258 Pro-atherogenic miR-103 inhibits endothelial proliferation by targeting IncWDR59
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Background: Endothelial cell (EC) maladaptation to disturbed blood flow at predilection sites of atherosclerosis is characterized by inflammation and defective regeneration. The lncRNA 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 injury by miR-103-mediated suppression of KLF4, the role of Dicer-regulated Wnt and Notch1 signaling in EC regeneration is unclear.

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

Methods: Microarrays, rapid amplification of cDNA ends and K4-K6 domain analysis of lncRNAs were performed. lncRNA binding sites were predicted by RNAHybrid. Endothelial GW182 was immunoprecipitated to study miRNA-mediated lncRNA targeting. Antisense oligonucleotides to inhibit miRNAs and to block the interaction between a specific miRNA and one of its targets (target site nomprecipitated to study miRNA-mediated lncRNA targeting). Anti-sense oligonucleotides to inhibit Wnt and Notch1 signaling. PCR to analyze gene expression, and immunofluorescence staining to assess Ki-67, Notch1 and β-catenin expression in vitro and in ApoE-/mice with a conditional knockout of Dicer in ECs (EC-Dicer-/ mice).

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 anti-Notch1 promoted EC regeneration, reduced apoptosis, upregulated the expression of the arterial 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 TSβ-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.

259 Circulating long-non coding RNA LIPCAR and left ventricular diastolic function in patients with uncomplicated type 2 diabetes mellitus
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Background: Heart disease is the leading cause of mortality in type 2 diabetes mellitus (T2DM). 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 patients with T2DM during subclinical stages. Long non-coding RNAs have been proposed as potential biomarkers of cardiovascular disease. In particular, circulating microatonic long non-coding RNAs uc022bqs.1 (LIPCAR) has been recently associated with cardiac remodeling and cardiovascular dysfunction.

Purpose: Our aim was to analyse LIPCAR as potential biomarker of early cardiac alterations in patients with well-controlled T2DM of short duration.

Method: Forty-eight T2DM men with well-controlled T2DM of short duration and without structural heart disease or cardiac schema were eligible. A complete panel of clinical, biochemical and metabolic characteristics was measured. Left ventricular (LV) dimensions and function were measured by magnetic resonance imaging (MRI). RNA was isolated from serum samples using miRNasyl kit. LIPCAR level was quantified using qRT-PCR.

Results: Univariate regression analysis indicated that LIPCAR was associated with parameters of LV diastolic function, including the peak filling rate of the early filling phase (E), the peak (E-decmax) and mean (E-decmean) deceleration gradients of E and the ratio E/A peak flow (P < 0.0105 for all associations). There was no association between LIPCAR and parameters of LV dimensions or systolic function. 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 < 0.050 for all models). E/A peak flow levels were significantly lower in those patients in the fourth quartile compared to those in the lowest quartiles (P = 0.043). T2DM population was divided in two groups: 24 T2DM patients with normal LV diastolic function and 24 T2DM patients with type I LV diastolic dysfunction. In multivariate logistic regression models, LIPCAR was positively associated with LV diastolic dysfunction, independently of confounding factors (P < 0.050 for all models). Circulating levels of LIPCAR were significantly elevated in T2DM patients with LV diastolic dysfunction (P = 0.002).

Conclusion: 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.
Endothelial cell adenosine deaminase acting on RNA-1 is critically involved in vascular development and homeostasis in vivo

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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, ADAR1flox/flox mice were inbred with Tie2-Cre mice, in which the expression of Cre recombinase is driven by endothelial cell (EC) specific promoter of angiopoietin 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, ADAR1flox/flox mice were inbred 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 littermate 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-associated transcripts and innate immune response, possibly due to activation of the cytosolic dsRNA receptors TLR3 and MDAS.

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

Synthetic transcription in perivascular adipose tissue function in health and obesity

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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, ±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. Neural 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 SR59203A (100μM). A model of obesity was set-up by feeding 57% ± 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 replicating 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 SR59203A reduced the anti-contractile effect. Complete inhibition of the effects of the solution transfer could be achieved by incubation of exogenous PVAT with SR59203A. 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