A multi-omic 'meta-analysis' of the smooth muscle phenotypic transition in vascular disease to identify disease-promoting mechanisms, prognostic biomarkers and therapeutic targets

Oxford supervisors: Professor Nicola Smart[1], Associate Professor Gillian Douglas[2], Professor Kim Dora[3]
Novo Nordisk supervisor: Dr Nils Rorsman[4]

Departments:

1. Department of Physiology, Anatomy and Genetics
2. Division of Cardiovascular Medicine, Radcliffe Department of Medicine
3. Department of Pharmacology
4. Novo Nordisk Research Centre Oxford

Project outline

Background. Progression of vascular disease, notably atherosclerosis, systemic and pulmonary hypertension, diabetic vasculopathy and abdominal aortic aneurysm (AAA), is critically determined by the response of arterial smooth muscle cells (SMCs). In their fully differentiated, contractile state, SMCs confer stability and regulate vascular tone. In disease, however, growth factors secreted by damaged endothelium and immune cells, induce a 'contractile to synthetic' phenotypic SMC switch. Chronic SMC dedifferentiation impairs contractile function, leads to vascular thickening and stiffness, and exacerbates inflammation, to promote plaque instability and susceptibility to AAA (Munshaw et al., 2021 and 2023). While inhibiting SMC phenotypic transformation has proven potential to attenuate progression of vascular disease, there remains a paucity of therapeutic targets. Genome-wide association studies (GWAS) have yielded insights into genetic predisposition e.g. mutations in the atheroprotective endocytic receptor, LRP1 or transcription factor, TCF21. However, the challenge in the post-genomic era remains to elucidate the molecular mechanisms through which GWAS loci influence pathogenesis, and to identify targets for disease prevention. Treatment options are further confounded by the lack of prognostic biomarkers based on SMC phenotype, rather than inflammation, to predict clinical trajectory. Single cell 'omics' approaches have transformed our ability to dissect heterogenous SMC responses to disease, albeit most preclinical studies focus on single disease models/arterial sites and fail to validate in human-relevant systems, while clinical studies are hampered by limited tissue availability (especially healthy control vessels), as well as variable disease severity and patient characteristics.

Hypothesis: A high resolution, multi-species 'meta-analysis', integrating scRNA-seq data sets across diverse arteries and disease conditions, will reveal key regulators of SMC phenotypic transitions, some with conserved function between vessels and others with organotypic roles. This approach will identify novel disease-promoting mechanisms, prognostic biomarkers and therapeutic targets.
Description of the work to be undertaken: We will systematically curate, extract, integrate, and analyse the SMC populations from published scRNA-seq data sets of conduit (coronary), elastic (aortic), microvascular, cerebral, systemic and pulmonary arteries from a range of murine and rat

disease models, as well as from healthy and diseased human arteries. Co-profiling of bulk and single-nucleus chromatin accessibility (ATAC-seq) will further elaborate regulatory mechanisms. Harmonizing molecular signatures across data sets will reveal common, as well as organotypic, disease-specific, SMC molecular traits associated with phenotypic modulation, and an array of putative therapeutic targets. Of the key regulatory mechanisms identified, we anticipate evidence to support an emerging hypothesis that altered metabolism, notably increased glycolytic flux and decreased glucose oxidation, drives SMC phenotypic modulation. GWAS-implicated alleles will also be prioritised. Validation of selected novel pathways will start with expression profiling across a range of human and murine vascular disease samples, to shortlist candidates for functional interrogation. Our established human SMC phenotypic switching model will then be used for medium-throughput arrayed siRNA assays against shortlisted candidate regulators, to identify genetic manipulations that ameliorate or exacerbate phenotype modulation. Significantly altered marker expression and morphological changes identified by automated imaging will be the primary readouts, with functional assessment (e.g. proliferation, migration) and evaluation in an endothelial-SMC co-culture model used to fully characterise lead hits. Proteomic analysis of the SMC secretome will confirm putative biomarkers, identified from the meta-analysis to associate with disease progression, for validation in clinical samples from the OxAMI and OxAAA cohorts. While target validation in human-based systems will ensure translational relevance, our expertise will allow for in vivo testing of leading candidate therapies in murine models of disease, such as atherosclerosis, AAA or vein grafting, and establish a solid basis for future clinical applications.

Supervisor's recent relevant publications:

1. Munshaw S, Redpath AN, Pike BT, **Smart N** (2023). Thymosin β4 preserves vascular smooth muscle phenotype in atherosclerosis via regulation of low density lipoprotein related protein 1 (LRP1). *Int Immunopharmacol*. 115:109702.
2. Munshaw S, Bruche S, Redpath AN, [.....] Fischer R, Channon KM, **Smart N** (2021). Thymosin β4 protects against aortic aneurysm via endocytic regulation of growth factor signaling. *J Clin Invest*. 131(10):e127884.
3. **Dora KA**, Borysova L, [….] **Smart N**, Ascione R (2022). Human coronary microvascular contractile dysfunction associates with viable synthetic smooth muscle cells. *Cardiovasc Res*. 118(8):1978-1992.
4. **Douglas G**, et al. (2020). A key role for the novel coronary artery disease gene JCAD in atherosclerosis via shear stress mechanotransduction. *Cardiovasc Res*. 2020 Sep 1;116(11):1863-1874.
5. Lupu IE, De Val S, **Smart N** (2020). Coronary vessel formation in development and disease: mechanisms and insights for therapy. *Nat Rev Cardiol*.17(12):790-806.