range of vascular pathologies including vascular inflammation and atherosclerosis. While TNF- α has shown to regulate Tie receptors, the chronic impact IL-1 β has on Ang-1/Tie receptor signalling pathway and vascular function has not been investigated.

Aim To examine the impact IL-1 β has on Tie receptor expression and Angiopoietin-1 induced PI3Kinase/AKT activity in endothelial cells.

Method Primary Human Umbilical Vein Endothelial Cells (HUVEC) were stimulated with 25ng/ml of IL-1 β in the presence or absence of 100ng/ml of human recombinant Ang-1. The treatment times ranged from 0 to 48h. Cell lysates from the treated cells were then subjected to Western blotting to analyze Tie receptor levels and phospho-AKT (pAKT), a signaling molecule associated with Ang-1 cellular transduction. The levels of target proteins were compared between reactions by quantifying mean intensity of bands.

In addition, cells were treated with Ang-1 in the absence or presence of IL-1 β at various time points. The cell viability assay was performed on the treated cells by following the manufactures protocol. The mean percentage of live to dead cells was calculated from three random fields for each treatment.

Data for the Tie receptor and AKT analysis is presented as means and SEM of three independent experiments. Statistical significance represented with $p < 0.05$ using Student's t-test. Data for the cell viability assay is presented as means and SEM of two independent experiments.

Results A significant reduction in the levels of Tie-1 receptor was observed at 3h ($58.6 \pm 8.6\%$) in HUVECs treated with IL-1 β . The cytokine was able to maintain significant low levels of Tie1 up until 48h of treatment, whereas the changes in levels of Tie-2 were insignificant. Interestingly, IL-1 β significantly reduced Ang1-induced pAKT activity from 3h onwards with maximum reduction of $73.4 \pm 12.8\%$ observed at 48h. The cell viability assays showed reduction in the percentage live to dead cells between Ang-1 and Ang1 + IL-1 β for chronic time points tested.

Conclusion Long term exposure of Interleukin-1 β is capable of altering Tie-1 levels and increasing the Tie-2: Tie-1 ratio in endothelial cells. In contracts, IL-1 β reduces Ang-1-PI3Kinase/AKT signalling and endothelium cell viability. This opposing phenomenon observed where IL-1 β impairs Ang-1 protective ability suggests and different mechanism of regulation, independent of the Tie-1 receptor.

Conflict of Interest None

BS21

STABILISATION OF SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) TO INHIBIT HUMAN SAPHENOUS VEIN SMOOTH MUSCLE CELL PROLIFERATION AND VASCULAR STENOSIS

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Introduction Suppressor of cytokine signalling 3 (SOCS3) limits multiple signalling pathways involved in vascular inflammation and remodelling responsible for neointimal hyperplasia and vein graft failure. However, SOCS3 function is limited by its short biological half-life, suggesting that SOCS3 stabilisation might prove an effective therapeutic strategy. Through

identification of SOCS3 ubiquitination sites, we have engineered a novel SOCS3 transgene resistant to proteasomal degradation and assessed its ability to limit signalling pathways and processes responsible for neointimal hyperplasia.

Methods Flag-tagged SOCS3 transiently expressed in HEK293 cells was immunoprecipitated with anti-Flag antibody and fractionated by SDS-PAGE for trypsin digestion. Tryptic peptides were analysed by liquid chromatography and tandem mass spectrometry, and ubiquitination sites identified from di-Gly-modified SOCS3 peptides. Smooth muscle cells (SMCs) and endothelial cells (ECs) from human saphenous vein (HSV) were transduced with recombinant lentiviruses (LVs) with MOIs ranging from 3.6-22.2 tfu/cell. Ubiquitination was assessed by immunoprecipitation of soluble cell lysates and immunoblotting. Half-lives of SOCS3 transgenes were determined by immunoblotting following HSVSMC incubation $\pm$ protein synthesis inhibitor emetine. HSVSMC proliferation was assessed by cell counting. Finally, effects of WT and Lys-less SOCS3 gene delivery on cell signalling was determined by assessing phosphorylation of STAT3 (Tyr705) and ERK1/2 (Thr202/Tyr204) by immunoblotting.

Results Analysis of recombinant SOCS3 immunoprecipitated from HEK293 cells revealed that 8 of the 9 Lys residues in human SOCS3 were ubiquitinated. A mutated SOCS3 in which all 9 Lys residues were mutated to Arg was resistant to ubiquitination compared to wild type (WT) SOCS3. LV transduction of WT and Lys-less SOCS3 in HSVSMCs and ECs was highly efficient with $>90\%$ of cells expressing SOCS3 transgene after 48 hours. Lys-less SOCS3 was significantly more stable, displaying a biological half-life >4 hours versus <4 hours for WT ($n=6$, $p < 0.001$). Despite these differences, WT and Lys-less SOCS3 were functionally equivalent in their ability to selectively inhibit STAT3 phosphorylation in response to either sIL-6R α /IL-6 ($74 \pm 6\%$ and $80 \pm 7\%$ inhibition respectively; $n=4$, $p < 0.05$ versus controls) or PDGF-BB ($67 \pm 17\%$ and $72 \pm 18\%$ inhibition respectively; $n=5$) but not ERK1/2 phosphorylation. However, while WT and Lys-less SOCS3 each inhibited cell proliferation in response to sIL-6R α /IL-6 ($83 \pm 29\%$ and $89 \pm 22\%$ inhibition respectively; $n=4$, $p < 0.05$ versus controls) only Lys-less SOCS3 significantly inhibited PDGF-BB-stimulated proliferation ($67 \pm 11\%$ inhibition; $n=4$, $p < 0.05$ versus controls).

Conclusions These results provide evidence for possible therapeutic targeting of SOCS3 stabilisation to limit HSVSMC dysfunction responsible for vein graft failure.

Conflict of Interest None

BS22

TRPV4 IS A PHOSPHOLIPASE C COUPLED RECEPTOR WHEN MITOCHONDRIA ARE DEPOLARISED IN INTACT ENDOTHELIAL CELLS

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Mitochondria are significant regulators of intracellular Ca²⁺ signalling in the vascular endothelium. Depolarization of the mitochondrial membrane potential ($\Delta\psi_m$) inhibits IP₃-mediated Ca²⁺ release and may regulate Ca²⁺ influx across the plasma membrane. However, precisely how Ca²⁺ influx is regulated by mitochondria in the endothelium is unknown. To examine mitochondrial regulation of Ca²⁺ influx, the interaction of TRPV4-mediated Ca²⁺ influx with

mitochondria was examined in the endothelium in intact blood vessels. In controls, TRPV4 activation with GSK1016790A(GSK) generated repetitive Ca^{2+} oscillations that required Ca^{2+} influx. When the $\Delta\psi_m$ was depolarised, by the uncoupler carbonyl cyanide m-chlorophenyl hydrazine (CCCP) or the complex I inhibitor rotenone, TRPV4 activation generated a much larger Ca^{2+} rise and propagating multicellular Ca^{2+} waves. The ATP synthase inhibitor oligomycin did not potentiate TRPV4 mediated Ca^{2+} influx. GSK-evoked Ca^{2+} waves, that occurred when mitochondria were depolarised, persisted in a Ca^{2+} free extracellular solution i.e. were independent of Ca^{2+} influx. These signals were blocked by the TRPV4 channel blocker HC067047 (HC067), the SERCA inhibitor cyclopiazonic acid, the phospholipase C (PLC) blocker U73122 and the inositol triphosphate receptor (IP3R) blocker caffeine. These observations suggest that TRPV4 may directly activate Ca^{2+} release from the internal store. The large propagating waves were inhibited by the pannexin blocker probenecid and the extracellular ATP blockers suramin and apyrase. These results highlight a previously unknown role of mitochondria in shaping TRPV4 mediated Ca^{2+} signalling and show that TRPV4 may trigger ATP release via a pannexin hemichannel when mitochondria are depolarised.

Conflict of Interest n/a

BS23

MRNA EXPRESSION PROFILING OF DUAL SPECIFICITY PHOSPHATASES (DUSPs) IN THE HYPERTENSIVE HEART

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Introduction DUSPs dephosphorylate Ser/Thr and Tyr residues, most notably in the mitogen-activated protein kinases (MAPKs) that are activated by dual phosphorylation in a T-X-Y motif. Whereas some DUSPs preferentially dephosphorylate nuclear MAPKs (DUSP1/2/4/5), others dephosphorylate cytoplasmic MAPKs, including growth-promoting ERK1/2 (DUSP6/7/9) and stress-responsive p38-MAPKs or JNKs (DUSP8/10/16). MAPKs regulate cell growth/survival/death and are implicated in hypertensive heart disease. DUSPs may fine-tune these pathways and influence cardiac remodelling. Here, we assessed expression profiles of cardiac DUSP mRNAs in human heart failure and hypertensive rodents.

Methods Cardiac DUSP (DUSPs 1, 2, 4, 5, 6, 7, 8, 9, 10 and 16) mRNA expression was assessed in an archive of human left ventricular tissue from control (n=12) and non-ischemic heart failure (n=12) individuals. Rodent hypertension models were also studied: spontaneously hypertensive rats (SHRs; 11-13 weeks, n=8) were compared to age-matched WKY controls (n=8), and C57BL/6J male mice (10 wks) were infused with vehicle (acidified-PBS, n=16) or 0.8 mg/kg/d angiotensin II (AngII) for 24h (n=11) or 7d (n=12) using osmotic minipumps. In all studies, cardiac DUSP mRNA expression was assessed by qPCR relative to GAPDH. Statistical tests used a 1-way ANOVA with Holm-Sidak post-test (mouse studies) and unpaired t-tests (human and rat studies).

Results All DUSPs were detected in human hearts except DUSP5; DUSP7 was significantly suppressed (0.53 ± 0.045 -fold, $p=0.0065$) in failing hearts. All DUSPs were detected in rat or mouse hearts. With chronic hypertension in SHRs, Dusp2

(1.39 ± 0.10 -fold, $p=0.0090$), Dusp4 (1.90 ± 0.17 -fold, $p=0.0004$), Dusp6 (1.31 ± 0.15 -fold, $p=0.0190$), Dusp7 (1.48 ± 0.05 -fold, $p<0.0001$), Dusp8 (2.01 ± 0.15 -fold, $p=0.0024$) and Dusp16 (1.22 ± 0.05 -fold, $p=0.0295$) were all upregulated relative to WKY controls, whilst Dusp9 was decreased (0.33 ± 0.08 -fold, $p=0.0232$). In contrast, in the acute model of murine hypertension with AngII-treatment, there were significant increases in Dusp8 (1.48 ± 0.12 fold, $p=0.0003$), Dusp10 (1.54 ± 0.08 -fold, $p<0.0001$) and Dusp16 (1.37 ± 0.06 -fold, $p=0.0306$) abundance at 24h, whilst only Dusp6 (1.47 ± 0.11 -fold, $p=0.0013$) was elevated at 7 days relative to vehicle controls.

Conclusion DUSPs are expressed in human and rodent hearts. With acute adaptation to hypertension, changes in DUSP profiles potentially modulate activities of stress-responsive MAPKs. However, in established hypertension and human heart failure, changes in DUSP profiles potentially modulate activities of cytoplasmic ERKs. Thus, DUSPs are likely to contribute to development of hypertensive heart disease.

Conflict of Interest None

BS24

INVESTIGATING THE ROLE OF SALT-INDUCIBLE KINASE 2 (SIK2) IN HEART

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Introduction Heart failure (HF) is characterised by an inadequate cardiac pumping ability and is one of the major causes of death worldwide. Numerous conditions lead to HF, such as myocardial infarction (MI). After MI, an extensive death of cardiac cells combined with limited regenerative capacity causes a pathological remodelling of the left ventricle, characterized by substantial fibrosis and hypertrophy. Replacement of damaged tissue or limitation of pathological progression are not achievable with current treatments. Thus, novel therapeutic approaches to address this unmet medical need are still required.

We decided to explore the unknown role of Salt-inducible kinase 2 (SIK2) in cardiac cells to potentially identify a novel target for HF therapy. Recently, this kinase has been identified as a key modulator of Hippo pathway-mediated stimulation of cell proliferation in *Drosophila* and a putative mediator of cardiac hypertrophy progression in response to chronic high-salt intake.

Methods and Results Firstly, we demonstrated that SIK2 protein is expressed in both fibroblasts and cardiomyocytes. We also identified a variation of its expression during the different developmental stages of the organism, displaying a greater level during the neonatal phase, and an elevated expression during HF progression, underlining the decisive importance of this kinase in myocardial tissue organization and remodelling.

We performed an in-silico analysis to unroll the mediators of this effect, which suggested that SIK2 activity is mainly mediated by either Hippo or Akt pathway. Exploiting an adenovirus overexpression system, we observed evidence of increased Akt phosphorylation levels. We also found that SIK2 overexpression in primary neonatal rat cardiomyocytes causes activation of LATS, one of the main components of the Hippo pathway, suggesting a potential SIK2-mediated regulation of the Hippo pathway. These alterations did not cause