Basic science

BS1 REPROGRAMMING HUMAN MACROPHAGES WITH DRUG X: POTENTIAL MECHANISMS FOR STABILISATION OF ATHEROSCLEROTIC PLAQUES

¹Klaudia Kocsy, ¹Endre Kiss-Toth, ²Jessica Redgrave, ²Arshad Majid, ³Sheila Francis. ¹Department of Infection, Immunity & Cardiovascular Disease, The University of Sheffield, Sheffield, UK; ²Department of Neuroscience, The University of Sheffield, Sheffield, UK; ³The University of Sheffield, Sheffield, UK

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Introduction Ischemic stroke is commonly caused by large artery atherosclerosis. Patients with a high atherosclerotic burden (stenosis) and inflamed or ulcerated plaque are at increased risk of early recurrent ischaemic events. Treatment with anti-inflammatory agents may therefore reduce stroke incidence and recurrence in patients with this condition, but in vitro human studies of the possible mechanisms are lacking. We hypothesised that an anti-inflammatory compound (denoted drug X*) alters the expression of macrophage specific genes, including scavenger receptors in in vitro models of human carotid plaque macrophages.

Methods CD14+ monocytes were collected from whole blood donations (volunteers) and differentiated to monocyte derived macrophages (MDMs). Macrophage polarisation with M1 (100 ng/ml LPS + 20 ng/ml INF- γ) and M2a (20 ng/ml IL-4) markers was performed in complete media supplemented with 3 different clinically relevant concentrations of drug X.

Gene expression (RT-qPCR) analysis (n=10) was carried out in the differentiated hMDMs to test for macrophage specific genes and scavenger receptors. Western Blot analysis (n=7) and immunofluorescence stain (IF) (n=5) was performed on drug X- treated MDMs to validate changes in the expression of Oxidized Low Density Lipoprotein Receptor 1 (OLR1). The physiological effect of drug X was also tested on oxLDL (25 μ g/ml) uptake by macrophages (n=5).

Results Our data suggest that drug X may re-program macrophages to a less inflammatory state (an M2 state) by significantly altering Mannose Receptor C-type 1 (MRC1) gene expression (p:0.0183). Drug X also alters the expression level of Macrophage Scavenger Receptor 1 (MSR1) gene, provoking the pacification of the M1 state and resulting in a beneficial effect on LDL uptake (p: 0.0845). Using OLR1 gene expression as a readout, in both M1 and M2a macrophages, the average OLR1 expression was reduced by treatment with drug X (p: 0.0003). This implies that M1 macrophages can bind less oxLDL which could reduce the formation of atherosclerotic plaque in the vessel wall. OLR1 protein expression was also reduced (p:0.0003) following 24-hour exposure of the macrophages to drug X. In functional tests, oxLDL uptake by drug X treated hMDMs was reduced by 44% (p:0.003).

Conclusions The gene expression of macrophage specific genes and scavenger receptors in human M1 and M2a macrophages was significantly altered following 24-hour exposure to clinically relevant concentrations of drug X in vitro. Drug X reduced 'active' and pathogenic cell behaviours e.g., lipid uptake and promoted the polarisation to the M2 state.

These data demonstrate that human macrophages can be reprogrammed to a less pathogenic state in vitro and they provide a potential mechanism for the effectiveness of drug X in the stabilisation of carotid atherosclerotic plaques in humans.

* Drug X is used for ongoing IP/patent assessment
Conflict of Interest No

BS2 MECHANOSENSOR PIEZO1 IN SKELETAL MUSCLE PERICYTES

¹Yilizila Abudushalamu, ¹Hema Viswambharan, ²Romana Mughal, ¹Richard Cubbon, ¹Mark Kearney, ¹David Beech, ¹Piruthivi Sukumar. ¹Discovery and Translational Science Department Leeds Institute of Cardiovascular and Metabolic Medic, Leeds, UK; ²Department of Optometry and Vision Sciences School of Applied Sciences University of Huddersfield

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Introduction Pericytes (PC) are mural cells which are present in blood microvessels. They play an important part in the formation and stabilization of new blood vessels. They are essential for vascular development and cardiovascular homeostasis. They wrap around the endothelial cells (EC) and endothelial-pericyte communication is both via physical contact and paracrine signalling.¹ Piezo1, a cation-permeable pore-forming ion channel protein, has recently been described as mechanosensors in many mammalian cells including EC. Shear stress sensing by endothelial Piezo1 is essential for angiogenesis and development of regular vascular architecture during embryogenesis.² Moreover, endothelial Piezo1 regulates blood pressure and exercise-induced mesenteric vasoconstriction.³ PC of skeletal muscle respond to exercise and contribute significantly to angiogenesis and myogenesis. Certain types of exercise can increase and others can reduce or maintain the PC numbers and coverage.^{4 5 6} As both EC and PC are prone to mechanical stress produced by blood flow and pressure, we hypothesise that Piezo1 may play important roles in PC physiology too. Hence, we aim to evaluate the expression and functions of Piezo1 in PC using in vivo and in vitro models and experiments.

Methods We have developed a novel transgenic mouse model – tamoxifen-inducible PC-specific knockout of Piezo1 (PP-/-). We developed a method to isolate PC from mouse skeletal muscle (skmPC) and obtained human brain vascular PC (HBVP) from commercial sources. Expression and functionality of Piezo1 was analysed by RT-PCR and Ca²⁺ influx assay. Using Yoda1 (Piezo1 agonist) and Yoda1 analogues as pharmacological tools and Piezo1-specific siRNA for gene silencing, we performed functional experiments to elucidate the role of Piezo1 in PC.

Results We confirmed the purity of PC population using flow cytometry and PC specific antibody markers. The flow cytometric analysis showed >98% of isolated mouse skeletal muscle PC in culture. We found different populations of primary PC and HBVP express functional Piezo1, demonstrated by Yoda1-induced Ca²⁺ influx. RT-PCR, Ca²⁺ influx assay and gene-rescue experiments confirmed the PC-specific knockdown of Piezo1 in our transgenic mouse model. Piezo1 mRNA expression was reduced via siRNA-mediated silencing of Piezo1 in HBVP. Transcriptomics analysis showed chemical activation of Piezo1 leads to a perturbation in interleukin (IL)-mediated signaling in HBVP. We found that Piezo1 activation leads to downregulation of IL33, IL1RAP and VCAM-1 mRNA, upregulation of IL1RL1, ICAM-1 mRNA levels. Complementary work on murine skmPC confirmed the role of Piezo1 activation in IL signalling pathways.

Conclusions In summary, we demonstrated that the primary skmPC and HBVP express functional Piezo1. Activation of

Piezo1 alters inflammatory signalling which may have implications on PC pathophysiology. We plan to investigate this observation further to understand how Piezo1-mediated signalling influences PC-EC communication in exercise and general health.

Conflict of Interest no

BS3

NOVEL STRATEGY USING HESC-DERIVED CARDIOMYOCYTES TO EXPLORE THE CRITICAL IMPORTANCE OF THE APELIN RECEPTOR IN THE CARDIOVASCULAR SYSTEM

¹Robyn Macrae, ¹Maria Colzani, ²Emma Robinson, ¹Semih Bayraktar, ¹Thomas Williams, ¹Rhoda Kuc, ¹Janet Maguire, ¹Sanjay Sinha, ¹Anthony Davenport. ¹University of Cambridge, Cambridge, UK; ²University of Colorado

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Introduction Apelin receptor activation promotes beneficial vasodilation and positive inotropy¹ and is critical for early cardiac development.² In cardiovascular disease states, including heart failure, expression of apelin receptor endogenous peptide ligands, apelin and Elabela, are downregulated.¹ We aim to use human embryonic stem cell (hESC)-derived cardiomyocytes (hESC-CMs) as a model to interrogate the role of the apelin receptor in the cardiovascular system and the potential of targeting the receptor therapeutically. Here, we have generated a system to inducibly knockdown the apelin receptor in hESC-CMs to examine the effects on differentiation of hESC to cardiomyocyte, and the effects on function of the resulting hESC-CMs.

Methods A short hairpin RNA based apelin receptor (shAPLNR) inducible knockdown system was generated in pluripotent hESCs, utilising the sOPTiKD system as described previously,³ before differentiating to hESC-CMs. Inclusion of tetracycline in the culture medium was used to induce knockdown, with knockdown efficiency determined by qRT-PCR and radioligand binding. RNA-sequencing analysis and a panel of phenotypic assays were performed to examine effects of apelin receptor knockdown on cardiomyocyte differentiation and function.

Results We have previously demonstrated that hESC-derived cardiomyocytes express the apelinergic system at a similar level to adult cardiomyocytes (Bmax hESC-CMs 21 fmol/mg versus adult 14 fmol/mg). Apelin receptor expression was significantly reduced in hESCs and hESC-CMs by tetracycline inclusion at both RNA and protein level ($84.2\% \pm 4.3$ and $82.8\% \pm 3.9$ compared to control in hESCs and hESC-CMs, respectively). Interestingly, efficiency of differentiation to cardiomyocyte was reduced compared to control cells ($77.8\% \pm 5.5$ cardiomyocyte control vs $22.2\% \pm 7.4$ cardiomyocyte knockdown). RNA-sequencing analysis revealed 272 differentially expressed genes involved in pathways related to electrophysiological signalling, adhesion and the cytoskeleton.

Discussion We have successfully shown knockdown of apelin receptor expression in hESC-CMs. To our knowledge, this is the first use of this system to knockdown expression of a GPCR. This system allows detailed characterisation of apelin receptor activation in cardiovascular development and disease pathogenesis, providing an innovative approach to develop strategies to target the apelin receptor therapeutically in cardiovascular disease, where novel treatments are urgently needed.

Conflict of Interest None

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BS4

MODELLING OF STROKE RISK: AN EPIGENETIC AND IN VITRO STUDY

¹Natalia Cullell, ²Cristina Gallego-Fábrega, ²Jara Cárcel, ²Elena Muiño, ²Laia Lluçà-Carol, ²Miquel Lledós, ³Robert Beal, ³Stephen White, ⁴Manel Esteller, ⁴Manuel Castro de Moura, ⁵Jerzy Krupinski, ⁶Israel Fernández-Cadenas. ¹Fundació Docència i Recerca MutuaTerrassa / Biomedical Research Institute Sant Pau, Terrassa, Spain; ²Biomedical Research Institute Sant Pau; ³Manchester Metropolitan University; ⁴Josep Carreras Leukaemia Research Institute; ⁵Fundació Docència i Recerca MutuaTerrassa; ⁶Biomedical Research Institute Sant Pau, Fundació Docència i Recerca MutuaTerrassa

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Background and Aims 35 loci have been associated with stroke in Genome-Wide association studies (GWAS). We previously found an altered DNA methylation pattern in one gene identified in GWAS to be associated with stroke risk (ZFHX3). DNA methylation in this gene was causally associated with atherothrombotic stroke. Our aim is to determine whether genes associated with stroke risk in GWAS are also linked with stroke susceptibility through epigenetic regulation. Moreover, to study the implication of these genes in the atherosclerotic process using an in vitro model.

Methods DNA methylation was assessed in 253 ischemic stroke patients and 43 controls using the Infinium 450K BeadChip and EPIC BeadChip. We selected all the CpG-sites located in the 35 loci previously associated with stroke in the Megastroke + Uk Biobank cohorts. The significant associations were evaluated in an in vitro model of human coronary artery endothelial cells exposed to normal laminar flow (LSS) and atherogenic flow environments: oscillatory (OSS) and elevated laminar shear stress (ESS).

Results 134 CpG-sites located in 27 different loci were associated with stroke ($p < 0.05$) and 8 CpG-sites remained significant after Bonferroni adjustment. These CpG-sites corresponded to 6 different genes: ZFHX3, SH2B3, SMARCA4, TSPAN2, ILF3 and CDK6. All of them presented hypomethylation in stroke patients compared with controls. We found a significant increased expression of ZFHX3 and CDK6 in ESS compared to LSS. Expression of ILF3 was 60% higher in OSS and 40% lower in ESS compared to LSS. The phosphorylation status of ZFHX3 and SMARCA4 was found to be increased in the OSS environment.

Conclusions Our findings indicate that epigenetic regulation of genes that are risk factors for stroke is associated with stroke susceptibility.

Conflict of Interest No

BS5

EFFECTS OF HYPOKALAEMIA ON ELECTROPHYSIOLOGICAL AND CALCIUM HANDLING OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIAC ANISOTROPIC SHEETS

Bimal Gurung. *The Chinese University of Hong Kong, Sha Tin, Hong Kong (China)*

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Introduction Hypokalaemia, defined as extracellular concentration $[K^+]$ below 3.5 mM, can cause cardiac arrhythmias by