# Downregulation of endothelial PLXNA4 under pro-atherosclerotic conditions diminishes vascular integrity enabling monocyte transendothelial migration

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**Background and Aims**: Atherosclerotic plaque (AP) is a complex pathological formation, containing numerous interacting cell types. Our understanding of the cellular composition and dynamics of AP is mainly based on the evaluation of a limited number of known markers and therefore may be incomplete and biased. Here we employ single cell transcriptomics (scRNAseq) to identify and define cell types in human plaques and to reconstruct their lineage trees and relationships.

**Methods**: We have performed scRNAseq on atherosclerotic plaques obtained from 3 (discovery cohort) and 6 (validation cohort) carotid endarterectomy patients. We have employed the scRNAseq based on CEL-seq2/SORT-seq protocol coupled with fluorescence-activated cell sorting. This method allowed us to specifically select viable and nucleated single cells. Finally we have used RaceID and StemID algorithm to identify common and rare cell types and to perform the lineage tracing analysis.
**Results**: We have identified 15 different cell subtypes, among others endothelial cells (EC), macrophages and smooth muscle cells (SMC). Besides these major types we were able to detect less frequent cell populations - including ACKR1+ venular endothelial cells. Notably, we were able to find individual cells and cell clusters co-expressing both EC and SMC genes, such as SPARC, COL6A1, PECAM1 and CD34. These cells exhibited increased median transcriptome entropy and connectivity, relative to other EC and SMC clusters - altogether supporting their progenitor role and suggesting the plasticity of cell identity in AP.
**Conclusions**: ScRNAseq enabled us to identify and define cell subtypes present in human AP. Our data suggest the cellular lineage plasticity in human AP.

**E-poster session**
**SaaG: Advances in endothelium biology**

**EAS19-0647.**
**EARLY DIABETES INDUCES ALTERATIONS IN ENDOTHELIAL PROGENITOR CELL PHENOTYPE AND HOMING IN MICE SUSCEPTIBLE TO ATHEROSCLEROSIS**

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**Background and Aims**: To evaluate number, viability and function of circulating endothelial progenitor cells (EPCs) with role in the pathogenesis of aortic valve disease, particularly in diabetic mice with *atherosclerosis*.

**Methods**: A diabetic ApoE$^{-/-}$ mouse model was used to identify early and progressive changes, at 4 or 7 days after the last streptozotocin or citrate buffer injection, when diet was switched from standard chow to atherogenic diet. Circulating EPCs were identified by CD34 and VEGFR2 staining and the expression of a4b1, aVb3, aVb5, b1, a5 integrins and *CXCR4* chemokine receptor on EPC surface were assessed by flow cytometry. EPC recruitment in aortic valve was measured by fluorescence microscopy of CD34$^{+}$ cells in valve sections.
**Results**: Atherogenic diet induced EPC apoptosis, regardless of the presence of diabetes, as shown by significant higher caspase-3 levels. However, at both tested time points, diabetic mice had significantly lower blood EPC levels than the corresponding non-diabetic animals. EPCs from diabetic mice expressed α4β1 and αVβ3 integrins at a lower level, the rest of integrins and *CXCR4* being seemingly unaffected by diabetes or diet. Aortic valves from mice fed 7 days with atherogenic diet presented a significantly higher number of EPCs recruited and diabetes abolished EPC recruitment at this time point.
**Conclusions**: Reduced EPC number and expression of α4β1 and αVβ3 integrins on EPCs at 4 and 7 days after diabetes induction in atherosclerosis-prone mice resulted in lower recruitment of EPCs in the aortic valve.
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**E-poster session**
**SaaG: New frontiers in endothelium biology**

**EAS19-0739.**
**DOWNREGULATION OF ENDOTHELIAL PLXNA4 UNDER PRO-ATHEROSCLEROTIC CONDITIONS DIMINISHES VASCULAR INTEGRITY ENABLING MONOCYTE TRANSENDOTHELIAL MIGRATION**

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**Background and Aims**: Atherosclerosis is a systemic inflammatory disease, characterized by the accumulation of macrophages in the vascular wall. Neuroimmune guidance cues (NGCs), originally identified to regulate neuronal and vascular patterning, are significant players in leukocyte trafficking. We set out to investigate the expression of NGCs and their receptors in human endothelial cells and monocytes under pro-atherogenic conditions and to clarify their function in atherosclerosis-related processes

**Methods**: Expression of NGC genes in human primary monocytes and endothelial cells cultured in the absence or presence of TNFα or IL1β was determined using RT-PCR. Immunohistochemical staining to determine protein expression of PLXNA4 in human aortic sections with varying stages of atherosclerosis are currently conducted. *In vitro* silencing of PLXNA4 in endothelial cells enabled us to investigate the role of PLXNA4 in endothelial function and monocyte migration.
**Results**: Several NGCs are significantly up- or downregulated under pro-atherogenic conditions. A particularly interesting concurrent downregulation occurred in the receptor PLXNA4 in endothelial cells and its ligand SEMA3A in monocytes. *In vitro*, silencing of endothelial PLXNA4 markedly induced an inflammatory elongated morphological change. Indeed, endothelial cells with reduced PLXNA4 displayed increased expression of ICAM1, impaired barrier function and increased RAC1 activity. Importantly, we observed an increase in transendothelial monocyte migration with reduced endothelial PLXNA4 expression.
**Conclusions**: We show that PLXNA4 has important anti-inflammatory properties. Loss of PLXNA4 affects endothelial integrity and monocyte transendothelial migration. These studies provide novel insights into the immune-modulatory roles of semaphorin ligands and their plexin receptors in atherosclerosis.

**E-poster session**
**SaaG: New frontiers in endothelium biology**

**EAS19-0195.**
**ENDOGLIN PLAYS ROLE IN CHOLESTEROL-INDUCED ENDOTHELIAL DYSFUNCTION AND MONOCYTE TRAFFICKING IN HUMAN AORTIC ENDOTHELIAL CELLS**

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