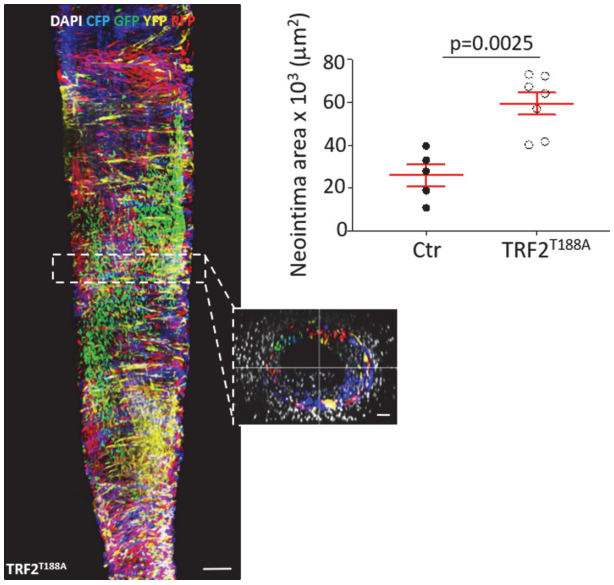

Abstract BS34 Figure 1

driving inflammation through immune cell recruitment in vascular disease. Targeting senescence and senescence-associated inflammation in particular, could limit injury-induced neointima formation and neoatherosclerosis.

Conflict of Interest No

BS35 PONTIN REGULATES CARDIAC REMODELLING BY MODULATING THE HIPPO PATHWAY IN CARDIOMYOCYTES

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The development of heart failure (HF) is characterised by adverse remodelling events such as hypertrophy, fibrosis, and apoptosis, which together can alter the size, shape, and function of the heart. Identification of new factors that are involved in these processes is important in developing new strategies for HF therapy. Pontin (RUVBL1) is a member of the AAA+ protein family that regulates embryonic zebrafish heart development; however, its role in the adult mammalian heart is unknown. Our previous studies have found that Pontin can induce the activity of the major downstream effector of the Hippo pathway YAP, which is known to play an essential role in mediating cardiac remodelling. Thus, in this study, we aim to investigate the roles of Pontin in mediating adverse cardiac remodelling.

Pontin knock-down (KD) and overexpression (OE) in primary neonatal rat cardiomyocytes (NRCM) were achieved by siRNA-mediated gene silencing and adenoviral-mediated overexpression, respectively. We found that Pontin KD negatively modulates NRCM proliferation, represented by a significant reduction in Ki67-positive cells and EdU-incorporation compared to control. Moreover, Pontin KD significantly reduces the level of active YAP, nuclear YAP localisation, and YAP transcriptional activity in NRCM. In contrast, Pontin OE induced NRCM proliferation by approximately 1.6 folds as determined by Ki67 staining and EdU incorporation assay. YAP activity and nuclear translocation were also significantly increased following Pontin OE. We also found that Pontin OE reduced cardiomyocyte hypertrophy and apoptosis (TUNEL staining) in response to Angiotensin II stimulation, whereas KD of Pontin increased Angiotensin-stimulated hypertrophy and apoptosis.

To study the role of Pontin in vivo, we generated a mouse model with an inducible cardiomyocyte-specific knockout of Pontin (PontinicKO) by crossing Pontinflox/flox mice with αMHC-MerCreMer transgenic mice. Induction of Pontin ablation was achieved by intraperitoneal injection with tamoxifen. We found that PontinicKO mice exhibited a severe cardiomyopathy phenotype at four weeks after tamoxifen injection, which was characterised by a significant reduction of ejection fraction, increased cardiac fibrosis and hypertrophy, and profound cardiomyocyte apoptosis.

In conclusion, our study has identified Pontin as a key regulator of cardiac remodelling, likely by modulating the Hippo pathway. Further studies are needed to explore the therapeutic potentials of Pontin in controlling cardiac remodelling.

Conflict of Interest None

BS36 SELECTIVE ACTIVATION OF PRIMED VASCULAR SMOOTH MUSCLE CELLS

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Dysregulation of vascular smooth muscle cells (VSMCs) is a hallmark of vascular diseases including aneurysm and atherosclerosis. VSMCs in healthy vessels maintain a contractile and quiescent phenotype, but can be 'activated' and undergo phenotypic switching to a synthetic, proliferative state when under stress. Previously, we have shown that VSMC expansion after carotid ligation and in atherosclerotic lesions is oligoclonal, meaning activation is restricted to few VSMCs (Chappell et al., 2016, PMID: 27682618). Further to this, we identified a small population of Sca1+ VSMCs with reduced expression of contractile markers in healthy vasculature (Dobnikar et al., 2018, PMID: 30385745), which we hypothesize may have increased responsiveness. However, the mechanisms behind selective VSMC activation and the relevance of Sca1+ cells in disease are yet to be elucidated.

To investigate VSMC activation dynamics, we used a murine carotid ligation injury model, which acutely induces VSMC proliferation. We conducted single-cell RNA-sequencing (scRNA-seq) 5 days after injury, corresponding with the onset of proliferation. Dimensionality reduction showed that injury resulted in a gradient of VSMC phenotypes from contractile to proliferative, with no evidence of a distinct response by a dedicated progenitor population. To characterise this gradual phenotypic change, we performed trajectory analysis, where cells are ordered in 'pseudotime' based on gene expression similarity. Trajectory inference identified two injury response paths of which only one was associated with the transition to active cell proliferation, alongside extracellular matrix organization, and cell adhesion. The second path was enriched for genes associated with protein refolding.

Sca1+ cells mapped to the proliferation-associated path and positioned prior to cells expressing cell cycle markers in pseudotime, supporting the hypothesis that Sca1+ cells represent a primed stage.

As VSMCs expand clonally in vitro as in vivo, we conducted functional analysis of SCA1+ VSMCs using an in vitro model of clonal proliferation. While proliferation was not restricted to SCA1+ cells, attachment and clonal expansion of SCA1+ cells was increased and temporally advanced compared to SCA1- VSMCs. As genes differentially expressed along the proliferation associated path were also associated with the GO terms of cytoskeletal regulation and migration, we explored functional differences of SCA1+ VSMCs via imaging flow cytometry of F-actin and ROCK1. SCA1+ VSMCs from healthy vessels had a marked reduction in both proteins, suggestive of a less contractile and more migratory phenotype, in line with the hypothesis that these cells are primed.

These findings cannot be directly translated to human disease as a human SCA1 orthologue is unknown. Therefore, we conducted scRNA-seq of the healthy human aorta, with the aim of identifying an equivalent population. Single-cell profiling of the human aorta also revealed a gradient of VSMC phenotypes, and trajectory analysis indicated remarkably similar gene expression changes to those that occur in the mouse injury model.

Our findings elucidate mechanisms underlying selective VSMC activation by characterizing the associated transcriptional signature and suggest that phenotypic switching is advanced in VSMCs that are 'primed' in a cell-intrinsic manner. Analysis of human scRNA-seq data indicates that this primed population is relevant in disease, and that functional testing of identified candidate drivers can enable targeted treatments of VSMC dysregulation in atherosclerosis.

Conflict of Interest none

Young investigators award

A C-REL DRIVES ATHEROSCLEROSIS AT SITES OF DISTURBED BLOOD FLOW

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Atherosclerosis is an inflammatory disease that develops preferentially at bends and branches of the vasculature exposed to disturbed flow and low shear stress (LSS). These mechanical conditions modify endothelial cell (EC) physiology by regulating proliferation, inflammation and other fundamental processes. Shear stress alters multiple transcriptional programs, including those regulated by the NF-κB family of transcription factors. Although some members of the NF-κB pathway are known to respond to shear, the influence of this haemodynamic force on the c-Rel NF-κB subunit and its role in atherogenesis are still unknown.

The expression and function of c-Rel was studied using human umbilical vein EC (HUVEC) and human coronary artery EC (HCAEC) exposed to LSS or high shear stress (HSS) using *in vitro* flow systems. Western blotting revealed that LSS strongly induced c-Rel expression in HUVEC and HCAEC. Gene silencing coupled to transcriptome profiling demonstrated that c-Rel promotes pathogenic EC processes (inflammation, proliferation) via induction of p38 MAP kinase and non-canonical p100/p52 NF-κB signalling.

Consistently, immunofluorescent *en face* staining of murine aortas revealed a striking enrichment of c-Rel at LSS regions compared to HSS regions. Genetic deletion of c-Rel, either specifically in EC or in the whole body, rescued EC function, reduced arterial inflammation and decreased atherosclerotic lesion area in hypercholesterolemic AAV-PCSK9-treated mice, indicating that c-Rel promotes atherosclerosis by inducing EC pathological changes.

Our data demonstrate that c-Rel promotes EC pathophysiological changes at LSS regions and is a driver of atherosclerosis via activation of MAPK and non-canonical NF-κB pathways. These data identify c-Rel as a novel therapeutic target to reduce atherosclerosis.

B SAFETY AND OUTCOMES OF A HIGH INTENSITY EXERCISE PROGRAMME IN YOUNG PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY: THE SAFE-HCM STUDY

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Background Moderate intensity exercise training in older patients with hypertrophic cardiomyopathy (HCM) can improve functional capacity, without significant harm. However, younger patients are attracted to high intensity training (HIT) regimes.

Purpose To assess the feasibility, safety and outcomes of an individually tailored, HIT programme in young patients with HCM, and whether observed benefits are sustained at 6 months.

Methods Eighty patients with HCM (45.7y±8.6) underwent baseline clinical and psychological assessment. Individuals were randomised to a 12-week HIT programme (n=40) or usual care (n=40). Baseline evaluation was repeated at 12 weeks (T12). Feasibility, safety, health and psychological benefits were assessed. At 12-weeks individuals were encouraged to continue with the frequency and intensity of physical activity (PA) achieved at the end of the cardiac rehabilitation programme. Participants in the exercise arm were invited to follow-up at 6 months (T6m).

Results The majority (83%) of participants completed the 12-week study. Reasons for refusal included travel, work and family commitments. Resource requirements were similar to other programmes. All individuals felt supported, more confident to exercise, and found educational materials clear and informative. At T12 there was no significant difference between groups in the composite arrhythmia safety outcome (p=0.99). There was no significant difference between groups in episodes of non-sustained ventricular tachycardia (NSVT) (p=0.573) or ectopic burden (p=0.729). The indices of exercise capacity were significantly improved in the exercise compared to the control group; peak VO2 (+3.7 ml/kg/min [CI 1.1,6.3], p=0.006), VO2/kg at anaerobic threshold (VO2/