac fibrosis. MicroRNA sequencing revealed that knockdown of lnc-fibrogen resulted in up-regulation of miR29a. RNA pull-down assay using biotinylated miR-29a showed its strong physical interaction with lnc-fibrogen. In addition, luciferase reporter assays in HuVF using constructs with luc-WT lnc-fibrogen and luc-lnc-fibrogen mutant with miR-29a binding site deletion showed that miR-29a mimic reduced the activity of luc-WT lnc-fibrogen, but not luc-lnc-fibrogen mutant, suggesting the functional interaction between lnc-fibrogen and mi29a. Importantly, the treatment of miR-29a target site blocker prevented the increment of ECM and HuVF proliferation induced by ectopic lnc-fibrogen expression. Taken together, these data suggest that lnc-fibrogen modulates cardiac fibrosis by sponging miR-29a.
Conclusions: The present study identified a novel, cardiac fibroblast-enriched lncRNA lnc-fibrogen, which promotes cardiac fibrosis by sponging anti-fibrotic miR-29a. These results suggest that targeting lnc-fibrogen could be a potential novel therapeutic approach to treat or prevent cardiac fibrosis and heart failure.

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microRNA-210 is necessary for exercise-induced cardiomyocyte proliferation and mediates the benefical effect of exercise against cardiac ischemia-reperfusion injury

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Background: Exercise can induce physiological cardiac growth, and protect against cardiac ischemia-reperfusion injury (IRI). microRNA-210 (miR-210) is up-regulated in exercised heart, however, its role in exercise-induced physiological cardiac growth and whether it could mediate the protective effect of exercise against cardiac IRI remains unclear.
Purpose: We aimed to study the role of miR-210 in exercise-induced physiological cardiac growth, and to unravel the molecular mechanism of miR-210 mediating the beneficial effect of exercise against cardiac IRI.
Methods: Physiological cardiac growth was induced by 8 weeks of swimming exercise in rats. miR-210 knockout (KO) rats and wide type (WT) littermates were utilized to investigate whether miR-210 mediates exercise-induced cardiac growth. Myocardial hypertrophy and cardiomyocyte proliferation were determined by WGA staining and Ki67/α-actinin immunolabelling, respectively. Then, the effects of miR-210 in cardiomyocyte proliferation and apoptosis were investigated in neonatal rat cardiomyocytes transfected with miR-210 mimics or inhibitors. Furthermore, the target genes of miR-210 were identified using luciferase reporter assay, Western blot, and function rescue experiment. Finally, miR-210 KO rats and WT littermates were subjected to acute cardiac IRI after 8 weeks of swimming exercise to clarify whether miR-210 mediates the beneficial effect of exercise against IRI.
Results: Exercise induced physiological cardiac growth in both miR-210 knockout rats and WT littermates, as evidenced by increased heart weight/body weight ratio and heart weight/tibia length ratio and enlarged myocardial cross-sectional area. However, exercise-induced cardiomyocyte proliferation was decreased in miR-210 KO rats, indicating that miR-210 is necessary for exercise-induced cardiomyocyte proliferation. In neonatal rat cardiomyocytes, Ki67/α-actinin and EdU/α-actinin immunolabellings showed that miR-210 promoted cardiomyocyte proliferation, and Tunel staining showed that miR-210 prevented oxygen glucose deprivation/reperfusion (OGDR)-induced cardiomyocyte apoptosis. Furthermore, we identified CDK10, APC, EFNA3, and PTPN1 as target genes of miR-210 in the regulation of cardiomyocyte proliferation and apoptosis. Finally, we demonstrated that the protective effect of exercise against cardiac IRI was totally absent in miR-210 KO rats, suggesting that miR-210 is necessary for exercise-induced cardioprotection.
Conclusions: miR-210 is necessary for exercise-induced cardiomyocyte prolifer-