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# Single-Cell Spatial Transcriptomics Reveals Cellular and Molecular Heterogeneity of Non-Small Cell Lung Cancer

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**Abstract**

Recent breakthroughs in single-cell spatial transcriptome technology have transformed biomedical research. This cutting-edge technology equips scientists to dive into the molecular details of human tissues and organs like never before. Seemingly overnight, researchers gained the power to visualize nearly every single gene in every single cell with spatial resolution, a monumental leap from previous methods, which were limited to a few genes in a few cell types. This revolution promises to deliver unprecedented insights into complex diseases such as cancer. Quickly becoming a critical tool for rapidly advancing precision medicine, the technology is helping unravel the mysteries of the local cellular environment's influence on the function and behavior of individual cells. Such fine-tuned resolution is crucial for defining baseline and condition-dependent gene expression levels across multiple cell types in healthy and diseased tissues. Since specific tissue environments depend on the various cell types of present, their proximity to each other, and the timing of signals, the ability to gain a more precise understanding of the spatial context of molecular events in intact tissues is critical. Single-cell spatial transcriptomics provides visualization and quantitation of that context at the highest possible resolution. Here we applied this revolutionary technology to explore tumor heterogeneity in non-small cell lung cancer (NSCLC) tissues.

**Keywords:** Single-cell; Spatial transcriptomics; Precision medicine; Cellular heterogeneity; NSCLC

**Abbreviations:** NSCLC: Non-Small Cell Lung Cancer; TME: Tumor Microenvironment; UMAP: Uniform Manifold Approximation and Projection; TLS: Tertiary Lymphoid Structures

## Introduction

Cellular heterogeneity dictates the fate of all tissues in both normal development and the pathogenesis of human disease. Defining this heterogeneity has primarily been focused on gene expression profiles in single cells [1,2]. Single-cell analysis has become a critical tool for researchers studying complex biological systems and disease mechanisms [3]. Significant strides have been made in our understanding of cancer, stem cell biology, neuroscience, developmental biology, and infectious diseases by leveraging single-cell analyses [4]. Single-cell analyses are so powerful because they can effectively capture and characterize the cellular heterogeneity and dynamics that characterize human health and disease mechanisms. The biological nuances and complexity that single-cell analyses uncover are paving the way for novel drugs and diagnostics and are likely to drive the future of precision and personalized medicine.

Complex tissues with high cellular heterogeneity require single cell technologies both at the transcriptomic and spatial level to fully interrogate the cell types within them. Simultaneous detection of multiple markers is critical to obtain meaningful information from patient samples using single cell platforms. Single cell technologies enable precise analysis of heterogeneous tissues to elucidate key therapeutic targets and biomarkers. In addition, studying target gene expression with spatial context is vital for understanding the cellular organization and function within the tissue [5].

The tumor microenvironment (TME) is a network of complex interactions between the tumor cells, immune cells, endothelial cells, fibroblasts, and the surrounding extracellular matrix. Immunotherapies including immune checkpoint blockade

have demonstrated therapeutic efficacy and durable responses for several tumor types, however most patients are either nonresponsive or develop resistance to immunotherapies due to immunosuppressive TME [6]. For developing next generation immunotherapies, it is important to identify reliable biomarkers for predicting treatment response. Correlating immune cell population and their activation states in treated tumors to the therapeutic response and clinical outcome will be the key route to lead us there [7].

Single-cell transcriptomics, or scRNA-seq, is widely used for analyzing the transcriptome of single-cell populations. With scRNA-seq, gene expression profiling can explore genotype-phenotype relationships at the fine-grained single-cell level. Minute changes in a single cell can often lead to system-wide changes. For example, a cancer cell can undergo mutations that render it resistant to therapy, leading to changes in the composition of the entire cellular population of the tumor during treatment. Recent years have seen scRNA-seq integrated into biomedical research, and huge scope still remains for the application of this technology in the future.

Spatial transcriptomics enables researchers to measure all gene activity in a sample, and map where each gene activity is occurring relative to all other activity. Retaining spatial context when studying the molecular information of a tissue allows researchers to visualize changes happening in situ and begin to piece together complex cause-and-effect relationships between cellular changes. Integrating spatial technology adds another layer of complexity into single-cell analysis. It is essential that the sample preparation and cell capture methods are capable of retaining the original spatial localization of each cell within a tissue. The analysis can then be mapped back to the original tissue section, allowing visualization of the molecular information in situ [8,9].

## Results and Discussion

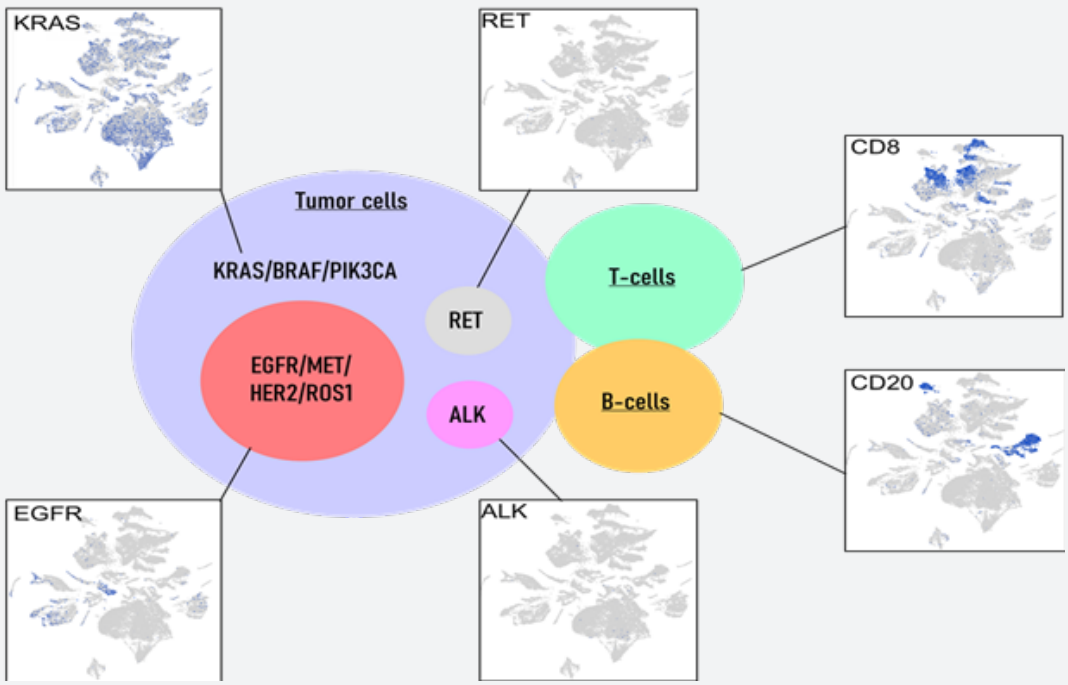


**Figure 1: Visualization of single-cell and multigene expression data to elucidate spatially-defined cellular and molecular subtypes in NSCLC tissues. Each cell type is represented by a molecular marker(s) where the size of the colored circle indicates how abundant a certain cell type is, i.e. the larger the circle, the higher the estimated proportion of the indicated cell type. The cell types presented here are KRAS/BRAF/PIK3CA-positive tumor cells, EGFR/MET/HER2/ROS1-coexpressing tumor cells, ALK-expressing tumor cells, RET-positive tumor cells, and CD8+ T cells, and CD20+ B cells.**

The combination of single-cell and spatial transcriptomics provides an unprecedented opportunity to navigate cellular landscape in complex tumor tissues. Evaluating gene signatures of the tumor cells and infiltrating immune cells can provide prognostic and predictive information. In order to examine a variety of NSCLC tumors at scale, we took advantage of the Chromium Single Cell Gene Expression Flex assay (10x Genomics, Inc.) and its built-in multiplexing capability to profile hundreds of thousands of cells from 3 unique human donors on a single chip, totaling 34,321 cells were recovered. Distinguishing cell types and subpopulations is a fundamental task in the bioinformatic analysis of spatial transcriptomics data. This can be resolved


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with the help of clustering analysis where spatially variable genes can be discovered and data dimensions can be clustered within a tissue. Our scRNA-seq spatial transcriptomics analyses have demonstrated successful visualization and spatial mapping of tumor markers and immune cell markers within the TME (Figure 1). We merged the single-cell transcriptomic profiles collected from different samples for a uniform manifold approximation and projection (UMAP)-based cell clustering analysis, the cell types of which were further annotated by using known cell type-specific gene markers. In total, 25 distinct cell clusters were identified and annotated, including subtypes of tumor, T cells, B cells, cancer stem cells, and TME.

To identify the clinical impact of these cell types in NSCLC, we selected the 9 driver genes that are under NCCN/ASCO/FDA guidelines for therapeutic biomarkers. We found that the genes of KRAS, BRAF and PIK3CA were widespread expressed in tumor cells, with high degree of co-localization in the same cell cluster. On the other hand, EGFR, HER2, MET and ROS1 genes were co-expressed restrictively in certain areas, indicating that not all tumor cells expressing these driver genes. It is noteworthy that the expression of ALK and RET genes was limited and localized in distinct clusters, underlining the highly heterogeneous and dynamic tumor subtypes in NSCLC (Figure 1). The expression of the genes exclusively expressed in the T and B cell types, such as CD8 and CD20, respectively, were also identified, demonstrating the presence of tumor-infiltrating or tumor-resident lymphocytes in the microenvironment of NSCLC. Noting the significant association with immunotherapeutic outcome, we further explored on the subtypes of T and B cells and their biological characteristics in the TME of NSCLC.

We identified six classes of T cells: CD3, CD4, CD8, CD25, PD-1 and CTLA-4 (Figure 2). The cluster with co-expression of CTLA-4 and CD25 are possible Tregs, which are a part of larger CD4+ subpopulation. Distinct clusters with high expression level of CD3, CD8 or PD-1 were also identified. The B cells could be divided into two major groups: CD19+ and CD20+. A subset of B cells expresses both CD19 and CD20, while the rest express the CD19 only (Figure 2). We noticed that the CD20+ cells were mainly located at the tertiary lymphoid structures (TLS) of lung tumor tissues while CD19+ cells were not only located at the TLS but also randomly enriched within tumor tissues, suggesting CD19+ and CD20+ naïve-like B cells might function versatile in the lung cancers.

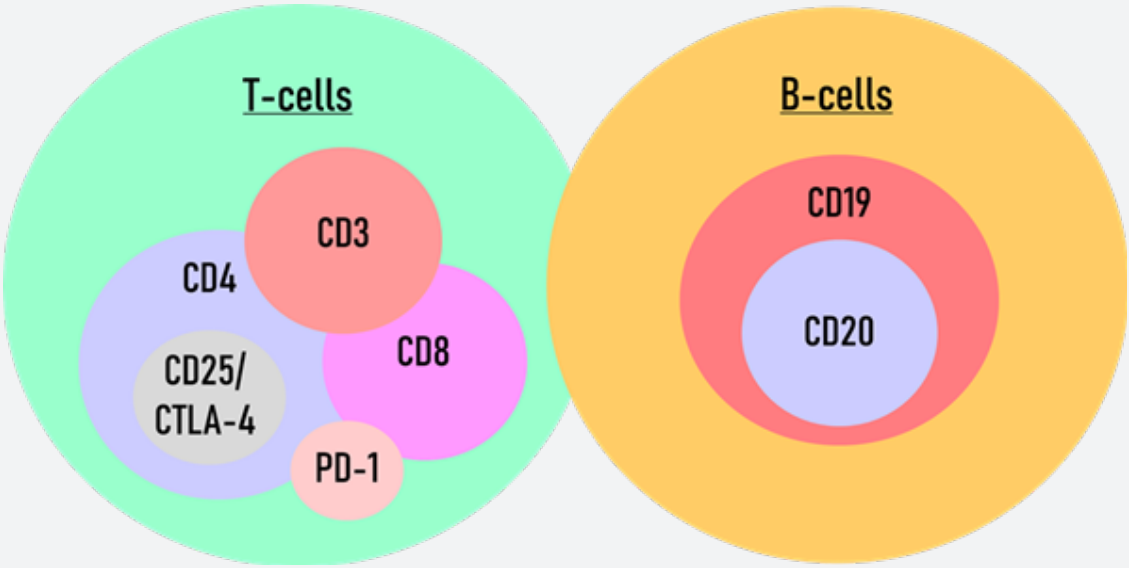


**Figure 2:** Main cell types of immune T-cell and B-cell subsets in TME of NSCLC tissues.

The cells in another cluster presented high expression of stem cell-related genes such as MYC, ABCG2 and KLF4, and we presumed them to be the origin of cancer cells during tumor progression (Figure 3A). We found that ABCG2 and KLF4 presented a co-expression pattern in one subcluster, whereas MYC was expressed in another subcluster. The theory of cancer stem cells assumes that not all tumor cells are present equally with regard to self-renewal, tumorigenesis, and maintenance potential [10]. Our findings here shed light on the hierarchical structure and heterogeneity of lung cancer stem cells. We also identified a class of myeloid-derived suppressor cells expressing CD11b, CD14 and CD33. Within this group, a subcluster expressed high levels of CD64 and CD80, suggesting tissue-associated macrophages played a vital role to create an immunosuppressive TME in NSCLC (Figure 3B).


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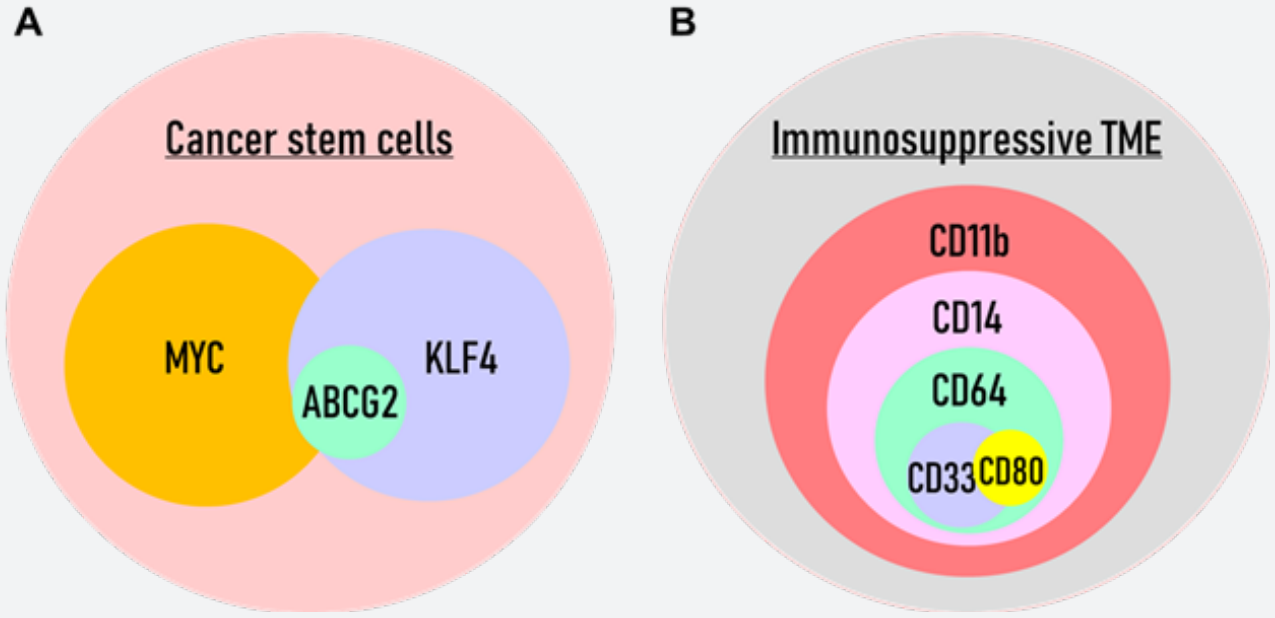


**Figure 3:** Spatial distribution and heterogeneity of the NSCLC TME. The subpopulations of various role types of (A) cancer stem cells, and (B) immunosuppressive TME are depicted as circular treemaps.

Taken together, our data highlighted the importance of using a single-cell spatial transcriptomics approach when identifying and tracking tumor subtypes in terms of tumor clonal evolution or tumor heterogeneity. This is further supported by observations of gene deregulation in several cell populations across tumor subtypes that would otherwise be masked in a bulk analysis. Additionally, the complexities of tumor and TME interactions and immunotherapeutic outcome are underscored by our study presented here.

## Conclusion

Single-cell spatial transcriptomics is an emerging technology that provides a roadmap of transcriptional activity within tissue sections. To better decipher domains or cell types that are spatially coherent in both gene expression and histology, a number of integrative approaches to combine gene expression, spatial location, histology, and H&E image have been developed. Integrating multi-modal information is expected to define cell types or domains accurately than using gene expression alone.

Investigations into the cellular mechanisms at work behind cancer progression benefit from the use of complementary technologies. While pseudobulk differential gene expression analysis of human lung tissue revealed several known markers associated with poor tumor prognosis, single cell data was able to provide the resolution needed to determine which cell type was responsible for that expression pattern. Furthermore, comparison of the gene expression profiles belonging to specific cell types revealed deregulation of canonical cell-type markers present in known cell

types across biological groups.

Cell typing atlases are typically based on profiles of healthy tissues, making it challenging to correctly annotate cell types that may be specific to certain disease states. Moving forward, the ability to profile larger numbers of cells from various disease states can improve cell-type classification and enable the construction of more complete references to help better understand the cellular dynamics that drive disease onset and progression.

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None.

## Conflict of Interest

The authors declare no conflicting interest.

## Ethics Approval and Consent to Participate

Not applicable.



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## Consent for Publication

Not applicable.

## Availability of Data

Data is available from the corresponding author on reasonable request..

## References


1. Pollen A, Nowakowski T, Shuga J, Xiaohui W, Anne AL, et al. (2014) Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. Nat Biotechnol 32(10): 1053-1058.
2. Nowakowski T, Bhaduri A, Pollen A, Beatriz A, Mohammed A Mostajo R, et al. (2017) Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. Science 358(6368): 1318-1323.
3. Vandereyken K, Sifrim A, Thienpont B, Theirry V (2023) Methods and applications for single-cell and spatial multi-omics. Nat Rev Genet 24(8): 494-515.
4. Hodzik E (2016) Single-cell analysis: Advances and future perspectives. Bosn J Basic Med Sci 16(4): 313-314.
5. Marx V (2021) Method of the Year: spatially resolved transcriptomics. Nature Methods 18(1): 9-14.
6. Bai R, Chen N, Li L, Nawen D, Ling B, et al. (2020) Mechanisms of cancer resistance to immunotherapy. Front Oncol 10: 1290-1290.
7. Lyons YA, Wu SY, Overwijk WW, Keith AB, Anil KS (2017) Immune cell profiling in cancer: molecular approaches to cell-specific identification. NPJ Precis Oncol 1(1): 26.
8. Longo SK, Guo MG, Ji AL, Paul AK (2021) Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat Rev Genet 22: 627-644.
9. Piwecka M, Rajewsky N, Rybak-Wolf A (2023) Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease. Nat Rev Neurol 19(6): 346-362.
10. Kontostathis G, Zoidakis J, Anagnou NP, Papa KI, Vlachou A, et al. (2016) Proteomics approaches in cervical cancer: focus on the discovery of biomarkers for diagnosis and drug treatment monitoring. Expert Rev Proteomics 13(8): 731-745.



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