Piezo1 alters inflammatory signalling which may have implications on PC pathophysiology. We plan to investigate this observation further to understand how Piezo1-mediated signalling influences PC-EC communication in exercise and general health.

Conflict of Interest no

BS3 NOVEL STRATEGY USING HESC-DERIVED CARDIOMYOCYTES TO EXPLORE THE CRITICAL IMPORTANCE OF THE APELIN RECEPTOR IN THE CARDIOVASCULAR SYSTEM

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Introduction Apelin receptor activation promotes beneficial vasodilation and positive inotropy[1] and is critical for early cardiac development.[2] In cardiovascular disease states, including heart failure, expression of apelin receptor endogenous peptide ligands, apelin and Elabela, are downregulated.[1] We aim to use human embryonic stem cell (hESC)-derived cardiomyocytes (hESC-CMs) as a model to interrogate the role of the apelin receptor in the cardiovascular system and the potential of targeting the receptor therapeutically. Here, we have generated a system to inducibly knockdown the apelin receptor in hESC-CMs to examine the effects on differentiation of hESC to cardiomyocyte, and the effects on function of the resulting hESC-CMs.

Methods A short hairpin RNA based apelin receptor (shAPLNR) inducible knockdown system was generated in pluripotent hESCs, utilising the sOPTiKD system as described previously,[3] before differentiating to hESC-CMs. Inclusion of tetracycline in the culture medium was used to induce knockdown, with knockdown efficiency determined by qRT-PCR and radioligand binding. RNA-sequencing analysis and a panel of phenotypic assays were performed to examine effects of apelin receptor knockdown on cardiomyocyte differentiation and function.

Results We have previously demonstrated that hESC-derived cardiomyocytes express the apelinergic system at a similar level to adult cardiomyocytes (Bmax hESC-CMs 21 fmol/mg versus adult 14 fmol/mg). Apelin receptor expression was significantly reduced in hESCs and hESC-CMs by tetracycline inclusion at both RNA and protein level (84.2% ± 4.3 and 82.8% ± 3.9 compared to control in hESCs and hESC-CMs, respectively). Interestingly, efficiency of differentiation to cardiomyocyte was reduced compared to control cells (77.8% ± 5.5 cardiomyocyte control vs 22.2% ± 7.4 cardiomyocyte knockdown). RNA-sequencing analysis revealed 272 differentially expressed genes involved in pathways related to electrophysiological signalling, adhesion and the cytoskeleton.

Discussion We have successfully shown knockdown of apelin receptor expression in hESC-CMs. To our knowledge, this is the first use of this system to knockdown expression of a GPCR. This system allows detailed characterisation of apelin receptor activation in cardiovascular development and disease pathogenesis, providing an innovative approach to develop strategies to target the apelin receptor therapeutically in cardiovascular disease, where novel treatments are urgently needed.

Conflict of Interest None

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BS4 MODELLING OF STROKE RISK: AN EPIGENETIC AND IN VITRO STUDY

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Background and Aims 35 loci have been associated with stroke in Genome-Wide association studies (GWAS). We previously found an altered DNA methylation pattern in one gene identified in GWAS to be associated with stroke risk (ZFHX3). DNA methylation in this gene was causally associated with atherothrombotic stroke. Our aim is to determine whether genes associated with stroke risk in GWAS are also linked with stroke susceptibility through epigenetic regulation. Moreover, to study the implication of these genes in the atherosclerotic process using an in vitro model.

Methods DNA methylation was assessed in 253 ischemic stroke patients and 43 controls using the Infinium 450KBeadChip and EPICBeadChip. We selected all the CpG-sites located in the 35 loci previously associated with stroke in the Megastroke + Uk Biobank cohorts. The significant associations were evaluated in an in vitro model of human coronary artery endothelial cells exposed to normal laminar flow (LSS) and atherogenic flow environments: oscillatory (OSS) and elevated laminar shear stress (ESS).

Results 134 CpG-sites located in 27 different loci were associated with stroke ($p<0.05$) and 8 CpG-sites remained significant after Bonferroni adjustment. These CpG-sites corresponded to 6 different genes: ZFHX3, SH2B3, SMARCA4, TSPAN2, ILF3 and CDK6. All of them presented hypomethylation in stroke patients compared with controls. We found a significant increased expression of ZFHX3 and CDK6 in ESS compared to LSS. Expression of ILF3 was 60% higher in OSS and 40% lower in ESS compared to LSS. The phosphorylation status of ZFHX3 and SMARCA4 was found to be increased in the OSS environment.

Conclusions Our findings indicate that epigenetic regulation of genes that are risk factors for stroke is associated with stroke susceptibility.

Conflict of Interest No

BS5 EFFECTS OF HYPOKALAEMIA ON ELECTROPHYSIOLOGICAL AND CALCIUM HANDLING OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIAC ANISOTROPIC SHEETS

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Introduction Hypokalaemia, defined as extracellular concentration [K+] below 3.5 mM, can cause cardiac arrhythmias by