The main effector of the renin angiotensin system (RAS), angiotensin II (AngII), is a key mediator of this remodelling. AngII has previously been shown to upregulate microRNA-132 (miR-132) expression in rat VSMC which may contribute to AngII-mediated vascular remodelling. The counter-regulatory axis of the RAS, consisting of ACE2/Ang-(1–7)/Mas has been reported to inhibit proliferation and migration of VSMC in vitro and neointimal (NI) formation in vivo. Here we investigated the effects of an alternative peptide of the counter-regulatory RAS, Ang-(1–9), on NI formation in murine vein grafts and investigated the role of AngII and Ang-(1–9) on miR-132 expression and migration in human saphenous vein VSMCs.

Methods NI formation was induced by interposition of the vena cava into the carotid artery in C57bl6/J mice. Ang-(1–9) was delivered subcutaneously via minipump and NI formation quantified 28 days later. Primary human saphenous vein VSMCs (HSVSMC) were stimulated with AngII alone or in combination with Ang-(1–9) and/or a MEK1/2 inhibitor (U1026). Expression of miR-132 was measured by qRT-PCR. ERK1/2 phosphorylation was assessed via western blot. Migration was assessed in HSVSMC via scratch wound assay following transfection of miR-132 mimic or scrambled control and/or incubation with U0126.

Results After 28 days Ang-(1–9) infusion significantly reduced NI formation in the grafted vessel of mice compared to control vein graft mice (29620 ± 3410 μm^2 vs 17640 ± 2049μm^2; $p<0.05$). In HSVSMCs AngII significantly upregulated miR-132. Co-incubation with Ang-(1–9) prevented these changes. AngII significantly increased ERK1/2 phosphorylation in HSVSMCs after 5 minutes, this was blocked with the addition of Ang-(1–9). Pharmacological inhibition of ERK1/2 phosphorylation attenuated AngII-induced miR-132 expression and attenuated AngII-induced HSVSMCs migration (70.3±3.52 vs 39.6±6.0% wound closure; $p<0.001$). Exogenous overexpression of miR-132 significantly increased HSVSMC migration compared to scrambled control-transfected cells (72.9±3.5 vs 46.1±3.4.2%; $p<0.001$). Blocking ERK1/2 phosphorylation in miR-132 overexpressing cells did not change HSVSMC migration.

Conclusion This study demonstrates that Ang-(1–9) reduces NI formation in a murine vein graft model and prevents AngII-induced ERK1/2 phosphorylation, miR-132 expression and HSVSMC migration in vitro. This study provides insight into the protective role of Ang-(1–9) in human VSMC and may highlight novel therapeutic targets in the setting of acute vascular injury.

Conflict of interest None

BS8 ESSENTIAL ROLE OF ENDOTHELIAL ADAR1 RNA EDITING IN VASCULAR INTEGRITY

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Introduction RNA editing is the binding to double-stranded RNA (dsRNA) of the adenosine deaminase acting on RNA (ADAR) family to catalyse the deamination of adenosine (A)-to- inosine (I). We have recently reported that ADAR1 is the main RNA editor in endothelial cells (ECs) and is dysregulated in human atherosclerotic heart disease. However, the role of ADAR1 in the mammalian vascular system has not been reported so far. The goal of the present study is to evaluate the role of EC-restricted ADAR1 in vascular homeostasis in vivo.

Methods Constitutional or inducible EC restricted ADAR1 ablation was achieved by crossing mice carrying a conditional (floxed) ADAR1 allele with either a Tie2-Cre or with a tamoxifen-inducible Cdh5-CreERT2 mouse line. EC function was assessed by the postnatal retinal angiogenesis model, barrier function assays and immunohistochemistry of lung and liver tissues. Cell culture assays, gene-silencing techniques, RT-qPCR, western blot and confocal microscopy were used to assess the ADAR1 effects in intracellular endothelial signalling.

Results The genetic loss of ADAR1 in ECs caused prenatal lethality at embryonic stage E13.5, demonstrating an essential role for endothelial ADAR1 in development. Postnatal ADAR1 ablation resulted in reduced vascular outgrowth, reduced vessel branching in the central vascular plexus and decreased filopodial protrusions from ECs at the angiogenic front of the vascular plexus compared with littermate control mice at P5. Furthermore, endothelial ADAR1 ablation in adult mice resulted in sudden death within 6–8 days after activation of Cre recombinase due to development of severe pleural effusions caused by widespread vascular leakage, indicating a disturbance of EC barrier function in lung microvasculature. Mechanistically, ADAR1-mediated RNA editing is essential for the metabolism of endogenous long-to-short dsRNAs, while silencing of endothelial ADAR1 resulted in accumulation of cytoplasmic long dsRNAs. Long dsRNAs are recognised as a danger-associated molecular pattern by the cytosolic innate immune sensing and signalling sensors. Consequently, the cytoplasmic accumulation of endogenous long dsRNAs resulted in activation of innate immune system in ECs, as assessed by the induction of interferon-β. Concomitantly, activation of the cytoplasmic dsRNA sensors resulted in dissociation of β-catenin from VE-cadherin in EC junctions and endocytosis of VE-cadherin.

Conclusion ADAR1-mediated RNA editing of long dsRNAs is essential for the long-to-short dsRNA metabolism and thus suppression of endogenous innate immune sensing and signalling. EC-restricted ADAR1 ablation results in embryonic lethality, impaired retinal angiogenesis at P5 and sudden death in adult mice due to pleural effusions, which all indicate that ADAR1-mediated RNA editing is indispensable for maintenance of vascular barrier integrity.

Conflict of interest none

BS9 KMT2C- A TETRALOGY OF FALLOT CANDIDATE GENE

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Congenital heart disease describes a group of defects resulting from aberrant heart development. Tetralogy of Fallot is the