TLR-7 ligand. Finally, treatment with the TLR-7 specific inhibitor, M5049, at a dose of 1.0 or 5.0 mg/kg/day via oral gavage prevented dilation of TAADs at four weeks compared with vehicle controls (9% vs 8% vs 23%; $P < .001$).

Conclusions: Self-RNAs released from stressed and dying cells is associated with chronically inflamed aortic tissue and promotes TAAD development via triggering TLR-7 signaling. Blocking TLR-7 signaling may represent a novel strategy to treat human TAADs.

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22-VIRC-522-AHA-VD

Integration of Coronary Artery Disease Genome-wide Association Studies with Bulk and Single-cell Transcriptomics from Atherosclerotic Plaques by Deconvolution, Reveals Novel Smooth Muscle Cell Genes

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Background: A central role of vascular smooth muscle cells (SMCs) in atherosclerosis has recently evolved that suggests causal genetic links to disease processes. Single cell sequencing studies of atherosclerotic plaques have identified multiple mesenchymal transition cell populations within the plaques. Here, we correlated cell fractions from plaques to coronary artery disease (CAD) related gene polymorphisms to identify novel SMC targets and study their influence on SMC function in atherosclerosis.

Methods: Deconvolution analysis was performed on bulk microarray data from carotid plaques in the Biobank of Karolinska Endarterectomies (BiKE; n = 127) using single cell sequencing data from coronary plaques (n = 5). CAD-associated genome-wide association studies loci associated with mesenchymal cell fractions were identified, followed by functional analyses of these genes in SMC in vitro using migration, proliferation and apoptosis assays.

Results: We identified five mesenchymal cell-specific genetic variants associated with CAD, BiKE patient symptomatology, and gene expression quantitative trait loci in BiKE plaque tissue and GTex normal arteries. These variants were harbored in genetic loci of *ARNTL*, *LDLR*, *MIA3*, *PAK1*, and *ARHGAP15*. Microarray analysis revealed increased expression of *ARHGAP15* and *PAK1* and decreased levels of *LDLR* in carotid plaques compared with normal arteries (n = 127 vs 10 respectively, Student *t* test). Immunohistochemistry demonstrated increased expression of corresponding proteins in the fibrous cap of plaques compared with normal arteries (n = 5). To investigate their function in SMCs, the genes were silenced using small interfering RNAs followed by migration, proliferation, and apoptosis assays. Preliminary results indicated that silencing of *MIA3*, *LDLR*, and *ARNTL* inhibited SMC proliferation.

Conclusions: The results of this project may reveal novel SMC-specific genetic links to the disease, which may serve as therapeutic targets to be explored for improved treatment of atherosclerosis.

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Transcriptional Profiling of Human Abdominal Aortic Aneurysm Tissue Reveals Distinct Extracellular Vesicle-Derived MicroRNA Cargo

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Background: Abdominal aortic aneurysm (AAA) contributes to significant postrupture mortality in aging populations. AAA management with watchful waiting and surgical repair is based on our limited understanding of disease processes, and current research foci including extracellular vesicles (EVs; nano-sized packages of proteins, RNAs, 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 (qEVoriginal columns 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 (HTG Molecular Diagnostics Inc.) and analyzed using Partek Genomics Suite (v.10) and MIENTURNET (19-11-25).

Results: Patients were selected for infrarenal AAA requiring surgical repair (AAA) or coronary artery disease requiring bypass graft 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 proaneurysmal 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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22-VIRC-456-AHA-VD

Single-cell and Spatially Resolved Transcriptome Analysis Reveals Cellular Heterogeneities 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 indicate 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 minipigs 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-