ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

REVIEW ARTICLE

Single-cell sequencing for lung cancer research: Progress and prospects

Zhongsha Li[1,2,3,4†], Wei Su[1,2,3,4†], Bingbing Bai[1,2,3,4†], Yuesong Wu[1,2,3,4], Baoyin Zhang[1,2,3,4], Dejuan Xiang[1,2,3,4], Gilwa Borjigin[1,2,3,4], Jinhai Deng[1,2,3,4,5], Jian Sun[1,2,3,4†*], and Mingzhu Yin[1,2,3,4†*]

[1]Clinical Research Center, Medical Pathology Center, Cancer Early Detection and Treatment Center and Translational Medicine Research Center, Chongqing University Three Gorges Hospital, Chongqing University, Wanzhou District, Chongqing, China

[2]Chongqing Technical Innovation Center for Quality Evaluation and Identification of Authentic Medicinal Herbs, Wanzhou District, Chongqing, China

[3]Chongqing University Three Gorges Hospital and Academy for Advanced Interdisciplinary Technology, CQU-Ferenc Krausz Nobel Laureate Scientific Workstation, Chongqing, China

[4]School of Medicine Chongqing University, Chongqing University, Shapingba District, Chongqing, China

[5]Richard Dimbleby Department of Cancer Research, Comprehensive Cancer Centre, Kings College London, London, United Kingdom

[†]*These authors contributed equally to this work.*

***Corresponding authors:**
Mingzhu Yin
(yinmingzhu@cqu.edu.cn)
Jian Sun
(sunjian@cqu.edu.cn)

Citation: Li Z, Su W, Bai B, *et al.* Single-cell sequencing for lung cancer research: Progress and prospects. *Eurasian J Med Oncol.* 2025;9(2):3-16.
doi: 10.36922/ejmo.6883

Received: December 3, 2024

1st revised: December 31, 2024

2nd revised: February 12, 2025

Accepted: February 27, 2025

Published online: March 12, 2025

Copyright: © 2025 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Lung cancer exhibits high morbidity and mortality rates, characterized by a heterogeneous genetic landscape and an immunosuppressive tumor microenvironment, despite recent therapeutic advances. High-resolution single-cell sequencing has facilitated a comprehensive analysis of malignant and host cell types, enhancing the understanding of lung cancer's heterogeneity and adaptability under basal conditions and in response to therapeutic intervention. In this review, we provide an overview of the utilization of single-cell sequencing technology in characterizing the microenvironment of lung cancer, facilitating early diagnosis, monitoring tumor progression, and elucidating mechanisms of drug resistance, thereby offering new insights into potential therapeutic interventions.

Keywords: Single-cell sequencing; Lung cancer; Heterogeneity; Tumor microenvironment; Drug resistance; Circulating tumor cells

1. Introduction

The high incidence and metastasis rates of lung cancer continue to pose significant health challenges and are the leading cause of cancer-related deaths globally, with an estimated 2 million new cases and 1.76 million deaths/year.[1] The poor prognosis of lung cancer is largely attributed to clinical factors, including late-stage diagnosis, high rate of metastasis, frequent recurrence, and the development of secondary drug resistance.[2-4] In the past, the management of advanced lung cancer primarily relied on cytotoxic chemotherapy.[5] However, in recent decades, significant advancements in therapeutic strategies have emerged, driven by the discovery of oncogenic driver mutations in

lung cancer.[6] As targeted therapy and immunotherapy have emerged as viable options, there has been improved progression-free survival (PFS) and overall survival (OS) in selected patients.[7] Despite advances in treatment, the late-stage diagnosis, high aggressiveness, frequent metastasis, and drug resistance continue to result in poor prognosis of lung cancer.[8]

Cancers are composed of complex multicellular structures that include not only malignant cancer cells but also various epithelial, immune, and stromal cells. This complex and heterogeneous system is commonly referred to as the tumor microenvironment (TME).[9,10] The severity of cancer largely depends on factors such as the regulation of immune responses within the TME, the remodeling of the extracellular matrix, and the process of neovascularization. Traditional bulk sequencing methods typically rely on analyzing the average gene expression levels within large cell populations, which can mask cellular heterogeneity.[10] In contrast, single-cell sequencing enables the direct analysis of gene expression at the individual cell level, facilitating the identification of distinct cellular subpopulations.[10] Single-cell sequencing technologies have revolutionized lung cancer research, offering new hope for more precise and effective treatments.[11] This review highlights the transformative impact of single-cell technologies in advancing lung cancer research and therapy.

2. Advances of single-cell sequencing technologies

Single-cell sequencing is a high-resolution, high-throughput technology that analyzes genetic material while eliminating batch effects and technical noise, enabling accurate characterization of cellular heterogeneity. Furthermore, there are low expression abundance genes that play an important role in tumor progression but are often overlooked by traditional sequencing technologies. Single-cell sequencing can detect these genes, which help guide personalized therapy for cancer patients in clinical practice, as well as for research into the mechanisms of disease onset. This technology has advanced rapidly in recent years, with notable milestones including the first single-cell transcriptomic analysis in 2009, the initial DNA sequencing of individual human cancer cells in 2011, and the first single-cell exome sequencing experiment in 2012.[12-14]

The standard workflow for single-cell sequencing comprises tissue preparation, single-cell isolation, library preparation, sequencing, and data analysis.[15] Among these steps, single-cell isolation is a critical step, as it determines the accuracy and amount of the amplified material. It can be achieved using methods such as limiting dilution, micromanipulation, laser capture microdissection (LCM), fluorescence-activated cell sorting (FACS), and microfluidic cell sorting. Capturing and isolating cells are the first and most critical step in single-cell sequencing. During this process, it is essential to maintain cell integrity to prevent cell loss or rupture, as these events can result in nucleic acid degradation, ultimately compromising the quality and accuracy of downstream genomic, transcriptomic, or epigenomic analyses. Limiting dilution involves the serial dilution of a cell suspension to progressively reduce its concentration, aiming to achieve single-cell capture in each well of a multiwell plate or sample chamber. This method is simple and cost-effective; however, it is characterized by low throughput and a significant risk of rare cell depletion, which restricts its utility in clinical and high-throughput research. Micromanipulation uses a micropipette under microscopic guidance to aspirate single cells. It is low-throughput, labor-intensive, and suited for isolating small cell numbers. Similarly, LCM uses a focused laser beam under a microscope to melt a thermoplastic ethylene-vinyl acetate membrane, creating localized adhesion to capture target cells, and while LCM offers exceptional spatial resolution that allows for the precise identification and isolation of specific cell populations within complex environments. However, like micromanipulation, it remains inefficient and time-consuming. FACS primarily involves labeling the surface or interior of cells with specific fluorescent dyes, and a flow cytometer emits a laser beam to excite these fluorescent labels, enabling the separation of different cell types based on their distinct fluorescence signals. Moreover, FACS can sort target cells with very low levels of marker expression and rapidly process and isolate large numbers of single cells, making it an ideal method for preparing large-scale single-cell sequencing samples. On the other hand, microfluidic cell sorting technology offers several advantages, including high throughput, high efficiency, and low reagent costs. However, it requires a high degree of cell size uniformity, and its associated equipment and consumables are relatively expensive, making it particularly well-suited for applications demanding high-precision and high-throughput single-cell analysis.

Single-cell sequencing encompasses a wide range of analyses, including single-cell genome sequencing, which investigates genetic variations and mutations; single-cell transcriptome sequencing, which examines RNA expression profiles; single-cell epigenome sequencing, which explores DNA modifications and chromatin accessibility; and single-cell multi-omics sequencing, which integrates multi-omics data across molecular layers. Other single-cell sequencing techniques also contribute to advancing research, as shown in Figure 1.

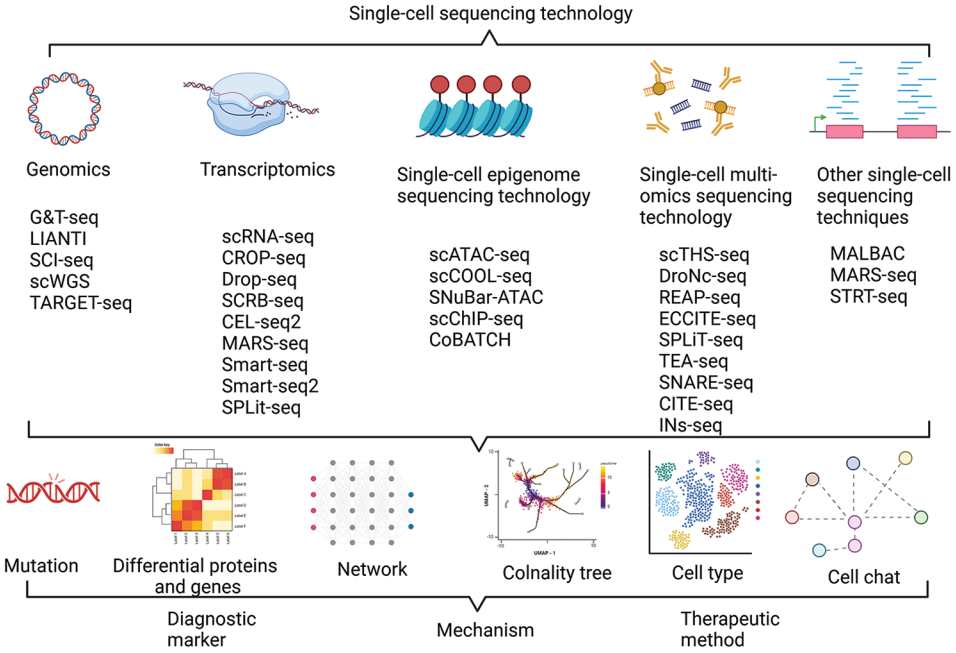


Figure 1. Overview of single-cell sequencing technologies. Figure created using Biorender.com

Abbreviations: G&T-seq: Genome and transcriptome sequencing; LIANTI: Linear amplification via transposon insertion; SCI-seq: Single-cell combinatorial indexing RNA sequencing; scWGS: Single-cell whole-genome sequencing; TARGET-seq: Targeted genomics and transcriptomics sequencing; scRNA-seq: Single-cell RNA sequencing; CROP-seq: CRISPR droplet sequencing; Drop-seq: Droplet sequencing; SCRB-seq: Single-cell RNA barcoding and sequencing; CEL-seq: Cell expression by linear amplification and sequencing; SMART-seq: Switching mechanism at the 5' end of RNA template sequencing; SPLit-seq: Split-pool ligation-based transcriptome sequencing; scATAC-seq: Single-cell assay for transposase-accessible chromatin sequencing; scCOOL-seq: Single-cell chromatin overall omic-scale landscape sequencing; SNuBar: Single-nucleus barcode-based; scChIP-seq: Single-cell chromatin immunoprecipitation sequencing; CoBATCH: Combinatorial barcoding and targeted chromatin release; scTHS-seq: Single-cell transposome hypersensitive site sequencing; DroNc-seq: Droplet-based nuclear single-cell RNA sequencing; REAP-seq: RNA expression and protein sequencing; ECCITE-seq: Expanded CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing; SNARE-seq: Single-nucleus chromatin accessibility and mRNA expression sequencing; CITE-seq: Cellular indexing or transcriptomes and epitopes by sequencing; Ins-seq: Intracellular staining and sequencing; MALBAC: Multiple annealing and looping-based amplification cycles; MARS-seq: Massively parallel RNA single-cell sequencing; STRT-seq: Single-cell tagged reverse transcription sequencing

DNA-based single-cell sequencing methods include whole-genome sequencing (WGS) and whole-exome sequencing (WES).[16,17] WGS entails fragmenting the entire genome and sequencing it without targeting specific DNA regions. Single-cell WGS can distinguish the unique genetic characteristics in different cell clones and allows researchers to track genomic changes over time. In addition, it facilitates the quantification of copy number variations (CNVs) and the characterization of structural variations (SVs) at the single-cell level.[18] However, WGS remains expensive due to its high resource and technology demands, and it struggles to detect low-frequency variants, which are rare mutations present in a small portion of the sample. In contrast, WES targets protein-coding regions, which harbor most disease-related variants. While WES is more cost-effective than WGS, it suffers from uneven coverage, often leaving certain genomic areas underrepresented.

Single-cell transcriptome sequencing is the most widely used approach in single-cell sequencing. scRNA-seq quantifies gene expression at the single-cell level without targeting specific transcripts. It captures RNA, converts it into cDNA, and employs high-throughput sequencing to generate transcriptomic profiles for individual cells.[19] RNA-based single-cell sequencing includes full-length transcript sequencing approaches (e.g., scNanoHi-C,[20] Quartz-Seq,[21] Smart-Seq,[22] and Smart-Seq2[23]), 3'/5'-end transcript sequencing technologies (e.g., CEL-Seq,[24] MARS-Seq,[25] Drop-Seq,[26] and InDrop[27]), and spatial RNA sequencing derived from single-cell RNA sequencing[27] (scRNA-seq). Among available platforms, droplet-based 10× Genomics Chromium and plate-based SMART-Seq are widely used. The 10× Genomics Chromium platform, which utilizes microfluidic droplet technology, is popular due to its high throughput and commercial availability. It encapsulates individual cells in Gel Bead-in-Emulsions, each containing

a uniquely barcoded gel bead. After cell lysis, mRNA binds to oligo(dT) primers and undergoes reverse transcription (RT), incorporating cell barcodes and unique molecular identifiers (UMIs). The reaction droplets are then broken, and the cDNA is polymerase chain reaction (PCR)-amplified into sequencing-ready libraries. Using Illumina sequencing technology, the system captures barcodes, UMIs, and mRNA sequences. Bioinformatics tools process the data for single-cell transcriptomic profiling and gene expression analyses. scRNA-seq offers the analysis of both coding and non-coding transcripts, allowing for the discovery of novel gene isoforms, alternative splicing events, and non-coding genes that may contribute to oncogenesis.[28,29]

With a deeper understanding of gene regulatory mechanisms, epigenetic modifications have been recognized as key contributors. These modifications include DNA base modifications and chromatin structural changes. At present, a wide range of sequencing technologies can measure chromatin SVs in epigenetics, from local to genome-wide levels. For example, a single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) identifies open chromatin regions at the single-cell level using Tn5 transposase, which inserts sequencing adapters into accessible chromatin.[30] The development of bulk ATAC-seq has resulted in three distinct scATAC-seq strategies, including microfluidics-based methods, split-and-pool combinatorial indexing, and droplet-based procedures. Single-cell isolation is achieved through microfluidics or droplet sorting, with barcoding used to distinguish DNA fragments. After amplification, library construction, and high-throughput sequencing, sequencing reads are mapped to a reference genome to identify chromatin accessibility, classify cell types, and infer gene regulatory networks. scATAC-seq is widely applied to fresh, frozen, and fixed tissues, providing valuable insights into chromatin dynamics, cellular heterogeneity, and gene regulation.[31] Single-cell epigenomic methods offer several significant advantages in studying complex biological systems. First, they enable the analysis of the epigenome in rare or difficult-to-isolate cell populations, which are often inaccessible to traditional bulk analysis techniques. Second, these methods facilitate the characterization of heterogeneous cell populations by allowing for the classification of individual cells into known types or the discovery of novel subpopulations with distinct epigenomic profiles. This approach enhances our understanding of cellular diversity and function within tissues. Finally, single-cell epigenomics provides insights into the long-range correlations and dynamics of epigenetic modifications across cell populations. By analyzing these dynamics, researchers can gain a mechanistic understanding of how epigenetic markers are established, maintained, and interpreted across different cellular contexts.[32]

The advent of single-cell multi-omics approaches has significantly enhanced our understanding of cancer biology, including diseases like lung cancer. These approaches integrate different molecular layers, such as the genome and transcriptome, transcriptome and epigenome, and transcriptome and proteome, among others. In single-cell genomics-plus-transcriptomics multi-omics technologies, genome and transcriptome sequencing (G&T-seq) enables the parallel analysis of mRNA and genomic DNA (gDNA) from the same single cell.[33] After cell lysis, polyadenylated mRNA is selectively captured using magnetic beads coated with oligo(dT) primers, effectively separating it from nuclear and mitochondrial DNA. The mRNA is then reverse transcribed using a Smart-seq2-based protocol, incorporating template switching for full-length cDNA synthesis and PCR amplification. This approach supports both short-read and long-read sequencing, with the latter being particularly useful for transcript isoform detection. Meanwhile, gDNA is amplified using either multiple displacement amplification, which provides uniform coverage with minimal bias, or displacement preamplification followed by PCR, which enhances genome-wide coverage for downstream sequencing.[34] By integrating transcriptomic and genomic data from the same cell, G&T-seq facilitates the study of genotype-phenotype relationships, cellular heterogeneity, and clonal evolution. Other single-cell genomics-plus-transcriptomics multi-omics technologies include direct RNA and DNA sequencing (DR-seq),[35] targeted genomics and transcriptomics sequencing (TARGET-seq),[36] and single-cell one-tube sequencing (scONE-seq).[37] These technologies are particularly valuable in cancer research, helping to uncover genomic instability and transcriptional dysregulation in diseases such as lung cancer.

According to the central dogma, the main steps of gene expression include transcription and protein synthesis. However, gene expression also involves complex post-transcriptional and translational regulatory mechanisms. Proteomics focuses on studying various properties of proteins, including their interactions, post-translational modifications, expression, and regulation. It examines protein changes within an organism in a holistic manner to explain individual physiological and metabolic activities. The most significant distinction between proteomics and traditional protein studies is that proteomics does not focus on studying one or a few proteins in isolation. Instead, it systematically investigates the overall response of proteins, their expression, and interactions under different

environmental conditions using correlative approaches. Single-cell proteomics refers to techniques capable of measuring protein expression levels within individual cells. Furthermore, integrating transcriptomics and proteomics provides deeper insights into gene expression and regulatory mechanisms.

Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) is a high-throughput, droplet microfluidics-based single-cell technology that integrates transcriptomic and proteomic data by simultaneously measuring mRNA and proteins. This method uses antibodies labeled with oligonucleotide barcodes to analyze proteins while capturing mRNA for sequencing. Antibody-derived DNA tags (ADTs) contain PCR handles, antibody barcodes, and poly(A) tails, allowing antibodies to bind specific target proteins on the cell surface. After cell sorting and lysis within droplets, the internal contents, including mRNA and surface proteins, are released. mRNA is captured using Drop-seq beads, which are beads coated with oligo(dT) primers that specifically bind to the poly(A) tails of mRNA. Simultaneously, antibody-derived oligonucleotides (bound to surface proteins) are also captured by these beads. A sequencing library is generated using cDNA molecules and PCR amplification, enabling the simultaneous quantification of mRNA and proteins through sequencing.[38] Multi-omics approaches allow the tracing of tumor cell clonal evolution, revealing epigenomic differences between primary and metastatic tumors. They also facilitate the study of key regulatory elements in the tumor immune microenvironment and uncover the epigenetic mechanisms of immune escape.

In addition, spatial genomics is rapidly emerging, as disease development involves dynamic changes in cells within specific spatial locations. Spatial omics enable the study of cellular interactions with surrounding cells and extracellular matrices. This allows for the identification of different cellular subpopulations and gene expression states in distinct tissue regions. By providing spatial dimensional information, it deepens the understanding of these processes and their underlying mechanisms. Spatial multi-omics technologies are advancing rapidly in single-cell sequencing, integrating molecular data with spatial information to analyze gene expression patterns and chromatin accessibility in tissue contexts. These technologies allow researchers to study not only gene expression but also cell–cell interactions and networks within their native tissue environments. Some prominent methods include array-based spatial transcriptomics, microfluidic deterministic barcoding strategies, DNA antibody tags, multiplex single-molecule fluorescent *in situ* hybridization, *in situ* sequencing, and mass spectrometry imaging.[34] For example, spatial ATAC–RNA-seq combines ATAC-seq, which studies chromatin accessibility, with RNA-seq, which captures gene expression, within the same tissue section while preserving spatial information. This method tags accessible chromatin regions and captures RNA, followed by spatial barcoding to map gene activity and chromatin states across tissue regions. This approach offers a comprehensive view of tissue-specific gene regulation and complex biological processes. Notably, *Nature* listed spatial multi-omics technologies as one of the seven technologies to watch in 2022, providing their invaluable insights into tissue organization and intercellular communication.[39]

3. Single-cell sequencing in lung cancer research

The TME is a highly intricate and dynamic network made up of tumor cells, immune cells, stromal cells, extracellular matrix elements, and a variety of signaling molecules.[40] It plays a crucial role in regulating tumor progression and response to therapy. Single-cell sequencing reveals cell types, phenotypic changes, and gene expression in lung cancer.[15,41]

3.1. Intratumor heterogeneity in lung cancer

Intratumor heterogeneity is mainly associated with clonal evolutionary trajectory, which is the main cause of drug resistance, metastatic progression, and adverse clinical outcomes.[42] scRNA-seq has become a pivotal technique in elucidating the intratumor heterogeneity (ITH) in lung cancer.[43] Tian *et al.*[44] analyzed approximately 5,000 single cells from primary tumors and adjacent normal tissues of 11 small cell lung cancer (SCLC) patients, discovering heterogeneity in cell cycle, immune response, hypoxia, epithelial-mesenchymal transition (EMT), and key transcription factors (TCFs) such as achaete-scute family BHLH TCF1 and neurogenic differentiation 1. Li *et al.*[43] used scRNA-seq to identify mixed-lineage tumor cells in non-SCLC, which co-expressed classical marker genes associated with adenocarcinoma (ADC), squamous cell carcinoma, and neuroendocrine tumor, demonstrating high cellular plasticity. Patients with high mixed-lineage features had worse prognoses, and aldo-keto reductase family 1 member B (AKR1B1) was identified as a potential therapeutic target. Chan *et al.*[45] found a highly heterogeneous SCLC population, including a recurrent phospholipase C gamma 2 (PLCG2)-high subpopulation with stem-like, pro-metastatic traits linked to poor prognosis, emphasizing the need for targeted therapies.

3.2. Epithelial cells

Epithelial cells, the origin of malignant cells, show a complex heterogeneity and plasticity in lung cancer.[46]

scRNA-seq has enabled a detailed analysis of these cells and their roles within the TME. In a study, it was observed that combined therapy resulted in a notable increase in normal lung epithelial cells, alongside the detection of residual cancer cells, even in patients who had achieved a complete pathological response. The epithelial cells were re-clustered into multiple populations, separating malignant cells from normal cells using the CopyKAT algorithm based on CNVs.[47] Lu *et al.*[48] found that S100P$^+$ epithelial cells are enriched in advanced lung cancer and correlate with poor prognosis. These cells exhibited higher CNV scores in advanced lung cancer and were shown to regulate cell migration, apoptosis, and autophagy via the nuclear factor kappa-B (NF-κB) and Wnt signaling pathways. These changes in biological pathways may provide new strategies for lung cancer treatment. Xu *et al.*[49] discovered that KRT81$^+$ tumor cells, a subpopulation of epithelial cells, are associated with adverse prognosis in lung adenocarcinoma (LUAD). These cells exhibited high activity in EMT and hypoxia pathways, indicating their high metastatic potential.[49] Li *et al.*[50] identified distinct cellular compositions in LUAD and lung squamous carcinoma (LUSC), with alveolar type 2 cells driving heterogeneity in LUAD and basal cells playing a similar role in LUSC.

3.3. Cancer-associated fibroblasts (CAFs)

CAFs are a heterogeneous population of activated fibroblasts within the TME, driving tumor metastasis, immune evasion, and treatment resistance.[51] scRNA-seq has identified three major fibroblast subpopulations in non-SCLC: adventitial fibroblasts, alveolar fibroblasts, and myofibroblasts. Alveolar and adventitial fibroblasts were localized to specific regions in normal lung tissue and were associated with higher OS rates in LUAD. In contrast, myofibroblasts were linked to poorer survival and were associated with factors such as loss of epithelial differentiation and TP53 mutations.[52,53] Myofibroblasts play a crucial role in tumor development and metastasis. scRNA-seq analysis revealed a fibroblast-to-myofibroblast transition in LUAD, with a significant increase in myofibroblast clusters within tumor tissue. These cells exhibited high expression of collagen genes (*COL3A1*, *COL5A1*, *COL5A2*, and *COL6A3*). In addition, these cells exhibited high activity in TGFβ and JAK/STAT signaling pathways, which are hallmark features of cancer-associated myofibroblasts.[54]

3.4. Immune cells

Immune cells play a crucial role in tumor progression and therapy response. scRNA-seq enables researchers to identify various immune cell types and study the mechanisms of immunosuppression, providing insights into cancer immune heterogeneity and diversity.[19] The immune atlas outlines an expanding range of immune cells, including lymphocytes, monocytes, macrophages, natural killer cells, and dendritic cells (DCs). For instance, stromal and immune cells undergo dynamic developmental and functional alterations, fostering a pro-tumor and immunosuppressive environment. This intricate crosstalk ultimately contributes to tumor growth and resistance to therapy.[55]

T cells are central to cancer immunotherapy. Joy A. Pai[56] revealed that in NSCLC patients, regulatory CD4$^+$ T cells (Treg), follicular helper CD4$^+$ T cells (TFH), and CD8$^+$ T cell subsets progressively exhaust as they approach the tumor. Wu *et al.*,[57] through scRNA-seq analysis of 42 tissue biopsies from stage III/IV NSCLC patients, mapped the cell type-specific transcriptome landscape of advanced NSCLCs, identifying rare immune cell types like follicular DCs and T helper 17 (Th17) cells.[57]

Current research indicates that tumor-associated macrophages are related to tumor metastasis, invasion, angiogenesis, and immunosuppression.[58] Guo *et al.*[59] found immune heterogeneity in macrophage (Mϕ) subtypes in NSCLC. The main Mϕ subtypes were fatty acid binding protein 4 (FABP4)-Mϕ in LUAD and secreted phosphoprotein-1 (SPP1)-Mϕ in LUSC. Tumor-infiltrating CD8$^+$ T cells undergo depletion; a high ratio of depleted to exhausted T cells is associated with a better prognosis in LUAD, and genetic markers of active tumor Tregs correlate with a poor prognosis in LUAD. Yu *et al.*[16] found that as NSCLC progressed, macrophage infiltration decreased, and macrophage function was altered. Low macrophage infiltration was found to be associated with low patient survival based on survival analysis. Tissue inhibitor of metalloproteinases 1 (TIMP1) has been reported to regulate metastatic foci metabolism by activating the PI3K/Akt pathway, and it has been demonstrated to be a potential biomarker for the pathogenesis of LUAD through protein fluorescence immunostaining experiments.[60] SPP1 is critical in driving macrophage polarization and facilitating lung cancer escape, highlighting its potential as a therapeutic target for LUAD.[61]

4. Prospective clinical applications of single-cell sequencing in lung cancer

With the widespread application of single-cell sequencing technology in lung cancer research, its potential for clinical translation has become more evident. Researchers are exploring its clinical integration to address medical challenges and support lung cancer treatment. Single-cell sequencing is being explored for its potential in early diagnosis, drug resistance monitoring, and personalized treatment of lung cancer.

4.1. Single-cell sequencing in the discovery of lung cancer biomarkers

Metastasis is the leading cause of death in lung cancer. Circulating tumor cells (CTCs) shed into the bloodstream contribute to metastasis. While most CTCs are eliminated by immune cells, some evade immune detection, survive, and form metastatic foci, increasing patient mortality. In 2007, CTCs were recognized as a tumor marker by the American Society of Clinical Oncology. They play a significant role in early diagnosis of tumors and are valuable for assessing treatment efficacy and prognosis. The examination of CTCs through innovative single-cell methodologies allows for the continuous observation of tumors and facilitates a thorough comprehension of tumor heterogeneity and the advancement of disease, thereby furnishing significant knowledge for early detection and tailored treatment.[19,62,63]

Single CTC-based sequencing is a promising minimally invasive method for early cancer diagnosis. Xu *et al.*[64] performed WES on a single CTC from an early-stage lung cancer patient and identified six novel gene mutations [human immunodeficiency virus type 1 enhancer binding protein 2 (*HIVEP2*), spermatogenesis associated 21 A (*SPATA21A*), tubulin gamma complex component 2 (*TUBGCP2*), potassium voltage-gated channel modifier subfamily G member 1 (*KCNG1*), microRNA 4756 (*MIR4756*), and acetylserotonin O-methyltransferase like (*ASMTL*)], which reveal genetic information about tumors at the single-cell level. Su *et al.*[65] investigated genomic alterations during chemotherapy by performing single-cell sequencing on CTCs from 48 SCLC patients. They found that most genetic mutations present in tumor tissues were precisely identified in CTCs, demonstrating considerable DNA heterogeneity. This indicates that single-cell genomic analysis of CTCs is a valuable method for tracking genetic variations and monitoring disease progression in SCLC. Chang *et al.*[66] found that CTCs in NSCLC patients receiving platinum-based chemotherapy carried cancer-driver mutations, including epidermal growth factor receptor (EGFR) and TP53, which were shared with primary and/or progressive tumors. CTCs also exhibited mutations in genes related to cell cycle regulation and stem cell traits, such as SH3KBP1-binding protein 1 (*SHKBP1*), nuclear mitotic apparatus protein 1 (*NUMA1*), zinc finger protein 143 (*ZNF143*), mucin-16 (*MUC16*), origin recognition complex subunit 1 (*ORC1*), paraoxonase 1 (*PON1*), and proline, glutamic acid, and leucine-rich protein 1 (*PELP1*). These mutations were linked to drug resistance and metastasis in NSCLC.

Transcriptomic analysis of individual CTCs using RNA sequencing is a promising technology for elucidating the molecular mechanisms involved in metastasis,[67] although research on lung cancer remains limited. In the analysis of CTCs obtained from NSCLC patients, single-cell 10× Chromium successfully identified diverse CTC phenotypes. The epithelial-like phenotype exhibited the expression of epithelial markers, along with markers of proliferation (Ki67), inflammation (IL-1β), and immune response pathways. The mesenchymal/invasive phenotype displayed markers such as vimentin, hypoxia-related genes, and genes associated with glycolysis. In addition, CTCs with a mesenchymal/stem cell-like phenotype demonstrated enrichment in genes such as ALDH13 and those linked to adipogenesis.[68] Beyond CTCs as biomarkers for lung cancer, chemokines such as CXC motif chemokine ligand 1 (CXCL1) and CXC motif chemokine ligand 2 (CXCL2) have also emerged as potential biomarkers. Using primary LUAD samples for scRNA-seq, researchers identified CXCL1 and CXCL2 and validated these findings through qRT-PCR, demonstrating a strong correlation between scRNA-seq and bulk RNA-seq data. In addition, an inverse relationship was observed between the expression levels of these chemokines and specific microRNAs (miR-532-5p and miR-1266-3p) in LUAD. Notably, miR-532-5p was detected in patients' plasma, underscoring its potential as a minimally invasive biomarker for early lung cancer detection.[69] Kagamu *et al.*[70] identified a novel $CD62L^{low}$ $CD4^+$ T-cell supercluster consisted of $CXCR3^+CCR4^-CCR6^+$ and $CXCR3^-CCR4^-CCR6^+$ cells, characterized by high interleukin-7 receptor and TCF7 expression, using single-cell RNA sequencing. The presence of this supercluster was significantly associated with PD-1 blockade efficacy, and its frequency in peripheral blood predicted patients' PFS and OS. In addition, a specific subpopulation of $CD4^+$ T cells, known as Th7R cells, was identified as capable of recovering from a state of T-cell depletion and is usually accompanied by a longer survival in patients with non-reduced levels of such cells. While patients with a PD-L1 tumor cell positivity of ≥1% are the main group to benefit from immune checkpoint inhibitor therapy, some PD-L1-negative patients also benefit from immunotherapy, suggesting the presence of additional potential biomarkers. These findings further highlight the critical role of $CD4^+$ T cells and their subpopulations in the development of personalized immunotherapy strategies, providing important new directions for precision treatment in advanced NSCLC. Zhong *et al.*[71] studied the characteristics of peripheral blood mononuclear cells in lung cancer patients before and after pembrolizumab treatment using single-cell RNA sequencing, discovering eight distinct cell types. The Cancer Genome Atlas (TCGA) data verified the link between the genes such as inhibitor of DNA binding 2 (*ID2*), phosphatidylinositol-4,5-

bisphosphate 3-kinase catalytic subunit delta (*PIK3CD*), and ubiquinol-cytochrome c reductase, complex III subunit X (*UQCR10*) and PD-1 expression, suggesting their potential as predictive markers for pembrolizumab efficacy in PD-L1-negative squamous lung cancer. In another study, Yudi Hu *et al.*[72] investigated the changes in smoking on T cells in primary NSCLC patients by integrating single-cell RNA-seq and bulk RNA-seq data. The results showed that smoking induced high expression of metallopeptidase domain 12 (ADAM12) in tumor-specific Tregs in untreated NSCLC patients, indicating ADAM12 as a potential biomarker and therapeutic target. Ruan *et al.*[73] found that the expression of CEA cell adhesion molecule 6 (CEACAM6), secretoglobin family 3A member 2 (SCGB3A2), and complement C3 (C3) was significantly elevated in tumor cells within the cerebrospinal fluid of patients with soft meningeal metastases from LUAD, suggesting these molecules as potential markers of tumor metastasis. A recent study integrated scRNA-seq and lipidomics to develop a lung cancer artificial intelligence detector (LCAID) v2.0, a lipid-targeted detection method for early lung cancer diagnosis. scRNA-seq revealed lipid metabolism dysregulation in tumor cells and identified nine lipid biomarkers through machine learning. Its accuracy and high specificity make it a valuable tool for early detection and large-scale screening of high-risk populations, providing a more personalized approach to lung cancer treatment.[74]

4.2. Single-cell sequencing for lung cancer prognostic evaluation

The application of single-cell sequencing in monitoring tumor dynamics offers unprecedented insights into cancer progression and treatment response. A midkine (MDK) is a protein that activates signaling pathways through ligand-dependent receptor interactions, resulting in specific biological responses. It is also a potent proangiogenic factor.[75] Overexpression of MDK (at both mRNA and protein levels) has been strongly linked to malignancy and poor prognosis.[76] Jiang *et al.*[77] identified two distinct subtypes using the LUAD scRNA-seq dataset, observing different prognostic and immunologic characteristics. Higher risk scores were associated with poorer survival outcomes and were more frequently linked to TP53 mutation, higher tumor mutation burden values, and PD-L1 upregulation. This model could serve as a potential biomarker for LUAD patients' risk stratification and treatment response prediction.[77] These findings highlight the critical role of scRNA-seq in refining prognostic models for LUAD patients and enhancing personalized therapeutic approaches to improve survival outcomes.

4.3. Single-cell sequencing in drug resistance and individualized therapy in lung cancer

Acquired drug resistance is a major factor contributing to poor prognosis and survival outcomes in cancer.[78,79] Disease recurrence is also caused by drug resistance, which can be due to genetic or epigenetic changes, modifications in drug targets, inhibition of apoptosis, increased drug efflux, and other cellular and molecular mechanisms.[80,81] Single-cell sequencing helps to understand the mechanisms of drug resistance in lung cancer. Through scRNA-seq and scATAC-seq analysis of cell line models and clinical specimens, Yukie Kashima[82] identified CD74 as an important factor in the transition of tumor cells from a naïve to a drug-tolerant state. CD74 upregulation confers resistance to the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) osimertinib and blocks apoptosis, enabling tumor regrowth. Salcher S[83] focused on tissue-resident neutrophils (TRNs) by analyzing 1,283,972 single cells integrated from 556 samples and 318 patients. The study revealed that genetic markers derived from TRNs were linked to treatment failure with anti-programmed cell death ligand 1 (PD-L1) therapy. In addition, researchers generated CTC-derived xenografts from patients with SCLC and performed single-cell RNA sequencing on both chemoresistant and chemosensitive CTC-derived xenografts, as well as patient-derived CTCs. The findings showed that cellular subpopulations underwent EMT after developing treatment resistance. In chemoresistant models, there was an increased expression of signaling pathway components previously associated with chemoresistance, such as MYC, mTOR, and Wnt.

The findings confirmed the presence of multiple cisplatin resistance pathways at intratumoral, intrapatient, and interpatient levels. These pathways were marked by the coexistence of distinct subpopulations of cells with heterogeneous gene expression.[84] A study found that in non-responsive tumors, the signaling mechanisms of T-cell receptors specific to mutated antigens (MANA) were significantly impaired. This reduction in signaling could weaken the immune system's ability to recognize and destroy tumor cells, contributing to treatment resistance and immune evasion. In addition, these clones demonstrated coordinated upregulation of checkpoint molecules, killer suppressor receptors, and inhibitors of T-cell activation. These observations offer valuable insights into resistance mechanisms that may be targeted to enhance the efficacy of PD-1 blockade.[85] He *et al.*[86] analyzed the role of circadian rhythm disruption (CRD) in tumors using three representative single-cell RNA-seq datasets of LUAD. The study showed that patients with LUAD who had high CRD scores tended to have a

poor prognosis. Furthermore, CRD was associated with resistance to anticancer treatments, such as chemotherapy and tyrosine kinase inhibitors. The proposed mechanism suggests that CRD facilitates the change of malignant cells into persistent, drug-resistant populations.

Despite its advantages, single-cell sequencing faces several challenges that must be addressed for broader and more reliable applications. Experimental limitations, such as amplification bias, differences in library sizes, and DNA damage during processing, can lead to inaccuracies in data interpretation. In addition, the lack of standardization across various techniques complicates the comparison and integration of results. The small starting material in single-cell sequencing also contributes to low capture efficiency and sequencing variability, further adding to the technical noise. Combined with high biological variation, these factors complicate data analysis. The large-scale, noisy, and sparse nature of single-cell data poses additional challenges for computational analysis, often requiring methods like imputation and clustering to handle missing values. Furthermore, low-quality data from broken, dead, or aggregated cells can interfere with downstream analysis, leading to misinterpretations. Many conventional bulk sequencing tools are not directly applicable, necessitating specialized methods for tasks such as cell clustering and gene regulatory network inference.[87] The high cost of single-cell sequencing remains a limitation, but advancements in sequencing technologies, library preparation, and computational tools are gradually overcoming these challenges. These innovations are making single-cell sequencing more affordable and accessible, paving the way for larger studies and broader clinical applications.

5. Conclusion

Single-cell sequencing technology provides a powerful tool for lung cancer research with its high-resolution and multidimensional analysis capabilities. It has demonstrated great potential in revealing lung cancer heterogeneity, resolving the TME, early diagnosis, invasion, and metastasis mechanisms, as well as exploring therapeutic and drug resistance. These advancements have greatly enhanced the understanding of lung cancer. In recent years, many studies have demonstrated the value of single-cell sequencing in lung cancer research. By integrating single-cell sequencing technologies, such as combining scRNA-seq with genomics, epigenomics, proteomics, metabolomics, and spatial transcriptomics, researchers can overcome the limitations of traditional sequencing methods while addressing certain shortcomings of single-cell sequencing itself.

Despite its significant advantages, many challenges hinder the widespread practical application of single-cell sequencing. For example, isolating a sufficient number of intact single cells from lung cancer tissue is a complex process, and mechanical or chemical manipulation may damage cells, reducing the precision of analyses. In addition, the high cost and complicated experimental procedures limit its application in broader clinical settings. The bioinformatics analysis of single-cell sequencing technology is also a great challenge. Processing and interpreting huge amounts of data require efficient and accurate algorithms, yet existing analytical techniques are not yet fully optimized to meet research needs. The development of more advanced algorithms to enhance the capability of single-cell analysis, especially in digitizing and visualizing the TME, is crucial for making findings more relevant to real-world scientific and clinical settings. Many of the current single-cell sequencing results provide only theoretical insights, necessitating further validation in larger patient populations, the development of targeted drugs, and the advancement of clinical translation. The refinement of these aspects will require extensive research and practice. As single-cell sequencing is still an emerging technology, its clinical application in identifying lung cancer biomarkers remains limited, with only a few biomarkers currently in the early stages of development. However, with continuous advancements in single-cell analysis and clinical validation, the potential of this technology in precision oncology remains promising, offering hope for more effective and personalized lung cancer treatments in the future.

Nevertheless, single-cell sequencing remains a hotspot for future lung cancer research. This technology has revolutionized lung cancer treatment by enabling the extraction of crucial insights from the TME. The advantages of single-cell sequencing are particularly evident in the context of immunotherapy, which is becoming the mainstream of cancer treatment. With the continuous advancement of sequencing technology and analysis methods, the application of single-cell sequencing will continue to expand. In the future, the integration of multi-omics data and single-cell technologies will further promote the development of lung cancer research and treatment. Single-cell sequencing is expected to become an indispensable tool, providing a stronger scientific foundation for improving the prognosis and quality of life of lung cancer patients while offering comprehensive and reliable data to support precision medicine and individualized treatment.

Acknowledgments

None.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82200070), the Chongqing Wanzhou Municipal Science and Health Joint Research Project (Grant No. wzwjw-kw2024008), the Chongqing Wanzhou Municipal Science and Health Joint Medical Research Project Key Program (Grant No. wzstc-kw2023001), the Innovation and Development Joint Fund of the Natural Science Foundation of Chongqing (No. CSTB2023NSCQ-LMX0037 and No. CSTB2023NSCQ-LMX0038), and the Natural Science Foundation of Chongqing, China (No. CSTB2024NSCQ-MSX0761).

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Mingzhu Yin, Jian Sun
Investigation: Wei Su, Bingbing Bai, Jinhai Deng, Yuesong Wu
Writing – original draft: Jian Sun, Zhongsha Li
Writing – review & editing: Wei Su, Bingbing Bai, Yuesong Wu, Baoyin Zhang, Dejuan Xiang, Gilwa Borjigin, Jinhai Deng

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. *Lancet (London, England)*. 2021;398(10299):535-554.
   doi: 10.1016/s0140-6736(21)00312-3
2. Gridelli C, Rossi A, Carbone DP, *et al*. Non-small-cell lung cancer. *Nat Rev Dis Prim*. 2015;1:15009.
   doi: 10.1038/nrdp.2015.9
3. Nooreldeen R, Bach H. Current and future development in lung cancer diagnosis. *In J Mol Sci*. 2021;22(16):8661.
   doi: 10.3390/ijms22168661
4. Li MY, Liu LZ, Dong M. Progress on pivotal role and application of exosome in lung cancer carcinogenesis, diagnosis, therapy and prognosis. *Mol Cancer*. 2021;20(1):22.
   doi: 10.1186/s12943-021-01312-y
5. Wauthoz N, Rosière R, Amighi K. Inhaled cytotoxic chemotherapy: Clinical challenges, recent developments, and future prospects. *Expert Opin Drug Deliv*. 2021;18(3):333-354.
   doi: 10.1080/17425247.2021.1829590
6. Otano I, Ucero AC, Zugazagoitia J, Paz-Ares L. At the crossroads of immunotherapy for oncogene-addicted subsets of NSCLC. *Nat Rev Clin Oncol*. 2023;20(3):143-159.
   doi: 10.1038/s41571-022-00718-x
7. McCoach CE. A cautionary analysis of immunotherapy prior to targeted therapy. *J Thoracic Oncol*. 2019;14(1):8-10.
   doi: 10.1016/j.jtho.2018.10.009
8. Cai J, Fang L, Huang Y, *et al*. Simultaneous overactivation of Wnt/β-catenin and TGFβ signalling by miR-128-3p confers chemoresistance-associated metastasis in NSCLC. *Nat Commun*. 2017;8:15870.
   doi: 10.1038/ncomms15870
9. Lambrechts D, Wauters E, Boeckx B, *et al*. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat Med*. 2018;24(8):1277-1289.
   doi: 10.1038/s41591-018-0096-5
10. Altorki NK, Markowitz GJ, Gao D, *et al*. The lung microenvironment: An important regulator of tumour growth and metastasis. *Nat Rev Cancer*. 2019;19(1):9-31.
   doi: 10.1038/s41568-018-0081-9
11. Bischoff P, Trinks A, Obermayer B, *et al*. Single-cell RNA sequencing reveals distinct tumor microenvironmental patterns in lung adenocarcinoma. *Oncogene*. 2021;40(50):6748-6758.
   doi: 10.1038/s41388-021-02054-3
12. Tang F, Barbacioru C, Wang Y, *et al*. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat Methods*. 2009;6(5):377-382.
   doi: 10.1038/nmeth.1315
13. Navin N, Kendall J, Troge J, *et al*. Tumour evolution inferred by single-cell sequencing. *Nature*. 2011;472(7341):90-94.
   doi: 10.1038/nature09807
14. Xu X, Hou Y, Yin X, *et al*. Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. *Cell*. 2012;148(5):886-895.
   doi: 10.1016/j.cell.2012.02.025
15. Zhang J, Song C, Tian Y, Yang X. Single-cell RNA sequencing in lung cancer: Revealing phenotype shaping of stromal cells in the microenvironment. *Front Immunol*. 2021;12:802080.
   doi: 10.3389/fimmu.2021.802080
16. Yu D, Zhang S, Liu Z, Xu L, Chen L, Xie L. Single-cell RNA sequencing analysis of gene regulatory network changes in the development of lung adenocarcinoma. *Biomolecules*.

2023;13(4):671.

doi: 10.3390/biom13040671

17. Zhang L, Lee M, Maslov AY, Montagna C, Vijg J, Dong X. Analyzing somatic mutations by single-cell whole-genome sequencing. *Nat Protoc*. 2024;19(2):487-516.

doi: 10.1038/s41596-023-00914-8

18. Funnell T, O'Flanagan CH, Williams MJ, *et al.* Single-cell genomic variation induced by mutational processes in cancer. *Nature*. 2022;612(7938):106-115.

doi: 10.1038/s41586-022-05249-0

19. Liao P, Huang Q, Zhang J, *et al*. How single-cell techniques help us look into lung cancer heterogeneity and immunotherapy. *Front Immunol*. 2023;14:1238454.

doi: 10.3389/fimmu.2023.1238454

20. Li W, Lu J, Lu P, *et al*. scNanoHi-C: A single-cell long-read concatemer sequencing method to reveal high-order chromatin structures within individual cells. *Nat Methods*. 2023;20(10):1493-1505.

doi: 10.1038/s41592-023-01978-w

21. Sasagawa Y, Nikaido I, Hayashi T, *et al*. Erratum to: Quartz-Seq: A highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity. *Genome Biol*. 2017;18(1):R31.

doi: 10.1186/s13059-017-1154-x

22. Ramsköld D, Luo S, Wang YC, *et al*. Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. *Nat Biotechnol*. 2012;30(8):777-782.

doi: 10.1038/nbt.2282

23. Picelli S, Björklund ÅK, Faridani OR, Sagasser S, Winberg G, Sandberg R. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nat Methods*. 2013;10(11):1096-1098.

doi: 10.1038/nmeth.2639

24. Hashimshony T, Wagner F, Sher N, Yanai I. CEL-Seq: Single-cell RNA-Seq by multiplexed linear amplification. *Cell Rep.* 2012;2(3):666-673.

doi: 10.1016/j.celrep.2012.08.003

25. Jaitin DA, Kenigsberg E, Keren-Shaul H, *et al*. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science.* 2014;343(6172):776-779.

doi: 10.1126/science.1247651

26. Macosko EZ, Basu A, Satija R, *et al*. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161(5):1202-1214.

doi: 10.1016/j.cell.2015.05.002

27. Klein AM, Mazutis L, Akartuna I, *et al*. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell*. 2015;161(5):1187-1201.

doi: 10.1016/j.cell.2015.04.044

28. Huang D, Ma N, Li X, *et al*. Advances in single-cell RNA sequencing and its applications in cancer research. *J Hematol Oncol*. 2023;16(1):98.

doi: 10.1186/s13045-023-01494-6

29. Li Y, Xu Q, Wu D, Chen G. Exploring additional valuable information from single-cell RNA-Seq data. *Front Cell Dev Biol*. 2020;8:593007.

doi: 10.3389/fcell.2020.593007

30. Buenrostro JD, Wu B, Litzenburger UM, *et al*. Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature*. 2015;523(7561):486-490.

doi: 10.1038/nature14590

31. Shi P, Nie Y, Yang J, Zhang W, Tang Z, Xu J. Fundamental and practical approaches for single-cell ATAC-seq analysis. *aBIOTECH*. 2022;3(3):212-223.

doi: 10.1007/s42994-022-00082-5

32. Schwartzman O, Tanay A. Single-cell epigenomics: Techniques and emerging applications. *Nat Rev Genet*. 2015;16(12):716-726.

doi: 10.1038/nrg3980

33. Macaulay IC, Haerty W, Kumar P, *et al*. G and T-seq: Parallel sequencing of single-cell genomes and transcriptomes. *Nat Methods*. 2015;12(6):519-522.

doi: 10.1038/nmeth.3370

34. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. *Nat Rev Genet*. 2023;24(8):494-515.

doi: 10.1038/s41576-023-00580-2

35. Dey SS, Kester L, Spanjaard B, Bienko M, Van Oudenaarden A. Integrated genome and transcriptome sequencing of the same cell. *Nat Biotechnol*. 2015;33(3): 285-289.

doi: 10.1038/nbt.3129

36. Rodriguez-Meira A, Buck G, Clark SA, *et al*. Unravelling intratumoral heterogeneity through high-sensitivity single-cell mutational analysis and parallel RNA sequencing. *Mol Cell*. 2019;73(6):1292.e8-1305.e8.

doi: 10.1016/j.molcel.2019.01.009

37. Yu L, Wang X, Mu Q, *et al*. scONE-seq: A single-cell multi-omics method enables simultaneous dissection of phenotype and genotype heterogeneity from frozen tumors. *Sci Adv*. 2023;9(1):eabp8901.

doi: 10.1126/sciadv.abp8901

38. He L, Wang W, Dang K, Ge Q, Zhao X. Integration of single-cell transcriptome and proteome technologies: Toward

spatial resolution levels. *View*. 2023;4(5):20230040.

doi: 10.1002/viw.20230040

39. Eisenstein M. Seven technologies to watch in 2022. *Nature*. 2022;601(7894):658-661.

doi: 10.1038/d41586-022-00163-x

40. Golo M, Newman PLH, Kempe D, Biro M. Mechanoimmunology in the solid tumor microenvironment. *Biochem Soc Transact*. 2024;52(3):1489-1502.

doi: 10.1042/bst20231427

41. Lei Y, Tang R, Xu J, *et al*. Applications of single-cell sequencing in cancer research: progress and perspectives. *J Hematol Oncol*. 2021;14(1):91.

doi: 10.1186/s13045-021-01105-2

42. Hinohara K, Polyak K. Intratumoral heterogeneity: More than just mutations. *Trends Cell Biol*. 2019;29(7):569-579.

doi: 10.1016/j.tcb.2019.03.003

43. Li Q, Wang R, Yang Z, *et al*. Molecular profiling of human non-small cell lung cancer by single-cell RNA-seq. *Genome Med*. 2022;14(1):87.

doi: 10.1186/s13073-022-01089-9

44. Tian Y, Li Q, Yang Z, *et al*. Single-cell transcriptomic profiling reveals the tumor heterogeneity of small-cell lung cancer. *Signal Transduct Target Ther*. 2022;7(1):346.

doi: 10.1038/s41392-022-01150-4

45. Chan JM, Quintanal-Villalonga Á, Gao VR, *et al*. Signatures of plasticity, metastasis, and immunosuppression in an atlas of human small cell lung cancer. *Cancer cell*. 2021;39(11):1479.e18-1496.e18.

doi: 10.1016/j.ccell.2021.09.008

46. Sainz de Aja J, Dost AFM, Kim CF. Alveolar progenitor cells and the origin of lung cancer. *J Intern Med*. 2021;289(5): 629-635.

doi: 10.1111/joim.13201

47. Hu J, Zhang L, Xia H, *et al*. Tumor microenvironment remodeling after neoadjuvant immunotherapy in non-small cell lung cancer revealed by single-cell RNA sequencing. *Genome Med*. 2023;15(1):14.

doi: 10.1186/s13073-023-01164-9

48. Lu H, Qian J, Cheng L, Shen Y, Chu T, Zhao C. Single-cell RNA-sequencing uncovers the dynamic changes of tumour immune microenvironment in advanced lung adenocarcinoma. *BMJ Open Respir Res*. 2023;10(1):e001878.

doi: 10.1136/bmjresp-2023-001878

49. Ran X, Tong L, Chenghao W, *et al*. Single-cell data analysis of malignant epithelial cell heterogeneity in lung adenocarcinoma for patient classification and prognosis prediction. *Heliyon*. 2023;9(9):e20164.

doi: 10.1016/j.heliyon.2023.e20164

50. Zhang L, Zhang Y, Wang C, *et al*. Integrated single-cell RNA sequencing analysis reveals distinct cellular and transcriptional modules associated with survival in lung cancer. *Signal Transduct Target Ther*. 2022;7(1):9.

doi: 10.1038/s41392-021-00824-9

51. Zhang H, Yue X, Chen Z, *et al*. Define cancer-associated fibroblasts (CAFs) in the tumor microenvironment: New opportunities in cancer immunotherapy and advances in clinical trials. *Mol Cancer*. 2023;22(1):159.

doi: 10.1186/s12943-023-01860-5

52. Hanley CJ, Waise S, Ellis MJ, *et al*. Single-cell analysis reveals prognostic fibroblast subpopulations linked to molecular and immunological subtypes of lung cancer. *Nat Commun*. 2023;14(1):387.

doi: 10.1038/s41467-023-35832-6

53. Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Front Biosci (Landmark Ed)*. 2010;15(1):166-179.

doi: 10.2741/3613

54. Dominguez CX, Müller S, Keerthivasan S, *et al*. Single-Cell RNA sequencing reveals stromal evolution into LRRC15+ myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov*. 2020;10(2):232-253.

doi: 10.1158/2159-8290.Cd-19-0644

55. Meier SL, Satpathy AT, Wells DK. Bystander T cells in cancer immunology and therapy. *Nat Cancer*. 2022;3(2):143-155.

doi: 10.1038/s43018-022-00335-8

56. Pai JA, Hellmann MD, Sauter JL, *et al*. Lineage tracing reveals clonal progenitors and long-term persistence of tumor-specific T cells during immune checkpoint blockade. *Cancer cell*. 2023;41(4):776.e7-790.e7.

doi: 10.1016/j.ccell.2023.03.009

57. Wu F, Fan J, He Y, *et al*. Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat Commun*. 2021;12(1):2540.

doi: 10.1038/s41467-021-22801-0

58. Xiang X, Wang J, Lu D, Xu X. Targeting tumor-associated macrophages to synergize tumor immunotherapy. *Signal Transduct Target Ther*. 2021;6(1):75.

doi: 10.1038/s41392-021-00484-9

59. Guo X, Zhang Y, Zheng L, *et al*. Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing. *Nat Med*. 2018;24(7):978-985.

doi: 10.1038/s41591-018-0045-3

60. Wang Z, Li Z, Zhou K, *et al*. Deciphering cell lineage specification of human lung adenocarcinoma with single-cell RNA sequencing. *Nat Commun*. 2021;12(1):6500.

doi: 10.1038/s41467-021-26770-2

61. Zhang Y, Du W, Chen Z, Xiang C. Upregulation of PD-L1 by SPP1 mediates macrophage polarization and facilitates immune escape in lung adenocarcinoma. *Exp Cell Res*. 2017;359(2):449-457.

doi: 10.1016/j.yexcr.2017.08.028

62. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: Current technology and clinical applications. *J Hematol Oncol*. 2022;15(1):131.

doi: 10.1186/s13045-022-01351-y

63. Li X, Li M, Huang M, *et al.* The multi-molecular mechanisms of tumor-targeted drug resistance in precision medicine. *Biomed Pharmacother/Biomed Pharmacother*. 2022;150:113064.

doi: 10.1016/j.biopha.2022.113064

64. Xu M, Zhao H, Chen J, *et al*. An integrated microfluidic chip and its clinical application for circulating tumor cell isolation and single-cell analysis. *Cytometry A*. 2020;97(1):46-53.

doi: 10.1002/cyto.a.23902

65. Su Z, Wang Z, Ni X, *et al*. Inferring the evolution and progression of small-cell lung cancer by single-cell sequencing of circulating tumor cells. *Clin Cancer Res*. 2019;25(16):5049-5060.

doi: 10.1158/1078-0432.Ccr-18-3571

66. Chang Y, Wang Y, Li B, *et al*. Whole-exome sequencing on circulating tumor cells explores platinum-drug resistance mutations in advanced non-small cell lung cancer. *Front Genet*. 2021;12:722078.

doi: 10.3389/fgene.2021.722078

67. Negishi R, Yamakawa H, Kobayashi T, *et al*. Transcriptomic profiling of single circulating tumor cells provides insight into human metastatic gastric cancer. *Commun Biol*. 2022;5(1):20.

doi: 10.1038/s42003-021-02937-x

68. Andrikou K, Rossi T, Verlicchi A, *et al*. Circulating tumour cells: Detection and application in advanced non-small cell lung cancer. *Int J Mol Sci*. 2023;24(22):16085.

doi: 10.3390/ijms242216085

69. Kim J, Xu Z, Marignani PA. Single-cell RNA sequencing for the identification of early-stage lung cancer biomarkers from circulating blood. *NPJ Genomic Med*. 2021;6(1):87.

doi: 10.1038/s41525-021-00248-y

70. Kagamu H, Yamasaki S, Kitano S, *et al.* Single-cell analysis reveals a CD4+ T-cell cluster that correlates with PD-1 blockade efficacy. *Cancer Res*. 2022;82(24):4641-4653.

doi: 10.1158/0008-5472.Can-22-0112

71. Zhong R, Zhang Y, Chen D, Cao S, Han B, Zhong H. Single-cell RNA sequencing reveals cellular and molecular immune profile in a pembrolizumab-responsive PD-L1-negative lung cancer patient. *Cancer Immunol Immunother*. 2021;70(8):2261-2274.

doi: 10.1007/s00262-021-02848-0

72. Hu Y, Xu C, Ren J, *et al*. Exposure to tobacco smoking induces a subset of activated tumor-resident tregs in non-small cell lung cancer. *Transl Oncol*. 2022;15(1):101261.

doi: 10.1016/j.tranon.2021.101261

73. Ruan H, Zhou Y, Shen J, *et al*. Circulating tumor cell characterization of lung cancer brain metastases in the cerebrospinal fluid through single-cell transcriptome analysis. *Clin Transl Med*. 2020;10(8):e246.

doi: 10.1002/ctm2.246

74. Wang G, Qiu M, Xing X, *et al*. Lung cancer scRNA-seq and lipidomics reveal aberrant lipid metabolism for early-stage diagnosis. *Sci Transl Med*. 2022;14(630):eabk2756.

doi: 10.1126/scitranslmed.abk2756

75. Tang Y, Kwiatkowski DJ, Henske EP. Midkine expression by stem-like tumor cells drives persistence to mTOR inhibition and an immune-suppressive microenvironment. *Nat Commun*. 2022;13(1):5018.

doi: 10.1038/s41467-022-32673-7

76. Filippou PS, Karagiannis GS, Constantinidou A. Midkine (MDK) growth factor: A key player in cancer progression and a promising therapeutic target. *Oncogene*. 2020;39(10):2040-2054.

doi: 10.1038/s41388-019-1124-8

77. Jiang A, Wang J, Liu N, *et al*. Integration of single-cell RNA sequencing and bulk RNA sequencing data to establish and validate a prognostic model for patients with lung adenocarcinoma. *Front Genet*. 2022;13:833797.

doi: 10.3389/fgene.2022.833797

78. Aleksakhina SN, Kashyap A, Imyanitov EN. Mechanisms of acquired tumor drug resistance. *Biochim Biophys Acta Rev Cancer*. 2019;1872(2):188310.

doi: 10.1016/j.bbcan.2019.188310

79. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature*. 2019;575(7782):299-309.

doi: 10.1038/s41586-019-1730-1

80. Zhang A, Miao K, Sun H, Deng CX. Tumor heterogeneity reshapes the tumor microenvironment to influence drug resistance. *Int J Biol Sci*. 2022;18(7):3019-3033.

doi: 10.7150/ijbs.72534

81. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. *Can Drug Resist*. 2019;2(2):141-160.

doi: 10.20517/cdr.2019.10

82. Kashima Y, Shibahara D, Suzuki A, *et al*. Single-cell analyses reveal diverse mechanisms of resistance to EGFR tyrosine kinase inhibitors in lung cancer. *Cancer Res*. 2021;81(18):4835-4848.

doi: 10.1158/0008-5472.Can-20-2811

83. Salcher S, Sturm G, Horvath L, *et al*. High-resolution single-cell atlas reveals diversity and plasticity of tissue-resident neutrophils in non-small cell lung cancer. *Cancer cell*. 2022;40(12):1503.e8-1520.e8.

doi: 10.1016/j.ccell.2022.10.008

84. Stewart CA, Gay CM, Xi Y, *et al*. Single-cell analyses reveal increased intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. *Nat Cancer*. 2020;1:423-436.

doi: 10.1038/s43018-019-0020-z

85. Caushi JX, Zhang J, Ji Z, *et al*. Author correction: Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature*. 2021;598(7881):E1.

doi: 10.1038/s41586-021-03893-6

86. He L, Fan Y, Zhang Y, *et al*. Single-cell transcriptomic analysis reveals circadian rhythm disruption associated with poor prognosis and drug-resistance in lung adenocarcinoma. *J Pineal Res*. 2022;73(1):e12803.

doi: 10.1111/jpi.12803

87. Chen G, Ning B, Shi T. Single-cell RNA-Seq technologies and related computational data analysis. *Front Genet*. 2019;10:317.

doi: 10.3389/fgene.2019.00317