Moreover, the temporal increase in the AF intensity at 565 ±20 nm wavelength during myogenic differentiation was similar to the AF profile of dissociated cells from arteriosclerotic vessels at this same wavelength. These data suggest that an AF photonic fingerprint of stem cell-derived myogenic progeny *in vitro* mimics that of vascular cells *ex vivo*. following IMT.

4 THE ROLE OF A NOVEL ANTI-ANGIOGENIC PROTEIN, FKBPL, IN ANGIOGENESIS ASSOCIATED WITH CARDIAC DYSFUNCTION

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People with diabetes have a five-fold higher incidence of cardiovascular disease, the leading cause of death globally. FKBPL is a novel angiogenesis-related protein, with a critical role in physiological and pathological angiogenesis. A first-in-class clinical FKBPL peptide mimetic, ALM201, has successfully completed clinical trials for treatment of solid tumours. FKBPL haploinsufficient (*Fkbpl*$^{\pm}$) mice, have a pro-angiogenic phenotype, accompanied by vascular dysfunction. Vascular dysfunction is associated with CVD and T2D.

In view of these findings, we now investigate a specific role for FKBPL in angiogenesis associated with cardiac dysfunction. In streptozotocin (STZ)-induced diabetic mice (50 mg/kg i.p. for 5 consecutive days), cardiac FKBPL mRNA levels were downregulated at 12 weeks compared to vehicle controls ($p<0.05$, $n=5$); this was associated with diastolic dysfunction (e.g. mitral valve E/A ratio). Similarly, in an experimental mouse model of myocardial infarction (MI) associated with severe cardiac ischaemia/hypoxia and increased angiogenesis, FKBPL mRNA ($p<0.05$) and protein levels ($p<0.01$) were downregulated versus sham controls ($n\geq3$). Complementary *in vitro* studies using human umbilical vein endothelial cells (HUVEC) demonstrated increased migration and differentiation following 24 hour exposure to hypoxia (1%) when compared to normoxia ($p<0.01$, $n=6$). In addition, FKBPL protein levels were downregulated following exposure to hypoxia ($p<0.01$, $n=6$), whilst activation of HIF-1α in normoxia by 24 hour DMOG treatment led to a two-fold reduction in FKBPL protein levels ($p<0.01$, $n=3$). Furthermore, HUVEC exposed to high glucose (30 mM for 24 hour) demonstrated downregulation of FKBPL compared to osmotic control ($p<0.05$, $n=3$). Interestingly, fenofibrate (50 μM) treatment was able to restore HUVEC levels of FKBPL in hypoxia ($p<0.01$, $n=3$). In conclusion, FKBPL may serve a key regulatory role in pathological angiogenesis associated with cardiac dysfunction and, as such, could be promising as a novel biomarker and therapeutic target in this disease setting.

5 HIF-1α DEPENDENT AND INDEPENDENT REGULATION OF PP2A IN HUMAN AORTIC SMOOTH MUSCLE CELLS UNDER HYPOXIA

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Aims Although hypoxia can modulate the phosphoprotein phosphates system, few studies have addressed if this is mediated through HIF. Therefore, we investigated the involvement of hypoxia-induced HIF-1α on:

- PP2A activity,
- post–translational modification of PP2Ac, and
- abundance of key enzymes involved in post–translational modification of PP2A in HASMC.

Methods and results HASMC and HAEC were cultured in cell type specific media for 24 hour under normoxic or hypoxic conditions (1% O_2) or following exposure to DMOG (100 μM). Effects on mRNA expression, phosphatase activity, post-translational modification and involvement of HIF-1α were assessed using RT-PCR, immunoblotting, an immunoprecipitation activity assay, ELISA and siRNA transfection. Hypoxia and DMOG decreased mRNA expression of HIF-1α and PPP2CA in HASMC and HAEC without altering cell viability. In HASMC hypoxia decreased phosphatase activity (total and PP2Ac) without affecting PP2Ac abundance, an effect mimicked by DMOG. Interestingly, hypoxia increased the level of phosphorylated and demethylated PP2Ac. The latter was associated with increased and decreased abundance of PME-1 and LCMT-1 respectively. Knockdown of HIF-1α prevented the hypoxia-mediated decrease in total phosphatase activity and mRNA expression of PPP2CA. However, it did not alter the effect of hypoxia on the abundance of pPP2Ac, DPP2Ac, LCMT-1 or PME-1.

Conclusion In HASMC, hypoxia inhibits PP2A activity through a HIF-1α dependent mechanism. In addition, PP2Ac undergoes HIF-1α independent phosphorylation and demethylation during hypoxia in keeping with changes in the abundance of PME-1 and LMCT-1. The post-translational modification of PP2Ac is consistent with altered assembly of the PP2A holoenzyme and inhibition of activity. Together these data indicate a complex interaction between hypoxia and the PP2A system which warrants further study.

6 THE ROLE OF A NOVEL ANGIOGENESIS RELATED PROTEIN, FKBPL, IN SPIRAL UTERINE ARTERY REMODELLING IMPORTANT FOR THE PATHOGENESIS OF PREECLAMPSIA

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Introduction Preeclampsia is a complication which occurs in 5%–6% of pregnancies, characterised by high blood pressure and/or other organ dysfunction in the third trimester of pregnancy. Preeclampsia has short-term risks for the mother and child, but is also associated with remote cardiovascular disease and/or type 2 diabetes mellitus in both. The pathogenesis of preeclampsia is unclear but it appears to be attributed to inappropriate remodelling of spiral uterine artery as a result of dysregulated trophoblast function. We investigated the involvement of novel regulator of developmental and pathological angiogenesis, FKBPL, and its role in the pathophysiology of preeclampsia.

Methods Trophoblast cells (HTR8.SV.neo, BeWo and JAR) were exposed to hypoxic (1%) or normoxic (21%) conditions

before wound scratch migration assays were performed, and FKBPL protein levels measured. BeWo cells were treated with the HIF-1α 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 REVASCULARISATION

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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