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 myocardial deformation in MI models and can be used to evaluate global and regional functional improvement from experimental treatments for MI.

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**

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Introduction Heart failure (HF) affects almost 1 million people in the 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 cardiomyocytes is still unknown.

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 cardiomyocytes (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.
compared to WT and MD FLT4, which exhibit mainly plasma membrane staining (Figure 1). TOF FLT4 PTVs are expressed, but at a lower level than WT, however, this can be augmented by treatments simulating low oxygen levels. Transcriptomic analyses revealed a subset of DEGs that are TOF FLT4-specific compared to both WT and MD expressing cells (TFSGs, Table 1). Gene ontology analysis showed that TFSGs were enriched for proteostatic, metabolic and developmental signalling processes. TFSGs were also compared with stably heart expressed developmental genes (SHDGs) and showed significant overlap when examined by permutation testing (Figure 2), directly linking in vitro and in vivo transcriptomic data. Inhibitors of all three proteostatic signalling pathways rescued TFSG expression changes, to differing degrees, confirming the mechanism of pathogenesis for FLT4 variants in TOF.

Conclusions We demonstrate a gain-of-function mechanism, with varying degrees of penetrance, that is responsible for the TOF phenotype. This contrasts with the already established dominant negative mechanism, that leads to Milroy lymphoedema with other FLT4 variants. Our results succinctly delineate the mechanisms of FLT4 pleiotropy in two unrelated cardiovascular conditions and suggest that targeting proteostatic signalling could identify potential pathways to therapeutic interventions in FLT4-associated TOF.

BS30 CRUCIAL FUNCTIONS OF ALPHA-ACTININ2 IN THE EMBRYONIC HEART

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Introduction Alpha-actinin is an integral protein of the Z-discs in heart and skeletal muscle cells, with important structural and signalling functions. Missense variants in alpha-actinin can cause inherited conditions, e.g. myopathies and cardiomyopathies. The underlying disease mechanisms are still unknown. To study the disease mechanisms of an alpha-actinin missense variant, which is known to cause Hypertrophic Cardiomyopathy in human patients, a mouse model was generated.

Methods Mice carrying the alpha-actinin missense variant were generated by CRISPR-Cas9 genome editing. The heterozygous adult mice were characterised by echocardiography and quantitative PCR. Hearts of homozygous embryos were analysed at E15.5 by high-resolution episcopic microscopy (HREM).

Results Mice carrying a single copy of the missense variant were viable and had normal appearance. Adult heterozygous mice showed no signs of cardiomyopathy on echocardiography. However, mature male mice displayed molecular signs of cardiomyopathy, such as induction of the fetal gene programme. No homozygous offspring could be generated. Embryonic lethality was confirmed and E15.5 was the latest stage homozygous pups were reliably found to be viable. At this timepoint, genotype distribution was within the expected Mendelian ratios. HREM of the hearts at this stage revealed increased right ventricular chamber size and decreased left atrial size, when compared to wildtype littermates. Membranous ventricular septal defects were observed in 3 out of 8 homozygous hearts. Further, these embryos displayed aortic stenosis and dysplastic leaflets of the pulmonary valve.

Conclusions Heterozygous adult mice only displayed sub-clinical signs of disease. In contrast, the missense variant is embryonic lethal in the homozygous setting and leads to a range of