Single-Cell Analysis for Cancer

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Abstract

Single-cell technologies have transformed cancer research by enabling high-resolution characterization of tumor heterogeneity and the microenvironment. These approaches uncover rare malignant cell states, clonal dynamics, and immunosuppressive subsets that shape tumor progression and therapeutic resistance. They have also advanced biomarker discovery, minimal residual disease monitoring, and prediction of immunotherapy response, opening new avenues for patient stratification and precision oncology. Yet, key challenges remain, including scalability, protocol standardization, and data privacy. Emerging innovations, such as spatial multi-omics, organoid-based systems, and artificial intelligence, promise to bridge these gaps and accelerate clinical translation. Collectively, single-cell technologies represent a paradigm shift in oncology, with profound implications for diagnosis, prognosis, and therapy.

Keywords

Single-cell sequencing · Tumor heterogeneity · Tumor microenvironment · Precision oncology · Spatial omics

10.1 Introduction

Cancer remains one of the leading causes of mortality worldwide, placing a substantial burden on global healthcare systems (Maomao et al. 2022; Klein 2021; Perkins et al. 2023). A major source of its complexity lies in its tumor heterogeneity, which encompasses genetic, epigenetic, and phenotypic variations both within a single tumor (intratumoral heterogeneity) and across patients (intertumoral

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heterogeneity) (Haffner et al. 2021). This heterogeneity shapes tumor progression, therapeutic response, and resistance. Furthermore, the dynamic interactions between malignant tumor cells and components of the tumor microenvironment (TME), including immune, stromal, and vascular cells, further contribute to the adaptive capacity and complexity of cancer (de Visser and Joyce 2023).

Traditional bulk tissue profiling techniques have provided invaluable insights, yet they average signals from thousands to millions of cells (Li et al. 2021). As a result, they may obscure rare populations or unique states that may critically influence tumor progression, metastasis, or therapy resistance. Bulk approaches also fail to resolve cell-type-specific responses or capture the spatial organization of cells within the TME, thereby limiting strategies for effective personalized treatments (Kuksin et al. 2021).

Single-cell technologies have transformed oncology by enabling high-resolution profiling of individual cells, offering unprecedented insights into tumor and TME heterogeneity (Kuksin et al. 2021). These approaches allow researchers to trace lineage relationships, identify resistant subpopulations such as persister cells, and dissect complex cell–cell interactions that drive cancer progression and therapeutic outcomes (Eyler et al. 2020; Baysoy et al. 2023; Russo et al. 2024). As such, single-cell analyses have become central to both fundamental cancer research and translational precision oncology.

10.2 Key Technologies at Single-Cell Level

10.2.1 ScRNA-seq and SnRNA-seq

Single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) are widely applied approaches for transcriptomic profiling at single-cell resolution. ScRNA-seq is best suited for fresh tumor tissues in which viable, dissociated cells can be isolated (Slyper et al. 2020).

However, many clinical tumor samples are preserved by freezing or fixation, making intact cell isolation challenging. In these contexts, snRNA-seq, which captures RNA from isolated nuclei, provides a more appropriate alternative (Nickason et al. 2025). This method has proven effective for reliable transcriptomic characterization of frozen samples, including solid tumors such as cervical squamous cell carcinoma (Ou et al. 2022), pancreatic ductal adenocarcinoma (Chen et al. 2025), and glioblastoma (Wang et al. 2024).

10.2.2 Multi-omics Single-Cell Technologies

To more fully elucidate the crosstalk between intracellular and intercellular mechanisms, multi-omics single-cell platforms, often described as multimodal omics approaches, have been established (Vandereyken et al. 2023).

Among these, single-cell transcriptomics is the most established and is often combined with other modalities to connect gene expression with phenotypic heterogeneity in an unbiased manner (Baysoy et al. 2023). For example, genome-transcriptome integration, as achieved by methods such as G&T-seq, enables parallel profiling of genomic DNA and mRNA from the same cell, thereby linking genomic alterations to transcriptional states (Macaulay et al. 2015). Similarly, combining transcriptome with epigenome through approaches such as scATAC-seq reveals chromatin accessibility, transcription factor activity, and the regulatory landscape that shape gene expression (Buenrostro et al. 2015). Another powerful strategy is the integration of transcriptome and proteome, exemplified by CITE-seq, which allows simultaneous measurement of unbiased gene expression profiles and multiplexed surface protein markers (Stoeckius et al. 2017). Collectively, these multimodal approaches provide novel insights into the molecular networks that govern cellular heterogeneity and cancer progression.

10.2.3 Spatial Multi-omics Technologies at Single-Cell Level

The single-cell multi-omics approaches described above require dissociation of cells from their native tissue, thereby losing information on the physical interactions that occur within and between cells (Browaeys et al. 2020; Efremova et al. 2020). To address this limitation, emerging spatial multi-omics technologies at the single-cell level aim to map molecular features and cell–cell interactions within intact tissues at a genome-wide scale (Baysoy et al. 2023; Tang et al. 2023).

Among these, spatial transcriptomics is the most advanced, enabling gene expression profile to be directly mapped onto tissue sections while preserving the spatial context of cellular populations. Techniques such as 10x Visium, Slide-seq (Rodriques et al. 2019), and MERFISH (Chen et al. 2015) have become widely adopted for studying the spatial organization of tumors and other biological systems. More recently, spatial transcriptomics has been combined with additional omics layers, giving rise to integrated platforms that profile genome, epigenome, and proteome features in situ. Notable examples include Slide-DNA-seq (Zhao et al. 2022), Spatial ATAC-seq (Deng et al. 2022a), Spatial ATAC-RNA-seq (Zhang et al. 2023), Spatial CUT&Tag (Deng et al. 2022b), SM-Omics (Vickovic et al. 2022), and Spatial CITE-seq (Liu et al. 2023a).

10.2.4 Data Integration Frameworks and Computational Tools

As single-cell multi-omics data become increasingly complex, effective data integration is essential. The primary goal is to achieve robust and sensitive identification of cell types or cell states, thereby facilitating insights into the regulatory mechanisms and functional diversity of cells (Hao et al. 2021). Argelaguet et al. categorized single-cell data integration strategies into three main classes: horizontal,

vertical, and diagonal integration, each tailored to different experimental and analytical contexts (Argelaguet et al. 2021).

Accordingly, computational tools for single-cell multi-omics can be broadly divided into matched and unmatched pipelines. Matched pipelines analyze multiple omics layers measured simultaneously from the same cell, exemplified by tools such as Seurat v4 (Hao et al. 2021) and SCHEMA (Singh et al. 2021). In contrast, unmatched pipelines integrate unaligned datasets generated from separate experiments (Baysoy et al. 2023), with representative tools including LIGER (Welch et al. 2019), GLUE (Cao and Gao 2022), and Seurat v5 (Hao et al. 2024).

10.3 Tumor Heterogeneity Uncovered by Single-Cell Technologies

10.3.1 Intratumoral Heterogeneity: Genetic, Epigenetic, and Transcriptional Diversity

Intratumoral heterogeneity is a major driver of cancer complexity and treatment failure, arising from a variety of sources such as genetic mutations, epigenetic modifications, and transcriptional variability (Mathur et al. 2024). Recent developments in single-cell technologies have enabled high-resolution profiling of this heterogeneity, resulting in the creation of single-cell atlases across multiple tumor types. These efforts have provided valuable insights into the nature of intratumoral heterogeneity, uncovered novel regulatory mechanisms in cancer cells, and offered powerful tools for advancing tumor biology.

For example, Mathur et al. combined 3D neuronavigation during surgical resection with scATAC-seq and scRNA-seq to reveal the sources and spatial patterning of intratumoral heterogeneity, thereby identifying potential therapeutic targets to overcome heterogeneity-driven resistance (Mathur et al. 2024). Similarly, by integrating scRNA-seq and scATAC-seq data from human ovarian and endometrial cancers, Regner et al. identified newly emergent regulatory elements in malignant cells that activate hallmark cancer pathways, underscoring the pivotal contribution of intratumoral heterogeneity (Regner et al. 2021). Taken together, these findings highlight the transformative potential of single-cell analysis for dissecting tumor heterogeneity and informing therapeutic strategies.

10.3.2 Tumor Cell State Identification and Plasticity

Single-cell technologies have uncovered rare and transient cell states that remain hidden in bulk analyses (Shaffer et al. 2017; Tombor et al. 2021). These states, including drug-tolerant persister cells (Shen et al. 2019, 2020a; Wang et al. 2025), cancer stem-like cells (Chan et al. 2021), and invasive phenotypes (Li et al. 2023), are key drivers of tumor progression and therapeutic resistance (Pu et al. 2023; Shen et al. 2020b).

In melanoma, for example, Rambow et al. performed scRNA-seq to profile malignant tumor cells from patients with BRAF mutations and identified four distinct drug-tolerant transcriptional states. Among these, a neural crest stem cell-like state emerged as a key driver of therapeutic resistance (Rambow et al. 2018), illustrating how cellular heterogeneity shapes treatment outcomes. Likewise, in small-cell lung carcinoma (SCLC), Chan et al. analyzed tumors from 21 patients using scRNA-seq and revealed biological complexities not captured by bulk-level sequencing. Their findings showed that SCLC—particularly the SCLC-N subtype—exhibits greater heterogeneity than lung adenocarcinomas and displays substantial subtype plasticity, including interconversion between SCLC-A and SCLC-N subtypes (Chan et al. 2021). These studies underscore the dynamic nature of tumor evolution and highlight the power of single-cell approaches to resolve clinically relevant heterogeneity.

10.3.3 Evolutionary Trajectories and Clonal Architecture

Single-cell sequencing has revealed the evolutionary trajectory of tumors, capturing the spatial and temporal progression of genetic and transcriptional changes (Nolan et al. 2023). Tumor clonal evolution is not a linear process but rather a dynamic interplay among genetic alterations, epigenetic modifications, and microenvironmental influences (Eyler et al. 2020).

For instance, Heiser et al. integrated single-cell and spatial transcriptomic data from colorectal cancers to delineate patient-specific tumor evolutionary trajectories, while simultaneously mapping alterations in the TME and clonal architecture. By stratifying tumors based on their evolutionary trajectories, the study sheds light on the coordinated evolution of colorectal cancer and its tumor microenvironment, offering a conceptual framework that may guide the development of more precise targeted therapies (Heiser et al. 2023). In parallel, single-cell-based lineage tracing enables visualization of tumor evolution at clonal resolution, providing a powerful approach to study tumor progression and identify novel opportunities for therapeutic intervention (Yang et al. 2022a).

10.4 Tumor Microenvironment at Single-Cell Resolution

10.4.1 Characterizing Immune Cell Diversity in Tumors

TME comprises a complex mix of immune, stromal, and vascular cells (de Visser and Joyce 2023). The composition and functional state of immune populations within the TME critically influence tumor growth, metastasis, and response to treatment (Chan et al. 2021). Single-cell data allows for high-resolution characterization of immune subsets, providing insights into their activation, differentiation, and roles in tumor progression (Li et al. 2023).

For example, Chen et al. applied scRNA-seq to analyze the TME in nasopharyngeal carcinoma, generating a refined classification of immune cell populations (Chen et al. 2020). Myeloid cells were subdivided into macrophages, monocytes, plasmacytoid dendritic cells (pDCs), and dendritic cells (DCs); T/NK lineages were categorized into $CD8^+$ T cells, conventional $CD4^+$ T cells, regulatory T cells (Tregs), dysfunctional T cells, and natural killer (NK) cells; and B-cell compartments into B cells, germinal center B cells, $FCRL4^+$ memory B cells, and plasma cells (Chen et al. 2020). Notably, this study also identified novel immune subsets, including $CLEC9A^+$ dendritic cells, thereby establishing a paradigm for immune cell classification within the TME based on single-cell technologies.

10.4.2 Tumor-Infiltrating Lymphocytes and Immunosuppressive Cells

Tumor-infiltrating lymphocytes (TILs) are well-established biomarkers of antitumor immunity and can be routinely assessed in clinical practice using standard hematoxylin and eosin staining (Passaro et al. 2024; Shin et al. 2017). Bridging basic research and clinical translation, single-cell technologies have been instrumental in defining the diversity of TIL subpopulations within the TME. For instance, Zheng et al. performed scRNA-seq on 316 patients across 21 tumor types to construct a pan-cancer RNA atlas of T cells, enabling a systematic comparison of their heterogeneity and dynamics (Zheng et al. 2021). This study revealed that distinct T-cell subsets correlate with patient-specific characteristics, highlighting potential determinants of the TME and providing a foundation for the rational design of precision T-cell-based immunotherapies. Similarly, Yang et al. profiled TIL-derived B cells from 649 patients across 19 tumor types using single-cell RNA sequencing, generating a comprehensive pan-cancer B-cell RNA atlas (Yang et al. 2024). Their analysis uncovered two tumor-enriched subpopulations—stress-response memory B cells and tumor-associated atypical B cells—with prognostic potential across cancers, providing a foundation for deeper exploration of B-cell diversity and function in oncology.

Not all immune populations, however, exert antitumor activity. Immunosuppressive subsets, such as Tregs, myeloid-derived suppressor cells, and tumor-associated macrophages, can attenuate immune response and promote tumor progression (Bremnes et al. 2016). Single-cell technologies have accelerated research in this domain as well, enabling detailed dissection of the heterogeneity and functional states of these inhibitory populations. For instance, Alvisi et al. employed scRNA-seq and complementary approaches to show that Tregs are hyperactivated in intrahepatic cholangiocarcinoma, resulting in severely restricted immune cell infiltration and consequently, a profoundly compromised antitumor immune response (Alvisi et al. 2022).

10.4.3 Response and Resistance to Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs), specifically anti-PD-1 and anti-CTLA-4 antibodies, act by targeting the dysfunctional immune system and promoting CD8-positive T cells killing cancer cells, thereby revolutionizing the landscape of cancer immunotherapy (Carlino et al. 2021; Wang et al. 2023a). However, patient responses to ICI therapy exhibit significant heterogeneity, which leads to inconsistent clinical outcomes (Dall'Olio et al. 2021). Fortunately, single-cell analyses have been increasingly applied to identify predictive biomarkers of response, such as specific immune cell subsets or the expression patterns of immune checkpoint molecules (Zhang et al. 2021; Yang et al. 2022b; Liu et al. 2022, 2025).

For instance, Liu et al. conducted a meta-analysis of published single-cell datasets and revealed that $CXCL13^+$ $CD8^+$ T cells are positively associated with favorable responses to ICI therapy, with treatment further enriching this subset in responsive tumors (Liu et al. 2022). Zhang et al. performed scRNA-seq on patients with advanced triple-negative breast cancer and identified $CXCL13^+$ $CD8^+$ T cells as key mediators of effective responses to anti-PD-1 therapy. They further showed that paclitaxel treatment diminished the abundance of this T-cell subset, thereby impacting clinical outcomes (Zhang et al. 2021). Collectively, these findings underscore the power of single-cell technologies in resolving immune heterogeneity and advancing the development of robust biomarkers to guide precision immunotherapy.

10.4.4 Predicting Immunotherapy Outcomes Using Single-Cell Data

The predictive potential of single-cell analyses in immunotherapy is immense (Liu et al. 2024). By integrating single-cell sequencing with advanced bioinformatics techniques, it is now possible to forecast patient responses based on the cellular composition and functional states of the immune cells in the TME (Hu et al. 2023).

For example, scRNA-seq and TCR-seq analyses of tumor samples from 234 NSCLC patients treated with neoadjuvant chemo-immunotherapy revealed striking differences in the immune landscape between responders and non-responders (Liu et al. 2025). Samples from patients achieving a major pathological response (MPR) displayed elevated levels of $FGFBP2^+$ NK/NK-like T cells, memory B cells, and effector T cells, whereas non-MPR ones were enriched for $CCR8^+$ regulatory T cells (Liu et al. 2025). In another study, Hu et al. applied scRNA-seq to profile tumors from NSCLC patients receiving neoadjuvant PD-1 blockade combined with chemotherapy, identifying $FCRL4^+FCRL5^+$ memory B cells and $CD16^+CX3CR1^+$ monocytes as predictive biomarkers of immunotherapy response (Hu et al. 2023). Together, these findings underscore how single-cell technologies can explore immune heterogeneity and uncover cellular predictors of immunotherapy efficacy.

10.5 Clinical Applications and Translational Insights

10.5.1 Biomarker Discovery and Patient Stratification

The ability to identify rare cell populations and their associated molecular signatures is crucial for developing effective biomarkers (Passaro et al. 2024). Single-cell technologies have facilitated the identification of biomarkers capable of predicting disease progression, therapeutic response, and relapse (Wang et al. 2023b; Li et al. 2024; Wu et al. 2024; Deng et al. 2024; Dong et al. 2024). For example, through an integrative analysis of bulk, single cell, and spatial transcriptomics validated by fluorescent staining, Li et al. identified PSME2 as a pan-cancer biomarker correlated with M1 macrophage infiltration (Li et al. 2024). In osteosarcoma models, PSME2 overexpression notably suppressed tumor cell proliferation, migration, and invasion (Li et al. 2024). These results indicate that PSME2, reflecting M1 macrophage infiltration, could serve both as a prognostic marker and a potential therapeutic target.

Moreover, single-cell profiling can be used to stratify patients based on their tumor's cellular makeup. For instance, a recent large-scale scRNA-seq analysis of normal breast tissue and primary breast tumors showed that, relative to normal epithelial cells, luminal progenitor cells display increased sensitivity to neoadjuvant chemotherapy, PARP inhibitors, and immunotherapy (Gao et al. 2024). Additionally, the study conducted both in vitro and in vivo validation of PLK1 as an LP-subtype-specific therapeutic target, thereby laying the foundation for precise prognosis and patient stratification in breast cancer (Gao et al. 2024).

10.5.2 Monitoring Minimal Residual Disease

Minimal residual disease (MRD) represents the minute fraction of malignant cells that escape eradication following therapy and lie beyond the detection limits of existing imaging and screening approaches (Pantel and Alix-Panabières 2019; Costa et al. 2022). Detecting MRD is closely linked to subsequent cancer recurrence and can provide an early warning period of months before clinical relapse is apparent (Medford et al. 2023). Consequently, the study of MRD carries significant clinical implications for early intervention and improved patient management. Due to their sensitivity, single-cell technologies have become powerful tools for detecting and characterizing MRD.

Because MRD is routinely applied in the clinical management of hematologic malignancies—such as leukemia, lymphoma, and multiple myeloma—single-cell technologies have been more widely investigated in these cancers (Ledergor et al. 2018; Cui et al. 2024; Zhang et al. 2022). For example, Zhang et al. employed scRNA-seq in combination with B-cell receptor sequencing to investigate B-cell acute lymphoblastic leukemia and discovered that inhibition of hypoxia signaling pathways sensitizes leukemic cells to chemotherapy (Zhang et al. 2022). Furthermore, MRD-related studies in solid tumors are equally important. For

instance, Lemaitre et al. applied single-cell spatial transcriptomics to hepatocellular carcinoma patients and revealed that PD-L1$^+$ macrophages maintain MRD by activating TGFβ signaling in stem-like cancer cells, indicating that disrupting this interaction could prevent MRD-driven HCC relapse (Lemaitre et al. 2024). Together, these studies illustrate how single-cell technologies are reshaping MRD research and hold the promise to enable earlier detection, deeper mechanistic insight, and more effective intervention strategies across both hematologic and solid tumors.

10.5.3 Single-Cell-Based Precision Oncology

Progress in single-cell sequencing is transforming cancer research and is expected to become a cornerstone of translational oncology (Melnekoff and Laganà 2022). By enabling the profiling of individual tumor cells, single-cell analysis reveals key mutations, gene-expression patterns, and drug sensitivities that are specific to each patient's tumor (Ding et al. 2022; Krishna et al. 2021; Franken et al. 2024; Valdes-Mora et al. 2018). These insights hold substantial promise for precision oncology, guiding the development of personalized treatment regimens.

For instance, Sinha et al. leveraged publicly available bulk and single-cell expression datasets from large-scale drug screens to build a computational pipeline, PERCEPTION, tailored for precision oncology (Sinha et al. 2024). This framework accurately predicted drug responses in cultured cell lines and patient-derived primary tumor cells and was further validated in clinical trials for multiple myeloma and breast cancer. Importantly, it also captured the emergence of resistance in lung cancer patients treated with tyrosine kinase inhibitors. This study represents how single-cell omics can be leveraged to stratify patients, anticipate therapeutic resistance, and inform clinical decision-making.

10.5.4 Single-Cell Profiling in Circulating Tumor Cells

Circulating tumor cells (CTCs) are rare cancer cells that have slough off from the primary tumor into the bloodstream to circulate, and their presence is associated with metastasis (Hu et al. 2023). By using single-cell profiling for CTCs, researchers can track the evolution of metastatic disease, evaluate metastatic potential, identify markers for early detection of relapse, and help clinicians for treatment personalization (Pailler et al. 2019; Lei et al. 2021; Zhu et al. 2018; Zhang et al. 2024; Lin et al. 2021).

For example, Liu et al. performed scRNA-seq on CTCs, primary tumors, and metastatic lesions from patients with pancreatic ductal adenocarcinoma, revealing that platelet-derived RGS18 protects CTCs from NK cell-mediated immune surveillance through engagement of the HLA-E:CD94-NKG2A immune checkpoint axis. This study represents a paradigm for understanding how CTCs evade immune surveillance (Liu et al. 2023b). Similarly, Sun et al. conducted scRNA-seq across CTCs from multiple cancer types and discovered that CTCs primarily evade NK

cell-mediated innate immune surveillance through CD155–TIGIT immune checkpoint interactions (Sun et al. 2025). Together, these studies highlight the critical role of single-cell analysis of CTCs in uncovering mechanisms of immune evasion and guiding the development of targeted therapies for metastatic cancer.

10.6 Perspectives

10.6.1 Technical Limitations and Scalability Issues

Despite the transformative potential, single-cell technologies face several technical hurdles that limit their broader application in oncology. A major challenge lies in sample quality, as single cells need to be isolated rapidly and accurately with minimal damage. Mechanical dissociation can compromise cell integrity, while enzymatic digestion used to release single cells from tissue may alter cell viability or transcriptional states (Ding et al. 2020). Furthermore, technical noise introduced during library preparation, amplification, or sequencing can obscure true biological variation, complicating downstream interpretation (Hwang et al. 2018).

Beyond technical precision, scalability represents another critical bottleneck. Current single-cell platforms remain resource-intensive in both cost and computational demand, restricting their application in large patient cohorts or longitudinal clinical trials (Li et al. 2020). The high expenses associated with sequencing depth and multimodal profiling further impede their integration into routine diagnostics. Overcoming these challenges will require innovations in microfluidic technologies, cost-efficient library preparation, and high-throughput automation pipelines that can lower costs while preserving accuracy (Ziegenhain et al. 2017). Without such improvements, the scalability of single-cell technologies in real-world clinical settings will remain a formidable barrier.

10.6.2 Standardization and Reproducibility in Clinical Settings

The clinical application of single-cell technologies remains limited due to the absence of standardized experimental and analytical procedures (Lähnemann et al. 2020). Differences in tissue handling, dissociation protocols, sequencing depth, and data processing pipelines often yield divergent outcomes (Mereu et al. 2020). Such variability can obscure biological signals and reduce reproducibility across laboratories. The absence of universally accepted reference standards makes it difficult to benchmark emerging methods, slowing the pace of clinical adoption.

To overcome these barriers, harmonization of protocols and quality control frameworks is urgently needed (You et al. 2024). Establishing standardized guidelines for tissue collection, preservation, sequencing, and data analysis will ensure comparability across studies and clinical centers. Consistent quality control and regulatory frameworks will be essential for integrating single-cell profiling into precision oncology and for building the trust necessary to apply these tools in clinical decision-making.

10.6.3 Data Privacy and Ethical Concerns in Single-Cell Cancer Data

The generation of high-resolution single-cell datasets raises important challenges in data governance and ethics (Wang et al. 2023c). Because these datasets contain highly detailed genetic information, they pose a risk of patient re-identification. Robust frameworks for data encryption, secure storage, and controlled sharing will therefore be essential to protecting patient privacy (Gürsoy et al. 2020; Tadi et al. 2024).

Beyond privacy, broader ethical considerations must also be addressed. The use of patient-derived samples necessitates transparent and comprehensive informed consent processes, explicitly outlining potential risks of tissue collection, intended use of the data, and policies for data sharing within the research community (Lee et al. 2024). Addressing these issues will require a multidisciplinary effort, uniting oncologists, ethicists, policymakers, and patient advocates to establish ethical and legal frameworks that protect patient rights and sustain public trust while fostering scientific innovation.

10.6.4 Future Perspectives: Organoid Modeling and AI Integration

Future directions in single-cell cancer research will increasingly rely on the convergence of advanced experimental platforms and computational innovations. Organoid models, when integrated with spatial multi-omics technologies, provide unprecedented spatial and functional insights into tumor ecosystems (Passaro et al. 2024; Fukushima et al. 2025). These integrative approaches enhance the precision of biomarker-driven drug development and help overcome challenges posed by intratumoral heterogeneity, ultimately enabling the design of more personalized and effective therapeutic strategies (Passaro et al. 2024).

In parallel, rapid progress in artificial intelligence, particularly with large language models (Ding et al. 2024), has begun to reshape single-cell data analyses. The intersection of single-cell profiling and large language models offers intuitive, user-friendly tools that reduce technical barriers, allowing researchers without extensive computational expertise to explore and interpret complex single-cell datasets with greater efficiency (Ding et al. 2024; Yang et al. 2022c; Hou and Ji 2024).

Looking ahead, single-cell technologies are poised to transform both cancer research and precision oncology (Chan et al. 2021; Li et al. 2023). By integrating state-of-the-art experimental approaches with advanced computational models, these tools will deepen our understanding of tumor heterogeneity, tumor–microenvironment interactions, and therapeutic resistance. Ultimately, the integration of single-cell profiling into clinical practice holds the promise to move oncology from generalized treatment paradigms toward truly individualized strategies that anticipate disease trajectories and optimize therapeutic outcomes.

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