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miR-218 and miR-34a drive persistent myocardial oxidative stress by targeting chromatin remodelers DNMT3b and SIRT1: a new mechanistic insight in diabetic cardiomyopathy

F. Cosentino1, E. Legchenko1, R. Kueffner2, G. Hansmann1

1Karolinska Institute, Cardiology Unit, Stockholm, Sweden; 2University of Zurich, Veterinary physiology, Zurich, Switzerland; 3Second University of Naples, Pharmacology, Naples, Italy; 4Sapienza University of Rome, Department of Clinical and Molecular Medicine, Rome, Italy; 5Cardiovascular Research, Physiology Institute, University of Zurich, Zurich, Switzerland

Background: A 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 (miR) and chromatin changes is emerging as a key driver of cardiovascular damage. Purpose: In the present study we investigate 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 echocardiography. Micro-Ultrasond 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-218 and miR-34a were profoundly dysregulated in the diabetic heart, and IGC did not affect their expression. We found that miR-218 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 normalization blunted ROS production while restoring cardiac function in diabetic mice. Conclusion: We show here that a complex epigenetic machinery involving miR-218/34a and DNMT3b/SIRT1 axes may explain persistent p66Shc overexpression and subsequent oxidative burst in the diabetic heart. These findings provide molecular insights to understand the lack of benefit of glycemic control on diabetic cardiomyopathy phenotype.

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Effects of miRNAs modulated by endurance training on cardiomyocyte excitability

M. Bonzanni1; A. Napoli2; S. Landi3; A. Bucoli3; G. Vernillo2; M. Baruscotti1; A. La Torre2; E. Legchenko1; R. Kueffner2; G. Hansmann1

1University of Milan, Biosciences, Milan, Italy; 2University of Milan, Biomedical Sciences for Health, Milan, Italy

Despite the well-established benefits of endurance training, several studies showed that a large fraction of veteran endurance athletes has a significantly higher risk to develop arrhythmias than aged-matched sedentary subjects. To dissect the mechanism underlying this increased risk, we focused on the potential modulatory role of microRNAs (miRNAs) 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/d at 23 cm/s. We collected plasma from both humans and mice and collected intrasinal node (SAN), atria and ventricles from mice, and then quantified miRNA levels in each tissue. Among 84 cardiac-related miRNAs tested, a downregulation (p < 0.05) of muscle-specific miR-1 (6 fold) and miR206 (2 fold) appeared in athletes’ plasma (n=20) compared to non-athletes (n=14). Downregulation by 11 fold of miR-1 was confirmed in the plasma of trained (n = 6) vs non-trained mice (n=5), while no change in the level of miR-206 was evident. We found a cardiac-specific regional modulation of these miRNAs never reported before. In particular, we found an upregulation of both miR-206 (+132 fold) and miR-1 (+3 fold) 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). Upregulation 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 55% reduction in heart rate accompanied by a reduction in the pacemaker if density (−52%) caused by downregulation of the CHM4 isofrom. 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.

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Different transcriptional and microRNA expression signatures in the healthy heart (RV vs. LV) and the failing, pressure-overload right ventricle (SuHx model)

E. Legchenko1, R. Kueffner2, G. Hansmann1

1Hannover Medical School, Pediatric Cardiology and Critical Care, Hannover, Germany; 2Heinrich Zentrum Munch - German Research Center for Environment and Health, Munich, Germany

Background and Hypothesis: Pulmonary vascular disease (PVD) is characterized by progressive obliteration of pulmonary arteries leading to increased pulmonary vascular resistance, pulmonary arterial hypertension (PAH), right ventricle (RV) failure, and death in ~25-60% of patients 5yrs after diagnosis. We hypothesized that distinct mRNA/microRNA expression patterns can be found in the failing, hypertensive RV. Given the known developmental differences between RV and left ventricle (LV), we proposed certain genes to be differentially expressed (RV vs. LV) even in the healthy state.

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/vois) 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 VEGFR3 blocker SUGEN1416 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: RV/ LV ratios developed severe PAH and RV failure vs. ConNx and ConHx, respectively: RVSP (~25 vs. 15mmHg), RV WSP (~12 vs. 6mmHg), RV EDV (~10 vs. 5mmHg), RV ESV (~5 vs. 3mmHg), RV SV (~9 vs. 3mmHg), RV IVC (~15 vs. 8mmHg), RV weight (~25 vs. 15mg). 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/PVD, RV Failure) and controls, in a comprehensive, unbiased approach. In the ongoing data analysis, we identified mRNA signature patterns (mRNA, miRNA) that will expand 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.