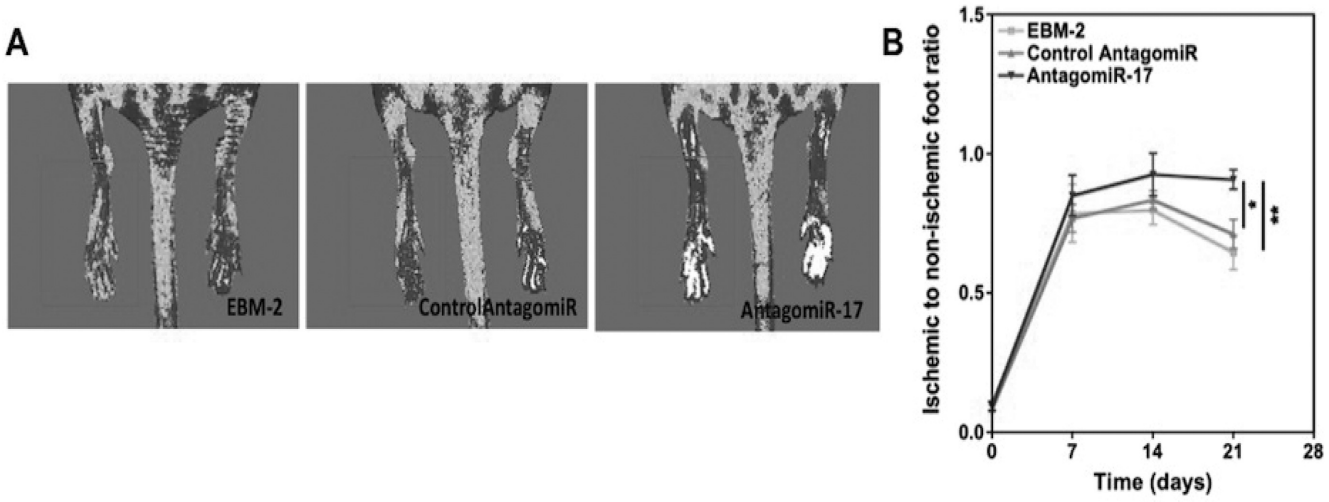

Abstract 198 Figure 1

significantly inhibited VSMC proliferation and migration, while knockdown of miR-22 dramatically promoted VSMC proliferation and migration, respectively. Ecotropic virus integration site 1 protein homolog (EVI-1), a transcription factor localised to the nucleus and binds DNA through specific conserved sequences of GACAAGATA with the potential to interact with both corepressors and coactivators, was predicted as one of the top targets of miR-22 by using several computational miRNA target prediction tools, and was negatively regulated by miR-22 in VSMCs. The luciferase activity of the wild-type, but not the miR-22 binding site mutants of EVI-1 3â€™-UTR reporter was substantially repressed by miR-22 overexpression, confirming the EVI-1 is one of the functional targets of miR-22 in VSMCs. Data from co-transfection experiments also revealed that miR-22 inhibited VSMC proliferation and migration through repressing EVI-1 gene expression. Importantly, perivascular enforced expression of miR-22 in the injured vessels significantly reduced EVI-1 expression levels, decreased VSMC proliferation, and inhibited neointima formation in wire-injured femoral arteries.

Conclusions/implications Our data have demonstrated that miR-22 is an important regulator in VSMC functions and neointima hyperplasia, suggesting its potential therapeutic application for vascular diseases.

198 MICRORNA-17 AS THE TARGET OF IMMOBILIZED VASCULAR ENDOTHELIAL GROWTH FACTOR IN ENDOTHELIAL CELL SURVIVAL UNDER ISCHAEMIC CONDITIONS

[1]Sezin Aday*, [1]Marie Besnier, [2]Janet Zoldan, [3]Laura Carreto, [1]Jaimy Saif, [4]Robert Langer, [1]Costanza Emanueli, [5]Lino Ferreira. *[1]University of Bristol; [2]The University of Texas at Austin; [3]University of Aveiro; [4]Massachusetts Institute of Technology; [5]CNC-Center for Neuroscience and Cell Biology; *Presenting Author*

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The development of tools to control the cell activity after transplantation is of utmost importance in Regenerative Medicine. For clinical efficacy, it is imperative that cells survive and engraft into the host tissue. However, a significant number of cells (typically >70% in few days) die or are lost within hours after transplantation. Therefore, the implementation of new strategies to promote cell survival after transplantation is crucial.

Both cord blood-derived endothelial progenitor cells (CB-EPCs) and VEGF-A have been proposed as therapeutic tools to induce angiogenesis for the treatment of ischemic diseases. VEGF-A promotes the formation of microvessels that differ structurally and functionally depending on its presentation. Free VEGF forms big, leaky vessels while immobilized VEGF facilitates the formation of highly branched networks. Immobilization of VEGF alters VEGFR-2 signaling and subsequent downstream pathways. Improved understanding of the molecular targets underlying the action mechanism of immobilized VEGF might help to develop new platforms to increase post-transplantation cell survival and regenerative capacity.

We showed that conjugated VEGF modulates cord blood-derived endothelial cell (CB-EC) activity by decreasing the expression miR-17 both *in vitro* and *in vivo*. To mimic the down-regulation of miR-17 by immobilized VEGF and understand the effect of miR-17 down-regulation on cell survival, CB-ECs were transfected with antagomiR-17. AntagomiR-17 increased CB-EC survival at least 1.5 times (n = 6) compared to scrambled antagomiR or pro-angiogenic miRNAs reported in the literature (e.g. miR-424 and miR-132) and sprout formation on Matrigel at least 2 times (n = 5) under hypoxia conditions (0.1% O_2). *In vivo*, antagomiR-17 accelerated hemodynamic recovery of the whole limb (n = 12) in unilateral limb ischemia obtained by occlusion of the left femoral artery (Figure1).

Blood flow recovery evaluated by Laser Doppler analysis was significantly higher 21 days after surgery in antagomiR-17 group compared to scrambled antagomiR and EBM-2 groups. Immunohistochemical analyses showed around 1.4 times increase in the capillary density of skeletal muscle in antagomiR-17 condition. In order to determine the gene target and potential pathway involved in the biological effect of antagomiR-17, next generation mRNA sequencing and data mining were used. Gene expression results and siRNA experiments on CB-ECs revealed *ZNF652* and *CDKN1A* as the targets of antagomiR-17-mediated EC survival. Interestingly, *CDKN1A* was reported in the literature as an anti-apoptotic molecule.

Overall, our results show that the immobilized VEGF increases the EC survival both *in vitro* and *in vivo* by down-regulating miR-17, of which main gene targets are *CDKN1A* and *ZNF652* to exert its function in the survival of the cells.

199 A NOVEL SELECTIVE INHIBITIOR FOR PLASMA MEMBRANE CALCIUM ATPASE 4 IMPROVES VEGF-MEDIATED ANGIOGENESIS

[1]Sathishkumar Kurusamy*, [2]Dolores Lopez-Maderuelo, [3]Robert Little, [1]David Cadagan, [1]Clare Murcott, [1]Rhiannon Baggott, [3]Delvac Oceandy, [1]Farjana Begam Rowther, [1]Weiguang Wang, [4]Ludwig Neyses, [3]Elizabeth Cartwright, [5]Juan Miguel Redondo, [1]Angel luis Armesilla. *[1]University of Wolverhampton; [2]Centro Nacional de Investigaciones Cardiovasculares; [3]Institute of Cardiovascular Sciences, University of Manchester; [4]Institute of Cardiovascular Sciences, University of Manchester, UK and University of Luxemburg, Luxemburg; [5]Centro Nacional de Investigaciones Cardiovasculares; *Presenting Author*

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Cardiovascular diseases such as ischaemic heart disease, peripheral arterial disease and stroke are leading causes of death worldwide. Therapeutic angiogenesis, which can improve the formation of new blood vessels in the ischaemic organ, provides a valuable tool for treating cardiovascular diseases. Angiogenesis, a complex blood vessel formation process, involves the participation of several pro- and anti-angiogenic factors. Among them, pro-angiogenic factor Vascular Endothelial Growth Factor (VEGF) has been identified to play a critical role in pathological angiogenesis. Pre-clinical studies demonstrate that VEGF-based pro-angiogenic therapies result in successful reperfusion of the ischaemic organ in animal models. In our previous study, we reported a novel role for the Plasma Membrane Calcium ATPase 4 (PMCA4) as a negative regulator of angiogenic processes mediated by VEGF. Here, we hypothesised that selective inhibition of PMCA4 with the small molecule aurintricarboxylic acid (ATA) will improve VEGF-driven angiogenesis *in vitro* and an *in vivo* model of mouse limb ischaemia.

Consistent with this hypothesis, we demonstrate in this work that inhibition of PMCA4 by treatment with ATA significantly increases the activity of calcineurin/NFAT pathway and the subsequent expression of the NFAT-dependent, pro-angiogenic protein RCAN1.4 in VEGF-stimulated endothelial cells. Additionally, ATA treatment reduces the level of membrane-associated calcineurin, suggesting that enhancement of calcineurin signalling is the result of a disruption of the interaction between PMCA and calcineurin. Moreover, ATA treatment strongly enhances endothelial cell motility and capillary-like formation in matrigel assay. Furthermore, ATA significantly enhances MLEC motility in PMCA4 +/+ (wild type), but not in PMCA4 -/- (knock out) cells, conforming that ATA-mediated inhibition of PMCA4 is implicated in the increase of migration exerted by ATA. Importantly, long-term exposure of endothelial cells to ATA has no changes in cell viability, highlighting the potential clinical application of ATA. In this sense, Post-ischaemic reperfusion of ischaemic limbs in animals treated with ATA is significantly higher than in control-treated animals. The data from this study indicated that modulation of the activity of PMCA4 by treatment with ATA might have important clinical applications to promote blood vessel formation in human diseases associated with insufficient angiogenesis.

200 DEDIFFERENTIATED OR REBORN AGAIN? ELUCIDATING THE CHROMATIN REMODELLING MECHANISMS DURING ENDOTHELIAL CELL REPROGRAMMING FOR CARDIOVASCULAR THERAPY

Rachel Caines*, Sophia Kelaini, David Grieve, Alan Stitt, Andriana Margariti. *Queen's University Belfast; *Presenting Author*

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The vascular endothelium is central to cardiovascular homeostasis. Repair and regeneration of endothelial cells (ECs) has been an important research focus for a number of years. The recent ability to derive ECs through cell reprogramming has opened new avenues. Reprogramming somatic cells to ECs is in its infancy, but the road ahead looks very promising.

A new reprogramming strategy has ruled out safety issues concerned with teratoma formation. Cells exposed to reprogramming factors for 4 days become epigenetically primed and have been defined as Partially induced Pluripotent Stem (PiPS) cells. They do not transverse pluripotency, and so do not form tumours. They have shown the ability to be differentiated into ECs by culture conditions. Efficiency of reprogramming has increased from 0.01% to 30–40% in the case of PiPS-ECs, but there is scope for improvement as the underlying mechanisms are still unclear. The role of epigenetics in reprogramming has come to the forefront recently and the ability to generate a homogenous and functional EC population will be best sought through chromatin remodelling mechanisms.

A protein found to be crucial in 4-day reprogramming was SETSIP, or SET similar protein. SETSIP has high sequence homology to SET with an additional 10 amino acids at the N-terminus. SET plays roles in chromatin remodelling as a transcriptional regulator and roles in differentiation, apoptosis and cell cycle progression. The study aims to elucidate a robust and efficient protocol for the production of a homogenous and functional EC population for use in personalised cardiovascular medicine.

SETSIP has been overexpressed and knocked out of early PiPS-ECs and iPS-ECs to observe the effect on EC reprogramming. Luciferase assays have been undertaken to understand the EC specific pathways regulated by SETSIP. Experiments have also been performed to establish the effect of treatment with VEGF on SETSIP expression and EC differentiation. Future work will involve the employment of CRISPR technology to create a SETSIP deficient cell line to observe the differentiation potential of the cells and phenotype of any derived ECs.

SETSIP was found to translocate to the cell nuclei, and capable of regulating expression of important EC markers. The functional consequences of this were assessed *in vitro* and *in vivo* where SETSIP was found to be important for the formation of vascular tubules. Furthermore, epigenetic modulators such as CBP/p300 were identified as potential mediators of the gene regulatory effects of SETSIP in ECs. These results represent an important step forward in understanding the process of EC reprogramming for use in regenerative medicine.

These findings provide knowledge of the intricate processes during EC reprogramming not only to support the scientific validity of the newly generated ECs but also to ensure the safety of bringing cellular reprogramming to the bedside of cardiovascular patients.