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.

REFERENCES

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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 $\pm$ 18, Nox4 siRNA 96 $\pm$ 23 arbitrary units/au; n=9, p<0.001, tube formation: control siRNA 6.9 $\pm$ 1.2, Nox4 siRNA 4.6 $\pm$ 0.7 au; n=9, p<0.001) and -overexpression (migration: EV 149 $\pm$ 21, OE 204 $\pm$ 25 au; n=6, p<0.01; tube formation: EV 732 $\pm$ 33, OE 1024 $\pm$ 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 $\pm$ 0.27, Nox4 siRNA 0.39 $\pm$ 0.17 ischaemic/control limb ratio; n=6, p<0.05) and -overexpressing (EV 0.34 $\pm$ 0.09, OE 0.61