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Pro-atherogenic miR-103 inhibits endothelial proliferation by targeting IncWDR59
D. De Gonzalo Calvo1; F. Schottmer2; C. Bang2; R. Zimmer2; A. Schober1
1Institute for Molecular and Translational Therapeutic Strategies (IMTTS), Hannover, Germany; 2Ludwig-Maximilians University, Munich, Germany.

Background: Endothelial cell (EC) maladaptation during subclinical stages of atherosclerosis is characterized by inflammation and defective regeneration. The RNase Dicer is essential for the production of microRNAs, which play a crucial role in EC maladaptation. EC-derived Dicer promotes atherosclerosis and suppresses KLF4, Notch1, and Wnt signaling. While Dicer increases EC inflammation by miR-103-mediated suppression of KLF4, the role of Dicer-regulated Wnt and Notch1 signaling in EC regeneration is unclear.

Methods: We test the hypothesis that Dicer impairs EC regeneration through miRNA-mediated suppression of long non-coding RNAs (IncRNAs) that promote Wnt and Notch1 signaling.

Results: In EC-Dicer-/- mice, endothelial Notch1 and β-catenin activation and EC proliferation were increased in atherosclerotic arteries. The novel IncWDR59 was the most significantly upregulated IncRNA in EC-Dicer-/- mice and its sequence contained a putative miR-103 binding site. Inhibition of miR-103 increased the expression of IncWDR59 in ECs and overexpressing miR-103 increased the enrichment of IncWDR59 in the RNA-induced silencing complex. Blocking the interaction between miR-103 and IncWDR59 by TSBs promoted EC proliferation, reduced apoptosis, upregulated the expression of the artherosclerotic marker SOX17, and increased Notch1 and β-catenin activity. Inhibiting Notch1 and β-catenin activity decreased EC proliferation and increased apoptosis. Blocking Notch1 but not silencing β-catenin abolished the TSB-mediated increase of EC proliferation and SOX17 expression.

Conclusions: MiR-103 targets the novel IncRNA IncWDR59 in ECs and, thereby, impairs EC proliferation by inhibiting Notch1 activity. This mechanism may contribute to the pro-atherogenic effects of Dicer and blocking the interaction between miR-103 and IncWDR59 might be a promising therapeutic strategy against atherosclerosis.

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Circulating long-non coding RNA LIPCAR and left ventricular diastolic function in patients with uncomplicated type 2 diabetes mellitus
D. De Gonzalo Calvo1; F. Schottmer2; C. Bang2; R. Zimmer2; A. Schober1
1Institute for Molecular and Translational Therapeutic Strategies (IMTTS), Hannover, Germany; 2Ludwig-Maximilians University, Munich, Germany.

Background: Cardiac dysfunction is often unrecognized in T2DM early stages due to the absence of clinical symptoms. There is a clinical need of biomarkers to predict and/or monitor cardiac alterations in T2DM patients with LV diastolic dysfunction. In multivariate linear regression models, LIPCAR was inversely associated with LV diastolic function, measured as the E/A peak flow, independently of possible confounders (P < 0.05 for all models). Circulating levels of LIPCAR were significantly elevated in T2DM patients with LV diastolic dysfunction (P = 0.002).

Method: The level of serum LIPCAR is predictive of LV diastolic function in patients with uncomplicated T2DM and may be helpful in the evaluation of early cardiac alterations in T2DM.

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Circulating thrombospondin-1 inhibits coronary blood flow reserve in aging hearts through a CD-47-dependent decrease in NO in coronary arterioles
A. Leblanc; C.D. Nevitt; G. McKenzie; K. Christian; J. Austin; S. Henrique
Cardiovascular Innovation Institute, Louisville, United States of America.

Intro: In healthy adults, nitric oxide (NO) mediates the vasodilation of coronary arterioles in response to increased myocardial perfusion demand. Aging and cardiovascular disease are associated with the loss of NO signaling and a decline in the ability to increase coronary blood flow (BF), or CFR. Thrombospondin-1 (Tsb-1), a secreted protein, has been shown to limit NO-dependent vasodilation in peripheral vascular beds via formation of superoxide (O2-). CD47 blocking antibodies are currently in use in pre-clinical trials as an anti-angogenic therapy for cancer, but we wanted to determine if inhibiting CD47 would be effective in reversing NO-dependent coronary microvascular dysfunction.

Purpose: Establish the relationship between circulating Tsb-1, CD47 signaling, coronary perfusion, and aging. The present study tests the hypothesis that blocking CD47 will improve NO-mediated vasoreactivity in coronary arterioles from aged individuals, resulting in improved CFR.

Methods: Isolated coronary arterioles from young (4 month) or old (24 month) female Fischer-344 rats (n=7/group) were challenged with the NO donor DNa-NONOate (1x10^-7)-1x10^-5M) and vessel relaxation and O2- production was measured before and after Tsb-1, CD47, and/or tempol and catalase exposure. In vivo CFR was determined through injected microspheres at baseline and post-dobutamine following control IgG or aCD47 incubation (45 min, n=4/group).

Results: Isolated arterioles from young and old rats relax similarly to exogenous NO at baseline, but addition of a physiological concentration of Thbs-1 (2.25µM)-inhibited NO-mediated vasodilation by 24% in old rats (58.0 ± 2.4, Thbs-1: 65.5 ± 2.8) whereas young vessels were unaffected. Similarly, Thbs-1 increased O2- production in coronary arterioles from rats of both ages, but this was exaggerated in old rats (AL young: 12.1 ± 16, old 20.7 ± 2). Tempol and catalase showed limited ability to improve vasodilation in the presence of Thbs-1, but the addition of aCD47 blocking antibody completely restored NO-dependent vasodilation in isolated arterioles from aged rats and attenuated O2- production (5% relaxation). Thbs-1 + aCD47: 84.0 ± 6.0. Further, aCD47 treatment increased CFR from 9.6 ± 9.3 (control IgG) to 84.0 ± 23% in distal section of the left ventricle dissected to apex in intact animals.

Conclusions: These findings suggest that the influence of Thbs-1 and CD47 on coronary perfusion and vasoreactivity increases with aging. Based on these results, humanized CD47 blocking antibodies may be used to therapeutically target coronary microvascular dysfunction associated with aging.
261 Endothelial cell adenosine deaminase acting on RNA-1 is critically involved in vascular development and homeostasis in vivo

FF. Luxellia; A. Gatioua; P. Groteb; C. Amrhenia; A. Doddballapura; T. Braunb; A. Zeibertc; S. Dimmeleid; K. Stellosf

Background: Adenosine deaminase acting on RNA-1 (ADAR1) binds to double-stranded RNAs and mediates adenosine (A) to inosine (I) RNA editing, which is a widespread post-transcriptional mechanism in mammals that affects several coding and regulatory RNAs, by altering their sequence and structure. However, the role of ADAR1 in vascular system has not been reported so far.

Methods and Results: To investigate the role of ADAR1 in vascular development, ADAR1floxb/lox mice were mated with Tie2-Cre mice, in which the expression of Cre recombinase is driven by endothelial cell (EC) specific promoter of angiopeptin receptor. The EC-restricted ADAR1 knockout mice resulted in embryonic death at E13.5, suggesting an essential role for endothelial ADAR1 in embryonic development. To evaluate the role of ADAR1 in postnatal retinal vascular development, ADAR1floxb/lox mice were mated with mice carrying a tamoxifen-inducible VE-Cadherin-Cre transgene (Cdh5-CreERT2) creating an inducible endothelial cell-restricted ADAR1 knockout (iEC-ADAR1 KO) mouse model. Postnatal ADAR1 ablation resulted in 24 ± 4% reduced vascular outgrowth, 18 ± 7% reduced vessel branching in the central vascular plexus and 39 ± 11% decreased filopodial protrusions from endothelial cells at the angiogenic front of the vascular plexus compared with litterate control mice at P5 (all P < 0.05). Furthermore, endothelial cell ablation of ADAR1 in 8-week-old mice resulted in formation of pleural effusion and ascites, indicating a disturbance of endothelial cell barrier function, and in death within 6-8 days after ADAR1 ablation (log rank P = 0.001 of the Kaplan-Meier survival curve for n=12 mice per group). TUNEL with CD31 counterstain revealed the presence of apoptotic lung endothelial cells, indicating that ADAR1 plays a critical role in endothelial cell homeostasis in vivo. Mechanistically gene set enrichment analysis of transcriptome expression after ADAR1 knockdown in HUVECs revealed that the most important biological function affected is apoptosis and that ADAR1 downregulation is strongly associated with upregulation of interferon-regulated transcripts and innate immune response, possibly due to activation of the cytokine dsRNA receptors TLR3 and MD2.

Conclusion: The RNA editor ADAR1 is critically involved in vascular development and homeostasis in vivo.

262 Sympathetic transmission in perivascular adipose tissue function in health and obesity

S. Saxton; S.B. Withers; J. Ohanian; AM. Heagerty
University of Manchester, Institute of Cardiovascular Sciences, Manchester, United Kingdom

Background & Aims: Healthy perivascular adipose tissue (PVAT) exerts an anti-contractile effect on resistance arteries which is vital in regulating arterial tone. Activation of β3-adrenoceptors by the neurotransmitter, noradrenaline, may be implicated in the anti-contractile effect of PVAT. In obesity the anti-contractile effect is lost, leading to the development of hypertension. Accordingly, we have investigated the effect of sympathetic nerve stimulation (SNS) within healthy and obese PVAT on the anti-contractile effect, and have identified the mechanisms involved.

Methods: Electrical field stimulation (EFS) profiles of healthy C57 mouse mesenteric arteries (<200μm, 2-4/PVAT) were characterised using wire myography (0.1-30Hz, 20V, 0.2ms pulse duration, 4s train duration). To demonstrate the release of an anti-contractile factor, the solution surrounding stimulated exogenous PVAT was transferred to a PVAT denuded vessel. Neuronal inhibition using tetrodotoxin (TTX, 1μM), or sympathetic denervation using 6-hydroxydopamine (6-OHDA, 2μM) were performed. β3-adrenoceptor function was investigated using the agonist CL-316,243 (10μM) and antagonist SR5920A (100μM). A model of obesity was set up by feeding C57s a 60%-fat diet over a period of 10-12 weeks. EFS profiles of healthy arteries were compared to arteries from aged-matched obese mice, and the β3-adrenoceptor agonist CL-316,243 was tested.

Results: During EFS PVAT elicits a reproducible anti-contractile effect, which is replicated using exogenous PVAT. Solution transfer from stimulated exogenous PVAT to a →PVAT vessel significantly reduced contraction, confirming that stimulated PVAT releases a transferable anti-contractile factor. Neural inhibition using TTX, or sympathetic denervation with 6-OHDA, abolished all anti-contractile activity implicating sympathetic nerves in release of anti-contractile factors. β3-adrenoceptor agonist CL-316,243 enhanced the anti-contractile effect, and β3-adrenoceptor antagonist SR5920A reduced the anti-contractile effect. Complete inhibition of the effects of the solution transfer could be achieved by incubation of exogenous PVAT with SR5920A. In arteries from obese mice, the EFS-induced anti-contractile effect was lost, and could not be restored using CL-316,243.

Conclusions: These results demonstrate that SNS in PVAT elicits an anti-contractile effect by activation of adipocyte β3-adrenoceptors, triggering the release of vasodilators. In obesity, β3-adrenoceptors may have become desensitised, resulting in a loss of function, and leading to hypertension.

WITHDRAWN.