adhesion of leukocyte subsets to vascular wall were severely impaired in Adar2-/-/tg mice. Leukocyte transmigration was also diminished by >2-fold in Adar2-/-/tg and in iEC-ADAR2 KO mice in response to IL-6 or ischemia. Similar results were obtained for leukocyte rolling, adhesion and infiltration after acute (4h) and chronic (3d; 21d) ischemia. IL-6-inflamed cremaster muscles showed that rolling and adhesion of leukocyte subsets to vascular wall were severely impaired in Adar2-/-/tg mice. Leukocyte transmigration was also diminished by >2-fold in Adar2-/-/tg and in iEC-ADAR2 KO mice in response to IL-6 or ischemia. Similar results were obtained for leukocyte rolling, adhesion and infiltration after acute (4h) and chronic (3d; 21d) ischemia from iEC-ADAR2 KO mice and human ischemic tissues. Next we studied how ADAR2 controls IL6ST expression. ADAR2-deficient EC miRNAome revealed the upregulation of a conserved group of miRNAs targeting the IL6ST mRNA including miR-199a-5p and miR-335-3p. In a single nucleotide level, miR-199a-5p and miR-335 directly disrupted Drosha recruitment to both and thus inhibited their maturation process. Accordingly, rescue experiments using miRNA-inhibitors restored IL6ST levels after ADAR2 deficiency.

**Conclusion**

Taking together, inhibition of the microRNA maturation process by ADAR2-mediated RNA editing is integral for IL-6 trans-signalling in vascular endothelium and subsequent leukocyte trafficking to ischemic tissues in mice and humans.

**Conflict of interest**

n/a

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**Abstract BS49 Figure 1**

(A) Relative expression of apelin receptor (APLNR) gene in hESC-derived cardiomyocytes (CM). Expression is displayed relative to 18S. (B) Saturation binding curve for beating hESC-derived cardiomyocytes when incubated with increasing concentrations of [125I]apelin-13.
Medial calcification is the formation of mineralised tissue within the smooth muscle layer of the vessel wall, and frequently occurs in patients with chronic kidney disease. Calcification within the medial layer of the vessel wall can reduce aortic and arterial elasticity, which impairs cardiovascular haemodynamics and results in a significantly elevated risk of morbidity and mortality in the form of hypertension, cardiac hypertrophy and sudden cardiac death. Protein kinase Cα (PKCα) belongs to the PKC family of serine/threonine kinases and we recently discovered that knocking-down PKCα expression increases high phosphate-induced mineral deposition by vascular smooth muscle cells (VSMCs) in vitro. This study tests the hypothesis that PKCα regulates uraemia-induced medial calcification in vivo.

PKCα-/- mice were generated on the calcification-susceptible DBA/2 background (PKCα-/-) using CRISPR/Cas9 technology. To induce uraemia, wild-type DBA/2 and PKCα-/- mice underwent a two-stage subtotal nephrectomy and were fed a high phosphate (1.5%) diet for 8 weeks. Renal function was measured by blood urea nitrogen (BUN). Calcification in the ascending aorta/aortic arch and abdominal aorta were analysed and quantified by micro CT and histology. On average, 68.6 ± 3% (SD, n=3) of renal mass was removed from wild-type and 65 ± 3.2% (n=5) was removed from PKCα-/- mice (P>0.05). Loss of PKCα significantly increased uraemia-induced medial calcification in the abdominal aorta (20-fold increase, P<0.05) when compared to wild-type controls; there is also a trend for calcification to be increased in the ascending aorta/aortic arch of PKCα-/- mice. Whilst there is a trend for BUN levels to be elevated in PKCα-/- mice (1.3-fold increase compared to wild-type controls, P=0.1), there is no correlation between BUN levels and the extent of calcification in these mice.

We have shown previously that inhibiting transforming growth factor-β (TGF-β) signalling with SB431542 prevents the increase in calcification observed in PKCα-siRNA treated VSMCs. Therefore, to determine the mechanism by which loss of PKCα exerts its effects we examined the relationship between PKCα and TGF-β signalling in vitro and in vivo. Our results show that knock-down of PKCα using siRNA increased TGF-β1-induced Smad2 phosphorylation in VSMCs in vitro (P<0.05). Furthermore, phosphorylated Smad2 immunostaining was detected throughout calcified aortic arches from PKCα-/- mice. In contrast, only small areas of phosphorylated Smad2 immunostaining was detected in calcified wild-type controls.

In conclusion, our study suggests that PKCα may play a protective role in uraemia-induced medial calcification. The PKCα/TGFβ signalling axis could therefore represent a new therapeutic target for uraemia-induced medial calcification.

Conflict of interest None