Discovery of novel atherosclerosis targets through delineating the arterial gene regulatory pathways that change in response to pathological cardiovascular stimuli

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Project outline

Background: Endothelial cells (ECs) form the inner lining of all blood vessels, and play a key role in maintaining homeostasis. However, endothelial dysfunction is a hallmark of many human diseases, including atherosclerosis, diabetes and hypertension, with ECs critical mediators of inflammatory responses. The switch from a healthy to pathological phenotype involves the concurrent repression of healthy arterial endothelial identity genes and activation of inflammatory and mesenchymal genes, therefore a clearer understanding of the mechanisms that drive this switch from healthy homeostatic to activated pathological endothelium is essential to identify new targets and develop novel therapies.

Hypothesis: Analysis of arterial gene enhancer behaviour in response to pathological stimuli will identify new regulatory signalling and transcriptional pathways and provide novel targets for therapy.

Key pilot data: My laboratory has recently identified and verified *in vivo* a group of arterial enhancers driving expression of arterial identity genes. These key genes are not only essential for human arterial identity but are also dysregulated during the development of atherosclerosis. Ongoing work in our lab is now using these enhancers to determine the pathways regulating arterial identity during embryonic development. The project proposed here will sit alongside this work, focusing on pathways involved in atherosclerosis .

Aim 1: *To examine the behaviour of different arterial identity enhancers in human arterial ECs exposed to pathological stimuli e.g altered flow and glucose levels, oxLDL, drivers of EndoMT and inflammation.*
Aim 2: *Identify the key transcriptional regulators altering arterial gene expression in these conditions.*
Aim 3: *Verify key pathways in human tissue and animal models of pathological vasculature.*
Aim 4: *Fully characterize the downstream consequences of knock-down of one or more key pathways on endothelial cell phenotype, and investigate links to human diseases via GWAS.*

Description of Work: We have already identified a number of active mammalian arterial enhancers. These are all functionally conserved between human and mice (including active enhancer marks in

human coronary arterial ECs and adult mouse aorta) and drive arterial-specific patterns of gene expression during embryonic development in transgenic zebrafish and mice embryos. For **Aim 1**, we will transfect human ECs (immortalized human arterial ECs (telo-HAECs) and primary human coronary arterial ECs (HCAECs)) with arterial enhancer:GFP transgenes, and monitor their behaviour in response to changes in flow (using our state-of-the-art flow chamber), altered glucose levels, inflammatory metabolic stimuli such as TNF-α and oxLDL, and during endoMT transition stimulated by TGF-β. In **Aim 2**, we will mix standard enhancer analysis (motif mutation, phylogenic footprinting, siRNA/CRISPR-Cas9 knock-down of candidate regulators) with proteomics (using a label-free WT vs mutant motif capture approach to limit noise and maximise discovery) and chemical screens (reading out levels of enhancer:GFP in human cells after treatment). This will identify the key transcriptional regulators, and cognate signalling pathways, affecting arterial gene activity in response to these pathological stimuli, focusing on motifs that recur across enhancers with similar pathological responses. Key pathways will be verified in human disease datasets and tissue. Expression patterns will be assessed using tissue samples by histological analysis and interrogation of published datasets e.g. sc-ATAC and sequencing data from human atherosclerosis plaques as well as mouse models (e.g. artherosclerosis induced by AAV-mPCSK9, altering flow by partial carotid ligation) in mouse enhancer:lacZ lines already established in my laboratory. Lastly, in **Aim 4** we will use CRISPR-Cas9 to generate human endothelial cells lacking the most important verified pathway(s) in order to investigate the downstream consequences on endothelial cell phenotype as assessed by sc-RNAseq and ATAC-seq. We will also investigate associations between components of key pathways and coronary artery disease associated loci using GWAS summary information (derived from a case-control sample size of over 1 million).

Supervisor's recent relevant publications:

Sarah De Val

1. Neal A, Nornes S, Louphrasitthiphol P, Sacilotto S, Preston MD, Fleisinger L, Payne S and **De Val S** (2021). ETS factors are required but not sufficient for specific patterns of enhancer activity in different endothelial subtypes. Dev Biol, May; 473:1-14. PMID: 33453264, DOI: 10.1016/j.ydbio.2021.01.002
2. Payne S, Gunadasa-Rohling M, Neal A, Redpath AN, Patel J, Chouliaras K, Ratnayaka I, Smart N*, **De Val S*** (2019). Regulatory pathways governing murine coronary vessel development are dysregulated after myocardial infarction in the adult heart. Nat Commun, Jul 22;10(1):3276. PMID: 31332177, DOI: 10.1038/s41467-019-10710-2
3. Neal A, Nornes S, Payne S, Wallace MD, Fritzsche M, Louphrasitthiphol P, Wilkinson RN, Chouliaras KM, Liu K, Plant K, Sholapurkar R, Ratnayaka I, Herzog W, Bond G, Chico T, Bou-Gharios G, **De Val S** (2019). Vein identity requires BMP signaling through Alk3. Nat Commun. Jan 28;10(1):453. PMID: 30692543, DOI: 10.1038/s41467-019-08315-w
4. Sacilotto N, Chouliaras KM, Nikitenko LL, Lu YW, Fritzsche M, Wallace MD, Nornes S, Garcia-Moreno F, Payne S, Bridges E, Liu K, Biggs D, Ratnayaka I, Herbert SP, Molnar Z, Harris AL, Davies B, Bond GL, Bou-Gharios G, Schwarz JJ and **De Val S** (2016). MEF2 transcription factors are key regulators of sprouting angiogenesis. Genes Dev. 30:2297-2309. PMID: 27898394, DOI: 10.1101/gad.290619.116

Gillian Douglas

1. Wood A, Antonopoulos A, Chuaiphichai S, Kyriakou T, Diaz R, Al Hussaini A, Marsh AM, Sian M, Meisuria M, McCann G, Rashbrook VS, Drydale E, Draycott S, Polkinghorne MD, Akoumianakis I, Antoniades C, Watkins H, Channon KM, Adlam D, **Douglas G** (2022). PHACTR1 modulates vascular compliance but not endothelial function: a translational study. *Cardiovasc Res*. Jun 2:cvac092. PMID: 35653516, DOI: 10.1093/cvr/cvac092

2. **Douglas G**, Mehta V, Al Haj Zen A, Akoumianakis I, Goel A, Rashbrook VS, Trelfa L, Donovan L, Drydale E, Chuaiphichai S, Antoniades C, Watkins H, Kyriakou T, Tzima E, Channon KM (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. PMID: 31584065, DOI: 10.1093/cvr/cvz263
3. Chuaiphichai S, Rashbrook VS, Hale AB, Trelfa L, Patel J, McNeill E, Lygate CA, Channon KM, **Douglas G** (2018). Endothelial Cell Tetrahydrobiopterin Modulates Sensitivity to Ang (Angiotensin) II-Induced Vascular Remodeling, Blood Pressure, and Abdominal Aortic Aneurysm. *Hypertension*. Jul;72(1):128-138. PMID: 29844152, DOI: 10.1161/HYPERTENSIONAHA.118.11144
4. **Douglas G**, Hale, AB, Patel J, Chuaiphichai S, Haj Zen A, Rashbrook VS, Trelfa L, Crabtree MJ, McNeill E, Channon KM (2018). Roles for endothelial cell and macrophage Gch1 and tetrahydrobiopterin in atherosclerosis progression. *Cardiovascular Research*. 2018: 114 (10) 1385-1399. PMID: 29596571, DOI: 10.1093/cvr/cvy078
5. Chuaiphichai S. Crabtree MJ, McNeill E, Hale AB, Trelfa L, Channon KM, **Douglas G** (2017). A Key Role for Tetrahydrobiopterin-Dependent Endothelial NOS Regulation in Vascular Resistance Arteries: Studies in Endothelial Cell Tetrahydrobiopterin-Deficient Mice. *British Journal of Pharmacology* 2017;174 (8): 657-671. PMID: 28128438, DOI: 10.1111/bph.13728