and localise to atherosclerotic plaques in experimental atherosclerosis. These targeted NPs have the potential to amplify fluorescent signal for imaging and carry a therapeutic cargo for targeted drug delivery to atherosclerotic plaques.

BS16 THE RNA-BINDING PROTEIN ANKHD1 PROVIDES VASOPROTECTION VIA ENHANCING ENOS AND PTGIS

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Introduction Atherosclerosis is a common cardiovascular disease characterised by lipid and inflammatory cell accumulation in the subendothelium of arteries. This disease develops predominantly at branches and bends exposed to disturbed blood flow, whereas regions exposed to uniform flow generating unidirectional high shear stress (HSS) are protected. The mechanisms of atheroprotection by HSS are not fully understood but they involve induction of the vasoprotective molecules endothelial nitric oxide synthase (eNOS) and prostacyclin synthase (PTGIS). The RNA binding protein ANKHD1 has not been studied previously in vascular endothelium. Here we report that ANKHD1 is induced in healthy endothelium by HSS and protects arteries by enhancing the expression of eNOS and PTGIS.

Methods Human Umbilical Vein Endothelial Cells (HUVECs) and Human Coronary Artery Endothelial Cells (HCAECs) were exposed to HSS (~11 dyne/cm2) or low shear stress (LSS[~4 dyne/cm2]) using the orbital shaker system for 72 hours. ANKHD1 function was analysed in HUVECs or HCAECs by gene silencing followed by quantitation of eNOS and PTGIS by qRT-PCR. ANKHD1 interaction and regulation of eNOS and PTGIS were performed by RNA-immunoprecipetation assay and RNA stability assay (Actinomysin D [ActD] experiment) respectively. The vascular phenotype of Ankhd1-/- mice and Ankhd1+/+ (wild-type) mice was assessed macroscopically (aged 20 weeks) and via en-face staining of enos and Ptgis in HSS and LSS areas of the aortic arch.

Results ANKHD1 was significantly upregulated by HSS in primary human EC (HUVECs: p=0.0022, N=5; and HCAECs: p= 0.0095, N=4). ANKHD1 gene silencing downregulated eNOS (N=5, p=0.0297) and PTGIS (N=5, p= 0.0003), indicating that ANKHD1 is a positive regulator of these vasoprotective molecules. At a mechanistic level, RNA-immunoprecipitation assay showed binding of ANKHD1 to eNOS and PTGIS in HUVECs, and ActD-chase experiments showed that ANKHD1 positively regulates eNOS mRNA stability. In mice, Ankhd1 positively controlled aortic diameter (0.8343 mm in Ankhd1-/- vs 0.9318 mm in Ankhd1+/+, N=13–12, p=0.0011). A closer inspection by en face staining revealed that ANKHD1 was enriched at HSS regions of the mouse aorta (p=0.0446, N=5). At a functional level, en-face staining showed that the levels of enos (n=8–11, p=0.0425) and Ptgis (N=6–13, p= 0.0045) and the number of ECs (N=9–15, p=0.0044) were reduced in Ankhd1-/- mice compared to Ankhd1+/+, indicating that Ankhd1 promotes EC function at HSS regions of the aorta.

Conclusions ANKHD1 plays a key role in HSS-upregulation of eNOS and PTGIS by interacting with these RNAs and promoting their stability. This suggests the involvement of ANKHD1 in vascular homeostasis and the protection of HSS regions from atherosclerosis.

BS17 GLYCOCALYX DEPTH ASSESSMENT IN PIG BLOOD VESSELS FOR VEIN GRAFT APPLICATIONS

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Introduction Human saphenous veins (hSV) are routinely used during coronary artery bypass grafting, however around 50% of grafted veins occlude 5–10 years post-surgery, often due to platelet aggregation and restenosis. All blood vessels are lined with endothelial cells which are coated in glycocalyx (eGlx), a gel-like layer of macromolecules at the interface between blood and the vessel wall, that have a role in mechanosensing blood flow, and preventing platelet aggregation. Direct comparisons between eGlx depth in different vessels could prove essential for future studies aiming to improve patency rates. Porcine arteriovenous graft models are used in translational studies to test bioengineered conduits, and it is therefore necessary to detect eGlx in this species. This study aims to screen lectins to optimise a staining protocol for eGlx, and then measure eGlx depth in venous and arterial porcine tissue, and in a pig arteriovenous graft model, to establish any differences between vessel types, and within the graft, which could influence their behaviour and longevity in the grafting environment.

Methods and Results Seven biotinylated lectins were assessed for eGlx staining specificity in formalin-fixed paraffin-embedded venous and arterial sections. Lycopersicon esculentum (tomato) (LEL) was deemed optimum, and fluorescent dual-staining with LEL and Octadecyl Rhodamine B Chloride (R18, for cell membranes) enabled confocal fluorescence imaging and peak-to-peak intensity profile analysis to be used as a measure of eGlx depth. The eGlx layer was significantly thicker in carotid arteries compared to jugular veins (301.5 ±33.8 nm vs. 242.3 ± 41.1 nm, p<0.05, n=6 per vessel). For the graft experiment, decellularised hSV were either seeded with porcine endothelial colony-forming cells or left unseeded as control, before being grafted into pig right carotid arteries for 4 weeks; only patent grafts were assessed for eGlx depth (n=4 and n=3 respectively). Seeded grafts had a significantly thicker eGlx in the proximal graft region (325.9 ±16.1 nm) compared to controls (240.8 ± 15.1 nm; p<0.01). Notably, control grafts had significantly reduced eGlx depth compared to its native proximal artery (p<0.05), while the seeded samples showed no difference. On average, native carotid arteries proximal to the graft had significantly increased eGlx depth compared to the distal arteries (315.5 ± 21.2 vs. 265.5 ± 35.5 nm, p<0.05, n=7).

Conclusions The confocal fluorescence profile peak-to-peak method was optimised here for the first time in large porcine vessels using LEL. The eGlx depth differences found between pig veins and arteries, as well as in native tissue and grafts, may influence the vein graft occlusion. The direct effect of eGlx thickness on graft long-term outcomes needs to be determined in further animal studies to assess whether eGlx graft management improves patency rates.