First Giant Steps Toward a Cell Atlas of Atherosclerosis

Hanrui Zhang, Nancy R. Zhang, Mingyao Li, Muredach P. Reilly

Atherosclerosis—a chronic inflammatory disease of the artery wall—is the underlying cause of major clinical cardiovascular diseases.[1–3] Cells within atherosclerotic lesions are heterogeneous and dynamic.[1–3] Their pathological features have been characterized by histology and flow cytometry and more recently, by bulk-tissue omics profiling. Despite this progress, our knowledge of cell types and their roles in atherogenesis remains incomplete because of masking of differences across cells when using genomic measurement at bulk level.[4] Single-cell RNA sequencing (scRNA-seq) has catalyzed a revolution in understanding of cellular heterogeneity in organ systems and diseases.[5] In the current issue, studies by Winkels et al[6] and Cohain et al[7] advance our understanding of leukocyte heterogeneity in atherosclerosis using scRNA-seq providing great promise for discovery of novel molecular mechanisms and therapeutic targets.

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Single-Cell Transcriptome Profiling of Murine Atherosclerosis

Using a 10x genomics scRNA-seq platform, Winkels et al[6] characterized CD45$^+$ leukocytes from aortas of *Apoe*$^{-/-}$ mice fed chow or Western type diet for 12 weeks. A total of 11 leukocyte subpopulations were distinguished, including T cells (5), B cells (2), NK (natural killer) cells (1), monocytes (2), and macrophages (1), and confirmed by a 35-marker mass cytometry panel. A subsequent B-cell–focused analysis identified and functionally validated 3 subpopulations. They also applied a deconvolution method[8] leveraging mouse scRNA-seq data to infer human atherosclerosis cell composition in bulk mRNA expression data of 126 human carotid plaques from the Biobank of Karolinska Endarterectomies (BiKE). The proportion of deconvoluted memory T cells was inversely associated, albeit weakly, with the rate of cardiovascular disease events, providing a hint that unbiased analyses may identify leukocyte subpopulations that drive adverse clinical events.

Cohain et al[7] performed scRNA-seq, using the Drop-seq platform, to characterize CD45$^+$ leukocytes from aortas of *Ldlr*$^{-/-}$ mice fed chow or high-fat diets for 11 weeks. A total of 13 leukocyte subpopulations were identified, including T cells (4), B cells (1), NK cells (1), granulocytes (1), mast cells (1), dendritic cells (1), monocytes (1), and macrophages (3). They subsequently focused on the 3 distinct macrophage subpopulations—inflammatory, resident like, and a newly described TREM2^{high} (triggering receptor expressed on myeloid cells 2) population confirmed their presence in advanced atherosclerosis in *Ldlr*$^{-/-}$ mice and in the *Apoe*$^{-/-}$ mice and validated expression of macrophage subpopulation markers in human carotid plaques by immunohistochemistry. Notably, the gene expression signature of atherosclerosis-associated *TREM2*^{high} macrophages also shows similarities to the recently described TREM2-dependent disease-associated microglia in Alzheimer disease, supporting speculation that pathogenesis of Alzheimer disease and atherosclerosis may share myeloid molecular mechanisms.[9] These analyses also distinguished dendritic cells from macrophages—populations that are challenging to separate using conventional marker-based approaches.

Both studies used relatively standard analysis pipelines, including CellRanger (https://github.com/10XGenomics/cellranger) and the scRNA-seq analysis package SEURAT (https://github.com/satijalab/seurat). Both uncovered leukocyte subpopulations and their transcriptome signatures in healthy and atherosclerotic aortas of mouse models with some preliminary validation of selected clusters and translation to human. Although Cohain et al[7] and Winkels et al[6] agree on the major cell types found in murine atherosclerosis, the cell type clusters reported from the 2 studies have subtle but important differences. These apparent differences may be because of difference in animal models and cell numbers used for the initial cluster identification, the subject-to-subject variation between mice, or artifacts of data acquisition and analytic strategies. Winkels et al[6] estimated cell type proportions by deconvolution and emphasized the selection bias of scRNA-seq using digestion-based sample preparation, which apparently underestimated the proportion of myeloid cells. Overall, these differences highlight the sensitivity of subpopulation discovery and quantification to the model and clustering algorithm and underscore the need for rigorous and reproducible findings in this nascent field. Despite limitations, these articles do highlight the potential power of single-cell transcriptome analysis to transform our understanding of plaque biology in atherosclerosis.

Next Steps Toward the Cell Atlas of Atherosclerosis

Key next steps include a focus on single-cell discovery in human atherosclerosis, combining single-cell analysis with functional and phenotypic characterization in mechanistic studies, adapting novel single-cell profiling technologies and

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computational tools and defining genetic influences on cell subpopulations and functions in disease.

There are several reasons to focus initial single-cell discoveries on human atherosclerosis and then to study mechanisms of such discoveries in appropriate animal models: (1) human and mouse leukocyte subpopulations and functions are not identical; (2) typical mouse models of atherosclerosis do not develop complex features of advanced human disease; (3) mouse atherosclerosis genetic models themselves, as noted in both papers,[6,7] may influence the proportion and distribution of leukocyte subpopulations; and (4) genetic regulation of leukocyte biology and atherosclerosis can differ between human and mouse. For example, the majority of genetic association signals for human disease lie in noncoding regions with cell-specific regulatory features, such as enhancers and long noncoding RNAs, which are often not conserved outside of primates.[10] Thus, integration of human discovery with mechanistic studies in model systems is required to advance clinical translation.

Atherosclerosis progression and resolution are characterized by dynamic cellular transitions within a complex and evolving microenvironment. Integrating single-cell genomics data into a temporal, spatial, and functional context is important for the dissection of plaque biology. For example, it is possible to use computational approaches to infer cellular localization by integrating scRNA-seq data with positional gene expression profiles derived from a gene expression atlas.[11] Combining single-cell analysis and genetic approaches, such as lineage tracing, in model systems is a powerful strategy to dissect cellular dynamics and transdifferentiation. Computational methods are evolving for inferring cellular origins[11] and dynamic trajectories from large numbers of human single-cell profiles,[12] although better algorithms are still needed. Identification of novel cell types or subpopulations in plaques within a specific spatial and temporal context will reveal novel genetic and molecular mechanisms. Such knowledge will be critical for advancing mechanism-based therapeutics, including those proved efficacious in recent clinical trials,[13] for safe therapeutic targeting of inflammatory processes in atherosclerotic cardiovascular disease.

Our ultimate goal is to understand the regulatory landscape of human atherosclerosis so that we can define the contribution of cellular subpopulations and their interactions to cardiovascular disease outcomes. By adapting advanced single-cell technologies with new measurement capabilities and increased scale, including single-cell and single-nucleus omics profiling, we are empowered to build a large resource of single-cell multiomics profiling of human atherosclerosis to (1) define cell subsets for functional and mechanistic studies; (2) address how germline and somatic genetic variants[14] alter cell type diversity and function; (3) investigate the potential to target cell subpopulations, their unique cell surface markers, functional genes, and master regulators in clinical atherosclerosis; and (4) to map circulating and lesion cell subpopulations as risk stratification tools for clinical application.

Challenges and Opportunities

Limited materials and difficulties in large-scale, rapid biospecimen preparation raise technical and analytic challenges in single-cell profiling of human atherosclerosis. Cell type identification and classification are an important step that can be especially challenging when focusing on subtypes within a relatively homogeneous cell population. For example, the 3 macrophage populations reported by Cohain et al,[7] but not by Winkels et al,[6] have overlapping distributions in gene expression that may reflect a continuum of cell states. As applied by Winkels et al,[6] computational tools[8] for deconvoluting bulk RNA-seq datasets using scRNA-seq can overcome underrepresentation of cell types in digestion-based scRNA-seq, as noted for myeloid cells.[6] However, mouse atherosclerosis scRNA-seq data need to be validated against human lesion scRNA-seq for such application. Cell type identification becomes even more challenging when data comes from multiple human subjects, where batch effects and genetic differences can create spurious clusters. Computational cell type classification and cell differentiation trajectory analysis using data from single-cell genomic assays is an active area of research, and better methods for handling technical noise, batch effects, and imputation of gene expression for lowly expressed genes will allow more automated, reproducible results.[15] Tools developed in other fields can be applied; for example, in cancer, both intratumor heterogeneity and intersubject variation have long been investigated side-by-side and have proved useful in predicting disease outcomes.[16] New technologies for in situ RNA sequencing, such as MERFISH (multiplexed error-robust fluorescence in situ hybridization) and spatial transcriptomics,[4] can achieve resolution to map transcriptomic features to anatomic or morphological features. Emerging protocols using fixed cells or nuclei isolated from frozen or lightly fixed tissue open the possibility of using archival materials,[4] which can provide invaluable insights into the genetic mechanisms and translational potential.

In summary, the time is now to build a cell atlas of atherosclerosis to drive discovery through mechanism-based translation to clinical application. This scientific endeavor will revise and refine our understanding of the plaque biology, propel translational applications, and drive precision medicine and therapeutics.

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Disclosures

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