The role of Plasma Membrane ATPase 4 (PMCA4) in vascular remodelling during Abdominal Aortic Aneurysm formation

Kinza Khan[1], Miguel R Campanero[2,3], James M Cotton[4,] Juan Miguel Redondo[3,5], Angel L Armesilla[1,3]

[1]Cardiovascular Molecular Pharmacology Laboratory, School of Pharmacy, Research Institute in Healthcare Science, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK; [2]Department of Cancer Biology, Instituto de Investigaciones Biomedicas Alberto Sols, CSIC-UAM, Madrid, 28029, Spain; [3]CIBERCV; [4]Department of Cardiology, Heart and Lung Centre, New Cross Hospital, Wolverhampton, UK; [5]Gene Regulation in Cardiovascular Remodelling and Inflammation Group, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain;

Introduction: An Abdominal Aortic Aneurysm (AAA) is a permanent localised dilation of the abdominal aorta, classified by a diameter of ≥3.0 cm. Predominant in men aged >65 years, this chronic degenerative disease accounts for 6,000 deaths per year in the UK. Inflammatory infiltration and enzymatic destruction of the elastic lamellae and extracellular matrix (ECM) proteins constitute the key histopathological features. An imbalance in the expression of ECM remodelling proteins, such as matrix metalloproteinases and their inhibitors, contributes to the progressive weakening of the vascular wall. AAAs are also associated with modulation and apoptosis of vascular smooth muscle cells (VSMCs). Understanding the molecular mechanisms behind this remodelling process, may identify potential novel candidates for therapeutic intervention.

Plasma Membrane Calcium ATPase 4 (PMCA4) belongs to a family of transmembrane receptors that extrude calcium from the cytosol to the extracellular environment. There are four isoforms (PMCA1-4) encoded by the *ATP2B1-4* genes. PMCA4 has been identified as a negative regulator of VEGF-induced angiogenesis in our laboratory. Vascular remodelling during aneurysm formation includes increased angiogenesis alongside the above-mentioned extracellular matrix degradation and chronic inflammation. With PMCA1 and PMCA4 being the major isoforms expressed in aortic tissue, we aim to determine whether they have a role during pathological remodelling of aneurysmal disease.

Methods: Cell culture, qRT-PCR, Western Blot, siRNA-mediated gene silencing

Results: Here we examined the role of IL1β on the expression of PMCA4 using qRT-PCR. We show a time- and dose-dependent downregulation of *ATP2B4* mRNA expression in human primary Aortic Endothelial Cells (AoEC). This translated to a reduction in protein levels, confirmed using western blot studies. To determine the functional consequences of reduced PMCA4 expression, we screened an array of genes related to the extracellular matrix. si-RNA mediated knockdown of PMCA4 in the presence of an inflammatory stimulus, led to a 3.27-fold and 4.37-fold enhancement on the IL1β-induced expression of the metalloproteinase *ADAMTS1* (P= 0.025915) and the cell adhesion molecule *SELP* (P= 0.007897), respectively.

Conclusion: AAA current treatment involves surveillance until the risk of rupture outweighs the risk of surgery. A complete understanding of the molecular and cellular mechanisms implicated in aortic dilation and AAA progression will aid the development of therapeutic strategies. Our results demonstrate that inflammatory stimuli reduce the levels of PMCA4 in AoEC. Reduced expression of PMCA4 leads to increased expression of proteins related to ECM remodelling, suggesting a role for PMCA4 during AAA progression.