Basic Science – Cardiac Diseases, Arrhythmias

Identification of transcription factors driving fibroblast differentiation towards an inflammatory phenotype by single-nucleus multiomics

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Introduction: Atrial fibrillation (AF) is highly prevalent in the aged population, contributing to ischemic stroke and HF development. Atrial myopathy (AM), characterized by electrical and structural remodeling, including atrial enlargement, provides the substrate for AF. AM is associated with changes in cell type composition, interactions, and phenotypes. These changes in the atria and the transition to AF require reprogramming in cell transcriptomes mediated by interactions between transcription factors (TFs), cis-regulatory elements (CRE) and the epigenome. The role of this regulome in AM and the evolution to AF is yet to be established.

Aims: To investigate cellular, transcriptional, and epigenetic changes underlying the transitions between AM and AF in human.

Methods: We applied multimodal profiling (paired snRNA-seq and snATAC-seq, 10x Genomics Multiome) to atrial tissue from 42 individuals with/without AF, and snRNA-seq to an additional 18 individuals to generate a cell-type-specific map of the atrial regulome. To study AM evolution and progression to AF, 24 LA samples were grouped by AF or sinus rhythm (SR) phenotype and by left atrial volume index (LAVI: a marker of AM): AF + high LAVI, SR + high LAVI, AF + low LAVI, and SR + low LAVI.

Results: Integration of snRNA-seq and Multiome data from 60 samples (24 LA, 36 RA) yielded 25,119 and 50,535 nuclei from LA and RA, respectively (Figure 1A and 1B). AF was associated with decreased cardiomyocytes (CM) and increased fibroblasts (FB) in LA. LA appendage cell transcriptomes overlapped with the LA free wall (Human Heart Cell Atlas), confirming the relevance of this region for analysis (Figure 1C). Analysis of CM and FB identified distinct chromatin remodeling and enrichment for transcription factor motifs. An inflammatory PTGDS+ FB (PTGDS, prostaglandin D2 synthase, an enzyme linked to inflammation) state (Figure 1D) was enriched in the high LAVI group irrespective of the presence of AF. Cell-type-specific enhancer-driven regulon (eRegulon), including TFs GLIS3/TWIST2/TBX20, displayed strong enrichment at both the transcriptomic and epigenetic levels in FB. Supporting a role in AM-associated fibrosis, target genes of this eRegulon were involved in biological processes (BPs), including matrix organization and response to TGF-β. Furthermore, trajectory analysis showed that the basal FB state differentiated towards PTGDS+ FB, with the expression of TWIST2 and TBX20 increased across pseudotime.

Conclusions: Integration of snRNA-seq and snATAC-seq identifies cell-type-specific molecular alterations driving AF, highlighting regulome and transcription factor changes as key mediators of atrial remodeling during AM and AF.

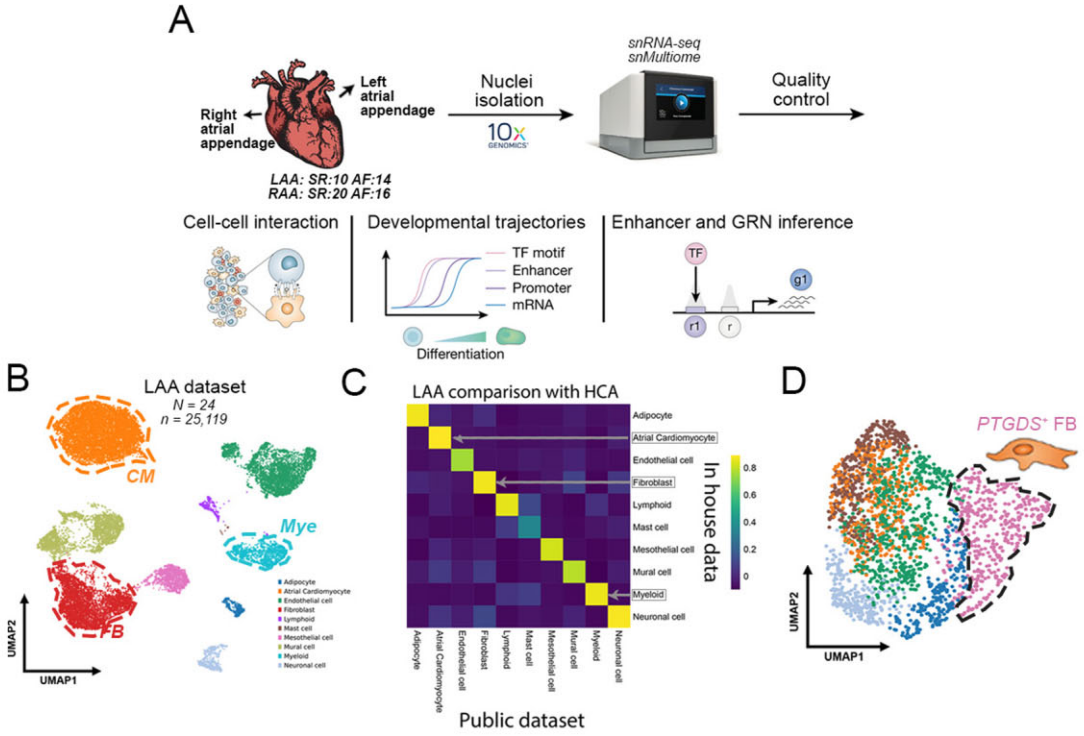

Figure 1 snMultiome (single-nucleus RNA-seq and single-nucleus ATAC-seq) for the study of transcriptomic and epigenetic remodeling in the left and right atrial appendage (LAA and RAA) in AF. (A) Schematic representation of the samples used in this study, sequencing experiments and downstream bioinformatic analysis. (**B**) UMAP visualizations where dots correspond to individual nuclei for 25,119 nuclei profiled with snRNA-seq in LAA. n = # nuclei. N = # patients. (**C**) Comparison of the generated snRNA-seq atlas to the published human heart cell atlas (HCA, left atria only) for transcriptomic profiles. (**D**) UMAP of subtypes of cardiac FB.