175 INHIBITON OF TUMOUR NECROSIS FACTOR ALPHA SIGNALLING IMPROVES VASCULAR REMODELLING AND DECREASES THE PRO-INFLAMMATORY AND CYTOTOXIC PHENOTYPE OF PERIPHERAL NATURAL KILLER CELLS IN A MODEL OF CHRONIC HYPERTENSION IN PREGNANCY

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Objective Pregnancy induces extensive yet relatively rapid remodelling of the cardiovascular (CV) system, however, little is known about this adaptation in women with pre-existing CV disease. We have previously characterised the stroke prone spontaneously hypertensive rat (SHRSP) as a model of deficient uterine artery remodelling and identified an increase in pro-inflammatory TNFÎ ± relative to the normotensive WKY strain during pregnancy.

Design and method SHRSP were treated with etanercept (0.8 mg/kg) or vehicle at gestational day (GD) 0, 6, 12 and 18. Animals were sacrificed at GD18. SHRSP, SHRSP treated with etanercept (ETN) and WKY (n = 6) were used for vascular studies. An independent set of animals (n = 6) were used for flow cytometry analysis.

Results Etanercept significantly reduced systolic blood pressure in the SHRSP after GD 12 (Î”SBP GD 10–21 SHRSP 12.0 ± 4.17 vs. ETN 25.8 ± 4.27 mmHg; $p < 0.05$). Analysis of GD18 uterine arteries showed that etanercept significantly reduced uterine artery contractile ability (SHRSP 57.3 ± 8.75 vs. ETN 35.2 ± 2.19 kPa; $p < 0.01$) and increased carbachol response (SHRSP 13.8 ± 3.8% vs. ETN 40.1 ± 3.25%; $p < 0.05$). Characterisation of uteroplacental blood flow using Doppler showed that etanercept significantly reduced resistance index relative to SHRSP (SHRSP 0.79 ± 0.02 vs. ETN 0.61 ± 0.02 resistance index; $p < 0.01$). Etanercept significantly increased litter size in the SHRSP (SHRSP 7.80 ± 0.44 vs. ETN 12.75 ± 0.94 fetuses), reduced resorption frequency (SHRSP 66.7% vs. ETN 25.0% dams with resorption) and decreased premature glycogen cell loss from the placenta. Further, we sought to identify the source of excess TNFÎ ± in the SHRSP. Inflammatory natural killer (NK) cells ($CD3^-CD161^+$) were significantly increased in the SHRSP relative to the WKY in the placenta (WKY 11.6 ± 2.39 vs. SHRSP 659.8 ± 201.2 cells/mg; $p < 0.01$). Etanercept reduced the percentage of NK cells which produced TNFÎ ± in the maternal circulation and placenta in the SHRSP. Additionally, etanercept significantly reduced the number of $CD161^+$ NK cells in the placenta of the SHRSP (SHRSP 659.8 ± 201.2 vs. ETN 148.0 ± 12.62 cells/mg; $p < 0.01$) by inducing a phenotypic switch to a granzyme B_{low}, $CD161_{low}$ population.

Conclusions Etanercept improves uterine artery function and pregnancy outcome in the SHRSP. We propose that this is through the limitation of both damaging TNFÎ ± release and cytotoxicity from NK cells.

176 A HISTONE DEACETYLASE 7-DERIVED 7 AMINO ACID PEPTIDE ACTS AS A PHOSPHORYLATION CARRIER

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Histone deacetylase 7 (HDAC7) belongs to class II HDAC family, playing a pivotal role in the maintenance of endothelium integrity. There are 8 splicing variants in mouse HDAC7 mRNAs. Within the 5â€™ terminal non-coding area of some variants, there exist some short open reading frames (sORFs). Whether these sORFs can be translated and their potential roles in cellular physiology remain unclear. In this study, we demonstrated that one sORF encoding a 7 amino acids (aa)-peptide could be translated in vascular progenitor cells (VPCs) in response to vascular endothelial cell growth factor (VEGF). The 7aa-peptide (7A) could be phosphorylated at serine residue via MEKK1. Importantly, the phosphorylated 7A (7Ap) could transfer the phosphorylation group to the Threonine residue of the 14–3–3Î³ protein in a cell free in-gel buffer system. The *in vitro* functional analyses revealed that 7A enhanced VEGF-induced VPC migration and differentiation toward endothelial cell (EC) lineage, in which MEKK1 and 14–3–3Î³ served as the upstream kinase and the downstream effector respectively. Knockdown of either MEKK1 or 14–3–3Î³ attenuated VEGF-induced VPC migration and differentiation. Exogenous 7Ap could rescue the effect of VEGF on the *MEKK1* siRNA-transfected but not on the *14–3–3Î³* siRNA-transfected VPCs. The *in vivo* studies showed that 7A especially 7Ap induced capillary vessel formation within Matrigel plug assays, increased re-endothelialization and suppressed neointima formation in the femoral artery injury model, and promoted the foot blood perfusion recovery in the hindlimb ischemia model via increasing $Sca1^+$ cell niche formation. These results indicate that the sORFs within the non-coding area can be translated under some circumstances and that the 7aa-peptide may play an important role in cellular processes like migration and differentiation via acting as a phosphorylation carrier.

177 ENDOTHELIAL MICROPARTICLES: NOVEL REGULATORS OF VASCULAR CALCIFICATION *IN VITRO*

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Endothelial microparticles (EMPs) are complex structures with pleiotropic properties and are emerging as an index of endothelial damage; however, further work to determine the effect of EMPs on vascular smooth muscle cells (VSMC) is needed. We have shown that elevated EMPs are detected in Systemic Lupus Erythematosus (SLE) and carotid artery disease patients, who present accelerated vascular ageing and calcification. This study aims to investigate the molecular components of EMPs and whether they modulate vascular calcification and osteogenic differentiation of VSMCs *in vitro*.

EMPs were generated *in vitro* using human aortic endothelial cells (AoEMPs) by serum starvation (24 h) followed by TNF-alpha stimulation (10 ng/ml; 24 h), isolated by ultracentrifugation and quantified using flow cytometry. Human coronary artery smooth muscle cells (HCASMCs) were incubated with 10^6 AoEMPs/ml in osteoinductive media (5 mM BGP and 2.6 mM $CaCl_2$) for 21 days. Calcification was assessed by alizarin red staining and calcium deposition assays. Conditioned media was collected at 7, 14 and 21 days to identify markers of bone metabolism using Bioplex array

technology and ELISA. AoEMPs were also subjected to proteomic and miR screening to identify relevant molecules and pathways.

AoEMP-treated HCASMCs showed enhanced calcification after 21 days, by both alizarin red staining ($p < 0.005$) and calcium deposition assays ($p < 0.05$). In addition, secreted osteocalcin and osteoprotegerin levels were elevated in AoEMP-treated cells after 7 and 21 days respectively. ELISA determined that HGF, a key protein in vascular calcification, was elevated in media from the AoEMP-treated group compared to controls at 14 and 21 days. HGF levels were also higher in SLE patient plasma ($p < 0.05$) compared to healthy controls, suggesting that HGF may be involved in the vascular calcification observed in SLE patients. Furthermore, proteomic screening identified HGF in AoEMPs, whilst miR screening highlighted miR-3148 in both AoEMPs and SLE plasma and is predicted to target osteoprotegerin gene expression.

We conclude that AoEMPs enhance calcification and the reprogramming of HCASMCs to an osteogenic pathway *in vitro*, which may be in part, linked with HGF and miR-3148, supporting their role in vascular calcification in SLE and carotid artery disease. Further studies are required to determine how AoEMPs contribute to pathophysiological mechanisms *in vivo* and whether the circulating property of AoEMPs may represent a new biomarker in vascular calcification.

178 CAVEOLIN-3 A NOVEL REGULATOR OF RYANODINE RECEPTOR; A RELATIONSHIP THAT IS PERTURBED IN THE OBESE HEART

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Background Obesity is the leading cause of insulin resistance and a key factor underlying the development of type 2 diabetes.[1] Impaired excitation-contraction (EC) coupling is a hallmark of both type 1 and type 2 diabetes.[2] EC coupling is maintained by the finely tuned process termed calcium induced calcium release involving a number of proteins including the ryanodine receptor (RyR2). Our group has previously reported an interaction between caveolin-3 (Cav3) and RyR,[3] however, the functional consequences of this interaction are unknown. Cav3, a small integral membrane protein is involved in orchestrating an array of signalling pathways and is a negative regulator of nitric oxide synthase (NOS) and thus nitric oxide (NO) production. Significantly, post-translational modification of RyRs, S-nitrosylation, the addition of nitroso group, has been linked to 'leaky' channels. We hypothesize that the formation of a Cav3-RyR2 complex has a cardioprotective role regulating NOS activity within cardiac myocytes by maintaining the nitroso-redox state of the receptor and that this relationship is perturbed in the obese heart.

Methods Tissue was lysed from the left ventricle fromrats fed a high fat diet (40% HFD) and age-matched control rats on normal chow (10% diet) and probed for protein expression levels using western blotting. The SPRY domain of RyR2 was recombinantly expressed and purified from *E.coli*, and recombinant full length Cav3 was expressed and purified from yeast (*Pischia Pastoris*). Interaction between full-length recombinant Cav3 and SPRY domain was analysed by microscale thermophoresis (MST).

Results In the pre-diabetic heart (obesity) we have determined a down-regulation of Cav3 expression ($P < 0.05$), elevated levels of NOS3 ($P < 0.01$) but no change to RyR2 levels in the left ventricle. Our bioinformatics analyses identified multiple putative caveolin binding motifs (CBMs) within the RyR1 and RyR2 primary sequences that are conserved. The recent publication of a high resolution 3-D structure of skeletal RyR1 allowed us to determine that one of the CBMs is localised to a SPRY domain and sequence alignment with RyR2 found this region is conserved. We have successfully purified the RyR2 SPRY domain and full-length Cav3. Preliminary data indicate a possible interaction between recombinant Cav3 and the SPRY domain using microscale thermophoresis.

Conclusion Our study shows down-regulation of Cav3 and upregulation of NOS3 with no change in RyR2 expression level in the obese rat model. Functional studies are now underway to investigate the implications of an imbalance between NOS3 and Cav3 in terms of RyR2 calcium release. Further, biophysical and structural studies are now necessary to investigate the putative SPRY-Cav3 interaction and determine if other regions of RyR2 mediate Cav3-RyR2 binding.

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179 NEUROVASCULAR FUNCTION IN ATHEROSCLEROSIS

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Cardiovascular and neuronal dysfunction have to a large extent been treated as separate disease categories from both research and clinical perspectives. However, there is now growing evidence that pathological changes in the shared circulatory system may be key drivers of both cardiovascular and neuronal dysfunction. It is speculated that compromised circulatory function, as can be seen in inflammatory vascular conditions such as atherosclerosis, may impact the regulation of cerebrovascular blood flow in response to dynamically changing neuronal metabolic demands also known as neurovascular coupling. With impaired neurovascular function being a pathogenic factor underlying cerebrovascular pathology, here we aim to establish if atherosclerosis elicits any alterations in the neurovascular function.

Paigen Diet fed ApoE -/- mice fitted with a stable cranial window over the right somatosensory cortex combined with state of the art multi-modal neurovascular imaging comprised of 2D-optical imaging spectroscopy (OIS) to measure evoked blood flow, volume, and oxygenation changes and electrophysiology to record neuronal activity. Any neurovascular breakdown will then be further investigated using high resolution multi-photon microscopy and immunohistochemistry to identify cellular deficits and potential molecular targets. Functional magnetic resonance imaging (fMRI) will also be used later to assess any sub-cortical effects that 2D-OIS and electrophysiology cannot detect, which will assist in combining highly