proliferation in response to flow were identified. Future work will validate the role of these genes in endothelial proliferation and atherosclerosis using mammalian models, and human cells and tissues.

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Conflict of Interest none

BS16 THE ROLE OF PARASYMPATHETIC NERVOUS SYSTEM IN THE INFARCT-LIMITING EFFECT OF SGLT2 INHIBITORS

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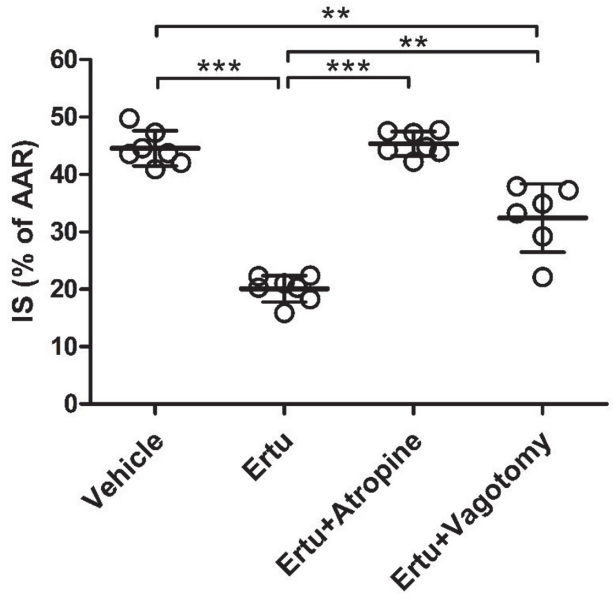
Introduction As long-term outcome in patients with acute myocardial infarction (MI) is predicted by final infarct size (IS), reducing IS is of paramount importance. Recent experimental studies have demonstrated a strong infarct-sparing effect of SGLT2 inhibitors – a class of drugs which have proved to be safe and beneficial in patients with heart failure. Repurposing SGLT2 inhibitors for the benefit of patients presenting with an acute MI should be preceded by investigation of the underlying mechanisms of infarct limitation. Experimental and clinical data indicate a potential role for autonomic modulation in these mechanisms, specifically sympatho-inhibition. The aim of this study was to evaluate the role of parasympathetic tone in the infarct-sparing effect of SGLT2 inhibitors.

Methods Twenty seven Sprague Dawley rats were fed with the diet containing the SGLT2 inhibitor Ertugliflozin or vehicle for 3 days. Myocardial ischaemia/reperfusion injury was caused by a 40-min left anterior descending coronary artery occlusion followed by 2 hours of reperfusion under isoflurane anaesthesia (4% for induction and 1.5-2% for maintenance). Two groups of animals, pre-treated with Ertugliflozin, were subjected to parasympathetic denervation prior to myocardial ischaemia, either with the muscarinic receptor antagonist, atropine sulfate i.v. (2 mg/kg bolus, then 1 mg/kg/h), or bilateral cervical vagotomy (figure 1).



Abstract BS16 Figure 1 The effect of parasympathetic denervation on acute cardioprotection by SGLT2 inhibitor Ertugliflozin (Ertu). IS – infarct size, AAR – area at risk. ** - p<0.01, *** - p<0.001.

Results Pre-treatment with Ertugliflozin reduced IS by 63% (p<0.001). Blocking muscarinic receptors with atropine abolished the infarct-limiting effect of Ertugliflozin (IS=45±2%, p>0.05 vs. vehicle, p<0.001 vs. ertugliflozin), whereas bilateral mechanical vagotomy only attenuated cardioprotection (IS=32±5%, p<0.01 vs vehicle and Ertugliflozin).

Conclusion These results suggest that the Infarct-limiting effect of SGLT2 inhibitor Ertugliflozin may be mediated via M-cholinoreceptors.

Conflict of Interest No

BS17 RNA BINDING PROTEIN MULTIPLE SPLICING (RBPMS) DRIVES A CONTRACTILE SPLICING NETWORK IN HUMAN EMBRYONIC STEM CELL DERIVED VASCULAR SMOOTH MUSCLE CELLS

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10.1136/heartjnl-2021-BCS.215

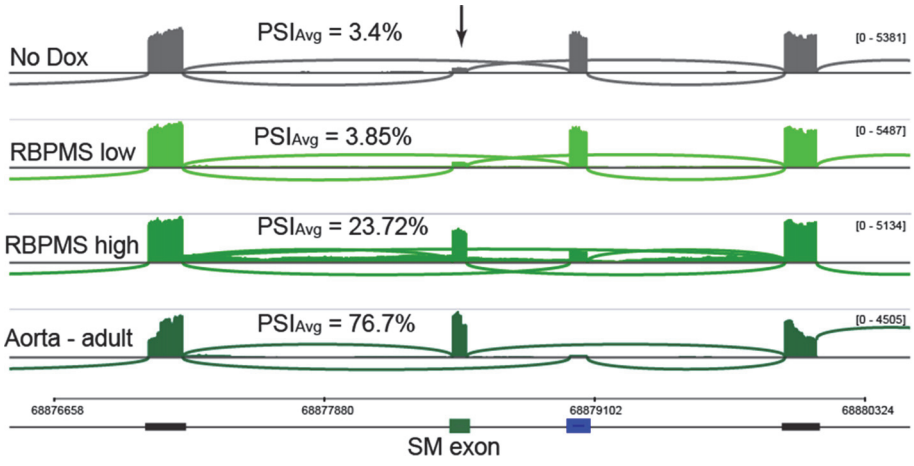
Introduction Contractile Vascular Smooth Muscle cells (VSMCs) lining the walls of major arteries express a unique network of alternative splicing (AS) patterns in critical VSMC regulatory and functional genes (SM-AS). The regulation and impact of SM-AS on VSMC phenotype and behaviour, however, remain poorly understood. Previously, we uncovered a master splicing regulator, RNA Binding Protein Multiple Splicing (RBPMS), a factor highly expressed in tissue VSMCs. RBPMS contributes to about 20% of SM-AS seen in contractile VSMCs.[1 2]

Aim To understand the contribution of RBPMS and the SM-AS splicing network to VSMC function.

Basic Methods We have used human embryonic stem cell derived VSMCs (hES-VSMC)[3] – a system compatible with stable genetic manipulation and facile molecular and phenotypic assessment. We generated hESC clones housing Doxycycline-inducible[4] RBPMS alongside GFP at the pUCAAVS genomic safe harbour. Notably, induction from this locus was heterogeneous with GFP intensity acting as an indicator of RBPMS expression. This uniquely lends itself to downstream SM-AS and phenotypic comparison of cells with varying levels of RBPMS expression within the same population. For SM-AS assessment, mRNA-seq was performed on flow assisted cytometric sorted hES-VSMCs based on GFP expression followed by splicing network analyses using rMATS[5], Matt[6] and gene ontology (stringDB). For phenotype assessment, mitogen-induced proliferation of GFP high and low cells was monitored by estimating a cell-tracer dye dilution using flow cytometry (figure 2A). Motility was examined using live cell imaging followed by cell tracking analysis over 24 hours (ibidi) (figure 2B).

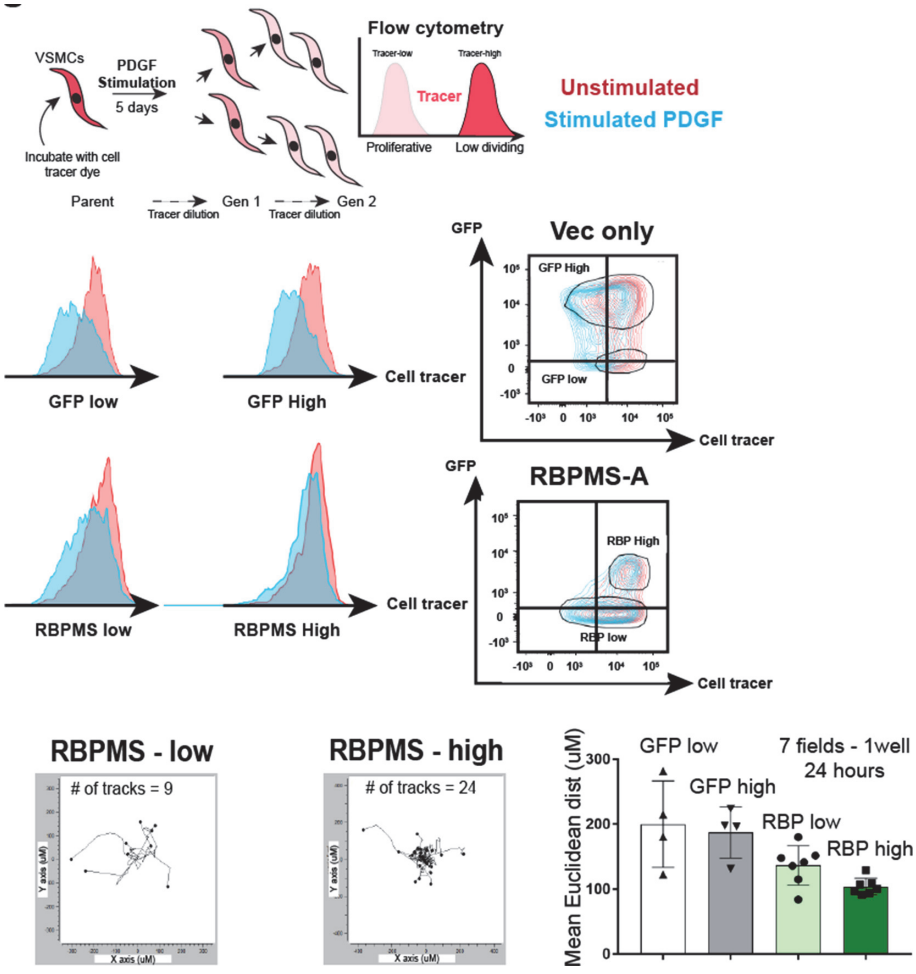
Results and Conclusions We observe that RBPMS-high cells possessed distinct splicing profiles from RBPMS-low cells. Statistical comparison of the two groups using rMATS5, revealed

a network of AS events triggered by RBPMS showing SM-AS patterns akin to contractile, tissue VSMCs in major targets including ACTN1 (figure 1), TPM1, CALD1, SMTN and focal adhesion factors like VCL. Gene ontology analyses indicate focal and adherens junction, cytoskeletal and contractile filament proteins as highly represented gene categories in this



ACTN1 – sashimi plot of mRNA-seq exon reads showing PSIAvg – Average Percentage Spliced In or inclusion percentage of the Smooth Muscle- SM exon.

Abstract BS17 Figure 1



Abstract BS17 Figure 2 A) Proliferation; B) Motility

network. Phenotypic assessments suggest that RBPMS-high hES-VSMCs are less motile and less proliferative compared to RBPMS-low cells correlating with the phenotypic properties of mature VSMCs (figure 2).

Our findings indicate that RBPMS drives contractile splicing programs, possibly influencing phenotype by acting as a master regulatory hub for genes critical for VSMC identity and function.

Conflict of Interest None

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BS18 PROFILING ENDOTHELIAL GENE EXPRESSION IN CORONARY ATHEROSCLEROTIC PLAQUES IN A HUMAN-LIKE D374Y-PCSK9 HYPERLIPIDAEMIC PORCINE MODEL

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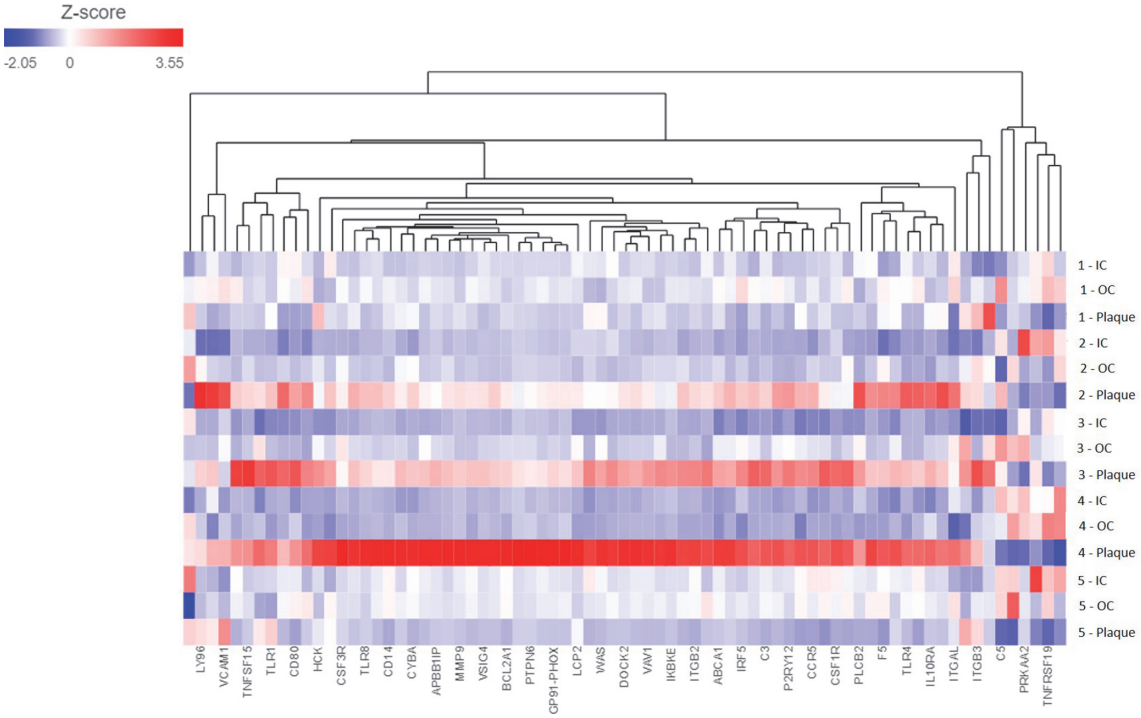
Introduction Endothelial dysfunction is central to the development of atherosclerosis. Previous approaches studying endothelial gene expression in relation to atherogenesis have utilised in vitro cell culture or in vivo models sampling endothelium from the carotid arteries or aortic arch. Endothelial gene expression studies in coronary atherosclerotic plaques in vivo



Abstract BS18 Figure 1

Abstract BS18 Table 1 Biological pathways to which differentially expressed genes belong to

Biological process related to atherosclerosis	Enrichment score	P-value	Genes [upregulated in endothelium overlying plaque] [downregulated in endothelium overlying plaque]
Toll-like receptor signaling pathway	14.0	8.54E-07	TLR1, TLR2, PIK3CD, CASP8, LY96, CD14, TLR3, TLR7, TLR4, TLR8, IKBKE, SPP1, IRF5, CCL4, CD80, CD86
Complement and coagulation cascades	11.5	9.92E-06	F5, C3, C1QA, C2, VSIG4, ITGB2, C5AR1, CR1, C5, F13A1
Cell adhesion molecules	8.3	2.42E-06	CD80, CD86, SLA-DMB, PDCD1LG2, ITGB2, MHC2, ITGAM, ITGA4, VCAM1, PTPRC, ITGAL, PD-L2, PD-L1, NCAM2
Leukocyte transendothelial migration	7.4	5.90E-04	MMP9, VAV1, PIK3CD, CYBA, GP91-PHOX, CXCR4, ITGAM, ITGB2, NOX2, VCAM1, ITGA4, NCF2, CXCL12
Chemokine signaling pathway	7.0	8.76E-04	ADCY7, CXCR4, CCL4, ARRB2, HCK, PIK3CD, VAV1, WAS, PLCB2, JAK3, DOCK2, SRC
Cholesterol metabolism	5.2	5.74E-03	ABCA1, PLTP, LIPA, SOAT1 , ANGPTL4
Platelet activation	4.9	7.41E-03	PLCB2, P2RY12, ADCY7, PIK3CD, PIK3CG, FCER1G, FERMT3, APBB1IP, ITGA2, ITGB3, LCP2
Cytokine-cytokine receptor interaction	4.4	0.01	CXCR4, MCP-1, CCL4, CCR5, CSF3R, CSF2RB, TNFSF15, CSF1R, IL10RA, TNFSF13, TNFRSF19, CXCL12
NF-kappa B signaling pathway	3.2	0.04	CD14, LY96, TLR4, BCL2A1, CCL4, VCAM1, CXCL12
Fluid shear stress and atherosclerosis	2.9	0.05	PIK3CD, ITGB3, HMOX1, MMP9, MCP1, VCAM1, CYBA, NCF2, PRKAA2