using siRNA increases VSMC mineralisation (both P<0.001). These increases are prevented by inhibiting TGFÎ[2] signalling with SB431542, suggesting cross-talk between FGF-2 and TGFÎ[2] signalling is crucial for the regulation of VSMC mineralisation. Syndecan-4 can also regulate FGF-2 signalling via protein kinase Cα (PKCα) activation. Biochemical inhibition of PKCα activity using G6976, or knocking-down PKCα expression increases VSMC mineralisation (both P<0.05); this increase is also prevented with SB431542. Finally, the ability of FGF-2 to inhibit VSMC mineralisation is reduced when PKCα expression is knocked-down.

In conclusion, our study has identified that syndecan-4/FGF-2 signalling is up-regulated in mineralising VSMCs to reduce TGFÎ[2] signalling and minimise further calcification. Syndecan-4 regulates FGF-2 signalling to prevent excessive mineralisation by (a) acting as a co-receptor for FGF-2 and inducing downstream signalling via FGFR and (b) interaction with PKα. The syndecan-4/FGF-2/TGFα signalling axis could therefore represent a new therapeutic target for vascular calcification.

211 CHARACTERISING FUNCTIONAL HETEROGENEITY IN HUMAN EPICARDIUM

[1]Sophie McManus*, [1]Laure Gambardella, [1]Victoria Moignard, [1]Dharini Iyer, [1]Bertie Gottgens, [2]Nicolas Le Novere, [1]Helle Jorgensen, [1]Sanjay Sinha. [1]*University of Cambridge*; [2]*Babraham Institute*

10.1136/heartjnl-2017-311726.209

Introduction The epicardium is a heterogeneous cell layer covering the mammalian heart. During embryogenesis, the epicardial lineage is essential to heart and vascular development, yielding cardiac fibroblasts and coronary vascular smooth muscle cells. The epicardium also has a trophic effect on developing cardiomyocytes. It is quiescent in adulthood but reactivates post-injury to a limited degree to yield cardiac fibroblasts, which allows fast healing, yet also causes fibrosis. Epicardial functional heterogeneity remains incompletely characterised.

Methods The Sinha group has derived a robust model of human epicardium (hpsc-epi) from human pluripotent stem cells; this was used for single-cell RNA sequencing (scRNA-seq). Immunohistochemistry and immunocytochemistry were used to validate scRNA-seq results in primary human foetal tissue. The candidate gene BNC1 has been investigated by siRNA-mediated knockdown studies *in vitro*.

Results Single-cell RNA-seq identified two main epicardial subpopulations in hpsc-epi: $WT1^{high}/BNC1^{high}/TCF21^{low}$ and $WT1^{low}/BNC1^{low}/TCF21^{high}$. Here we show validation of our scRNA-seq data in human foetal epicardium by immunohistochemistry in cryosections and human foetal epicardial explants, confirming our hpsc-epi model is representative of the *in vivo* situation. We show preliminary data from siRNA-mediated knockdown of BNC1, which indicate this gene may play a role in epicardial function, possibly in regulating cell migration in a model of epithelial-to-mesenchymal transition.

Implications Improved understanding of developmental epicardial regulation could pave the way towards harnessing epicardial potential in prospective strategies to aid revascularisation and regeneration of the injured heart.

212 SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) INTERACTION WITH CAVIN-1 LINKS SOCS3 FUNCTION AND CAVIN-1 STABILITY

[1]Jamie Williams*, [2]Nasser Alotaiq, [1]Tim Palmer. [1]*School of Pharmacy, University of Bradford*; [2]*Institute of Cardiovascular and Medical Sciences, University of Glasgow*

10.1136/heartjnl-2017-311726.210

Caveolae are lipid raft microdomains essential for the compartmentalisation and regulation of several signalling pathways e.g. JAK/STAT signalling. Disruption of caveolae is a significant factor in multiple disorders including muscular dystrophy, cardiovascular disease, and cancer. Central to caveolae stability is cavin-1 which couples caveolae to the microtubule network to prevent degradation of a key structural element i.e. caveolin-1, and caveolae disassembly. Via an unbiased quantitative proteomics screen, we have identified SOCS3, a negative regulator of JAK/STAT signalling, as a novel cavin-1 interactor.

SOCS3-cavin-1 interactions were characterised by immunoprecipitation assays and probing overlapping peptide arrays. SOCS3 bound to multiple regions within cavin-1, while a PEST motif within the C-terminal region of the SOCS3 SH2 domain was required for interaction with cavin-1 independently of its capacity to bind phospho-tyrosine. Biochemical analysis and confocal imaging also demonstrated that SOCS3 localisation within lipid raft microdomains and at the plasma membrane required cavin-1. Interestingly, SOCS3 does not ubiquitinate cavin-1 but instead supports cavin-1/caveolae stability. Moreover, genetic deletion of cavin-1 results in the loss of SOCS3-mediatied inhibition of cytokine signalling. Importantly, while the inhibitory function of SOCS3 relies on its induction, caveolae stabilisation occurs at basal SOCS3 expression levels. Thus, transmission electron microscopy demonstrated that SOCS3 knock-out endothelial cells show reduced levels of caveolae.

Our data suggest a novel role for SOCS3 in regulating caveolae assembly while cavin-1, acting as a scaffold-protein, might aid SOCS3-dependent regulation of JAK/STAT signalling. This is the first indication of a novel role for SOCS3 in caveola homeostasis and suggests that loss of caveolae represents a novel mechanism by which chronic activation of pro-inflammatory JAK/STAT signalling could be triggered in disease.

213 INCREASED BETA-AMYLOID PRODUCTION IS ASSOCIATED WITH DIABETES-INDUCED VASCULAR DYSFUNCTION

Paul Meakin*, Zofia Tuharska, Christopher McCaffery, Faisel Khan, Michael Ashford. *University of Dundee*

10.1136/heartjnl-2017-311726.211

Introduction β-amyloid (Aβ) is produced via the cleavage of amyloid precursor protein β-secretase (BACE1), resulting in the formation of amyloid plaques, a hallmark pathology of Alzheimer's disease (AD). AD, type 2 diabetes, obesity and cardiovascular disease appear intimately linked with endothelial dysfunction, inflammation, insulin resistance and elevated AÃƒÅ¸ levels all common features. Impaired endothelial function is represented by impaired endothelium-dependent, nitric-oxide (NO)-mediated relaxation. Tight regulation of vasoactive