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Single-cell sequencing and spatial transcriptomics: a new paradigm for understanding the cancer ecosystem

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Abstract

Cancer progression and treatment failure are profoundly influenced by the complex ecosystem of tumors, characterized by extensive cellular heterogeneity and a spatially organized microenvironment. Single-cell sequencing has revealed unprecedented diversity in malignant, immune, and stromal tumor populations, but lacks anatomical context. In contrast, spatial transcriptomics can map gene expression within tissue architecture, but typically at low cellular resolution. Integrative approaches now merge these advantages, enabling precise characterization of clonal dynamics, cellular communication, and the ecological niches that underlie metastasis and therapeutic resistance. This integrative approach provides a multidimensional view of tumor biology, highlighting context-dependent mechanisms of drug vulnerability and immune evasion. In this review, we explore how single-cell and spatial technologies are reshaping our understanding of the cancer ecosystem, integrating these two advanced technologies and advancing our progress toward spatially based precision oncology.

KEYWORDS

Single-cell sequencing; Spatial transcriptomics; Intratumoral heterogeneity; Tumor microenvironment; Immune evasion

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Introduction

Intratumoral heterogeneity (ITH) has emerged as a fundamental hallmark of cancer, profoundly shaping tumor evolution, therapeutic resistance, and disease recurrence. Genetic mutations, epigenetic alterations, metabolic reprogramming, and dynamic interactions with stromal and immune components collectively generate a mosaic of malignant subpopulations within a single tumor(1, 2). This complexity drives Darwinian selection, enabling specific clones to survive selective pressures imposed by chemotherapy, radiotherapy, or targeted agents, ultimately resulting in relapse or metastatic dissemination. Consequently, deciphering the architecture of ITH has become indispensable for understanding cancer progression and improving therapeutic outcomes. Traditional bulk omics and large-scale sequencing approaches have provided critical insights into the genomic, transcriptomic, and epigenomic landscapes of cancer(3). However, they rely on averaged signals derived from millions of cells, masking cellular diversity and spatial context. Such methods cannot delineate rare malignant clones, nor do they capture the interplay between malignant and non-malignant cells. Furthermore, tissue dissociation required for conventional sequencing eliminates the structural organization of the tumor microenvironment (TME), precluding analysis of how spatial arrangements of cells influence cancer behavior(4, 5). These limitations underscore the need for novel technologies capable of resolving cancer biology at both single-cell and tissue-architecture levels.

The advent of single-cell sequencing has transformed cancer research by enabling high-resolution analysis at the individual cell level. Tumor tissues are dissociated into single-cell suspensions and labeled with barcoded beads to generate gene expression profiles for each cell. Subsequent analyses of intratumoral heterogeneity, immune microenvironments, and mechanisms underlying drug resistance or metastasis have produced extensive datasets, offering a comprehensive and dynamic view of cancer biology at single-cell resolution(6, 7). Despite these advances, single-cell RNA sequencing (scRNA-seq) disrupts tissue architecture, resulting in the loss of crucial spatial context required to interpret intercellular communication(8). Spatial transcriptomics overcomes this limitation by capturing gene expression while preserving the native tissue structure. In this approach, tumor sections are permeabilized on glass slides, and released mRNAs are captured by spatially barcoded probes. Sequencing libraries are then constructed and analyzed using high-throughput sequencing. Bioinformatic reconstruction subsequently maps gene expression back to spatial coordinates within the tissue, enabling visualization and quantification of the entire transcriptome in its anatomical context(9-11). This approach uncovers intratumoral heterogeneity, microenvironmental organization, and cellular interactions with unprecedented clarity. The integration of single-cell sequencing with spatial transcriptomics has further enhanced resolution, allowing precise delineation of cellular niches, spatial gradients, and communication networks within tumors(12). Collectively, these innovations have ushered cancer research into a new era of multidimensional precision and refined biological insight (Figure 1). This review summarizes recent advances in the application of single-cell and spatial transcriptomic technologies in oncology, emphasizing their roles in decoding tumor heterogeneity, mapping cellular interactions, and identifying therapeutic vulnerabilities. By providing a comprehensive synthesis, it underscores the transformative potential of these technologies to reshape our understanding of the tumor ecosystem and guide the development of next-generation therapies.

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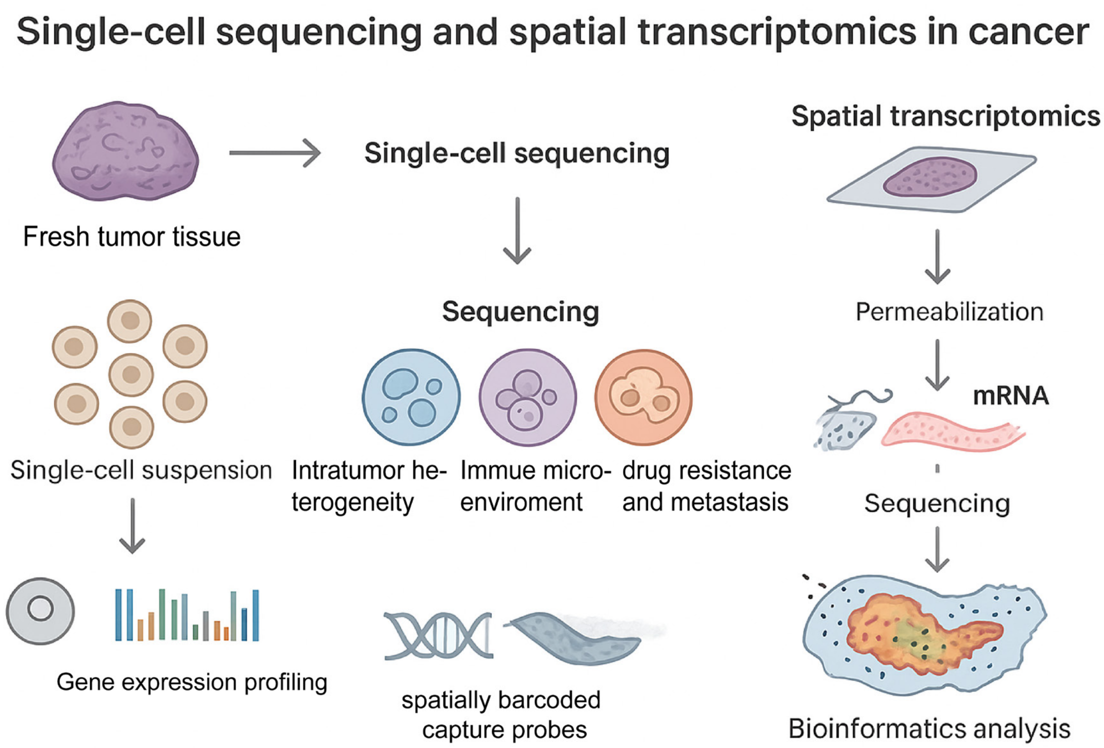

FIGURE 1

Single-cell sequencing and spatial transcriptomics workflow in cancer.

Fresh tumor tissue can be processed into single-cell suspensions for single-cell RNA sequencing, followed by barcoding, library preparation, and sequencing to obtain gene expression profiles of cell types. It can also be processed into tissue sections on spatially barcoded slides for spatial transcriptomics, where permeabilization captures mRNA followed by sequencing and bioinformatics analysis. These datasets can be integrated to interrogate intratumor heterogeneity, the immune microenvironment, and features related to drug resistance and metastasis.

Single-cell sequencing in cancer

Traditional cancer research has relied heavily on bulk genomic and transcriptomic profiling, which has provided critical insights into oncogenic drivers and molecular subtypes(13). However, such approaches average signals across diverse cell populations, masking rare yet functionally significant subclones. The inability to resolve cellular diversity has constrained our understanding of tumor evolution, adaptation to therapeutic stress, and interactions with the surrounding microenvironment. Overcoming these limitations requires technologies capable of capturing molecular variation at single-cell resolution. Single-cell sequencing has emerged as a transformative tool to meet this need. It has revealed profound intratumoral heterogeneity, with studies in liver, breast, and colorectal cancers identifying distinct cancer cell subtypes and their transcriptional profiles(14-16). This approach provides a fine-grained depiction of cellular diversity, uncovering mutations and transcriptional programs that drive malignant progression. By isolating and profiling individual cells, scRNA-seq distinguishes discrete transcriptional states within the same tumor, ranging from

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proliferative to drug-tolerant phenotypes(17). These discoveries have reshaped our understanding of clonal evolution, cellular plasticity, and therapy resistance. Beyond malignant compartments, scRNA-seq has mapped the complex immune landscape of tumors, revealing the heterogeneity of T cell exhaustion, macrophage polarization, and stromal remodeling(18). Collectively, these findings underscore the pivotal role of cellular diversity not only within cancer cells but also across the broader tumor ecosystem.

The application of single-cell approaches has also facilitated reconstruction of lineage hierarchies and developmental trajectories in cancer. Pseudotime analyses have mapped dynamic transitions from stem-like states to differentiated phenotypes, providing insights into tumor progression and relapse(19). Beyond transcriptomics, advances in single-cell multi-omics have extended this framework to integrate genomic alterations, chromatin accessibility, and protein expression(20). Such integration provides a more holistic perspective on the mechanisms underpinning intratumoral diversity and drug response. For example, linking somatic mutations with transcriptional states at the single-cell level has clarified how genetic lesions translate into functional heterogeneity(21). Similarly, combining epigenetic and proteomic measurements has deepened understanding of regulatory networks that control tumor plasticity(22). Collectively, single-cell sequencing has transformed cancer research by providing unprecedented resolution of tumor complexity. It has shifted the paradigm from population-level descriptions toward single-cell precision, enabling a more accurate understanding of disease mechanisms. Continued technological innovations and analytical frameworks are expected to further expand its utility, bridging basic discoveries with translational opportunities in oncology.

Core technology

Single-cell sequencing technologies are grounded in the principle of isolating individual cells and capturing their molecular content for high-resolution analysis. Unlike bulk sequencing, which generates averaged signals from heterogeneous populations, single-cell methods preserve cellular individuality(23). The typical workflow begins with single-cell isolation through microfluidic platforms, droplet-based systems, or well-based approaches. RNA or DNA of each cell is then barcoded during library preparation, enabling subsequent sequencing to trace molecular reads back to their cell of origin. This barcoding strategy forms the foundation of single-cell profiling, ensuring accurate reconstruction of cellular heterogeneity(24).

Building on this principle, a wide range of derivative technologies has been developed to expand the scope of single-cell analysis. scRNA-seq remains the most widely applied approach, offering transcriptome-wide coverage at unprecedented resolution. Variants such as Smart-seq provide full-length transcript capture, whereas droplet-based platforms like 10x Genomics Chromium prioritize high throughput(25). Beyond transcriptional profiling, single-cell ATAC-seq has enabled exploration of chromatin accessibility, uncovering regulatory landscapes that shape gene expression(26). Similarly, single-cell DNA sequencing has facilitated detection of somatic mutations and clonal architecture in tumors(27). More recently, integrative multi-omics approaches, including CITE-seq and scNMT-seq, have allowed simultaneous measurement of RNA, protein epitopes, epigenetic modifications, or methylation patterns within the same cell(28). These innovations have considerably broadened the interpretive power of single-cell research.

The downstream analysis of single-cell data is equally critical to extracting meaningful biological insights. After sequencing, raw reads undergo quality control, alignment, and barcode demultiplexing to generate expression matrices. Normalization strategies are then applied to reduce technical variability, followed by dimensionality reduction methods such as principal component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), or uniform manifold approximation and projection (UMAP). These steps enable visualization of cellular relationships and the identification of distinct clusters(29, 30). Clustering algorithms subsequently partition cells into transcriptionally defined populations, which can be annotated based on known marker genes. Advanced analyses include trajectory inference to reconstruct developmental lineages, gene regulatory network modeling to identify transcriptional drivers, and integration of multi-omics data to achieve syste(31)ms-level understanding. Increasingly, machine learning frameworks are being incorporated to handle the complexity and scale of single-cell datasets, facilitating robust classification and prediction(32, 33).

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Collectively, the core technologies of single-cell sequencing combine innovative isolation platforms, versatile profiling strategies, and sophisticated computational pipelines. This integration allows unprecedented characterization of tumor ecosystems, from decoding clonal diversity to mapping immune cell dynamics. Continued advances in both wet-lab protocols and computational methods will further enhance resolution, scalability, and accuracy, thereby consolidating the role of single-cell sequencing as a transformative tool in cancer research.

Application of single-cell sequencing in cancer

Single-cell sequencing reveals ITH in cancer

ITH manifests as clonal differences in tumor cell subpopulations and is a key factor in drug resistance. Single-cell sequencing can examine the genome, transcriptome, and epigenome at the individual cell level, revealing cellular heterogeneity lost in traditional mixed sample sequencing, revolutionizing our understanding of tumor genetic heterogeneity and the complexity of tumor progression. A critical aspect of ITH lies in the identification of distinct subclonal populations. Single-cell DNA sequencing allows precise reconstruction of clonal phylogenies, tracing the accumulation of somatic mutations across evolutionary lineages. Studies using this approach have uncovered branching evolutionary trajectories, where multiple subclones coexist and compete within the same lesion. Some of these subclones harbor driver mutations that confer growth advantages or therapeutic resistance, underscoring the importance of resolving clonal hierarchies. In various cancers, including breast, prostate, and liver cancer, single-cell technologies have successfully identified subclonal populations harboring unique copy number variations (CNAs), driver mutations, or phenotypic markers(34-36). In addition, single-cell sequencing can also identify subclonal functional heterogeneity in cancer, such as the differences in subclonal transcriptional profiles associated with platinum resistance in ovarian cancer and the high expression of the resistance gene TUBB3 in specific subclones in prostate cancer(37, 38). Such insights are vital for predicting tumor progression and for designing strategies aimed at eradicating resistant clones.

Beyond genetic heterogeneity, scRNA-seq has been pivotal in uncovering the transcriptional complexity of malignant cells. Studies have demonstrated the coexistence of proliferative, quiescent, and drug-tolerant states within a single tumor, reflecting dynamic adaptation to environmental stress(39). This high-resolution approach has enabled the identification of rare subpopulations with stem-like properties that frequently drive relapse after therapy. In esophageal cancer, cancer stem cells are recognized as critical mediators of tumor initiation and progression. By integrating scRNA-seq with bulk RNA-seq data, researchers have identified a cancer stem cell gene signature and developed a prognostic tumor stem cell marker signature (TSCMS) model(40). This strategy deepens understanding of the heterogeneity among cancer stem cell subpopulations and elucidates their mechanisms of drug resistance. Moreover, scRNA-seq has delineated cellular plasticity within malignant populations, illustrating how transitions between phenotypic states enhance metastatic potential(41). At the genomic level, single-cell DNA sequencing has clarified clonal evolution by reconstructing phylogenetic relationships among tumor cells. These analyses reveal the coexistence of dominant clones alongside multiple subclonal lineages, each characterized by distinct mutational landscapes. Such a framework offers mechanistic insights into how selective pressures, including therapeutic intervention, shape tumor evolution over time.

Importantly, ITH extends beyond malignant cells to encompass the TME. Single-cell approaches have revealed remarkable diversity among infiltrating immune cells, such as subsets of exhausted T cells, immunosuppressive macrophages, and functionally distinct natural killer (NK) cells. These immune compartments not only reflect tumor adaptation but also represent potential therapeutic targets. Understanding this cellular interplay has become essential for designing strategies that overcome resistance to immunotherapy. By uncovering hidden cellular diversity and clarifying evolutionary dynamics, this technology provides critical insights into mechanisms of treatment failure and recurrence.

Research on the immune microenvironment

Single-cell sequencing has provided unprecedented insights into the immune microenvironment of tumors, a key

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determinant of disease progression and therapeutic response. One of the most significant contributions of single-cell studies lies in the characterization of T cell exhaustion. Exhausted T cells display diminished effector function, sustained expression of inhibitory receptors, and altered transcriptional landscapes. Single-cell transcriptomic analyses have delineated progressive stages of exhaustion, ranging from progenitor-like to terminally dysfunctional phenotypes. These findings not only inform the mechanisms of immune evasion but also guide the development of checkpoint blockade strategies that aim to reinvigorate dysfunctional T cells. Multiple studies have analyzed the spatial distribution characteristics of exhausted subpopulations through single-cell sequencing and showed that in esophageal cancer and pancreatic cancer, exhausted CD8⊠ T cells (Tex) and regulatory T cells (Treg) are significantly enriched in tumor tissues and show a specific developmental trajectory(42, 43); there are 6 T cell subpopulations in ovarian cancer, and the degree of exhaustion is related to prognosis(44).

Tumor-associated macrophages (TAMs) represent another central focus of single-cell research. Rather than conforming to a simple M1–M2 dichotomy, TAMs display a spectrum of activation states with diverse roles in promoting angiogenesis, remodeling extracellular matrices, and suppressing cytotoxic immunity. Single-cell sequencing has mapped TAM subsets with unique transcriptional signatures and spatial distributions, thereby revealing their contribution to immune suppression and tumor progression. In ovarian cancer, scRNA-seq analysis revealed tumor cell heterogeneity and the infiltration of M2 TAMs within the TME(45). Combined with bulk RNA sequencing data, this underscores the critical role of TAM subpopulations in tumor progression. In breast cancer, scRNA-seq has identified multiple macrophage subpopulations, including specific subsets in both normal and tumor tissues(46). These subpopulations are involved in mammary gland development and tumorigenesis, providing a detailed understanding of the diversity and functional differentiation of TAMs. These discoveries underscore the necessity of targeting specific macrophage populations rather than broadly depleting the myeloid compartment.

NK cells, traditionally viewed as rapid responders against transformed cells, have also been redefined by single-cell approaches. Profiling NK cells within tumors has uncovered populations with impaired cytotoxicity, reduced cytokine production, and altered receptor expression, indicating functional exhaustion analogous to that observed in T cells. Within the breast cancer microenvironment, scRNA-seq identified six NK cell subsets with distinct functional states in ER+/HER2- and triple-negative breast cancers(47). In lung cancer, single-cell analysis revealed states of NK cell exhaustion and their association with reduced cytotoxic gene expression(48). Such insights have illuminated the mechanisms underlying NK cell dysfunction in solid tumors and have suggested avenues for restoring their antitumor activity. Collectively, single-cell sequencing has restructured understanding of the tumor immune microenvironment by resolving the heterogeneity of T cells, macrophages, and NK cells.

Mechanisms of drug resistance and metastasis

Conventional bulk analyses often mask rare but clinically relevant resistant clones, whereas single-cell approaches capture these populations in real time and trace their evolutionary dynamics. This level of resolution has provided compelling evidence that therapeutic resistance arises not only from genetic mutations but also from transcriptional plasticity and epigenetic reprogramming. By profiling tumor cells before and after treatment, single-cell studies have identified pre-existing resistant subpopulations that expand under selective pressure. These findings illustrate how a minor fraction of cells with distinct transcriptional states can survive therapy and eventually repopulate the tumor. In drug-resistant lung adenocarcinoma cells and tumor spheroids, single-cell sequencing revealed suppression of cell cycle–related pathways coupled with activation of metabolic and survival signaling, including TGF-β and MAPK cascades(49). Similarly, scRNA-seq analysis in bladder cancer detected elevated SLC1A6 expression in treatment-resistant cases, whose prognostic relevance was subsequently confirmed in a lung cancer cohort(50). Trajectory inference methods have further reconstructed the evolutionary lineages of resistant clones, demonstrating gradual transitions from drug-sensitive to drug-tolerant states. Collectively, these observations indicate that resistance evolves as a dynamic continuum shaped by both intrinsic cellular adaptations and extrinsic cues from the TME.

Single-cell approaches have also deepened understanding of metastatic dissemination. Comparative sequencing of

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primary and metastatic lesions has revealed subclones with distinct migratory or invasive signatures, as well as transcriptional programs that facilitate colonization of distant organs. In colorectal cancer, integration of single-cell and bulk sequencing has delineated the evolutionary trajectories underlying liver metastasis(51). In pancreatic cancer, single-cell sequencing uncovered that disseminated cells modulate T cell function through CITED4 upregulation, promoting hepatic colonization in pancreatic ductal adenocarcinoma and underscoring the contribution of the immune microenvironment to metastatic spread(52). Incorporation of spatial transcriptomic data has further clarified how interactions with stromal and immune compartments establish premetastatic niches. Notably, single-cell analyses have captured intermediate cell states with hybrid epithelial–mesenchymal features, highlighting the plasticity that drives metastatic progression. Moreover, joint genomic and transcriptomic profiling at single-cell resolution has illuminated how mutational landscapes intersect with transcriptional states to promote resistance and metastasis(53). This integrative framework provides mechanistic insight into the coupling of oncogenic alterations with adaptive gene expression programs, emphasizing the need to monitor evolutionary trajectories rather than rely solely on static molecular profiles.

Spatial transcriptomics in cancer

Spatial transcriptomics has emerged as a powerful complement to single-cell sequencing by preserving the spatial architecture of tissues while capturing gene expression patterns. In cancer biology, this capacity is particularly valuable because tumor behavior is not solely determined by the intrinsic properties of malignant cells but also by their interactions with stromal and immune components. Traditional methods such as laser-capture microdissection or immunohistochemistry provide partial information about spatial organization but lack genome-wide coverage and quantitative depth(54). In contrast, spatial transcriptomic platforms allow comprehensive profiling of transcriptomes in situ, thereby bridging the gap between molecular data and histological context.

The application of spatial transcriptomics in oncology has revealed the importance of microenvironmental cues in shaping tumor evolution. Cancer ecosystems comprise malignant clones, infiltrating immune cells, fibroblasts, and vascular elements that interact through signaling networks(55). Resolving these interactions requires technologies that not only detect transcriptional states but also map their positions within tissue landscapes. Spatial transcriptomics fulfills this need by enabling the identification of functional niches, gradients of signaling molecules, and cellular communities that drive disease progression. Moreover, the ability to integrate spatial data with single-cell sequencing has transformed the study of tumor heterogeneity. While dissociative single-cell methods excel at capturing transcriptional diversity, they fail to retain information about cellular neighborhoods and structural context. Spatial approaches restore this dimension, providing insights into how clonal dynamics, immune evasion, and therapeutic resistance are influenced by local microenvironments(56). These integrated datasets have already demonstrated that tumors with similar mutational profiles can display strikingly different spatial ecosystems, which in turn determine clinical outcomes.

As technologies advance, spatial transcriptomics continues to increase in resolution, sensitivity, and scalability. This progress promises to deliver unprecedented views of tumor organization, from large-scale tissue architecture to subcellular transcript localization. By placing molecular profiles into their native spatial context, these approaches are reshaping the understanding of how cancer ecosystems emerge, adapt, and respond to therapy. Ultimately, spatial transcriptomics offers a foundation for the development of precision oncology strategies that exploit not only genetic vulnerabilities but also the spatial logic of tumor biology.
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Core technology

Spatial transcriptomics has emerged as a transformative

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platform in cancer biology, offering transcriptomic profiling while preserving the spatial context of tissues. Unlike single-cell sequencing, which dissociates cells and disrupts native architecture, spatial approaches maintain the in situ organization of malignant and stromal compartments. This capability enables the reconstruction of tumor ecosystems with high fidelity and provides crucial insights into how microenvironmental cues influence cancer progression. By resolving the TME at high resolution, spatial transcriptomics elucidates interactions between cancer cells and surrounding immune or stromal populations, advancing understanding of metastasis, therapeutic response, and prognosis(57). In ovarian cancer, for instance, spatial transcriptomic analyses have revealed the cellular composition of the TME and the complexity of intercellular communication, both of which correlate with disease progression and treatment efficacy(58).

Among representative platforms, Visium (10x Genomics) has achieved widespread adoption due to its accessibility and compatibility with existing sequencing pipelines. It employs barcoded capture spots on glass slides, enabling transcriptome-wide profiling at moderate resolution, typically encompassing several to dozens of cells per spot. Although not at single-cell precision, Visium offers high sensitivity and genome-wide coverage, making it ideal for large-scale tissue mapping with robust molecular annotation(59). In contrast, Slide-seq achieves higher spatial resolution by using DNA-barcoded beads, each representing a defined spatial coordinate(60). This design enables transcript detection at near-cellular resolution and refines the delineation of heterogeneous niches within tumors. However, limited transcript capture per bead results in lower sensitivity, often requiring deeper sequencing to achieve adequate coverage.

High-definition spatial transcriptomics (HDST) further enhances spatial resolution, approaching subcellular levels through smaller barcoded capture spots. This fine-grained mapping allows visualization of transcript distribution near individual organelles, revealing intricate patterns of cellular interaction(61). Despite this precision, HDST faces sensitivity challenges, particularly in detecting low-abundance transcripts. The trade-off between resolution and sensitivity remains a critical consideration in experimental design, as HDST favors ultra-detailed spatial mapping at the expense of transcript yield. Beyond capture-based methods, in situ hybridization–based technologies such as multiplexed error-robust fluorescence in situ hybridization (MERFISH) offer single-molecule resolution with exceptional sensitivity. MERFISH uses combinatorial labeling and error-correction codes to image thousands of transcripts simultaneously within intact tissues. Unlike capture-array platforms, MERFISH directly visualizes individual transcripts in their native subcellular locations, providing unmatched precision in spatial gene expression analysis(62). However, its reliance on preselected gene panels limits scalability for whole-transcriptome discovery.

Comparative evaluation of these technologies reveals a continuum of trade-offs: Visium provides comprehensive transcriptome coverage with modest resolution; Slide-seq and HDST deliver higher spatial precision but reduced sensitivity; MERFISH achieves molecular-level detail at the cost of genomic breadth. The optimal platform depends on experimental objectives—whether mapping global expression landscapes, defining fine-grained spatial niches, or visualizing individual molecules. Collectively, these complementary technologies constitute the methodological foundation of spatial transcriptomics. Their integration enables multiscale dissection of tumor architecture, advancing understanding of intratumoral heterogeneity, microenvironmental regulation, and therapeutic vulnerability in cancer.

Application of spatial transcriptomics in cancer

Spatial transcriptomics reveals ITH in cancer

Spatial transcriptomics has provided unique opportunities to dissect ITH, a fundamental feature of cancer that drives progression, therapeutic resistance, and recurrence. Unlike dissociative single-cell approaches, spatial methods preserve the native architecture of tumors, making it possible to compare transcriptional programs across distinct regions such as the core and invasive edge. This spatial perspective has revealed how malignant subpopulations evolve within heterogeneous niches and how their interactions with the surrounding microenvironment shape disease trajectories. In hepatocellular carcinoma, spatial transcriptomics combined with single-cell sequencing has clarified the distribution characteristics of endothelial cells in different tumor regions (such as the core, invasion front, and stroma)(63). It has also

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verified the spatial expression patterns of collagen deposition and molecules such as POSTN (periostin), suggesting that endothelial cells participate in TME remodeling(64). One striking observation from spatial analyses is the transcriptional divergence between tumor cores and margins(65). Cells located in the central regions often exhibit hypoxia-associated gene expression, reduced proliferation, and stress-adaptive metabolic states. In contrast, cells at the invasive front display signatures of epithelial–mesenchymal transition, enhanced migratory potential, and dynamic interactions with immune or stromal components. This gradient underscores the adaptability of malignant populations and highlights the role of spatial positioning in dictating functional heterogeneity. In an analysis of oral squamous cell carcinoma, gene pathways related to the cell cycle and hypoxic stress were enriched in the TC region, indicating that cells in the core region are highly proliferative and hypoxic-adapted(66).

Moreover, spatial transcriptomics has illuminated how microenvironmental cues reinforce regional differences. The core frequently harbors necrotic areas and limited vascularization, imposing metabolic constraints that select for resilient cell states(67). At the periphery, interactions with fibroblasts, endothelial cells, and infiltrating immune populations foster phenotypes conducive to invasion and dissemination. Such findings demonstrate that ITH cannot be fully understood without considering the tissue landscape in which malignant cells reside. Integration of spatial transcriptomic data with mutational profiles has further clarified the interplay between genetic and non-genetic heterogeneity(68). While clonal mutations often establish the foundation of tumor evolution, spatially resolved analyses reveal that microenvironmental pressures dictate which transcriptional programs are activated. This layered view of ITH has been particularly informative for identifying subclones that dominate metastatic progression or persist after therapy. Collectively, spatial transcriptomics has advanced the analysis of ITH by linking cellular states to their precise locations within tumors. As methodologies achieve higher resolution and sensitivity, spatial analysis will continue to refine the understanding of ITH and uncover therapeutic vulnerabilities that remain invisible to conventional approaches.

Spatial interactions between immune cells and tumor cells

Spatial transcriptomics has profoundly advanced the understanding of how immune cells interact with tumor cells within their native tissue environment. The immune landscape of cancer is highly heterogeneous, shaped not only by the presence of distinct immune subsets but also by their physical proximity and functional communication with malignant populations. Conventional single-cell sequencing delineates immune diversity but fails to capture the spatial architecture that dictates immune surveillance or suppression. By preserving positional information, spatial approaches reveal how immune–tumor cell interactions orchestrate disease progression and therapeutic outcomes. In a study of pancreatic ductal adenocarcinoma, spatial transcriptomics combined with single-nucleus RNA sequencing assessed the spatial variation of immune cells in primary tumors and liver metastasis, showing that immune cells have different spatial patterns in metastatic lesions. In primary CNS lymphoma of the brain, spatial transcriptomics revealed the spatial heterogeneity of malignant B cell clones and the location of T cells upregulating immune checkpoint molecules (such as PD-1) in the TME, indicating the spatial origin of immune escape signals(69). One major contribution of spatial transcriptomics has been the delineation of immune exclusion versus infiltration patterns. Certain tumors exhibit dense immune cell clusters at the invasive margin but limited penetration into the core, indicating structural or molecular barriers that hinder effective antitumor immunity(70). Mapping these patterns has identified localized expression of chemokines, extracellular matrix components, and checkpoint ligands that confine immune cells to peripheral zones. Such insights explain why tumors with similar immune compositions may differ dramatically in responsiveness to immunotherapy.

Spatial analyses have further clarified the role of immune niches within tumor ecosystems. Cytotoxic T lymphocytes positioned adjacent to malignant cells frequently display exhaustion-associated transcriptional profiles, whereas those located in distal niches retain cytolytic capacity(71). Tumor-associated macrophages also occupy spatially defined regions where they either promote angiogenesis or suppress T cell function. In gastric cancer, spatial transcriptomics has characterized immune populations within tertiary lymphoid structures (TLS), demonstrating that their phenotypic and spatial heterogeneity depend on tumor localization and shape the immune microenvironment(72). These findings underscore that immune cell function is closely linked to

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spatial positioning. Moreover, integration of spatial transcriptomics with imaging-based methods has identified microdomains where NK cells, dendritic cells, and regulatory T cells cluster around tumor islets(73). These assemblies represent specialized communication hubs that either facilitate immune evasion or amplify antitumor responses. Deciphering such arrangements offers crucial insight for therapeutic innovation, including strategies that disrupt immunosuppressive niches or reposition effector cells toward tumor cores. Overall, spatial transcriptomics has redefined the study of immune–tumor cell interactions by connecting transcriptional states to their precise anatomical coordinates. This spatially resolved perspective elucidates the heterogeneity underlying variable treatment outcomes and guides the rational design of immunotherapies that harness spatial dynamics to restore effective antitumor immunity.

Integration of single-cell and spatial transcriptomics

The integration of single-cell sequencing with spatial transcriptomics has opened new avenues for decoding cancer ecosystems at multiple dimensions. Each technology provides unique strengths yet carries inherent limitations. Single-cell sequencing excels at resolving transcriptional heterogeneity with high sensitivity, capturing subtle states within malignant, stromal, or immune populations. However, the dissociative process disrupts spatial context and masks the microanatomical organization of these cells. Spatial transcriptomics, in contrast, preserves tissue architecture and locational relationships but often sacrifices resolution or sensitivity, particularly when profiling low-abundance transcripts. Combining both approaches creates a complementary framework that overcomes individual shortcomings while offering a more holistic view of tumor biology.

One major application of this integration lies in the reconstruction of high-resolution cellular maps. Single-cell sequencing generates detailed transcriptional profiles that can be computationally projected back onto spatial transcriptomic datasets, effectively assigning cellular identities to spatial coordinates. For example, in colorectal cancer, integrating scRNA-seq with spatial transcriptomics has identified spatial signatures of cellular communication networks (such as antigen presentation and phagocytosis pathways), revealing key signaling pathways within the inflammatory microenvironment(74). This strategy has enabled the delineation of TME with unprecedented granularity, revealing how malignant clones, immune subsets, and stromal elements are arranged into functional niches. Such integration has been pivotal in identifying regions of immune exclusion, gradients of metabolic stress, and zones of epithelial–mesenchymal plasticity within tumors.

Beyond static mapping, combined datasets facilitate the study of tumor evolution under therapeutic pressure. By tracing resistant subpopulations detected through single-cell analyses and localizing them within spatial landscapes, researchers can pinpoint ecological niches that support persistence(75). For example, integrated studies in lung and breast cancers have shown that drug-tolerant cells often occupy hypoxic or fibroblast-rich regions, underscoring the interplay between intrinsic transcriptional programs and extrinsic microenvironmental cues(76). These insights inform the design of interventions that disrupt protective niches rather than solely targeting resistant clones. The integration of single-cell and spatial approaches also enhances biomarker discovery. By coupling transcriptional resolution with locational context, researchers can identify prognostic markers not only based on gene expression levels but also on their spatial enrichment within clinically relevant regions. This dual perspective has improved the predictive value of biomarkers for immunotherapy response and metastatic risk.

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Conclusion

Single-cell sequencing and spatial transcriptomics have reshaped cancer research by enabling simultaneous dissection of cellular diversity and tissue architecture. These technologies have revealed hidden subclones, mapped immune and stromal heterogeneity, and clarified the spatial logic of tumor ecosystems. Their integration provides a multidimensional framework that links molecular states with microanatomical context, uncovering mechanisms of plasticity, immune evasion, and therapeutic resistance. Although current platforms face challenges related to resolution, sensitivity, and scalability, ongoing innovations promise to deliver increasingly precise maps of tumor organization. The combined application of single-cell and spatial approaches not only enhances biological interpretation but also accelerates the discovery of therapeutic vulnerabilities. As these technologies mature, they are expected to guide next-generation precision oncology by informing patient stratification, predicting treatment responses, and designing interventions that target both molecular alterations and spatial dynamics within the cancer ecosystem.

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