Background: Abdominal aortic aneurysm (AAA) contributes to significant postoperative mortality in aging populations. AAA management with watchful waiting and surgical repair is based on our limited understanding of disease processes, and current research focuses on extra-cellular vesicles (EVs). Nanosized packages of proteins, RNA, and lipids that facilitate intercellular communication may aid in developing novel AAA therapies. To characterize this regulatory cargo, we isolated EVs from human AAA tissue or control aortic punch biopsies and profiled EV content with microRNA (miRNA) sequencing to identify dysregulated pathways.

Methods: The study was approved by the University Health Network Research Ethics Board. EVs were isolated from human AAA or aortic punch tissue and enriched using size exclusion chromatography (qEV orginal column 70 nm, Izon Science Ltd.) (n = 3). EV size and concentration were determined using nanoparticle tracking analysis (NanoSight NS300, Malvern Panalytical Ltd.). EV-miRNA sequencing was performed with Illumina NextSeq (HTC Molecular Diagnostics Inc.) and analyzed using Partek Genomics Suite (v10) and MIENTURNET (19-11-25).

Results: Patients were selected for infrarenal AAA requiring surgical repair (AAA) or coronary artery disease requiring bypass surgery (control). EV size and concentration were confirmed with nanoparticle tracking analysis. Principal components and gene set analyses revealed distinct clustering of tissue types with 901 and 687 miRNAs enriched in AAA and control samples, respectively. Pathway prediction using established AAA miRNAs (eg, miR-122, miR-146a, and miR-503) identified significant interactions with proangiogenic signaling pathways (eg, PI3K-AKT, JAK-STAT, and HIF-1) as well as cell senescence and adhesion processes (false discovery rate of < 0.05).

Conclusions: EV-derived miRNAs from patients with AAA prominently associate with cell signaling, senescence, and adhesion pathways in aneurysm pathogenesis. To our knowledge, this is the first study to profile the EV-miRNA landscape in human AAA tissue. Further investigation will explore EV-miRNAs as mediators of communication between distinct vascular cell populations that contribute to AAA development.


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Single-cell and Spatially Resolved Transcriptome Analysis Reveals Cellular Heterogeneity and Novel Regulators of Atherosclerotic Plaque Destabilization

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Background: Cardiovascular diseases, including atherosclerosis, are the major cause of death in Western societies, still molecular mechanisms of plaque destabilization remain unclear. Long noncoding RNAs (lncRNAs) are one example of novel molecular modulators, as their expression is highly cell-type specific.

Methods: We used combined total (bulk) RNA and single cell (sc) RNA to study the transcriptome of advanced carotid artery lesions from patients undergoing carotid endarterectomy in our vascular surgery clinic. Additionally, we performed hybridization-based RNA in situ sequencing to identify where cluster-defining genes are located within the plaques.

Results: In this study, four sequencing datasets were investigated (total RNA from early vs late lesions from the same individual patient and unstable vs stable lesions from individual patients: two separate scRNA-seq datasets). Sixteen lncRNAs were cross-referenced between all four datasets. All of these lncRNAs presented a cell-type specific expression pattern, with 11 lncRNAs being significantly enriched in different smooth muscle cell clusters. We found all newly identified lncRNAs conserved in our scRNA-seq datasets of genetically mutated (LDLR+/−) Yucatan mini-pigs and the inducible carotid artery plaque rupture mice (ApoE−/−). The cluster-defining genes from the human scRNA-seq data were then located in human carotid artery tissue sections using the hybridization-