Acknowledgements: This project was supported by a University of Malaya and Ministry of Higher Education, Malaysia.

Background: Current research has demonstrated that monocyte/macrophages can coexist as diverse phenotypes which display an array of differing properties. The aim of this study is to robustly assess the ability of pro- and anti-inflammatory macrophages to polarise towards smooth muscle cell (SMC)-like and endothelial cell (EC)-like phenotypes.

Methods and Results: Human peripheral blood mononuclear cells (PBMC) were differentiated into pro- or anti-inflammatory macrophages through seven days culture with granulocyte-macrophage colony stimulating factors (GM-CSF) or M-CSF respectively. Utilising well-characterised SMC-related markers, pro-inflammatory macrophages demonstrated a significant up-regulation of mRNA and/or protein expression of caldesmon (2-fold;$p<0.05$), calmodulin (2-fold;$p<0.05$), smoothelin (3-fold;$p<0.05$), and vimentin (4-fold;$p<0.05$), compared to stimulated anti-inflammatory macrophages, all of which were further augmented upon three days PDGFBB and TGFβ co-stimulation. Interestingly, employing well-characterised endothelial cell-related markers, three days stimulation of anti-inflammatory macrophages with FGF2 or VEGFA induced a significant up-regulation of mRNA and protein expression for CD31 (PECAM1) (~2.5-fold;$p<0.05$) and VE-cadherin (4-fold;$p<0.05$), compared to stimulated pro-inflammatory macrophages. Despite no change/reduced in CDH5 (VE-cadherin) mRNA expression, protein expression was significantly increased upon VEGFA and FGF2 stimulation (3- fold and 7-fold respectively;$p<0.05$), suggesting a novel post-transcriptional regulatory mechanism.

Conclusions: These findings imply that blood derived macrophages in culture can exhibit smooth muscle cell- and endothelial cell-like features, which are pre-dominant in pro-inflammatory macrophages, and accentuated by SMC or EC-related growth factors. As such, divergent macrophages as sites of vascular injury may harbour the potential to trans-differentiate into VSMC-like and EC-like cells to facilitate healing and stabilisation of vascular pathologies such as atherosclerotic plaques and aortic aneurysms.

P23 CYCLIC-AMP INDUCED NUCLEAR ACTIN DYNAMICS DIVERGENTLY REGULATES PROLIFERATION AND MIGRATION OF VSMCs AND ECs

M McNeill, S White, G Sala-Newby, AC Newby, M Bond
Department of Translational Health Sciences, University of Bristol, U.K

Proliferation and migration of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) contribute positively and negatively, respectively, to intimal hyperplasia. Moreover, current generation anti-mitotics increase thrombotic risk by impairing endothelial regrowth. Hence, targets for VSMC-specific anti-proliferative and anti-migratory agents are required. Here we investigated the effect of cAMP-induced changes in nuclear actin monomer (G-actin) and how this controls proliferation and migration of VSMCs and ECs.

In VSMCs, but not ECs, cAMP significantly increased nuclear G-actin levels and inhibited proliferation and migration. Forced expression of non-polymerisable nuclear-targeted actin mutant (NLS-ACTIN-R62D) also significantly inhibited proliferation and migration in VSMCs but not ECs. To understand the underlying mechanisms, we investigated effects on pro-mitogenic transcription factors. Elevated cAMP and forced expression of NLS-ACTIN-R62D significantly inhibited nuclear translocation of the SRF co-factor MKL1 and activity of SRF and TEAD-dependent luciferase reporters in VSMCs. Silencing the nuclear-actin exporter (XPO6) or over expression the actin importer (IPO9) significantly increased nuclear actin levels, inhibited SRF and TEAD activity and inhibited proliferation and migration. Furthermore, depletion of nuclear G-actin with a constitutively-active mDIA2 mutant completely reversed the anti-mitogenic effects of cAMP in VSMC. In the human saphenous vein organ culture model, pharmacological MKL1 inhibition reduced intimal thickness (to $0.134\pm0.088\mu m$ of control, $p < 0.01$) and intimal proliferation (to $0.272\pm0.044\%$ of control, $p < 0.001$).

These data demonstrate cAMP-dependent increases in nuclear G-actin inactivate SRF and TEAD and inhibit proliferation and migration of VSMCs but not ECs. Further elucidation of this pathway could identify targets for specific inhibition of VSMC proliferation and migration.

P24 EARLY OVERNUTRITION IN RATS INDUCES ALTERATIONS IN THE CARDIOVASCULAR RESPONSE TO INSULIN IN ADULTHOOD

L Guerra-Menéndez[1,2], A Tejera-Muñoz[3], D González-Hedström[3,4], S Amor[3], B Martín-Carro[3], B Oltra[1], JA Paredes[1], R Arriazu[1], J Moratinos-Delgado[2], G Diéguez[1], AL García-Villalón[3], M Granado[3]
[1]*Basic Medical Science Department, Faculty of Medicine, CEU San Pablo University, Madrid, Spain;* [2]*Institute of Applied and Molecular Medicine (IMMA), Faculty of Medicine, CEU San Pablo University, Madrid, Spain;* [3]*Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Spain;* [4]*Pharmactive Biotech Products SL, Parque Científico de Madrid, Spain*

Acknowledgements: This work was funded by "San Pablo CEU- Banco Santander Project" with the support of the Institute of Applied and Molecular Medicine (IMMA) of Universidad San Pablo CEU and in collaboration with Universidad Autónoma of Madrid. Special thank to Domingo Barber, Paloma Martínez, Virginia García and Cruz Sádaba. Authors are grateful for the support provided by San Pablo CEU University.

The aim of this work was to study if early overnutrition in rats is associated with cardiovascular insulin resistance in adulthood. For that purpose we used the experimental model of litter reduction. At birth, rats were organized either in litters of 12 pups/mother (L12-controls) or in litters of 3 pups/mother (L3-Overfed). After weaning rats were fed ad libitum with a standard chow and sacrificed at the age of 6 months. After sacrifice, hearts were set into a Langendorff system whereby increasing insulin doses were administered and coronary perfusion pressure, heart rate and heart contractility were recorded. Likewise, 2mm rings of aorta were mounted in an organ bath whereby changes in vascular tension in response to increasing insulin concentrations were recorded. To assess the activation of the two main pathways involved in insulin intracellular signalling, total proteins were obtained from myocardial and arterial tissues and the MAPK/Akt expression and activation in response to insulin were analyzed. Myocardial contractility in response to insulin was significantly decreased in hearts from overfed rats due to a decreased activation of the PI3K/Akt. On the contrary, in the vascular reactivity experiments insulin induced a higher vasodilation in aorta segments from L3 rats that was not mediated by the activation of the PI3K/Akt pathway and the subsequent release of nitric oxide. In conclusion, overfeeding during lactation in rats induces alterations in vascular function in response to circulating hormones like insulin. This fact could be related with the cardiovascular alterations reported in this experimental mode

P25 MIR-103 PROMOTES ENDOTHELIAL MALADAPTATION AND ATHEROSCLEROSIS BY TARGETING LNCWDR59

L Natarelli[1], C Geißler, G Csaba[2], Y Wei[1], M Zhu[1], A di Francesco[3], P Hartmann[1], R Zimmer[2], A Schober[1]
[1]*Institut für Prophylaxe und Epidemiologie der KreislaufkrankheitenExperimental Vascular Medicine (IPEK), Institute for Cardiovascular Prevention, Ludwig-Maximilians University Munich, Munich, Pettenkoferstrasse 9; 80336 Munich, Germany;* [2]*Institute for Informatics, Ludwig-Maximilians University Munich, Oettingenstraße 67; 80538 Munich, Germany;* [3]*Department of Cardiac, Thoracic and Vascular Sciences, University of Padova, Via Giustiniani 2; 35128 Padova, Italy*

Background: Endothelial cell (EC) maladaptation to disturbed blood flow at athero-prone sites is characterized by chronic, low-grade EC injury and regeneration, which becomes defective upon additional damage by hyperlipidemia. The RNase Dicer promotes EC maladaptation by generating microRNAs (miRs), like miR-103. Here, were studied whether miR-103 affects EC maladaptation by regulating long non-coding RNAs (lncRNAs).

Methodology: RNA-seq and RACE-PCR were used to discover novel lncRNAs, endothelial-GW182 immunoprecipitation and target site blockers (TSBs) to study miRNA-lncRNA interactions, and Ki67 and phospho-γ-H2AX immunofluorescence staining to assess proliferation and DNA damage, respectively. Human lncWDR59 expression was examined in ECs and atherosclerotic lesions by qPCR and *in situ* hybridization.

Results: Several lncRNAs, such as lncWDR59, were upregulated in EC-*Dicer*$^{-/-}$ mice during atherosclerosis, which contain putative binding sites for miR-103. We determined the sequence of lncWDR59 and confirmed the regulation of its expression by miR-103. Hyperlipidemia and oxLDL inhibit EC proliferation and promote DNA damage through targeting of lncWDR59 by miR-103, which reduces Notch1 and β-catenin co-activation by preventing the binding between lncWDR59 and Notch1-inhibitor Numb. *In vivo*, blocking miR-103 and lncWDR59 interaction by treatment with TSBs promoted EC proliferation, reduced endothelial DNA damage and decreased atherosclerosis in *Apoe*$^{-/-}$ mice. Moreover, the functional role of lncWDR59 was conserved in human ECs and atherosclerotic plaques.

Conclusions: We found that lncWDR59 regulated by miR-103 controls the susceptibility of proliferating ECs to hyperlipidemia-induced DNA damage and thereby mediates EC regeneration at athero-prone sites. Hence, blocking miR-103 and lncWDR59 interaction might be a promising therapeutic strategy against atherosclerosis.

P26 MIR-101-3P CONTROLS *TRIB1* EXPRESSION IN HUMAN MACROPHAGES: A POTENTIAL TARGET IN ATHEROSCLEROTIC PLAQUES

C. Niespolo[1], J.S. Viloria[2,3], O.V. Perez[2], H. L. Wilson[1], E. Kiss-Toth[1]
[1]*Department of Infection, Immunity and Cardiovascular Diseases, University of Sheffield, UK;* [2]*Mind the Byte (formerly Intelligent Pharma), Barcelona, Spain;* [3]*University of Barcelona, Spain*

Acknowledgments: This work was funded by the European Marie Sklodowska Curie ITN Project TRAIN-TRIBBLES Research and Innovation Network (Grant No. 721532).

Background: miR-101-3p is a pro-inflammatory miRNA, with predominant roles in lipid metabolism, tumorigenesis and apoptosis. It enhances lipid retention in macrophages and promotes inflammation, by directly targeting ABCA1 and MKP-1, respectively[1,2]. By using bioinformatics, we identified TRIB1 as direct target of miR-101-3p. *TRIB1* deficiency has been linked to lipid disorders, increased atherosclerotic plaque formation and reduced differentiation of anti-inflammatory M2-macrophages[3]. It is also known that the mRNA of TRIB1 is highly unstable, exhibiting a half-life shorter than 1 hour, suggesting it may be regulated post-transcriptionally. We therefore investigated the interaction between TRIB1 and miR-101-3p and its consequences in macrophages.

Methods: miRanda3.2 target-prediction algorithm was used to predict miRNAs-targeting TRIB1. miRNA-target interactions were validated using a luciferase reporter assay in HEK293T cell line, employing miRNA mimic and inhibitor compounds as well as site-directed mutagenesis. RT-qPCR and western blotting were used to quantify endogenous gene expression and protein levels, after miRNA overexpression and inhibition in human monocytes-derived macrophages (MDMs).

Results: miR-101-3p impaired TRIB1-luciferase reporter gene activity, confirming its binding site on the 3'UTR of TRIB1. The overexpression of miR-101-3p in MDMs was associated with a decrease in endogenous TRIB1 and an increase in pro-inflammatory markers. Conversely, inhibition of miR-101-3p resulted in a partial rescue of TRIB1 expression.

Conclusions: Our results suggest that miR-101-3p is a novel regulator of TRIB1 in macrophages. Considering that miR-101-3p and TRIB1 are understood to regulate inflammation and lipid metabolism, miR-101-3p offers an interesting, potential target in atherosclerosis.

References

1. Zhang N., Lei J., Lei H., Ruan X., Liu Q., Chen Y. and Huang W. (2015). "MicroRNA-101 overexpression by IL-6 and TNF- α inhibits cholesterol efflux by suppressing ATP-binding cassette transporter A1 expression." *Exp Cell Res* **336**(1): 33–42.
2. Zhu Q. Y., Liu Q., Chen J. X., Lan K. and Ge B. X. (2010). "MicroRNA-101 targets MAPK phosphatase-1 to regulate the activation of MAPKs in macrophages." *J Immunol* **185**(12): 7435–7442.
3. Satoh T., Kidoya H., Naito H., Yamamoto M., Takemura N., Nakagawa K., Yoshioka Y., Morii E., Takakura N., Takeuchi O. and Akira S. (2013). "Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages." *Nature* **495**(7442): 524–528.