The molecular cues underpinning persistent myocardial damage despite intensive glycemic control (IGC) remain to be elucidated. Epigenetic regulation of gene expression by microRNAs (miRs) and chromatin changes is emerging as a key driver of cardiovascular disease. In particular, miR-21 and miR-34a have been implicated in multiple aspects of diabetic cardiomyopathy.

Effects of miRNAs modulated by endurance training on cardiomyocyte excitability

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Despite the well-established benefits of endurance training, several studies have shown that a large fraction of veteran endurance athletes have a significantly higher risk to develop arrhythmias than age-matched sedentary subjects. To dissect the mechanism underlying this increased risk, we focused on the potential modulatory role of microRNAs (miRs) that are able to alter the expression of ion channels and are also specifically modulated by exercise. In order to find miRNAs specifically modulated by the training regimen, we analyzed circulating miRNA levels in ultra-endurance athletes and in mice trained on the treadmill for 8 weeks. 5 d/wk for 1 h at 23 cm/s. We collected plasma from both humans and mice and collected sinoatrial node (SAN), atria and ventricles from mice, and then quantified miRNA levels in each tissue. Among 84 cardiac-related miRNAs tested, a downregulation of 3 fold was observed in the SAN, a downregulation of miR-1 in both atria (-11.5 fold) and ventricles (-45 fold), and an upregulation of miR-206 in ventricles (+3 fold). Downregulation of both miR-1 and miR-206 in the SAN have particularly attracted our attention due to the well-known bradycardia arising in this tissue in endurance athletes. Electrophysiological analysis was performed on primary cultures of spontaneously beating neonatal rat ventricular cardiomyocytes (NRVCs) transfected with either miR-1 or miR-206. In miR-1 overexpressing NRVCs there was a 3 fold reduction in rate accompanied by a reduction in the pump fraction (-25%) caused by downregulation of the HCN4 isoform. miR-206 overexpression in NRVCs did not cause any modification. In conclusion, miR-1 and miR-206 are modulated by training in both human and mice following endurance training. Alterations of miR-1 in particular have the ability to modulate cardiac excitability by altering ion channel expression levels.

Background: More intensive glycemic control in patients with diabetes mellitus did not reduce the occurrence of heart failure events. The molecular cues underpinning persistent myocardial damage despite intensive glycemic control (IGC) remain to be elucidated. Epigenetic regulation of gene expression by microRNAs (miRs) and chromatin changes is emerging as a key driver of cardiovascular disease. Purpose: In the present study we investigated whether epigenetic networks participate to persistent myocardial dysfunction despite IGC. Methods: Diabetes was induced in 4-6 months 129sv mice by streptozotocin. IGC in diabetic mice was achieved by slow-release insulin implants placed subcutaneously 3 weeks after the induction of diabetes and maintained for the following 3 weeks. Mouse miRNome profiling was investigated by real-time PCR array. DNA methylation was performed by bisulfite analysis of transcriptionally active CpG regions whereas chromatin immunoprecipitation (Chip) was employed to study histone modifications. Mitochondrial levels of superoxide anion (O2-) were detected by ESR spectroscopy. Left ventricular function was assessed by high resolution Micro-Ultrasound System (Vevo 2100, Visualsonics). Results: Mitochondrial oxidative stress was significantly increased in the diabetic heart and 3-week IGC did not revert this phenomenon. Consistently, normoglycemia restoration did not rescue left ventricular dysfunction, assessed by ejection fraction (EF) and fractional shortening (FS). miRNome analysis revealed that miR-21 and miR-34a were profoundly downregulated in the diabetic heart, and IGC did not affect their expression. We found that miR-21 and miR-34a respectively caused persistent downregulation of methyltransferase DNMT3b and deacetylase SIRT1 in the diabetic heart, even after IGC. Disturbed DNMT3b/SIRT1 axis triggered DNA demethylation and histone 3 acetylation, leading to enhanced transcription of the mitochondrial adaptor p66Shc, a key pro-oxidant gene. Interestingly, in vivo siRNA of p66Shc at the time of glucose overexpression blunted ROS production while restoring cardiac function in diabetic mice. Conclusions: We hypothesized that distinct miRNA/microRNA expression patterns can be found in the failing, hypertensive RV. Conclusions: The known developmental differences between RV and left ventricle (LV), we proposed certain genes to be differentially expressed (RV vs. LV)

Differential transcriptome and microRNA expression signatures in the healthy heart (RV vs. LV) and the failing, pressure-overloaded right ventricle (SuHx model)

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Methods: Age-matched, 6wks old Sprague Dawley rats (200g body weight) were divided into three groups: (1) control normoxia (ConNx, untreated), (2) control vehicle/hypoxia (ConHx, i.e. rats injected once subcutaneously with DMSO (vol/vol)) and exposed to chronic hypoxia for 3 weeks, followed by a 6wks in room air), (3) Sugen hypoxia (SuHx, i.e. rats, injected with the VEGF2 blocker SU5416 20mg/kg/dose s.c. dissolved in DMSO, and subsequently exposed to 3wks chronic hypoxia and 6wks room air). Hemodynamics, RV mass and volumes were assessed by cardiac catheterisation, Fulton’s index (Mass ratio RV/LV+ septum) and cardiac MRI. Expression studies (RNASeq, qPCR array) were performed on mRNA from RV and LV from 3-5 rats per group. Results: SuHx rats developed severe PAH and RV failure vs. ConNx and ConHx, respectively. RVD/EDV, RV EDV (40.5 vs. 25), RV ESV (87.8 vs. 34.9), RV EF (48 vs. 75%), EF (80 vs. 78% and 74.9 %). These miRNAs included miR-126-5p, all of which have not been reported to be involved in PAH. Currently, laser capture microdissection revealed 160 genes with differential expression in RVs among three groups (FDR 5%), including Pym1k, Myl7, Bgn, Cgfl. qPCR array revealed several miRNAs that were significantly up- or down-regulated in SuHx vs. ConNx and/or ConHx. These miRNAs included miR-126-5p, miR-140, miR-202-3p, all of which have not been reported to be involved in PAH. Current, laser capture microdissection of histological sections, and subsequent qPCR are being conducted on human explanted PAH heart-lung tissue. Conclusions: To the best of our knowledge, this is the first combined hemodynamic/systems biology study, comparing RV and LV mRNA and microRNA expression profiles in SuHx rats (PAH/PVH, RV Failure) and controls, in a comprehensive, unbiased approach. In the ongoing data analysis, we identified RNA signature patterns (mRNA, miRNA) that will explain our understanding of PAH and associated heart failure, that can lead the way to precision medicine in PAH, and may allow tailored therapies in the future.