Oral Presentations

1 DETECTION OF CALCIFICATION IN ATHEROSCLEROTIC PLAQUES USING OPTICAL IMAGING

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Introduction PET imaging, using the bone tracer $Na^{18}F$, allows the non-invasive location of atherosclerotic plaques that are at risk of rupture. However, the spatial resolution of PET is only 4–5 mm, limiting the mechanistic information this technique can provide.

Methods In this project, the use of fluorescence and Raman imaging to elucidate the mechanism of microcalcification within atherosclerotic plaques has been investigated.

Results and conclusion A fluorescent probe to specifically detect calcium has been synthesised: it has been shown to: selectively bind to hydroxyapatite (HAP), permit visualisation and quantification of HAP in both vascular and bone cell models, effectively stain cultured aortic sections and whole mouse aorta for OPT imaging.

It is believed that the biosynthetic pathway to HAP passes through a series of transitional states; each of these has different structural characteristics which can be studied using Raman spectroscopy. In particular, HAP has a strong characteristic Raman peak at 960 cm^{-1}. An increase in HAP concentration has been detected by Raman in both calcified cell models and aortic sections.

Building on these preliminary data, fluorescence and Raman imaging of both healthy and atherosclerotic tissue are planned.

2 PP2A: A GUARDIAN AT THE GATES?

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Endothelial junction proteins that regulate movement across the vascular system, are modulated through phosphorylation.[1] However, the role of protein phosphatase 2A (PP2A) mediated dephosphorylation in modulating permeability of the blood-brain barrier remains unclear. This study investigates the role of PP2A inhibition on VE-cadherin and PECAM-1 abundance, and whether it affects brain microvascular permeability.

Human brain microvascular endothelial cells (hBMECs) were exposed to okadaic acid (OA, 10 nM), or dimethylsulphoxide (DMSO; 0.01% v/v) for 24 hour. Protein expression was determined using immunoblotting. PP2Ac activity was measured by an immunoprecipitation assay (Millipore). Proteasomal degradation was investigated using MG-132 (2 μM). hCMEC/D3 cells were transfected with CIP2A and SET plasmids (pcDNA3.1) using polyfect. Transendothelial permeability was determined using FITC-dextran. Data are presented as mean ±S.E.M. (n=5) and analysed by one-way ANOVA with *post hoc* ($p<0.05$).

OA (PP2A inhibitor) reduced abundance of VE-cadherin to undetectable levels and decreased PECAM-1 abundance by 50% ($p<0.05$). OA decrease PP2A activity (58.9%±5.5%, $p<0.05$) without effecting protein abundance. OA increased demethylation of PP2Ac and reduced abundance of leucine carboxyl methyltransferase-1 (LCMT-1) ($p<0.05$); protein phosphatase methylesterase-1 (PME-1) abundance was not altered. Overexpression of the PP2A inhibitors CIP2A and SET decreased ($p<0.05$) VE-cadherin and PECAM-1 abundance compared to the pcDNA3.1 control. OA and overexpression of CIP2A and SET increased ($p<0.05$) transendothelial permeability.

In conclusion, inhibiting PP2A decreases VE-cadherin and PECAM-1 abundance due to proteasomal degradation. This loss is associated with increased microvascular permeability consistent with loosening of tight junctions. The inhibition of PP2A is not due to the loss of abundance but instead an increase in PP2A methylation by LCMT-1, preventing the assembly of the holoenzyme. As such, PP2A-mediated regulation of the blood brain barrier, might be a target of therapeutic value.

REFERENCE

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3 INJURY-ACTIVATED VASCULAR CELLS SHARE A COMMON PHOTONIC FINGERPRINT WITH STEM CELL-DERIVED MYOGENIC PROGENY FOLLOWING INTERROGATION USING A LAB-ON-A-DISC (LOAD) PLATFORM

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The accumulation of vascular smooth muscle (SM)-like cells within the intima contributes significantly to intimal medial thickening (IMT) and vascular remodelling typical of arteriosclerotic disease. The source of these cells remains controversial. Light has emerged as a powerful tool to interrogate cells label-free and facilitates discriminant observations both *in vitro* and *in vivo*. The auto-fluorescence (AF) profile of individual cells isolated from arteriosclerotic vessels, captured on V-cup array and interrogated across five wavelengths using a novel Lab-on-a-Disc platform, was significantly increased at the 565±20 nm wavelength concomitant with a reduction in Myh11 expression, when compared to differentiated vascular smooth muscle cells (SMC) from control vessels. *In vitro*, TGF-β1 promoted myogenic differentiation of murine bone-marrow derived $Sca1^+/CD44^+$mesenchymal stem cells (MSC) and murine $Sca1^+$ C3H 10 T1/2 cells concomitant with enrichment of the specific SMC epigenetic histone mark, H3K4me2 at the Myh11 promoter, Myh11 promoter transactivation and increased SMC differentiation marker mRNA and protein expression. Myogenic differentiation resulted in a significant increase in the AF intensity across 565±20 nm wavelength, an effect not observed for TGF-β1 treated RAMOS human B lymphocytes but mimicked by Notch activation of resident $Sca1^+$multipotent vascular stem cells (MVSCs) with Jagged1 and inhibited following elastin and collagen III depletion, respectively.

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