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

1. Bertocchi C, *et al*. *J. Signal Transduct* 2012;12:ID125295.

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.