Chapter 3
Single-Cell Approaches in Pulmonary Disease

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ABSTRACT

Pulmonary disorders asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and infections are also characterized by intricate cellular heterogeneity and dynamic microenvironments. Traditional bulk profiling important cellular variations that underlie disease initiation, progression, and therapeutic responses. Recent technical advances in single-cell methodologies, including single-cell RNA sequencing (scRNA-seq), mass cytometry, and spatial transcriptomics, have revolutionized the field of pulmonary biology by allowing the detection of low-abundance cell subsets, new biomarkers, and cell-to-cell interactions in lungs. These methods have uncovered immune epithelial crosstalk, cellular plasticity, and as-yet-unrecognized pathogenic subsets that mediate inflammation and tissue remodeling. In single-cell atlases of healthy and diseased lungs revealed unprecedented molecular signatures, opening

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the door to precision medicine in respiratory health. In this chapter, principles, applications, and future directions of single-cell strategies in pulmonary disease are discussed.

INTRODUCTION

Chronic lung diseases like asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, lung cancer, and other respiratory infections continue to be some of the most important causes of morbidity and mortality globally, an indication of lung biology's intricacy and susceptibility to environmental and genetic challenges, (Joshi, 2024). The lung is a structurally complex organ with epithelial, endothelial, stromal, and immune cell phenotypes, which dynamically interact to sustain respiratory homeostasis. Disease states typically occur not from homogeneous dysfunction in all cells but from heterogeneous and subtle changes within subsets of cells, as well as the derangement of intercellular communication networks, (Voicu et al., 2025). For decades, pulmonary research has disproportionately depended on bulk analysis tools such as transcriptomics, proteomics, and flow cytometry, which, while very useful in advancing our understanding, have underlying limitations. Bulk methods treat large groups of cells, generating mean molecular profiles that bury sparse but possibly important populations, hide dynamic changes, and destroy spatial data, (Xu et al., 2025). Thus, homology-between-sample downstream analyses may miss progenitor or stem-like cells governing regeneration, immune subsets governing inflammation, or specialized fibroblasts governing fibrosis. In addition, bulk analysis tends to produce erroneous impressions by implying that there are uniform changes in gene expression when only discrete cell subsets contribute to pathology, such as in fibrosis, where one subset of fibroblasts contributes to excess extracellular matrix deposition.

The absence of spatial resolution also provides insight into diseases in which pathological characteristics are greatly localized, such as airway remodeling in asthma, localized fibrotic foci in idiopathic pulmonary fibrosis, or tumor niches in lung cancer, (Savin, Zenkova, & Sen'kova, 2023). The constraints of bulk profiling have slowed the advancement toward elucidating the cellular foundation of pulmonary pathophysiology and defining specific therapeutic targets. The emergence of single-cell technologies over the past decade has revolutionized the field by enabling the high-resolution analysis of individual cells and their molecular programs within the lung microenvironment. Among these, single-cell RNA sequencing (scRNA-seq) has been transformative, allowing unbiased transcriptomic profiling of thousands of individual cells simultaneously and revealing rare populations, cellular states, and novel transcriptional signatures, (Wang et al., 2023). Complementary technologies,