Single-cell RNA-seq profiling of mouse endothelial cells in response to pulmonary arterial hypertension

Julie Rodor[1], Shiau-Haln Chen[1], Jessica P. Scanlon[1], João P. Monteiro[1], Axelle Caudrillier[1], Sweta Sweta[1], Katherine Ross Stewart[1], Alena Shmakova[1], Ross Dobie[2], Beth E.P Henderson[2], Kevin Stewart[1], Patrick W.F. Hadoke[1], Mark Southwood[3], Stephen D. Moore[3], Paul D. Upton[3], Nick W. Morrell[3], Ziwen Li[1], Stephen Y. Chan[4], Adam Handen[4], Robert Lafyatis[4], Laura P. M. H. de Rooij[5], Neil C. Henderson[2], Peter Carmeliet[5], Ana-Mishel Spiroski[1], Mairi Brittan[1], Andrew H. Baker[1]

Affiliations

1. The Queen's Medical Research Institute, Centre for Cardiovascular Science, University of Edinburgh, Edinburgh EH16 4TJ, UK.
2. The Queen's Medical Research Institute, Centre for Inflammation Research, University of Edinburgh, Edinburgh EH16 4TJ, UK.
3. Department of Medicine, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom.
4. Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, Blood Vascular Medicine Institute, Divisions of Cardiology and Rheumatology, Department of Medicine, University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
5. Laboratory of Angiogenesis and Vascular Metabolism, Center for Cancer Biology, and Department of Oncology and Leuven Cancer Institute (LKI), VIB and KU Leuven, 3000 Leuven, Belgium.

Abstract

Aims: Pulmonary arterial hypertension (PAH) is a rare progressive disease characterised by vascular remodelling. Endothelial cell (EC) dysfunction is a major regulator of these primary vascular changes, but little is known about the drivers behind their dysregulation. Given the heterogeneity of ECs in normal health and various diseases, we aim to characterise their transcriptional responses to PAH at single-cell resolution.

Methods & Results: We performed single-cell RNA-sequencing (scRNA-seq) on lung ECs isolated from an EC lineage-tracing mouse model that underwent both Control and Sugen5416/Hypoxia-induced PAH conditions. EC subpopulations corresponding to the pulmonary vessel types (artery, vein, capillary A, capillary B and lymphatic) were identified in both conditions. Globally, there was an up-regulation of genes associated with the class II major histocompatibility complex in PAH, confirmed by bulk RNA-sequencing. This suggests EC involvement in the disease inflammatory responses. Capillary B ECs showed a subpopulation-specific response, with genes involved in cell migration and angiogenesis up-regulated in PAH. Analysis with rat and human whole-lung PAH scRNA-seq datasets further confirmed the relevance of our findings in mouse. Similar up-regulation was observed in rat and human PAH for 51% of the up-regulated mouse genes. We also identified promising new therapeutics candidates to target EC dysfunction, such as CD74, showing *in vitro* knockdown leads to a loss of EC proliferation and barrier integrity. Finally, we used an *in silico* cell-ordering approach to identify zonation-dependent transcriptional changes across the arteriovenous axis in mouse PAH, including the upregulation of Sgk1 at the macro- and microvasculature junction.

Conclusion: This study provides high resolution insights into PAH-induced EC transcriptomic changes, identifying promising candidates to target endothelial dysfunction in PAH.