method and electron microscopy. Monocytes were migrated to MCP-1 across CAEC incubated with neutrophil-derived microparticles using a transendothelial migration assay. Cytokine release and adhesion molecule expression by hCAEC was investigated using a cytometric bead array and fluorescent antibody binding respectively. Microparticle adhesion to hCAECs was investigated under static and flow conditions.

Findings Neutrophil-derived microparticles were between 0.5 and 0.9μm. The most abundant surface markers were Annexin V with 20-30% positive microparticles, CD66c (10–15%) and CD18 (5–15%). Low levels of CD11b, CXCR2, and L-selectin were observed. More microparticles were produced in response to fMLP and aLDL compared to PBS. Microparticles bind to and induce MCP-1 release from hCAEC. Monocyte migration increased upon hCAEC incubation with neutrophil-derived microparticles and the extent of the increase was dependent on the stimulus used with aLDL>fMLP>PBS. This highlights a role for microparticles in endothelial activation.

11 THE MAMMALIAN STE20-LIKE KINASE 2 (MST2) MODULATES PATHOLOGICAL HYPERTROPHY BY ACTIVATING THE PROTO-ONCOGENE RAF1

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Presenter: Min Zi

Zi M*, S Prehar, A Maqsood, Y Reynolds-Khan, L Neyses, E Cartwright, D Oceandy. *Cardiovascular Medicine Research Group, School of Biomedicine, University of Manchester*

The novel idea that the molecular regulation of cardiac hypertrophy is closely related to tumour/cancer growth has emerged recently. One key finding was the identification of tumour suppressor RASSF1A as a powerful inhibitor of pathological hypertrophy. The mammalian STE20-like kinase 2 (Mst2) forms a molecular complex with RASSF1A and is also implicated in the development of various tumours. In contrast to Mst1, the role of Mst2 in the heart has not been precisely elucidated.

We used Mst2 knockout mice and isolated neonatal rat cardiomyocytes (NRCM) with an adenoviral mediated overexpression of Mst2 to investigate the role of Mst2 in the heart. Mst2-/- mice exhibited a significant reduction of hypertrophy in response to transverse aortic constriction (30% elevation in heart weight/tibia length ratio in Mst2-/- mice compared to 50% in wild type (WT), n=8, $P<0.05$). In agreement with the in vivo data, overexpression of Mst2 in NRCM significantly enhanced phenylephrine-induced cellular hypertrophy as indicated by cell size measurements and expression of the hypertrophic marker BNP. Since Mst2 physically interacts with Raf1, we investigated the activation of Raf1 and its downstream effector ERK1/2 in NRCM. Overexpression of Mst2 significantly increased the activation of Raf1 and ERK1/2 indicating that Mst2 positively regulates the pro-hypertrophic and pro cancer Raf1-ERK1/2 pathway. In conclusion, our data provide a key evidence of novel role of Mst2 as a positive regulator of cardiac hypertrophy by modulating the Raf1-ERK1/2 pathway. It also reinforces the notion that genes that are implicated in tumour growth/cancer are also important in myocardial hypertrophy.

12 PHARMACOLOGY OF HUMAN ETA AND ETB RECEPTOR SIGNALLING VIA G-PROTEIN AND BETA-ARRESTIN PATHWAYS

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Presenter: Janet Maguire

JJ Maguire, RE Kuc, VR Pell , AP Davenport. *Clinical Pharmacology Unit, University of Cambridge, Level 6 ACCI, Box 110 Addenbrooke's Hospital, Cambridge CB2 0QQ UK*

The endothelin (ET) system is altered in cardiovascular diseases and ET receptor antagonists are licenced for treatment of pulmonary arterial hypertension (PAH). Endothelins act via two G-protein-coupled receptors (GPCRs), ETA and ETB. However, it is recognised that GPCRs may also activate signalling pathways in a G-protein-independent manner via beta-arrestin. Ligand-specific pathway modulation (biassed agonism/antagonism) may have therapeutic application and therefore we have investigated the potential for pathway bias of ET agonists and antagonists. Concentration-response curves were constructed to ET agonists using ETA and ETB beta-arrestin assays. ET receptor antagonists were tested for their ability to block ET-1 responses in each assay and agonist-dependence of the ETA selective antagonist BQ123 was investigated. These data were compared to results from binding experiments in human heart (that expresses both subtypes) and to ETA-mediated vasoconstrictor experiments in human saphenous vein. The relative potency of ET peptides in the ETA and ETB beta-arrestin assays was as expected. Interestingly, for ETA, compared to ET-1, all other agonists tested were partial agonists. Differences from the known pharmacology of antagonists were also revealed in the beta-arrestin assays. Specifically, BQ123 behaved as a negative allosteric modulator in the ETA assay, exhibiting agonist-dependent affinities. Bosentan was a potent ETA-selective antagonist in the beta-arrestin assay in contrast to its non-selective profile determined in human heart. The apparent ETA beta-arrestin bias of bosentan may contribute to its clinical effectiveness in PAH but further investigation of a role for functional selectivity and biassed signalling via the ET receptors in cardiovascular disease is required.

13 FLOW INDUCED VESSEL REMODELLING IN THE CHICKEN EMBRYO IS ASSOCIATED WITH A SPECIFIC GENE EXPRESSION PROFILE

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Presenter: Emily Hoggar

[1]EC Hoggar, [1]M Placzek, [2]T J Chico. *[1]MRC Centre for Biomedical and Developmental Genetics, The University of Sheffield, Sheffield, United Kingdom; [2]NIHR Cardiovascular Biomedical Research Unit, Sheffield Teaching Hospitals, Sheffield, United Kingdom*

Collateral vessel development occurs by remodelling of pre-existing endothelial communications between occluded and neighbouring vascular territories to restore blood flow following occlusion of a large conducting artery. We have identified the genes involved in blood vessel remodelling in the chick embryo model of collateral vessel formation. In ovo ligation of the vitelline artery of E3 chick embryos occluded blood flow to the right side of the extra-embryonic vascular network. Collaterals arose by enlargement of pre-existing vessels and demonstrated active remodelling, peaking in number at 12h post ligation, followed by selection of a more efficient haemodynamic configuration of fewer, larger vessels over 48h. We characterised the global transcriptional changes at 4 and 12h post ligation and found 164 differentially expressed genes, unique to developing collateral vessels and therefore suggested to be driven by shear stress. Phosphodiesterase 10A (PDE10A) was highly up-regulated at 4h post-ligation. Local application of the PDE10A inhibitor Papaverine Hydrochloride had no effect on normal vessel diameter (ctrl 466±34μm, Papaverine [20uM] 600±90μm) but significantly impaired collateral vessel formation at 24h post-ligation (ctrl 109±9μm, Papaverine [20uM] 49±5 μm $P<0.0001$). Time course micrographs revealed significantly reduced collateral development from 6h post-ligation. In vitro proliferation assays using explanted collateral vessels, showed that flow-sensitised endothelial cells treated with papaverine, had a significantly lower proliferation index than controls at 12h post-ligation (ctrl 8.5%±0.5, Papaverine [20uM]) 2.5%±1.5 $P=0.04$). We conclude that developing collateral vessels demonstrate a unique gene expression profile. PDE10A is up-regulated during flow induced remodelling and pharmacologic inhibition significantly impairs this process.