Oral Presentations

1 MITOCHONDRIAL REGULATION OF EXOSOMAL TRANSPORTED MIRNA PLAY A CENTRAL ROLE IN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION AND MIGRATION ASSOCIATED WITH ATHEROGENESIS AND RE-STENOSIS

Zuhair Al Sulti*, Paul Coats. *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK*

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Vascular smooth muscle (VSM) cell proliferation and migration are the hallmark of atherosclerosis and re-stenosis. In this study, we have investigated the role of mitochondrial bioactivity in determining the content of exosomes released by vascular smooth muscle cells and the biological effect these exosomes have on vascular smooth muscle (VSM) cell proliferation and migration.

VSM cells were isolated from 12 week old male Sprague-Dawley rats. Experiments were undertaken using day zero isolated (contractile phenotype), 21 day cultured (synthetic phenotype) and 21 day mitochondrial incompetent (synthetic phenotype- Rho cell). The effect of balloon angioplasty on rat aorta structural remodelling was also studied.

Inhibition of mitochondrial network formation with the DRP1 inhibitor MDivi (10 uM) inhibited angioplasty-dependent remodelling. This observation was confirmed in VSM cell cultures where MDivi significantly reduced proliferation and migration. Total exosomal release was significantly greater in the 21 day cultured cells when compared with quiesced non-proliferating cells and 21 day cultured Rho cells (480±20 ng, 520±10 ng and 420±10 ng per ml respectively) and total exosomal RNA yield was 70.2±10.2 ng/ul, 118.7±2.4 ng/ul and 70.8±4.7 ng/ul respectively.

Mitochondrial function significantly influenced miRNA and mRNA measured in exosomes. When compared with the hyperproliferative synthetic VSM cell miR-21 expression was reduced by 88±12.1% and miR-145 expression increased by 73±19.8%. We also measured a 7-fold decrease and 6.6-fold decrease mTOR, PI3K and 4EBP1 respectively. A significant increase expression of P53, cdkn2a and ROS scavenging proteins including SOD1 and SOD2 were measured in 21 day cultured Rho cells vs. 21 day hyperproliferative VSM cells.

In this study we have further correlated VSM cell hyperproliferative phenotype with mitochondrial function. Moreover, further demonstrated mitochondrial function/VSM cell phenotype with exosomal release and cargo that potentially drives the hyperproliferative/migratory phenotype central to atherosclerosis and re-stenosis.

2 DEVELOPMENT OF A SERS-NANOPARTICLE BASED SYSTEM FOR THE MULTIPLEX DETECTION OF VASCULAR ADHESION MOLECULES

[1]Jonathan Noonan*, [2]Steven Asiala, [1]Gianluca Grassia, [1]Neil MacRitchie, [2]Kirsten Gracie, [2]Karen Faulds, [1]Iain B McInnes, [1]Paul Garside, [2]Duncan Graham, [1,3,4]Pasquale Maffia. *[1]Centre for Immunobiology, Institute of Infection, Immunity, and Inflammation, University of Glasgow, UK; [2]Centre for Molecular Nanometrology, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK; [3]BHF Centre of Excellence in Vascular Science and Medicine, University of Glasgow, UK; [4]Department of Pharmacy, University of Naples Federico II, Naples, Italy*

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Surface-enhanced Raman spectroscopy (SERS) is an emerging molecular imaging technique suitable for the highly sensitive simultaneous detection of multiple analytes. We aimed to apply SERS for the multiplex detection of adhesion molecules in human umbilical vein endothelial cells and macrophages *in vitro*, as a step towards applying this technology for simultaneously detecting multiple biomarkers of vascular inflammation and atherosclerotic plaque instability *in vivo*.

Immunofluorescent microscopy and flow cytometry identified the expression of intercellular adhesion molecule (ICAM)−1, vascular cell adhesion molecule (VCAM)−1 and p-Selectin on human umbilical vein endothelial cells (HUVECs) following stimulation with 10 ng/ml TNFα for 24 hours. Phorbol 12-myristate 13-acetate (PMA) differentiated THP-1 macrophage-like cells expressed only ICAM-1 in both unstimulated and 24 hours 10 ng/ml TNFα stimulated conditions.

Gold nanoparticles were PEGylated and functionalized with Raman reporters, in order to endow a specific pre-determined SERS signal, alongside antibodies against the desired adhesion molecule targets. This provided 'nanotags' configured as follows (antibody/Raman reporter); anti-ICAM-1/BPE; anti-VCAM-1/PYOT, anti-p-Selectin/PPY, IgG Isotype Control/DP.

TNFα stimulated HUVEC were exposed to a mixture of all nanotags simultaneously (final concentration of 20 ug/L) and were subsequently imaged using SERS microscopy. This rational allowed for the simultaneous detection and discrimination of ICAM-1, VCAM-1 and p-Selectin on activated HUVEC. Adhesion molecules were detectible as soon as 15 min following nanotag addition, while IgG control nanotags produced negligible signal at all concentrations and time points investigated. Following simultaneous exposure to all nanotags, SERS imaging identified the expression of ICAM-1 on PMA differentiated TNFα stimulated THP-1, with little-to-no contribution from anti-VCAM-1, anti-p-Selectin, or IgG control nanotags.

Here we have developed a SERS-based molecular imaging methodology for the multiplex detection of adhesion molecules *in vitro*.