# Refining Tumor Microenvironment Insights via Single-Cell and Spatial Transcriptomics for Precision Medicine

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**Abstract.** The heterogeneity and dynamic evolution of the tumor microenvironment (TME) are key drivers of tumor progression, immune evasion, and therapeutic resistance. Traditional transcriptomic approaches are limited by their reliance on population-averaged data and the lack of spatial context, making it challenging to fully dissect the complex interactions among tumor cells, immune infiltrates, and stromal components within the TME. In recent years, the synergistic advancement of single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) has revolutionized our ability to construct high-resolution cellular atlases and delineate spatially organized functional modules within the TME. These technologies have enabled the identification of novel cell subtypes, cell-cell communication pathways, and spatial niches associated with disease progression and therapy response. This review systematically compiles recent technological breakthroughs in the integration of single-cell and spatial multi-omics, highlights key discoveries in TME research, and discusses their transformative impact on the development of precision oncology. Additionally, it addresses current technical limitations and explores emerging strategies and future directions for advancing this rapidly evolving field.

**Keywords:** Single-cell transcriptomics, spatial transcriptomics, tumor microenvironment, precision medicine, multi-omics integrative analysis.

## 1. Introduction

The tumor microenvironment (TME) consists of a complex and ever-changing network of various cell types, extracellular matrix elements, and signaling molecules that affect tumor growth, spread, and treatment response. The development of single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics has transformed TME analysis by offering detailed insights into cellular diversity and spatial arrangement. Although scRNA-seq can identify unique cell populations and transcriptomic states, it does not provide spatial context, which is essential for comprehending cell-cell interactions. Spatial transcriptomics maintains spatial data, allowing for the visualization of gene expression patterns within tumor tissue. Combining these technologies provides a thorough understanding of the TME, revealing the influence of cellular diversity and spatial dynamics on tumor biology and treatment results.

Numerous cancer types have benefited from this integrative strategy, which has shed light on the mechanisms underpinning immune evasion and tumor growth. Researchers can find possible therapeutic targets and create plans to modify the TME for better cancer therapy results by mapping these interactions.

Combining single-cell and spatial transcriptomics provides a robust approach to understanding the complexities of the tumor microenvironment. This method improves our comprehension of tumor biology and offers potential for discovering new therapeutic targets and biomarkers for precision oncology [1]. As these technologies keep developing, they will certainly be crucial in enhancing our understanding of cancer and bettering patient treatment [2].

# 2. Relevent theories

## 2.1. Single-cell transcriptomics: From cellular heterogeneity to functional state analysis

Single-cell RNA sequencing (scRNA-seq) captures the transcriptomic profiles of individual cells within tissue samples, overcoming the population-averaging limitations of traditional sequencing techniques and providing a high-resolution perspective for deciphering the heterogeneity of the TME. This technology enables the systematic identification of diverse immune, stromal, and tumor cell subpopulations within the TME and reveals their functional state differentiation patterns. By integrating pseudotime analysis, scRNA-seq can dynamically trace the continuous evolution of cellular states, offering a theoretical foundation for targeting key functional nodes within the TME for therapeutic intervention.

### 2.1.1 Cellular subgroup heterogeneity in the tumor microenvironment (TME)

Single-cell RNA sequencing provides a detailed analysis of immune cell subtypes in the tumor microenvironment (TME), facilitating an accurate understanding of their functional diversity and clinical significance. In a study on metastatic melanoma patients, Barras et al. utilized single-cell transcriptomics and spatial proteomics to comprehensively analyze the heterogeneity of cellular subpopulations within the TME. Their findings revealed that patients who responded to tumor-infiltrating lymphocyte adoptive cell therapy (TIL-ACT) exhibited a higher baseline presence of activated T cells, macrophages, and dendritic cells, which formed a robust stimulatory interaction network within the tumor. After treatment, myeloid cells in responders underwent reprogramming, particularly transitioning into CXCL9+ macrophages, with an expanded interaction network between T cells and myeloid cells. These results suggest that a strong supportive immune cell network within the tumor is closely associated with a favorable response to TIL-ACT in metastatic melanoma patients [3].

Additionally, stromal and tumor cells exhibit significant heterogeneity. In a study on gastric signet-ring cell carcinoma (GSRC), researchers leveraged single-cell RNA sequencing to elucidate the interactions between stromal and tumor cells within the TME. The results indicated that GSRC cells exhibit unique cytological characteristics, including low cell adhesion and high immune evasion capacity. Moreover, the TME of GSRC was enriched with immunosuppressive stromal cells, which facilitated tumor growth and immune escape through the secretion of various cytokines and chemokines. Furthermore, differentially upregulated genes in GSRC cells were primarily enriched in aberrantly activated cancer-related and immune response signaling pathways, such as TNF-α, NF-κB, and TGF-β. These findings provide new insights into the aggressiveness and poor prognosis of GSRC, contributing to the development of targeted therapeutic strategies [4].

### 2.1.2 Cell dynamic functional state analysis: Pseudotime analysis

Pseudotime analysis is a computational technique employed to deduce cellular state changes in dynamic biological processes, especially using scRNA-seq data. By applying pseudotime analysis, researchers can reconstruct the trajectories of cellular differentiation, development, or other dynamic processes, revealing the continuous progression of cellular states. For example, in a study by Rosenberg et al., pseudotime analysis was utilized to uncover the transcriptional programs of four developmental lineages during central nervous system development in mice, providing important insights into early developmental processes [5].

Monocle3 is one of the most widely used tools for single-cell trajectory inference. It employs advanced algorithms to handle large-scale and complex single-cell datasets, with the capacity to process millions of single cells. The primary functions of Monocle3 include cell clustering, trajectory reconstruction, and differential expression analysis. By leveraging Monocle3 for pseudotime analysis, researchers can gain deeper insight into cellular state transitions and differentiation pathways in dynamic biological processes, providing valuable clues for understanding complex biological phenomena [6-7].

### 2.2. Spatial Transcriptomics: Mapping the Spatial Logic of Cellular Interactions

Although scRNA-seq has been widely used to analyze cellular states across various organisms and tissues, it fails to capture the microenvironmental context in which cells reside. Spatial transcriptomics (ST) has allowed scientists to assess the transcriptomic conditions of both target cells and their surrounding cells at the same time, broadening the field of single-cell biology. By analyzing multicellular microenvironments, ST reveals the reproducibility and functional organization of tissues under different physiological conditions.

#### 2.2.1 Spatial Heterogeneity Analysis of the Tumor Microenvironment

Unprecedented insights into the spatial organization of the TME have been made possible by recent developments in high-resolution profiling and spatial transcriptomics. Prognosis-associated cellular heterogeneity in papillary thyroid cancer has been identified by spatial transcriptomics, underscoring the influence of spatial distribution on treatment results and tumor prognosis [8]. In pancreatic ductal adenocarcinoma (PDAC), studies have demonstrated spatial ecotype heterogeneity across primary and metastatic sites, emphasizing tumor adaptability and the potential for personalized treatment [9].

Spatial profiling has demonstrated its predictive utility in breast cancer by connecting microenvironmental heterogeneity to genetic changes and clinical outcomes [10]. Tumor-infiltrating lymphocytes (TILs) and their interactions with tumor cells affect immunotherapy response and immune evasion in triple-negative breast cancer [11]. Furthermore, intricate TME patterns have been discovered by machine learning and AI-driven spatial analysis of pathology slides, opening up new possibilities for diagnosis and therapy stratification [12].

#### 2.2.2 Computationally Driven Spatial Transcriptomic Analysis

Recent advancements in computational methods have significantly enhanced spatial transcriptomic analysis by integrating multimodal data and improving predictive accuracy. Graph-based autoencoders like STACI combine spatial transcriptomics with chromatin imaging to identify molecular and functional alterations, enabling biomarker discovery in diseases such as Alzheimer's [13]. SpatialMap bridges scRNA-seq and image-based transcriptomics using generalized linear spatial models, accurately predicting unmeasured gene profiles across tissues and species [14]. Meanwhile, the BASS framework employs Bayesian hierarchical modeling to perform multi-scale spatial transcriptomic analysis, simultaneously clustering cell types and detecting spatial domains, revealing intricate tissue landscapes in regions like the cortex and hypothalamus [15].

## 3. Integrated analysis and application research

### 3.1. Integration of Single-Cell and Spatial Transcriptomics: Deconvolution and Mapping

The integration of single-cell and spatial transcriptomics is of great significance in modern biological research, particularly in the areas of deconvolution and mapping. By combining scRNA-seq data with spatial transcriptomic data, researchers can better understand the spatial distribution and functional characteristics of cells within tissues.

Deconvolution plays a crucial role in the analysis of spatial transcriptomic data. This technique enables the decomposition of mixed signals in spatial transcriptomics into contributions from different cell types, thereby achieving precise localization of cellular populations. For example, the SpatialDecon algorithm, which utilizes log-normal regression and background modeling, effectively quantifies cell populations in spatial gene expression studies [16]. Additionally, the STdGCN method integrates graph convolutional networks (GCNs) with scRNA-seq data to perform cell-type deconvolution in spatial transcriptomics, demonstrating outstanding performance in TME analysis [17].

Mapping techniques in spatial transcriptomics are used to predict the spatial distribution of unmeasured genes. Methods such as Tangram, gimVI, and SpaGE have demonstrated excellent performance in predicting the spatial expression of RNA transcripts [18]. By integrating spatial

transcriptomic data with scRNA-seq data, these methods enable transcriptome-wide spatial predictions in tissue sections.

Additionally, SpatialPrompt is a tool that integrates gene expression, spatial location, and scRNA-seq data to efficiently infer cell-type proportions at spatial locations, demonstrating superior performance across multiple datasets [19]. Meanwhile, Spatial-ID, by leveraging prior knowledge from scRNA-seq data and incorporating spatial information from spatial transcriptomics, enables comprehensive cell-type annotation in spatially resolved transcriptomic datasets [20].

GraphST, which combines graph neural networks (GNNs) with self-supervised contrastive learning, has been successfully applied across various tissue types and technological platforms, achieving superior spatial clustering and cell-type deconvolution [21]. The application of these methodologies not only enhances our understanding of tissue architecture but also provides novel insights into cell-cell interactions.

### 3.2. The Precision Medicine Value of TME Analysis

#### 3.2.1 Prediction of Immunotherapy Response and Overcoming Drug Resistance

The immune cell states in the TME affect the effectiveness of immunotherapy, and overcoming resistance requires a better knowledge of T-cell fatigue processes. The immune checkpoint molecules PD-1 and LAG-3 work together to worsen CD8+ T-cell fatigue within the TME, which reduces antitumor immunological responses. Single-cell transcriptomics studies have demonstrated that in mouse melanoma models, CD8+ T lymphocytes lacking both PD-1 and LAG-3 had a greater ability to eliminate tumors and a longer survival time. Broad T-cell receptor (TCR) clonotypic variety, effector-like gene enrichment, and elevated IFN-γ secretion are some of the distinctive transcriptional profiles of these dual-deficient T cells, which suggest enhanced functioning [22].

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The possibility of dual checkpoint targeting to overcome immunotherapy resistance is revealed by these studies, which emphasize the critical roles of PD-1 and LAG-3 in controlling T-cell exhaustion. Understanding these TME pathways can help create more potent immunotherapeutic approaches and increase patient response rates.

#### 3.2.2 Targeted Therapy and Microenvironment Remodeling

Dual blockade of immune checkpoint pathways effectively restores T-cell antitumor capacity, yet its precise mechanisms require further investigation. In a study on pancreatic ductal adenocarcinoma (PDAC), Moncada et al. integrated spatial transcriptomics (ST) and scRNA-seq to unveil spatial gene expression patterns within the tissue. They developed a multimodal cross-analysis method to precisely annotate cellular compositions in different tissue regions. The study revealed localized enrichment of specific ductal, macrophage, dendritic, and cancer cell subpopulations, along with unique co-enrichment patterns between different cell types. Furthermore, inflammatory fibroblasts were found to co-localize with cancer cells expressing stress-response gene modules, emphasizing the role of spatial multi-omics in identifying niche-dependent tumor progression signals. For instance, in KRAS-mutant tumor cells, the secretion of IL-6 was shown to activate the JAK/STAT pathway in adjacent stellate cells, inducing the formation of fibrotic stroma. Inhibitors targeting this interaction, such as IL-6R antagonists, significantly reduced tumor stiffness and enhanced chemotherapy delivery in preclinical models [24]. These findings support a paradigm shift in personalized therapy from

"tumor cell targeting" to "microenvironmental regulation", advancing therapeutic strategies aimed at disrupting key tumor-stroma interactions.

#### 3.2.3 Prognostic Biomarkers and Recurrence Monitoring

Dual targeting of PD-1 and LAG-3 has become a viable tactic for overcoming immunotherapy resistance by addressing immune tolerance within the TME. Liang et al. evaluated the spatial distribution of immune cells in tumor tissues from 104 patients both before and after treatment using multiplex immunofluorescence (mIF) and immunohistochemistry (IHC) in a neoadjuvant therapy (NAT) study on breast cancer. Their research showed that NAT effectiveness is significantly influenced by the distance between CD8+ T cells and tumor cells. Specifically, a significant relationship was observed between pathological complete response (pCR), disease-free survival (DFS), overall survival (OS), and the proportion of CD8+ T cells located within 20 micrometers of tumor cells (N20-CD8+ T cells). This association was confirmed for many subtypes of breast cancer and treatment approaches. Furthermore, a significant correlation between the N20-CD8+ T cell fraction and the chemokine CXCL9 expression level suggests that CXCL9 may be involved in the recruitment of CD8+ T cells to tumor locations. These findings highlight how crucial immune cell spatial distribution is for forecasting NAT effectiveness and developing individualized treatment plans [25].

## 4. Challenge

### 4.1. Complexity of Data Integration and Analysis

Combining single-cell RNA sequencing (scRNA-seq) with spatial transcriptomics (ST) data necessitates advanced computational techniques to precisely associate cell types and states with their spatial positions. However, existing integration approaches face limitations in accuracy and efficiency when handling high-dimensional, multimodal data, which can hinder a comprehensive understanding of cell-cell interactions and tissue architecture.

### 4.2. Balancing Spatial Resolution with Transcriptome Coverage

High-spatial-resolution techniques, such as fluorescence in situ hybridization-based methods, can provide subcellular-level localization information but are typically restricted to pre-designed gene panels, limiting full transcriptome coverage. In contrast, array-based ST platforms enable whole-transcriptome profiling but suffer from relatively low spatial resolution, making it difficult to resolve the heterogeneity of neighboring cells. Balancing spatial resolution and transcriptome coverage remains a critical challenge in technological advancements.

### 4.3. Tissue-Specific Constraints

Different tissue types may encounter unique technical challenges in spatial omics studies. For instance, certain tissues exhibit high autofluorescence, rapid RNA degradation, or difficulties in sectioning, all of which can affect data quality and resolution. Additionally, tissues lacking well-established reference atlases or those with unclear anatomical structures (such as certain tumor tissues) present challenges in data annotation and interpretation.

## 5. Conclusion

The integrative analysis of single-cell and spatial transcriptomics is reshaping the conceptual framework of TME. By enabling multidimensional analysis from molecular and cellular levels to spatial ecology, it provides unprecedented insights for precision medicine. Although challenges remain in standardizing technologies, integrating data, and validating clinical applications, this field is poised to become a central driving force in oncology research, ultimately revolutionizing individualized treatment decisions based on microenvironmental characteristics.

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