before wound scratch migration assays were performed, and FKBPL protein levels measured. BeWo cells were treated with the HIF-1 α activator, DMOG, for 24 hour before protein lysates were extracted for western blotting analysis. Colony forming efficiency and the number of holoclones, meroclones and paraclones of both HTR8.SV.neo and JAR trophoblast cells were determined in the presence of hypoxia or normoxia via clonogenic assay.

Results BeWo and JAR migration increased by approximately 40% following 24 hour exposure to hypoxia (n=6; BeWo, p<0.05; JAR, p<0.01), and FKBPL protein expression was downregulated (n=3; HTR8.SV.neo, p<0.01; BeWo, p<0.05; JAR, p<0.01), when compared to normoxia. DMOG treatment downregulated FKBPL protein levels in BeWo cells (n=3, p<0.01). JAR colony formation was reduced by approximately 70% in hypoxia (n=3, p<0.01); all colonies appeared to be holoclones. No change in colony formation was observed in HTR8.SV.neo cells; however, there was over two-fold reduction of holoclones, and an increase in differentiated colonies, meroclones plus paraclones (n=3, p<0.05).

Conclusion Our *in vitro* data suggest that FKBPL plays an important role in trophoblast functionality, which may extend to spiral uterine artery remodelling underlying the pathogenesis of pre-eclampsia.

7 ASSESSING ADENOVIRAL DELIVERY OF ANGIOTENSIN-(1-9) TO PREVENT HUMAN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION AND MIGRATION *IN VITRO* AND NEOINTIMA FORMATION *IN VIVO*

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Vein graft failure (VGF) following coronary artery bypass grafting occurs through proliferation and migration of vascular smooth muscle cells (VSMC) forming a neointima that blocks the graft lumen. Dysregulated renin angiotensin system (RAS) signalling promotes vascular remodelling through angiotensin II (Ang II) stimulating the angiotensin type 1 receptor. The counter-regulatory RAS peptide angiotensin-(1-9) [Ang-(1-9)], acts *via* the angiotensin type 2 receptor and inhibits VSMC proliferation and migration.¹ Here, gene transfer of Ang-(1-9) was investigated as a novel therapy to inhibit human saphenous vein VSMC (HSVSMC) migration and proliferation *in vitro* and neointima formation *in vivo*.

An adenoviral vector [RAdAng-(1-9)] was generated expressing a fusion protein that secretes Ang-(1-9) extracellularly following transduction.² Transgene expression was confirmed by immunoblotting. HSVSMC migration was evaluated by scratch wound assay. MTS assay was used to determine effects of conditioned media from RAdAng-(1-9) transduced HepG2 cells on HSVSMC proliferation.

Saline, RAdControl or RAdAng-(1-9) (1×10^{11} vp) was administered to C57/Bl6 mice intravenously. After 48 hours the endothelium of the left carotid arteries was denuded by wire injury. After 4 weeks, injured carotid arteries were subjected to histological staining and morphometric analysis performed.

Immunoblotting of cell lysates and conditioned media demonstrated RAdAng-(1-9)-transduced HSVSMC expressed and secreted the fusion protein. RAdAng-(1-9) transduction of

HSVSMC inhibited Ang II-induced migration as compared to RAdControl transduced cells [p<0.001]. Treating HSVSMC with conditioned media of RAdAng-(1-9) transduced HepG2 cells inhibited proliferation [p<0.05]. RAdAng-(1-9) delivered intravenously 48 hours before surgery significantly inhibited neointima formation 28 days after carotid wire injury [p<0.001].

These data demonstrate that adenoviral gene therapy with Ang-(1-9) can be used to inhibit HSVSMC migration and proliferation and neointima formation after acute vascular injury in mice.

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8 NADPH OXIDASE 4 IS A MAJOR REGULATOR OF CORD BLOOD-DERIVED ENDOTHELIAL COLONY-FORMING CELLS WHICH PROMOTES POSTISCHEMIC REVASCLARISATION

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Cord blood-derived endothelial colony-forming cells (CB-ECFCs) are a defined progenitor population with established roles in vascular homeostasis and angiogenesis, which possess low immunogenicity and high potential for allogeneic therapy. CB-ECFCs are subject to regulation by reactive oxygen species (ROS) and here we specifically investigated the role of the major ROS-producing enzyme, NOX4 NADPH oxidase, which is highly expressed in CB-ECFCs, in their vasoreparative function. Specifically, cells were assessed (1) *in vitro* under basal conditions, with pro-oxidative stimuli or modified NOX4 expression, using migration and tubulogenesis assays, and (2) *in vivo* using an established model of experimental hindlimb ischaemia in SCID mice to assess revascularisation. Pro-oxidant phorbol 12-myristate 13-acetate (PMA) increased cell migration and tubulogenesis, which was inhibited by the pan-Nox inhibitor VAS2870. Basal tube formation was also reduced by VAS2870, highlighting that function is enhanced by endogenous superoxide in a NOX-dependent manner. Complementary RT-PCR and Western blotting analysis found NOX4 to be the most highly expressed isoform in CB-ECFCs, with augmented expression confirmed following PMA treatment. NOX4-knockdown (migration: control siRNA 174 ± 18 , Nox4 siRNA 96 ± 23 arbitrary units/au; n=9, p<0.001, tube formation: control siRNA 6.9 ± 1.2 , Nox4 siRNA 4.6 ± 0.7 au; n=9, p<0.001) and -overexpression (migration: EV 149 ± 21 , OE 204 ± 25 au; n=6, p<0.01; tube formation: EV 732 ± 33 , OE 1024 ± 71 au; n=6, p<0.01) reduced and potentiated *in vitro* function, respectively. In a murine model of hindlimb ischaemia administration of NOX4-deficient (control siRNA 0.71 ± 0.27 , Nox4 siRNA 0.39 ± 0.17 ischaemic/control limb ratio; n=6, p<0.05) and -overexpressing (EV 0.34 ± 0.09 , OE 0.61

± 0.28 ischaemic/control limb ratio; $n=8$, $p<0.05$) CB-ECFCs into mouse ischaemic hindlimbs inhibited and promoted revascularisation whilst regulating host eNOS-associated angiogenic signalling. Together, these findings indicate a key role for NOX4 in CB-ECFCs, highlighting its potential as a target for enhancing their reparative function through therapeutic priming to support creation of a pro-reparative microenvironment and promotion of effective post ischaemic revascularisation.

9 INVESTIGATING THE COUNTER REGULATORY RENIN ANGIOTENSIN SYSTEM AXIS IN THE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RAT IN ISCHAEMIC STROKE

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Several studies have assessed the potential of targeting the renin angiotensin system (RAS) with therapeutics for ischaemic stroke. The counter regulatory RAS peptide, angiotensin-(1-9) has been shown to act via the angiotensin II type 2 receptor (AT₂R) to oppose detrimental effects of RAS dysregulation. We hypothesise that Ang-(1-9) may have a beneficial effect on stroke outcome in the spontaneously hypertensive stroke prone rat (SHRSP). Initial qPCR experiments have assessed temporal changes in RAS gene expression (angiotensin converting enzyme 2; ACE2, AT₂R; AGTR2, Mas receptor; Mas) following 35 min transient middle cerebral artery occlusion (tMCAO) followed by varying reperfusion times: no reperfusion ($n=4$), 2 hour ($n=4$) and 24 hour ($n=4$), compared to sham surgery ($n=7$).

In infarcted tissue, there was a significant 10- and 11-fold reduction in ACE2 and Mas expression respectively, 24 hour post tMCAO vs sham (RQ+RQ_{max}: ACE2: sham 1.0 ± 0.2 ; 24 hour post tMCAO 0.1 ± 0.01 , $p<0.01$, Mas: sham 1.0 ± 0.2 ; 24 hour post tMCAO 0.09 ± 0.03 $p<0.01$). However, in the same tissue, AGTR2 showed a 4-fold increase in expression after 35 min occlusion vs sham (RQ+RQ_{max}: sham 1.0 ± 0.3 ; 35 min MCAO 4.2 ± 0.2 , $p<0.05$). Additionally, in the sub-cortical remainder tissue, ACE2 and AGTR2 expression decreased by 2.5- and 5-fold respectively 24 hour post tMCAO (RQ+RQ_{max}: ACE2: sham 1.0 ± 0.1 ; 24 hour post tMCAO 0.4 ± 0.1 , $p<0.05$, AGTR2: sham 1.0 ± 0.4 ; 24 hour post tMCAO 0.2 ± 0.1 $p<0.05$).

These results demonstrate altered counter regulatory RAS gene expression in the ipsilateral hemisphere in the 24 hours following tMCAO in SHRSP. Additional experiments have demonstrated successful transduction of a control reporter gene-expressing, adeno-associated virus serotype 9 (AAV9) expressing enhanced green fluorescent protein (eGFP) (AAV9-eGFP) in the SHRSP brain via stereotactic delivery after both 4 and 7 days. Future studies will assess the therapeutic potential of Ang-(1-9) in tMCAO induced experimental stroke in SHRSP by delivering Ang-(1-9) via stereotactic delivery of an AAV9 vector.

10 ENDOGENOUS AND EXOGENOUS LOADING OF EXTRACELLULAR VESICLES FOR THERAPEUTIC DELIVERY OF RENIN-ANGIOTENSIN SYSTEM PEPTIDES IN CARDIOMYOCYTE HYPERTROPHY

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Introduction The RAS peptide angiotensin II (AngII) mediates cardiac hypertrophy. The counter-regulatory RAS axis peptide Angiotensin 1-7 [Ang-(1-7)] inhibits cardiomyocyte hypertrophy. EVs were purified from cardiomyocytes $\pm$ treatment with AngII or Ang-(1-7) to assess cardiomyocyte hypertrophy. EVs were loaded with Ang-(1-7) via electroporation for therapeutic delivery.

Methods H9c2 cardiomyocytes were untreated (control) or treated with AngII or Ang-(1-7). EVs were isolated from conditioned media by differential ultracentrifugation, characterised by BCA, western immunoblot, Nanosight and TEM and incubated with recipient cardiomyocytes. Next, cells were stained with F-Phalloidin actin and area measured. Gene expression of hypertrophy marker brain natriuretic peptide (BNP) was assessed by qRT-PCR. Control EVs were electroporated in the presence of Ang-(1-7) and levels determined by ELISA.

Results H9c2 cardiomyocyte-derived EV size was 101.0 ± 2.4 nm and EV markers CD63 and TSG-101 were consistently detected. EVs from AngII treated cardiomyocytes significantly increased recipient cardiomyocyte area compared to control EVs [control: $3291.1 \pm 90.1 \mu\text{m}^2$ vs AngII: $5252.3 \pm 125.4 \mu\text{m}^2$; $p<0.001$] and significantly increased BNP expression [$p<0.017$]. EVs isolated from Ang-(1-7) treated H9c2 cardiomyocytes significantly reduced AngII induced hypertrophy in recipient cardiomyocytes [AngII +Control EVs: $5566.3 \pm 139.0 \mu\text{m}^2$ vs AngII +Ang-(1-7) EVs: $4212.7 \pm 132.1 \mu\text{m}^2$; $p<0.01$]. Electroporation loaded EVs with Ang-(1-7) [naïve EVs: 0.0 pg/mL vs Ang-(1-7) EVs: $342.3 \pm 9.1 \text{ pg/mL}$; $p<0.001$]. Ang-(1-7) loaded EVs significantly reduced AngII induced hypertrophy in recipient cardiomyocytes [Naïve EVs: $4641.2 \pm 35.3 \mu\text{m}^2$ vs Ang-(1-7) EVs: $2758.4 \pm 20.1 \mu\text{m}^2$; $p<0.001$].

Conclusion EVs isolated from AngII treated H9c2 cardiomyocytes stimulate recipient cardiomyocyte hypertrophy. EVs isolated from Ang-(1-7) treated cardiomyocytes inhibit hypertrophy. Furthermore, EVs exogenously loaded with Ang-(1-7) inhibit cardiomyocyte hypertrophy. These findings have implications for understanding the role of the RAS and EV function in cardiomyocytes.

Poster Presentations

1 A SURVEY EVALUATING HEALTHCARE PROFESSIONALS' KNOWLEDGE AND PERCEPTIONS OF ELECTRONIC CIGARETTES

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