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UTILIZING SPATIAL TRANSCRIPTOMICS AND SINGLE-CELL SEQUENCING TO CREATE HIGH-RESOLUTION MAPS OF IMMUNE CELL INTERACTIONS IN HEALTHY AND DISEASED TISSUES

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ABSTRACT

The mammalian immune system is known to operate within a highly ordered tissue architecture in which the position of cells is related to physiological and pathological function. Classical bulk genomic analysis is known to not capture the importance of cellular heterogeneity and the microenvironment. With the advent of systems immunology through the combined use of single-cell RNA sequencing and spatial transcriptomics, it is now possible to generate detailed maps of immune interactions. According to this article, aims to distill the latest discoveries in the fields of dermatology, nephrology, cardiology, endocrinology, and oncology, and how these technologies are revealing the molecular geography of these tissues. These technologies, which allow us to visualize the interactions of ligand and receptor and rare immune subsets in their natural environment, are revolutionizing the understanding of wound healing, organ transplantation, ischemic damage, and tumor biology, and will soon usher in the era of precision medicine.

Keywords:

1. INTRODUCTION

1.1. Systems immunology in the age of single-cell and spatial technologies

Tissue physiological function is supported by complex interactions between different cell types, such as immune effector cells, stromal cells, and tissue parenchymal cells. Molecular profiling technologies, such as bulk RNA sequencing, traditionally gave a low-resolution view of tissue, averaging expression and obscuring important cell type heterogeneity. The introduction of scRNA sequencing resolved this issue by characterizing individual cell types and states (Ahmed et al., 2022). However, scRNA sequencing is a dissociated tissue approach, destroying the spatial context or seed and soil that is important in understanding how cells interact with each other in a juxtracrine and paracrine manner (Houser et al., 2023).

1.2. Need for high-resolution immune cell interaction maps in health and disease

The integration of scRNA-seq with spatial transcriptomics (ST) has been identified as one of the most promising advances in the field of systems immunology, enabling the spatial reconstruction of transcriptomic information at

subcellular resolution. In terms of understanding the pathogenesis of diseases, it has been recognized that maintaining spatial information is essential to understand how immune cells drive pathogenesis in particular spatial compartments or niches, including the tumor microenvironment (TME) (Yan et al., 2022), infarct border zone in the heart, or rejection sites in organ transplantation. The ability to maintain spatial information has enabled the identification of spatial biomarkers or therapeutic targets that were previously unknown to disjointed molecular or histological analysis (Nguyen et al., 2025).

1.3. Scope and objectives of the review

In this review, we will discuss the application of these technologies in multiple organ systems. We will review the technological basis of these technologies, the application in understanding healthy and diseased immune landscapes, and the implications for precision medicine. We will discuss how the intersection of single-cell and spatial data is revolutionizing our understanding of cell type heterogeneity, cell-cell communication networks, and the therapeutic potential of modulating microenvironmental niches (Mou et al., 2025).

2. Technological Foundations

2.1. Single-Cell Sequencing Technologies

Single-cell RNA-seq has progressed over the past decade since its introduction in 2009 to enable simultaneous profiling of thousands of cells using droplet-based methods such as 10x Genomics' Chromium (Ahmed et al., 2022). This has allowed for the unbiased discovery of rare cell populations and states, which can be hidden in bulk RNA-seq data. Smart-seq2 and CEL-seq2 have greatly improved sensitivity and scalability to enable full-length transcriptome and low-expression genes, such as those found in immunology (Cui et al., 2024).

Advances in single-cell epigenomics and proteomics in addition to transcriptomics, single-cell data now include epigenomics, such as single-cell ATAC-seq, and proteomics, such as CITE-seq. This allows a multi-omics view of cellular regulation. This provides a holistic view of the regulatory framework underlying immunity. This is because single-cell RNA and ATAC-seq can be used to study cell regulation by linking chromatin regulation and gene expression in real-time, enabling the study of the regulation of immune cell differentiation (Houser et al., 2023).

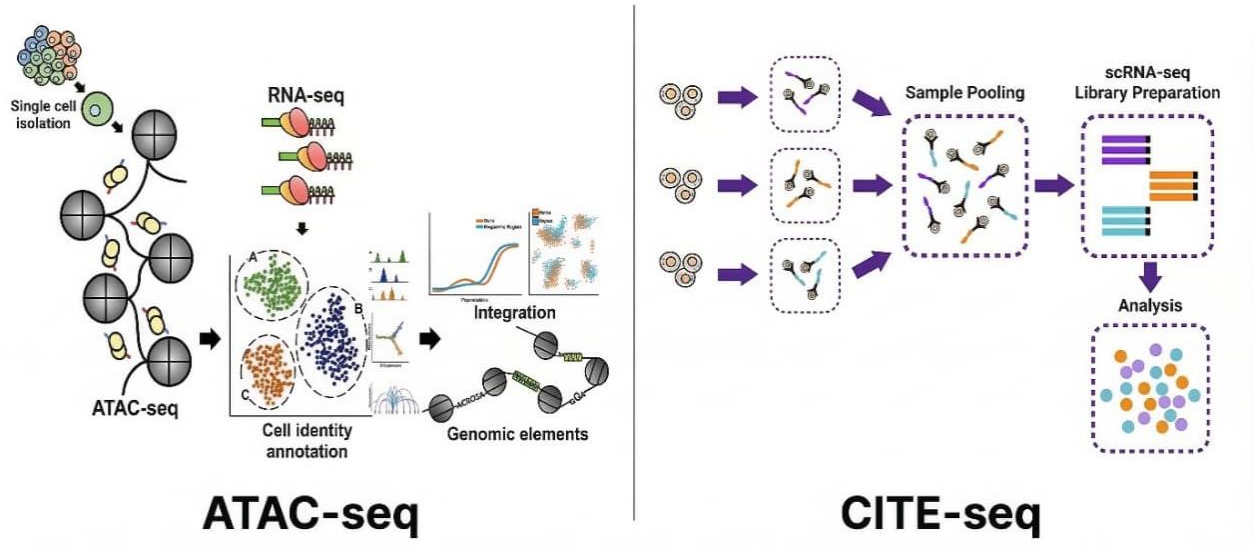

Fig 01. Integrated Single-Cell Multi-Omics Platform

Computational challenges and solutions the volume of data being generated by single-cell techniques has created considerable computational hurdles in terms of data storage

and management. However, scientists are increasingly making use of advanced bioinformatics techniques to re-analyze and integrate existing data, thus increasing the value of generated data. Dimensionality reduction techniques like UMAP and clustering are essential in making sense of single-cell data, although standardization of analysis techniques has been a challenge in making it clinically reproducible (Mou et al., 2025).

2.2. Spatial Transcriptomics Platforms

Slide-based (e.g., Visium) and Imaging-based (e.g., MERFISH, seqFISH) Current methodologies can be broadly classified into two types: sequencing-based and imaging-based methods. Sequencing-based methods, such as 10x Genomics Visium and Stereo-seq, make use of a spatially barcoded slide to read out mRNA transcripts. Imaging-based methods, such as MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization) and Xenium, make use of fluorescent in situ hybridization to detect a particular RNA target with high resolution (Moffit et al., 2016).

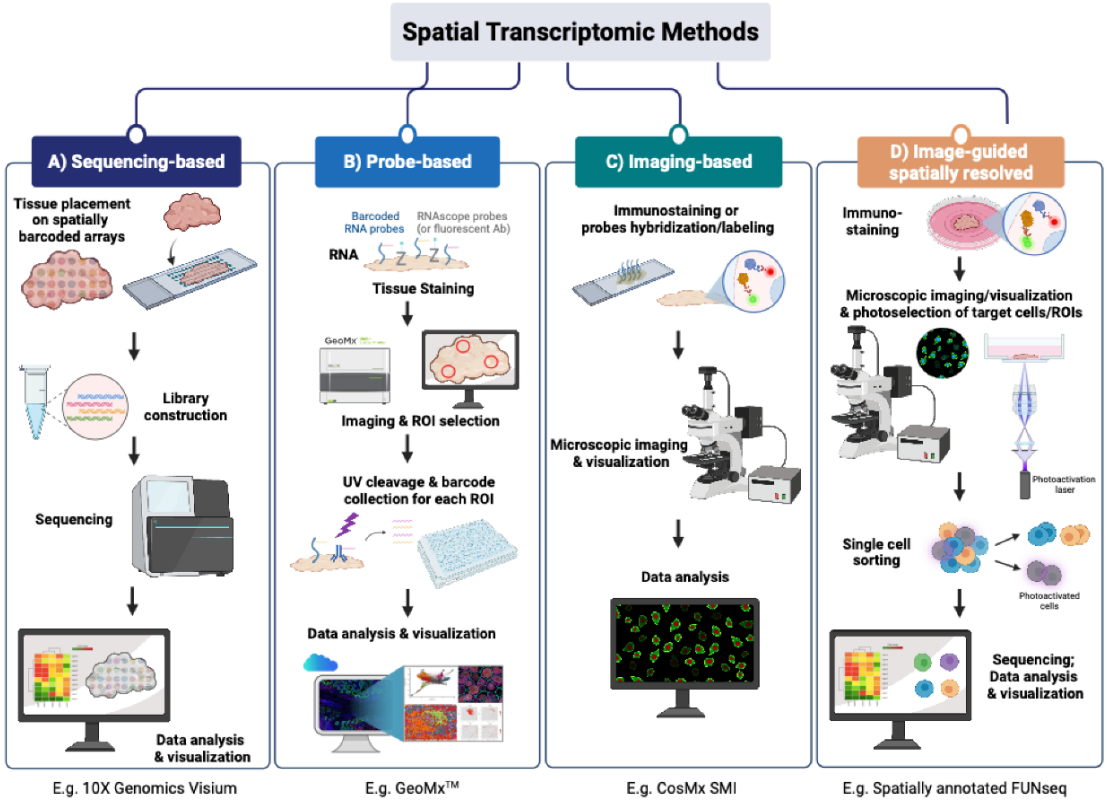

Figure 02. Four Main Types of Spatial Transcriptomic Methods

Visium provides a whole transcriptome view, but it has traditionally not offered single-cell resolution, while imaging-based methods offer high resolution but only for a pre-selected panel of genes (Vickovic et al., 2025). Resolution, throughput, and integration trade-offs One trade-off between resolution and throughput has been identified in that sequencing-based techniques have the advantage of genome-wide coverage but have lower resolution than imaging-based techniques (averaging 10 to 20 cells per spot in older versions), while imaging-based techniques have high resolution but are limited to a pre-defined panel of genes (ranging between 500 to 5000 genes). The newer technology Stereo-seq has been designed to have nanoscale resolution with the ability to capture tissues with a 13 cm x 13 cm field of view (Zhuang, 2021). Emerging spatial

proteomics and metabolomics spatial proteomics and metabolomics are on the horizon, which will give us a complete "molecular geography" of tissues. This will enable a simultaneous study of gene expression and protein localization, giving us a more holistic view of biological complexity. Spatial CITE-seq is on the horizon and will enable us to start mapping high-plex protein and whole transcriptome data at a cellular level, which is critical for validating if RNA expression leads to protein expression in an immunological context (Tang et al., 2009).

3. Integrating Single-Cell and Spatial Transcriptomics

3.1. Computational Integration Approaches

Integration strategies usually involve mapping scRNA-seq data, which provides a detailed parts list of different cell types present in a sample, onto spatial coordinates determined by ST platforms. Such an integration strategy enables researchers to visualize interactions between cells and metabolic crosstalk. Algorithmic tools can project single-cell identity onto spatial maps to elucidate the content of a spot that could contain multiple cells, thus enhancing the spatial resolution of sequencing-based ST data (Longo et al., 2021). Joint embedding, deconvolution, and graph-based methods including computational tools such as SPOTlight, Stereoscope, and others, utilize scRNA-seq data for the deconvolution of the cell composition of ST spots. These tools enable the determination of cell type composition as well as specific gene expression profiles in the spatial context, circumventing the resolution of array-based spatial technologies. Deep generative models, such as Spatial Scope, are also under development for the determination of single-cell resolution from the data, allowing for more precise cell mapping (Asp et al., 2020).

Benchmarking integration tools the absence of a systematic benchmarking process for ST technologies has made it difficult to determine appropriate methods. The development of standardized benchmarking frameworks and generation of cross-platform data are critical steps towards evaluating different integration tools. Current efforts are directed towards developing a standard evaluation framework to measure gene detection bias, diffusion profiles, and sensitivity across different platforms such as Visium and Xenium (Asp et al., 2020).

3.2. Data Harmonization and Cross-Platform Challenges

Batch effects, sparsity, and normalization the process of data integration sometimes faces the problem of batch effects and sparsity. Normalization methods have to be taken into account to ensure the accuracy of the data, especially in addressing the technical variability of spatial spots. The Sc transform method, which is part of the Seurat tool, and the spatial and morphological expression (SME) method have been developed to account for spatial context and variability in the composition of the tissue (Nguyen et al., 2025). Reference atlas building and annotation standards Building comprehensive references and annotation standards is essential for identifying different cell types in a consistent manner across various studies and platforms. Projects like the Human Cell Atlas are using these technologies to build hierarchical representations of different cell types in systems, organs, and tissues. Reference atlases are essential for identifying rare immune cell populations in diseased tissues where reference material is limited (Asp et al., 2019).

4. Systems Immunology in Healthy Tissues

4.1. Immune Cell Heterogeneity across Organs

Baseline immune cell atlas for homeostatic tissues scRNA-seq Baseline immune cell atlases for various tissues has been created using scRNA-seq tools, which have been useful in understanding the heterogeneity of immune cells in homeostatic tissues. For example, in the kidney, these tools have been useful in distinguishing between resident macrophages in the kidney (KRM) and infiltrating monocytes (Yao et al., 2016). Such an understanding is critical in distinguishing between pathological changes and physiological variations (Cui et al., 2024). Tissue-specific immune signatures and niches Studies have shown that there are tissue-specific immune signatures and niches. In the human heart, spatial analysis has

shown that there are immune niches in the epicardium that are rich in IgA and IgG, indicating a specific defense mechanism in the heart that is different from the general body defense mechanism (Kanemaru et al., 2023). In the kidney, specific macrophage populations are found in specific niches, such as those in the proximal tubule, indicating tissue-specific adaptation of macrophages (Nguyen et al., 2025).

4.2. Spatial Architecture of Immune Networks

Spatial neighborhoods and local interactions spatial transcriptomics enables the identification of spatial neighborhoods where certain immune cell populations reside and interact. In skin wound healing, certain populations of lymphocytes, such as RORgt+ gamma delta T-cells, have been found to reside at the wound edge and interact with epithelial cells to promote wound healing. This notion of "spatial neighborhoods" is an important aspect of how local interactions affect immune cell function, moving beyond simple presence/absence data (Houser et al., 2023). Functional implications of immune spatial organization the spatial organization of immune cells has some functional implications. In the development of the human heart, certain cell-cell interactions have been shown to regulate the development of the ventricular walls. The spatial organization of cells has implications for understanding the role of immune cells in the development of tissues, as opposed to immune defense mechanisms, such as the role of macrophages in the remodeling of the extracellular matrix by fibroblasts (Nguyen et al., 2025).

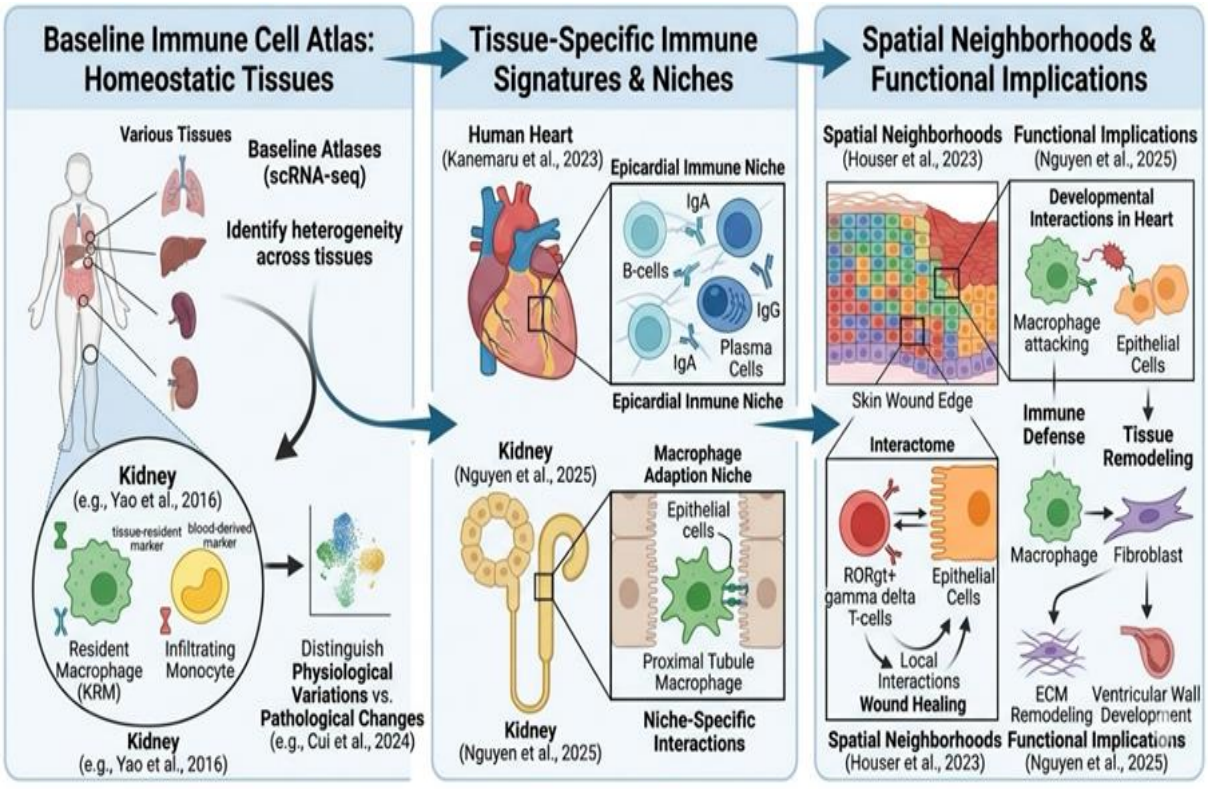

Fig 03. A Unified Single Cell and Spatial Immune Atlas Framework for Multi Tissue Heterogeneity and Niche Specific Function

5. Immune Cell Dynamics in Disease

5.1. Autoimmunity and Inflammatory Disorders

Altered immune cellular states and neighborhoods The ST technique has been used to identify the spatial distribution of cytokines, such as IL-17A and IL-13, which are associated with inflammation, such as psoriasis and atopic dermatitis, and has identified the specific T cells

responsible for inflammation, which can be "drivers" of inflammation, whereas other cells can be bystanders (Houser et al., 2023).

Spatially resolved dysregulated signaling circuits spatially resolved approaches have identified dysregulated signaling circuits in autoimmune diseases. For instance, in cutaneous lupus erythematosus (CLE), a "primed" pre-lesional phase characterized by signaling of type-I IFN has been found in normal-appearing skin. The signaling was found to be localized to the interfollicular dermo-epidermal junction, where there was interaction between keratinocytes and myeloid cells, implying the seed of inflammation in this disease (Houser et al., 2023).

5.2. Cancer Immune Microenvironment

Tumor Immune Cell Interaction Landscapes the TME is a microenvironment that is full of immune cell heterogeneity. Integrated maps have shown that various populations of Cancer-Associated Fibroblasts (CAFs) and Tumor-Associated Macrophages (TAMs) occupy specific niches in the tumor microenvironment. Various populations of CAFs have been shown in different parts of the tumor microenvironment, such as vascular CAFs and inflammatory CAFs (Yan et al., 2022). Spatial immunosuppression, exclusion, and hotspots of activation spatial analysis have revealed different mechanisms of immunosuppression, such as tumor-specific keratinocytes recruiting Tregs to exclude CD8+ T cells from tumor tissues. This "exclusion" is a key aspect of immunotherapy resistance and can only be visualized by spatial mapping, thus explaining how some tumors can have high levels of T cells but still not respond to immunotherapy (Yan et al., 2022).

5.3. Infectious and Fibrotic Diseases

Specific immune response in tissues to pathogens The scRNA-seq and ST studies of the cellular structure of the granulomas in the context of infectious diseases such as leprosy have shown that macrophages are specifically localized in the center of the granulomas, whereas T cells and dendritic cells are localized in the periphery of the granulomas, with non-immune cells such as fibroblasts and keratinocytes also being involved in the immune response through specific signaling pathways (Houser et al., 2023). Fibrosis and Immune-Stromal Cell Interactions In the context of kidney damage, the progression from fibrosis involves intricate immune cell–epithelial cell communication. An integrated analysis of this process identified the role of S100a8/A9 or Trem2+ macrophages in extracellular matrix remodeling that contributes to fibrosis. In the context of cardiac fibrosis, communication between CCR2+ macrophages and fibroblasts via IL-1 beta has been causally related to the progression of fibrosis, thereby establishing the spatial relationship for the progression from inflammation to fibrosis (Cui et al., 2024).

6. Functional Insights from High-Resolution Mapping

6.1. Cell–Cell Communication Networks

Ligand-receptor analysis in spatial context ST's capacity to understand ligand-receptor interactions in spatial terms is a strength of the model. This is particularly important in understanding how structural cells function as regulators in immunity. For instance, in skin cancer, tumor-specific keratinocytes have been shown to communicate with CAFs and macrophages in their local environment through beta-1 integrin ligands. This is no longer just about touching; it is about understanding the molecular language of the tissue niche (Houser et al., 2023). Inference of Inter-Cellular Signaling Dynamics By identifying ligand-receptor pairs, it is possible to infer intercellular dynamics such as the crosstalk between damaged proximal tubules and immune cells during acute kidney injury. This analysis extends beyond static gene sets, allowing for the dynamic network of communication that leads to disease, such as the IL-17A-mediated network between gamma delta T cells and epithelial cells during hypoxic wound adaptation (Cui et al., 2024).

6.2. Immune Trajectories and Differentiation Landscapes

Pseudotime and lineage relationships with spatial anchoring the pseudotime analysis of scRNA-seq data, when spatially anchored, has been shown to

reveal the differentiation trajectories of immune cells in tissue environments. In acute kidney injury, for example, trajectory analysis has been used to reveal the dynamic transition from proximal tubule cells to maladaptive states that are inflammatory in nature (Cui et al., 2024). Spatial constraints on cell fate decisions the spatial constraint is a key factor in cell fate decisions. The spatial distribution of the immune cells in the TME is a key determinant in the differentiation of immune cells into pro-tumor or anti-tumor cells. In islet transplantation, the spatial distribution of T cells and macrophages in the microenvironment has a significant impact on rejection or tolerance, implying that the "neighborhood" is responsible for the final function of the immune cell (Mou et al., 2025).

7. Clinical and Translational Applications

7.1. Biomarkers & Predictive Spatial Signatures

Spatial gene expression patterns as diagnostic tools spatial transcriptomics has helped identify spatial biomarkers which can function as diagnostic tools. In melanoma, for example, co-localization of certain keratinocytes expressing S100A8 with immune cells creates a diagnostic signature that is not apparent by standard histology (Houser et al., 2023). In kidney injuries, genes such as Havcr1 and Vcam1 have been identified as potential biomarkers for particular states of injury, which are more sensitive than standard serum markers (Cui et al., 2024). Prediction of response to immunotherapies these technologies have potential applications in the prediction of response to immunotherapies by characterizing the immunological profile of tumors. Technologies such as RashX can utilize information derived from scRNA-seq data to predict therapeutic responses for inflammatory skin diseases. This has potential applications for a future where spatial signatures can be used to determine biological therapy selection, moving beyond trial-and-error medicine (Houser et al., 2023).

7.2. Therapeutic Targeting via Spatially Resolved Maps

Targeting microenvironments in cancer and autoimmunity spatially resolved maps of the microenvironments in cancer and autoimmunity are providing the rationale for the development of targeted therapeutic strategies to target the relevant microenvironments. An example of such a strategy in cancer is the identification of the ligand-receptor pairs that attract Tregs to the tumor core, which provides the rationale to target immune tolerance in the tumor microenvironment (Yan et al., 2022). In kidney fibrosis, the role of macrophages in the remodeling of the ECM has provided a new therapeutic target that specifically targets the repair response in kidney fibrosis without the need to suppress the immune response in general (Kuppe et al., 2021). Spatial stratification of patients ultimately, these high-resolution maps will have the potential to improve patient stratification. This will be achieved by stratifying patients not only on the basis of the type of disease, but also on the basis of the spatial architecture of the disease, e.g., immune-excluded versus immune-inflamed tumors, and so on. This stratification will be especially important in diseases like sepsis-induced AKI and cancers, which are otherwise hard to treat (Ahmed et al., 2022).

8. Future Directions

To effectively exploit these tools' potential, there has been a focus on spatial multi-omics, which combines transcriptomics with spatial proteomics, metabolomics, and epigenomics. This is critical to determine if these RNA signatures translate into proteins and if these proteins can determine the chromatin states that lock cells into pathological states. Moreover, there has been a drive to go beyond these static states and infer temporal information to create four-dimensional models. This will include how immune cells behave over time, including interactions and movements, using methods such as time-series sampling and pseudo time trajectory analysis, which can already be used to study rapid responses to inflammation.

Moreover, the field is moving toward functional validation, for instance, with spatial CRISPR screening, which enables the study of gene perturbation in their spatial context to prove causality rather than relying on spatial correlations. However, there are still important barriers to be overcome for clinical translation, especially in terms of cost, workflow, and the need to accommodate formal-fixed paraffin-embedded material. This will require the establishment of benchmarking schemes, open-source data platforms, and ethical considerations of genetic data privacy. However, spatial transcriptomics and single-cell RNA sequencing will eventually become part of the standard clinical diagnostic repertoire, and this will be especially true once the technical and financial barriers to these approaches are overcome. This will have the potential to change the way medicine is practiced, from a more reactive approach to disease and its symptoms to a more proactive, mechanistic, or precision medicine, which could have a profound impact on patient outcome in diseases such as cancer, autoimmunity, and transplantation.

9. Conclusion

The integration of single-cell sequencing and spatial transcriptomics has revolutionized the field of systems immunology, moving from static and homogenized tissues to dynamic and high-resolution immune interactions. This technology has allowed for the visualization of the molecular geography of diseases, and this has been successful in the identification of rare cell populations, crucial ligand-receptor interactions, and the understanding of how the microenvironment of the tissue impacts immune cells.

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