Zooming into Cellular and Molecular Heterogeneity of Pulmonary Hypertension

What More Single-Cell Omics Can Offer

Pulmonary hypertension (PH) is a progressive disease of the lung vasculature associated with multifactorial etiology and poor prognosis that culminates in right-sided heart failure (1). The three prominent vascular cell types that constitute the major cellular component of the pulmonary vascular wall are endothelial cells (ECs), smooth muscle cells (SMCs), and adventitial fibroblasts (2). However, the precise composition and proportion of resident vascular cells and infiltrating cell types in the healthy and remodeled pulmonary vasculature, perivascular tissue, and lung interstitium have not been assessed so far.

The advent of single-cell genomics has offered the possibility to precisely map the cellular heterogeneity, the contribution of diverse lung cell types, and the underlying molecular signatures arbitrating the vascular remodeling process in PH. In recent studies, Saygin and colleagues employed single-cell RNA sequencing (scRNA-seq) on human idiopathic pulmonary arterial hypertension (IPAH; group 1 PH) lungs (3), and Hong and colleagues employed scRNA-seq on animal models of pulmonary arterial hypertension (PAH) (4); this has altogether commenced to divulge the species-specific and animal model–specific cellular composition and cell type–specific dysregulation of genes and pathways at single-cell resolution. The majority of experimental and preclinical studies in PAH are conducted via the exposure of rodents to Sugen–hypoxia or toxic alkaloid monocrotaline (MCT), which triggers mild to severe PAH when compared with the chronic stages of pulmonary arteriopathy in adults with human PAH (5).

scRNA-seq Highlights Model-Specific Alterations in Rodent PAH

In this issue of the *Journal*, Hong and colleagues (pp. 1006–1022) have offered a lung-wide perspective of the alterations in the proportion of specific pulmonary and distinct immune cell populations and their molecular phenotypes that are common or distinctly regulated between the two widely used animal models of PAH (4). Hong and colleagues performed scRNA-seq on Sugen–hypoxia and MCT–treated rat lungs and identified 28 distinct cell types expressing recognized markers for vascular, epithelial, stromal, lymphoid, and myeloid cell populations. One of the most dominant observations in this dataset is the significant shift in the composition of immune cell populations between both experimental PAH models, specifically the proportion of lung macrophage subtypes. Significant enrichment of interstitial macrophages was found in MCT but not in Sugen–hypoxia. On the contrary, alveolar macrophages were significantly elevated in Sugen–hypoxia, which also exhibited a larger transcriptomic shift in this scRNA-seq dataset. With regards to vascular cell-specific alterations in animal models of PAH, downregulation of BMPR2 and its downstream target ID1 was noted exclusively in endothelial (EA1) subpopulation in MCT PAH but not in Sugen–hypoxia. In addition to the commonly regulated genes across both models, differential expression analysis largely emphasizes the existence of both model-specific and cell type–specific dysregulation of transcriptional signatures and associated signaling pathways between MCT or Sugen–hypoxia. Considering the inevitable requirement for these animal models toward a better understanding of the pathologic sequelae, it is imperative to dissect the proportion of pathobiological features of human PAH recapitulated by the respective animal models of PAH further via single-cell multiomics approaches.

scRNA-Seq Reveals Vascular Cell Type–Specific Perturbations in Human PAH

Saygin and colleagues (3) first reported transcriptome profiling of human lung cell populations from three IPAH and six control lung explants at single-cell resolution. The authors identified 18 distinct cell clusters from both control and IPAH lungs, including major cell types of pulmonary vasculature (ECs, lymphatic ECs, SMCs, pericytes, and fibroblasts), respiratory epithelium, and immune cell populations. Intriguingly, the EC and pericyte/SMC clusters showed the most differentially expressed genes and dysregulated pathways among all the profiled lung cell types in the human dataset. Analysis of transcriptomic signatures revealed differential genes and pathways involved in the cell cluster comprising ECs (cardiovascular system development and blood vessel development) and pericytes/SMCs (circulatory system development and extracellular matrix and structure organization). Further analysis identified SOX18 as a key transcription factor elevated in IPAH ECs that may regulate EC transcriptome. Concisely, the human scRNA-seq dataset provided the first perspective of the cell type–specific transcriptional landscape in IPAH lungs and highlighted the significant transcriptomic alterations in IPAH ECs.

Challenges

A noteworthy difference between the scRNA-seq datasets generated in human IPAH (3) and rat PAH models (4) is the variable representation of vascular and immune cell types in both datasets. One major limitation that the single-cell studies will face is the undesired variability or bias in the cell-type composition of single-cell suspensions prepared from inherently complex tissues such as lungs. These issues arise from tissue dissociation protocols that are

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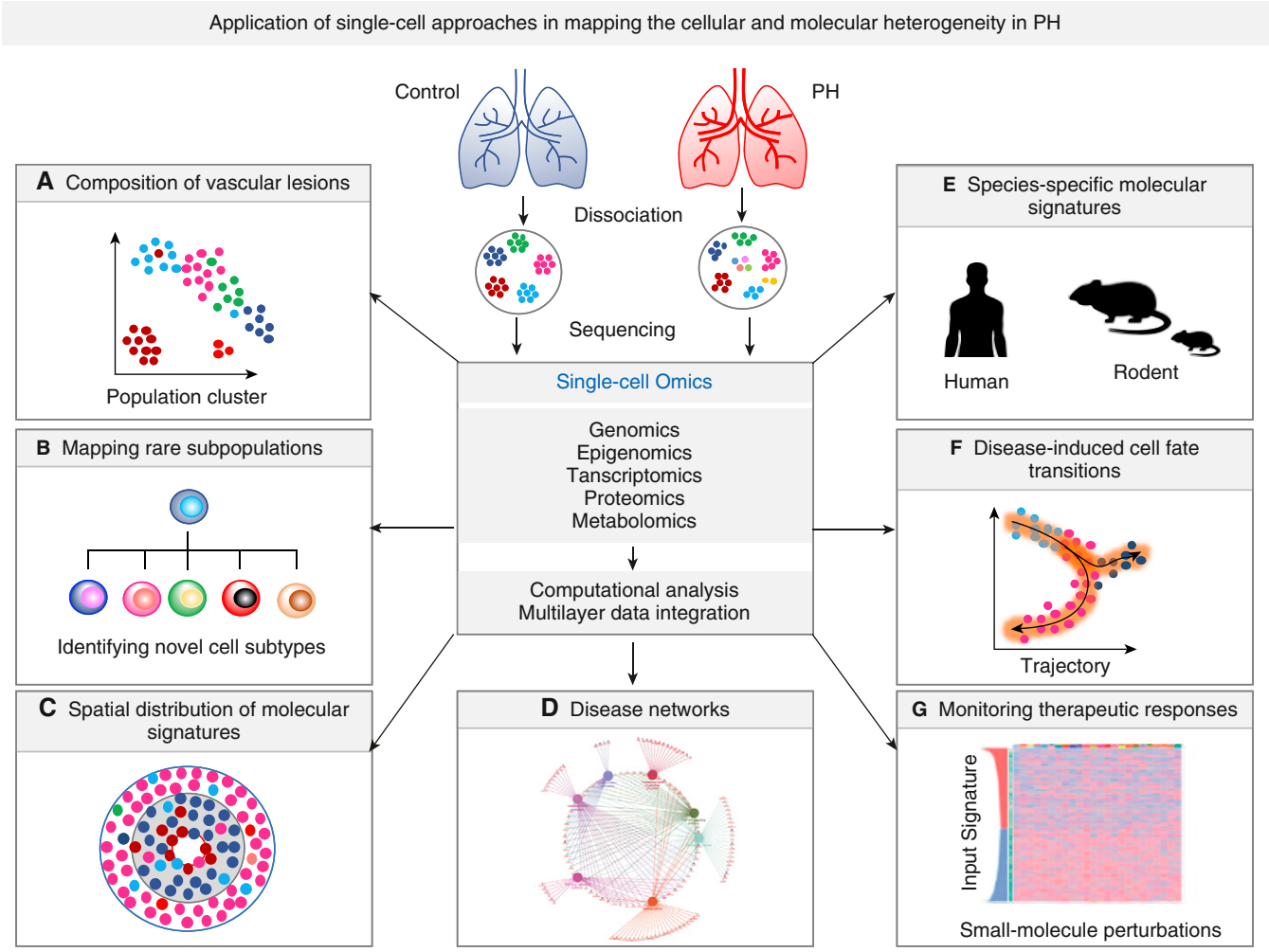

Figure 1. Unraveling the cellular heterogeneity and molecular signatures underlying pulmonary hypertension (PH) pathogenesis using single-cell approaches. (*A*) The cellular composition and molecular signatures underlying the observed phenotypic heterogeneity between normal and PH lungs can be mapped by employing single-cell approaches to assay the DNA, RNA, protein, and chromatin states at single-cell resolution. (*B*) Using the molecular signatures and new surface markers identified by single-cell profiling approaches, the differentially represented cellular subpopulations in PH can be further isolated by fluorescence-activated cell sorting and subjected to further validation of subpopulation-specific molecular signatures using custom gene expression panels. (*C*) Spatial distribution of the molecular signatures of specific subpopulations at the tissue scale can be achieved using spatial transcriptomics or fluorescent *in situ* RNA sequencing. (*D*) Integromic analysis of the data generated by multiomics approaches (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) will enable us to highlight the cell-specific molecular networks underlying the phenotypic transitions during the vascular remodeling process. (*E*) The data generated by multiomics approaches in different subgroups of human PH can be further compared with rodent datasets to reveal the species-specific molecular signatures that possess the translational value for prospective preclinical studies. (*F*) The disease-induced phenotypic state transition and the overall phenotypic plasticity of vascular cell types during subsequent stages of vascular remodeling process can be mapped by coupling *in vivo* transgenic strategies for lineage tracing and single-cell techniques in animal models of PH. (*G*) The disease-specific molecular signatures of PH-associated subpopulations (vascular or pulmonary or immune cells) can be further compared with the pharmacological perturbation signatures from the Connectivity Map databases to predict potential candidate drugs targeting the therapeutically viable PH-specific cellular subpopulations *in vivo*.

suboptimal for particular cell types, which can significantly confound the analyses and the subsequent interpretation of these valuable datasets. Prominently, there is also a huge concern regarding the reliability of molecular markers assigned to annotate and identify cell types in single-cell studies, which needs to be harmonized promptly. The next major constraint for conducting omics investigation in the human setting is the limited availability of characterized lung explants from patients undergoing transplantation for PAH, even if they represent a late stage of the disease.

Future Perspectives

Overall, the first single-cell transcriptomic datasets generated in both human (3) and animal models (4) have set a benchmark for future studies that further persuades to investigate and comprehend the species-specific and model-specific variability in the cellular composition of the lungs and right ventricle in PH (6). Apart from the disease-induced variability in the cellular composition of PH lungs (Figure 1A), the molecular signatures underlying the observed phenotypic heterogeneity can be mapped by integrating the datasets

generated by single-cell approaches that assay regulatory modifications in DNA, chromatin states (7), quantitative alterations in RNA, and protein at a single-cell resolution. Analysis of cell-specific molecular signatures can reveal potential cellular markers associated with the differentially represented subpopulations in PH lungs (Figure 1B). Spatial profiling methods, such as fluorescent *in situ* RNA sequencing (8), barcode *in situ* targeted sequencing (9), spatial transcriptomics (10), or multiplex immunofluorescence stainings (11), can be employed to comprehend the spatial distribution of cellular subpopulations in the development of angioobliterative vascular lesions. By integrating the datasets generated by multiomics single-cell approaches in rodent PH and different subgroups of human PH, integromic analysis (12) will aid us in unraveling the common, species-specific, cell-specific molecular networks and signaling hubs that possess the translational value for prospective preclinical studies (Figures 1D and 1E). In particular, transdifferentiation pathways and the plasticity of vascular cell types associated with the vascular remodeling process can be mapped by coupling *in vivo* single-cell techniques with transgenic strategies for lineage tracing (Figure 1F). Recent evidence has uncovered the presence of pharmacologically modulatable signaling hubs, transcriptional regulators, and epigenetic enzymes that are specifically dysregulated in PH vascular cells (13, 14). Furthermore, the PH-specific molecular signatures can be compared with the pharmacological perturbation signatures (15) to predict potential candidates for drug repositioning (Figure 1G) and to design therapeutic approaches targeting disease-specific subpopulations in PH. ■

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Prakash Chelladurai, Ph.D.
Max Planck Institute for Heart and Lung Research
German Center for Lung Research (DZL)
Cardio-Pulmonary Institute (CPI)
Bad Nauheim, Germany

Rajkumar Savai, P.D.
Max Planck Institute for Heart and Lung Research
German Center for Lung Research (DZL)
Cardio-Pulmonary Institute (CPI)
Bad Nauheim, Germany
and
Institute for Lung Health (ILH)
Justus Liebig University
Giessen, Germany

Soni Savai Pullamsetti, Ph.D.
Max Planck Institute for Heart and Lung Research
German Center for Lung Research (DZL)
Cardio-Pulmonary Institute (CPI)
Bad Nauheim, Germany
and
Department of Internal Medicine
German Center for Lung Research (DZL)
Cardio-Pulmonary Institute (CPI)
Justus Liebig University
Giessen, Germany

ORCID IDs: 0000-0002-8258-5115 (P.C.); 0000-0003-1538-2091 (R.S.); 0000-0003-0440-8831 (S.S.P.).

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