profile. While non-CAD-sEVs did not statistically differ from PBS nor untouched groups, CAD-sEVs increased the mRNA level of IL1a, IL1b, TNFa and decreased MRC1. Proteomics revealed that PF-sEVs from CAD patients carried higher amounts of pro-inflammatory molecules (ICAM-1 and IL18) compared to NonCAD control. Bioinformatics analysis showed that 861 miRNAs were decreased in the PF-sEVs from CAD patients compared to non-CAD. miRNA targets prediction and pathway analyses reported that clusters of deregulated miRNAs could regulate CD36 and SRB1 which were shown to be decreased in CAD-sEVs treated macrophages. Human PF-cells revealed a reduced expression of CD36 on PF-macrophages.
Conclusions We demonstrate, for the first time, that sEVs isolated from the PF of CAD patients induce a proinflammatory profile of human macrophages and that target crucial lipid metabolism pathways. These clinically relevant results could drive to decipher improved therapeutics able modulate the epicardial/myocardial immune response in CAD patients.

BS26 ROLE OF KMT2C, A HISTONE METHYLTRANSFERASE IN THE DEVELOPMENT OF COMPACTED MYOCARDIUM

Sabu Abraham. *University of Manchester, Core Technology Facility, 46 Grafton Street, Manchester, GTM M13 9NT UK*

10.1136/heartjnl-2022-BCS.206

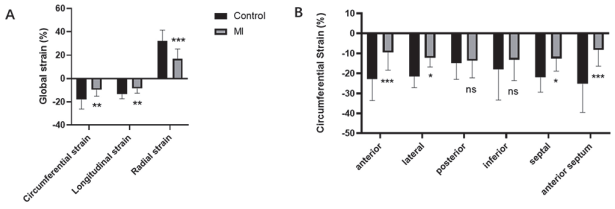
Epigenetic gene regulation has been increasingly established as a pivotal molecular mechanism driving heart development and its aberrant regulation has been implicated in congenital heart diseases. KMT2C is a histone methyltransferase enzyme that mediates the Histone 3 lysine 4 (H3K4) methylation that denotes active promoters and enhancers. Our previous work identified a number of de novo variants in KMT2C gene in nonsyndromic Tetralogy of Fallot patients. Global deletion of delta SET domain region of Kmt2c gene that harbour methyltransferase enzymatic activity resulted in neonatal lethality in mice. Histological analysis of knockout mice embryonic heart revealed ventricular septal defect (with and without an overriding aorta) with a low penetrance but also displayed a consistent phenotype resembling ventricular non-compaction. Embryonic hearts from the knockout mice at the e16.5 stage of development displayed a significantly thinner ($p<0.05$) compact myocardium of the left ventricle compared to the wild-type littermates. In order to get insights into the molecular mechanism for this phenotype, we carried out RNA sequencing experiments in ventricles of e16.5 embryonic hearts from mice with a homozygous deletion and wild type littermates. A significant decrease in gene expression is observed in many of the extracellular matrix (ECM) genes, especially elastin ($p<1.0E-6$), various subtypes of collagens, fibronectin, and integrins. We also found an altered expression of genes important for ECM homeostasis, e.g. MMPs, and ventricular trabeculation/compaction, e.g. Notch1. ECM is known to play important role in heart development, including trabeculation and formation of compacted myocardium. Our data suggest an important role played by Kmt2c in regulating ECM homeostasis and the formation of compacted myocardium.

BS27 ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION AND MYOCARDIAL DEFORMATION IN A REPERFUSED MOUSE MODEL OF MYOCARDIAL INFARCTION

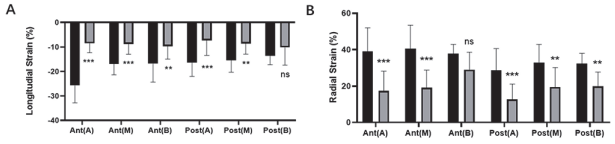
Zhiping Feng. *UCL, Paul O'Gorman Building, 72 Huntley Stree, Finchley, London, LND WC1E 6DD UK*

10.1136/heartjnl-2022-BCS.207

We evaluated the feasibility and accuracy of four-dimensional preclinical ultrasound (4D-US) and speckle-tracking imaging (STI) for monitoring changes in function post reperfused myocardial infarction (MI).
Methods Seventeen female mice (age = 10–12 wk) underwent ligation of the left anterior descending coronary artery. Cardiac MRI (Varian 9.4T) and echocardiographic images (Visualsonics 3100) were acquired at 2weeks (n=6) or8weeks (n=11) post-surgery. Ejection fraction was calculated and then compared between 4D-US, MRI, M-mode and Simpson's multi slice at each time point. Eight healthy mice and seventeen MI mice were used for STI strain analysis.
Results All ultrasound methods calculated ejection fractions that correlated with MRI. However, 4D-US provided the strongest agreement, outperforming M-mode and Simpson's multi slice (4D-US: $R2= 0.81$, M-mode: $R2= 0.55$, Simpson's: $R2= 0.73$) (table 1). STI-derived measures of global strain were significantly lower in the MI group in all dimensions ($P < 0.005$). (Figure 1 A) For regional strain analysis, circumferential strain values in MI were significantly lower in antero-lateral and septal regions compared with control mice ($P < 0.001$). (Figure 1 B). The longitudinal strain and radial



Abstract BS27 Figure 1 A, Differences in global strain between MI and control groups. B, Differences in regional strain in circumferential between MI and control groups. ns: not statistically significant; *P < 0.05 **P < 0.005, ***P < 0.001.



Abstract BS27 Figure 2 A-B, Differences in regional strain in longitudinal and radial between MI and control. Ant(A), anterior apical; Ant(M), anterior mid; Ant(B), anterior basal; Post(A), posterior apical; Post(M), posterior mid; Post(B), posterior basal. ns: not statistically significant; **P < 0.005; ***P < 0.0001.

Abstract BS27 Table 1 A comparison of ejection fraction derived from MRI and different echocardiography

Ejection fraction (%)	Mean	Error	Percent error	Correlation	R^2	P
MRI	49 ± 11					
4D-US	49 ± 10	0 ± 5	4 ± 3	Y = 0.8601*X + 7.037	0.8138	<0.0001
M-mode	46 ± 12	4 ± 10	8 ± 7	Y = 1.032*X - 5.567	0.5475	0.0007
Simpson's multi slice	49 ± 11	0 ± 6	4 ± 4	Y = 0.8212*X + 9.127	0.7306	<0.0001

strain were decreased in all segments of MI hearts compared with control mice except for one basal segment ($P < 0.001$) (figure 2 A-B). These reductions in regional contractility reflect the territory of the occluded coronary artery.

Conclusion This study demonstrates that 4D-US performs well against MRI and better than M-mode and Simpson's multi slice for left ventricle function analysis after MI. STI offers global and regional assessment of myocardium deformation in MI models and can be used to evaluate global and regional functional improvement from experimental treatments for MI.

BS28 SALT-INDUCIBLE KINASE 2 (SIK2) AS A NEW PUTATIVE THERAPEUTIC TARGET FOR HEART FAILURE

[1]Cecilia Facchi, [2]Min Zi, [3]Sukhpal Prehar, [2]Katharine King, [2]Alexandrea Njegic, [2]Xin Wang, [2]Elizabeth Cartwright, [3]Delvac Oceandy. *[1]University of Manchester, Oxford Rd, Manchester, GTM M13 9PL, UK; [2]The University of Manchester; [3]Division of Cardiovascular Sciences, FBMH, University of Manchester*

10.1136/heartjnl-2022-BCS.208

Introduction Heart failure (HF) affects almost 1 million people in UK. Myocardial infarction (MI), one of the leading causes of HF, results in the inadequate pumping ability of the heart. This is mainly due to a massive tissue remodelling, typified by cell death and subsequent formation of scar tissue. Despite advances in treatment options, current therapeutic approaches are unable to restore functional tissue and stop progression to HF. Regenerative medicine is a promising new strategy for HF treatment, with the aim of regenerating the heart post MI through stimulating the proliferation of resident cardiomyocytes. Salt-inducible kinase 2 (SIK2) is a key modulator of cell proliferation through Hippo and AKT pathways, which are both crucial for cardiac cells proliferation. However, the role of SIK2 in cardiac cells is still unknown. Therefore, the aim of this project is to unravel the role of SIK2 at cardiac level to potentially identify a new therapeutic target for HF.

Methods For in vivo experiments, MI was reproduced on global SIK2 knockout (SIK2-/-) mice through the ligation of the coronary artery. Cardiac function was evaluated with echocardiography analysis at 1 and 4 week after MI. Histology techniques were used to quantify infarct and cell size. In parallel, we set up two cellular models on isolated neonatal rat cardiomyoyctes (NRCM). Cells were either infected with adenovirus to overexpress SIK2 or treated with SIK2-specific inhibitor ARN-3236.

Results Our data demonstrated that SIK2 expression increased in HF mice. When we induced MI in SIK2-/- mice, we observed a trend of decreased infarct area and reduced level of hypertrophy, suggesting that the lack of SIK2 could have a protective effect. In vitro data further confirmed these findings, in which adenoviral-mediated SIK2 overexpression in cardiomyocytes induced cell enlargement and increased BNP production, indicating a stimulation of the hypertrophy response. To identify the possible underlying molecular mechanism, we first performed in silico analysis to identify possible SIK2 protein interactors. This analysis indicated that SIK2 might be associated with Hippo and AKT pathways. Experiments using cardiomyocytes overexpressing SIK2 confirmed this finding. We observed a decreased activity of YAP, which is one of the main effectors of the Hippo pathway. Conversely, we detected an increase in Akt phosphorylation, which suggests a SIK2-mediated stimulation of the pathway. Those effects were blunted with SIK2 inhibition following treatment with ARN-3236.

Conclusion In vitro and in vivo data suggested an important role of SIK2 in cardiomyocytes in relation to hypertrophy response, potentially through the modulation of Hippo and AKT pathway.

BS29 FLT4 PLEIOTROPY IN CONGENITAL CARDIAC OR LYMPHOVASCULAR DISEASE IS MEDIATED THROUGH DISTINCT CELLULAR MECHANISMS

[1]Richard Monaghan, [2]Simon Williams, [2]Bernard Keavney. *[1]University of Manchester, AV Hill Building, Oxford Road, Manchester, GTM M13 9PT, UK; [2]University of Manchester*

10.1136/heartjnl-2022-BCS.209

Background The FLT4 gene encodes vascular growth factor receptor 3 (VEGFR3). Studies by several groups have shown that genetic variation in FLT4 is implicated in susceptibility to sporadic, non-syndromic occurrences of the most prevalent cyanotic congenital heart disease, Tetralogy of Fallot (TOF). FLT4 is also implicated in Milroy disease (MD), the most common form of hereditary lymphoedema. MD is caused by dominant negative heterozygous FLT4 variants that ablate the activity of the kinase domain of the receptor in response to its ligands VEGFC and D, which is required for lymphangiogenesis. FLT4 genetic variation giving rise to TOF is distinct to MD, and the conditions do not have overlapping phenotypic features. The mechanism whereby FLT4 contributes to TOF risk has not been elucidated.

Methods FLT4 wild type (WT), a confirmed MD FLT4 variant, or two TOF-associated variants from previously published studies (either a missense de novo variant, DNV; or a protein truncating variant, PTV), with C-terminal V5 epitope tags, were expressed in primary human endothelial cells and their subcellular localisation was compared by immunofluorescence. Escape from nonsense-mediated decay of TOF FLT4 PTVs, and activation of proteostatic signalling of both TOF variants was assessed by immunoblotting techniques. Differentially expressed genes (DEGs) were examined by RNAseq analysis. Rescue of gene expression changes was investigated following treatment with chemical inhibitors of the three main pathways of proteostasis.

Results TOF FLT4 variants of both types display predominantly endoplasmic reticulum (ER)/perinuclear subcellular localisation, and activation of proteostatic signalling responses,