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Review

Integrative spatial multi-omics reveals the tumor ecosystem: From mechanistic insights to therapeutic strategies

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SUMMARY

Tumor heterogeneity and complex microenvironment interactions drive malignant progression and therapeutic resistance. Traditional omics technologies struggle to capture this complexity due to the loss of spatial molecular context. Spatial multi-omics technologies enable the simultaneous *in situ* analysis of gene expression, metabolic activity, and protein function within tissue, thereby transforming cancer research. This review systematically discusses the principles and applications of spatial transcriptomics, spatial metabolomics, spatial proteomics, and their integration. These approaches have revealed spatially driving mechanisms, such as the niche at the invasive front, dynamic evolution of the immune microenvironment, and cell-cell communication networks in metastatic spread. We also highlight their potential to promote precise tumor treatment including guiding patient stratification, optimizing treatment regimens, and overcoming drug resistance. Finally, we discuss the current challenges and future development directions. This study aims to clarify how spatial multi-omics deepens the understanding of tumor biology and accelerates clinical translation through multi-dimensional information integration.

INTRODUCTION

Cancer is a significant and pressing global health challenge, characterized by a highly intricate process of onset and progression.[1] It also exhibits considerable heterogeneity among different tumors, even within individual tumors, as well as a notable degree of genomic instability.[2] Despite extensive research efforts, a full understanding of the underlying molecular mechanisms remains elusive, and achieving effective therapeutic strategies continues to pose substantial challenges. Tumors exhibit profound variations across various biological levels, such as the genome, transcriptome, and metabolome.[3] Their progression is further driven by intricate cell-cell interactions,[4] metabolic reprogramming,[5] and spatial reorganization of tissue architecture,[6] complicating efforts to decipher the underlying oncogenic mechanisms. Therefore, the development of advanced research technologies capable of integrating diverse molecular data while maintaining the spatial context of the tumor microenvironment (TME) is critically important for advancing progress in the field of cancer systems biology.

Traditional histopathology relies primarily on morphological observations for tumor classification.[7] However, with advancements in omics technologies, comprehensive analytical approaches, including transcriptomics, proteomics, and metabolomics, have facilitated significant progress in tumor research. Notably, the introduction of next-generation sequencing (NGS) has enabled the large-scale collection of complex molecular data,[8] such as patterns of gene expression, genetic mutations, and epigenetic modifications. Single-cell technologies, including flow cytometry,[9] highly sensitive mass spectrometry,[10] and antibody-based labeling methods,[11] have enhanced the resolution of analysis to the level of individual cells. These innovations have significantly contributed to a deeper understanding of tumor heterogeneity and the identification of distinct cellular subpopulations.[12] However, a common limitation of these methods is the loss of spatial information. Because most of these techniques require the homogenization of tissue samples, the original spatial arrangement and context of the cells within the tissue are lost. This makes it challenging to delineate functional variations across different tissue regions. Single-cell RNA sequencing (scRNA-seq),[13] which is designed to uncover cellular diversity, involves the dissociation of tissues into individual cells. This dissociation severs the spatial relationships and physical proximity between cells in their native environments, thereby hindering the reconstruction of the original cellular interactions. Consequently, the lack of spatial context limits a deeper understanding of tumor complexity, cellular ecosystem dynamics, and microenvironmental crosstalk.

Spatial multi-omics technologies offer a crucial strategy for addressing the aforementioned challenges by enabling comprehensive profiling of multi-dimensional molecular changes during tumor progression while maintaining the original spatial architecture of the tissue.[14] Key representative approaches in this field include spatial transcriptomics (ST), such as 10× Visium and Slide-seq[15,16]; imaging mass spectrometry-based spatial metabolomics (SM), including techniques such as matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI)[17,18]; as well as multiplex fluorescence or mass spectrometry imaging (MSI)-based spatial proteomics (SP), exemplified by platforms such as multiplexed ion beam imaging by time of flight (MIBI-TOF) and co-detection by indexing (CODEX).[19,20] These advanced methodologies allow for the precise measurement of molecular features while simultaneously retaining their exact spatial locations, thus preventing the loss of critical contextual information that typically occurs during tissue dissociation. Leveraging their high-resolution spatial profiling capabilities, these technologies enable researchers to conduct unbiased analysis of the spatial organization, physical proximity, and functional interaction networks between tumor cells and surrounding immune and stromal cell populations across the entire tissue landscape.[21,22] Furthermore, spatial multi-omics approaches facilitate the integration of diverse data modalities using spatial coordinate systems and support the development of predictive models,[14] thereby providing a systematic framework for uncovering the spatial regulatory mechanisms that drive tumor heterogeneity and shape the TME.[23]

Thus, this review focuses on the applications and advantages of ST, SM, and SP technologies in cancer research. We further explore how integrated spatial multi-omics can provide a comprehensive three-dimensional analysis of genetic, metabolic, and proteomic profiles within tumors. It deciphers the spatial architecture of metabolic reprogramming and intercellular communication, identifies key biomarkers, and thereby reconstructs the molecular and spatial landscape of tumors, ultimately facilitating personalized and precision treatment through the integration of multi-dimensional data.

VARIOUS SPATIAL OMICS TECHNOLOGIES AND THEIR APPLICATIONS IN CANCER RESEARCH

Spatial omics technologies are essential for deciphering the mechanisms underlying cancer development and progression, and offer a distinct advantage in that they can precisely characterize the molecular features of tumors and their microenvironment while preserving the spatial context of the tissue. This enables the direct interpretation of the spatial patterns that govern tumor heterogeneity, cellular communication, and malignant evolution. The following sections explore how spatial omics technologies create value and unlock specific pathways for clinical applications by detailing the spatiotemporal dynamics of tumor molecules.

Spatial transcriptomics: Visualization of tumor genetic material at the 3D level

The core of ST lies in retaining the spatial location information of transcripts, thereby enabling unbiased and high-throughput analysis of whole-genome expression in intact tissues. Owing to its far-reaching influence, it was named the Breakthrough Technology of the Year in 2020.[24] Computational models based on ST data, such as the cell differentiation trajectory strategy CASCAT,[25] the computational method SpaTalk for inferring cell communication based on knowledge graphs and graph neural networks,[26] and the spatial model SpatialDM for quantifying ligand-receptor interactions jointly contribute to a deeper understanding of the spatial biological mechanisms of tumors.[27] Advancements in ST and analytical methods have revealed the spatial basis of cell type composition, maintenance of cellular heterogeneity, and intercellular communication, offering crucial insights into identifying novel therapeutic targets and deciphering drug resistance mechanisms.[14,28]

Principles and classifications of spatial transcriptomics technology

Spatial transcriptomic technologies can be primarily classified into two major categories based on their core detection principles: sequencing-based and imaging-based (Figure 1). Sequencing-based methods, such as 10× Visium and Slide-seq, associate transcripts with their spatial coordinates through spatial barcodes, enabling unbiased capture and quantification of the entire transcriptome. These approaches involve a compromise between the rate of data or sample processing and the level of detail or precision achieved.[15,29] For example, Visium is highly effective for capturing broad, comprehensive views of entire tissue sections, yet its spatial resolution is constrained by the physical dimensions of its probe capture locations (Figure 1A). In contrast, Slide-seq offers a resolution that approaches the single-cell level, but it is accompanied by drawbacks such as a relatively poor signal-to-noise ratio and the need for sophisticated data processing techniques. Imaging-based methods eliminate the requirement for library construction and sequencing. They conduct RNA imaging directly in tissues *in situ* (Figure 1B) through multiplex *in situ* hybridization (e.g., MERFISH) or *in situ* sequencing (e.g., STARmap).[30,31] Although these techniques can achieve subcellular resolution, gene throughput is restricted by a pre-designed panel, and the process is relatively technically complex. Furthermore, commercial platforms such as Xenium have improved the reliability and consistency of their workflows by refining probe designs and operational procedures,[32] while retaining high-resolution imaging capabilities. With regard to the selection of technology, it is essential to give full consideration to spatial resolution, detection throughput, sample compatibility, and the requirements for multimodal integration. For instance, Visium is suitable for systematically exploring heterogeneous tumor regions, whereas techniques such as MERFISH are more appropriate for conducting fine-grained validation of specific genes or cell interactions at the subcellular level. This provides precise support for research on tumor boundary delineation, inference of clonal evolution, and spatial visualization of therapeutic targets.

Computational integration and investigation into functional analysis

The computational combination of ST and scRNA-seq is a fundamental approach to addressing the resolution constraints inherent in ST.[33] For instance, the SPOTlight method employs nonnegative matrix factorization (NMF) to deconvolute and determine the

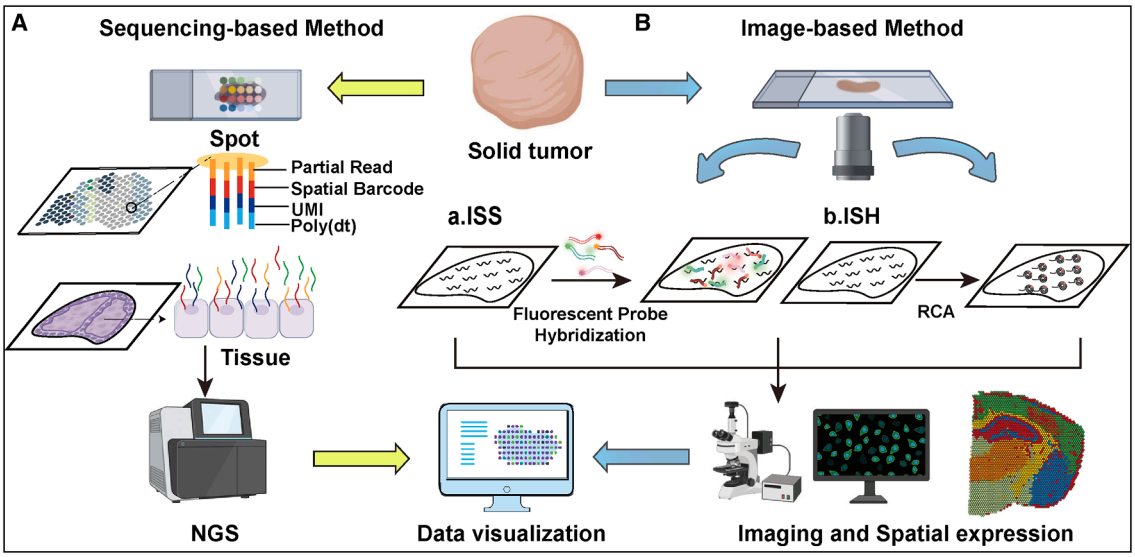

Figure 1. Spatial transcriptomics principles and advanced analytical tools

(A) Sequencing-based Method: Tissue sections are placed on microarrays or beads containing spatial barcodes. The capture probes bind to *in situ* cellular mRNA, and then high-throughput sequencing is employed to simultaneously measure the expression levels and spatial locations of transcripts (taking Visium as an example).

(B) Image-based Method: It mainly includes *in situ* hybridization (ISH) and *in situ* sequencing (ISS). ISH uses multiple fluorescently labeled short oligonucleotide probes to specifically hybridize with the RNA in tissues for imaging and localization; ISS performs rolling circle amplification (RCA) after the padlock probes are circularly ligated to cDNA, and then reads the RNA sequence information in the tissue *in situ* through sequencing reactions.

cellular makeup at each spatial capture location.[34] Similarly, Cell2location utilizes a Bayesian statistical model to achieve precise absolute spatial mapping of different cell types,[35] thereby significantly enhancing the characterization of cellular communities within the TME. These integrative approaches do more than create interactive maps of cellular distributions; they leverage spatial context to uncover the functional states of cells. Wu et al. integrated Visium technology with scRNA-seq to precisely characterize the spatial distribution of cell types within primary testicular diffuse large B-cell lymphoma (PT-DLBCL). Their study provided a detailed examination of the unique immune microenvironment of the tumor and uncovered the spatial regulatory mechanisms governing the expression of the key transcription factors E2F and CREB.[36] Xiao et al. utilized multimodal intersection analysis (MIA) to identify a distinct cluster of early metastatic epithelial cells within lung cancer tissues. They further delineated the spatial migration path of these cells, tracing their movement from the outer regions of the tumor toward the central core.[37] Such methodologies, which can be termed spatial functional annotation, enable dynamic tracking of cellular state evolution under microenvironmental pressures. This provides a powerful framework for investigating the spatiotemporal dynamics of tumor regulation with unprecedented precision.

With the continuous advancement of analytical tools such as SpatialDE for identifying spatially variable genes,[38] Giotto for characterizing cell neighborhoods,[39] and SpaGCN for delineating spatial domains,[40] ST has been substantially enhanced in its capacity to identify spatially variable genes, cellular neighborhoods, and tissue domains. These developments enable a more comprehensive understanding of the mechanisms underlying cancer cell communication. For instance, Arora et al. integrated multiple computational tools, including Seurat, CellChat, and scVelo/Dynamo, to conduct a comprehensive, multi-dimensional analysis of oral squamous cell carcinoma (OSCC). Their findings revealed that the tumor core region maintains cellular differentiation through epithelial stabilization signals such as those mediated by adhesion molecules and immune regulatory pathways. In contrast, the tumor's leading edge promotes the development of an invasive phenotype via extracellular matrix (ECM) remodeling, activation of epithelial-mesenchymal transition (EMT) signaling, and a pro-inflammatory microenvironment driven by cancer-associated fibroblasts (CAFs).[41] Similarly, through the combined application of MistyR and CellDegree analytical approaches, Zhou et al. demonstrated that *NAT10* serves as a potential therapeutic target in colon cancer (CC). Their findings revealed that *NAT10* enhances the interaction between epithelial cells and myeloid-derived suppressor cells (MDSCs), thereby promoting immune evasion, tumor progression, and treatment resistance.[42]

Beyond descriptive analysis, ST also leverages mathematical modeling and machine learning approaches to forecast the spatial evolution of tumors using available data. This forward-looking capability offers a novel way to comprehend the dynamic processes shaping the TME and establishes a scientific foundation for developing more targeted and individualized cancer treatment strategies. For instance, Quan et al. employed Visium data to identify critical genes linked to the Gleason score in prostate cancer, among which *RBM39* was identified, and they additionally confirmed its functional contributions to driving tumor progression.[43] Similarly, Xiao et al. constructed a preoperative

chemotherapy risk signature model (PCRSM) by applying machine learning techniques to integrate extensive single-cell and ST datasets, allowing for the precise forecasting of both prognosis and treatment responses in individuals with colorectal cancer.[44] In conclusion, ST has become an essential technology that leverages high-throughput experimental platforms, single-cell sequencing information, and sophisticated computational methods to comprehensively characterize tumor heterogeneity, map the spatial interactions between cells, and identify potential therapeutic targets. This approach provides a strong methodological background for the advancement of precision medicine.

Spatial metabolomics: Investigating metabolic reprogramming and interactions within the tumor microenvironment

Cancer cells undergo metabolic reprogramming to meet the increased demand for energy and biosynthetic precursors required for rapid proliferation. This metabolic rewiring establishes a distinct metabolic phenotype that is not only a hallmark of cancer but also critical for the acquisition of aggressive and malignant traits.[45] As a cutting-edge approach in the field of molecular imaging, SM combines detailed metabolite profiling with the ability to map molecules in their original tissue environment. Therefore, elucidating the spatial distribution and functional heterogeneity of metabolites within tumor tissues is essential for understanding cancer progression. SM has emerged as a cutting-edge molecular imaging approach that integrates comprehensive metabolite profiling with spatially resolved analysis, enabling the visualization of metabolite distributions directly within intact tissue sections. By preserving the native tissue architecture, SM provides critical insights into the spatial organization of metabolic activities and establishes a direct link between bioactive metabolites, tissue microenvironment, and physiological functions.

Principles and classifications of spatial metabolomics technology

The core technology driving SM is MSI, which enables the identification and measurement of metabolites without the need for chemical labeling or radioactive tracers.[46] In MSI-based SM, tissue sections are scanned in a raster pattern, and a mass spectrum is acquired at each sampling position (pixel). The spatial resolution and spatial accuracy of MSI are primarily determined by several technical factors, including the diameter of the sampling probe (or ionization beam), the raster step size during scanning, and the positioning precision of the motorized sample stage. Together, these parameters define the effective pixel size and determine how precisely metabolite signals can be correlated with histological structures.

Among the available MSI techniques, MALDI and DESI are the most widely employed methods. In MALDI-MSI,[17,18] a focused UV laser irradiates matrix-coated tissue surfaces to desorb and ionize analytes. The spatial resolution (typically 5–200 μm) is mainly determined by the laser spot diameter and the raster step size used during scanning. DESI-MSI generates ions by directing a focused stream of charged solvent droplets onto the tissue surface under ambient conditions, with spatial resolution (typically 50–200 μm) governed by the spray focus and solvent impact area. Both methods enable simultaneous acquisition of spatial distribution data for hundreds of metabolites in tissues, thereby facilitating qualitative analysis, relative quantification, and precise localization. In addition, secondary ion mass spectrometry (SIMS) offers superior spatial resolution by using a tightly focused primary ion beam to scan the sample surface, achieving sub-micrometer to nanometer resolution. This enables the visualization of metabolic turnover at the subcellular level, offering advantages over other techniques for studying compartmentalized metabolism.[47,48]

Spatial metabolomics: Monitoring metabolic reprogramming in cancer cells

The application of SM has significantly advanced biomarker discovery and the analysis of metabolic mechanisms in tumors and other diseases, demonstrating its substantial potential for use in clinical and biological research. As illustrated in Table 1, SM play a crucial role in identifying tumor biomarkers and elucidating metabolic reprogramming. For instance, amino acids not only serve as a key energy source for cancer cells but are also involved in regulating various biological processes.[60] In recent years, research has increasingly focused on amino acids and their metabolites to investigate their roles in tumorigenesis and tumor progression across diverse metabolic pathways and biological functions. Arginine, a vital amino acid implicated in cell proliferation, immune regulation, and cellular signaling has been associated with metabolic dysregulation in multiple types of cancer.[61] For example, Ma et al. used MALDI-MSI technology to find significant reprogramming of arginine and its metabolites spermine and spermidine in hepatocellular carcinoma tissues.[59] Furthermore, another study demonstrated that the levels of arginine, spermine, and spermidine were markedly elevated during the progression from cirrhosis to HCC, suggesting their potential as therapeutic targets and implicating a profound reconfiguration of the metabolic network in HCC.[58] Glutamine, another pivotal metabolite, plays a critical role in tumor energy and nitrogen metabolism.[62] Through the application of MSI in the study of esophageal cancer, investigators have confirmed that tumor tissues exhibit heightened levels of glutamine uptake. In addition, they identified a marked increase in the expression of glutaminase, a key regulatory enzyme that controls glutamine metabolism. These discoveries offer a more comprehensive understanding of the metabolic processes that contribute to the development of esophageal cancer.[49] Moreover, glutamine functions as an essential building block for the synthesis of both arginine and proline and is involved in the activity and function of lymphocytes.[63,64] Sun et al. conducted a metabolomic study and discovered that gastric cancer tissues and surrounding tumor-associated lymphoid areas exhibited excessive consumption of glutamine. This increased utilization leads to alterations in polyamine metabolic pathways. The researchers suggested that reducing the availability of glutamine may play a key role in disrupting the normal function of immune cells within the TME.[56] In conclusion, metabolomic analysis is a powerful tool for uncovering the complex metabolic processes involved in tumors.

Spatial metabolomics reveals distinct characteristics of the tumor microenvironment

The interface formed between the invading tumor cells and the surrounding stromal tissue is highly heterogeneous. This

Table 1. Applications of spatial metabolomics in cancer research utilizing multiple MSI techniques

Tumor Type	Tumor Subtype/ Comparative Tissue	Key Dysregulated Metabolites	Implicated Pathways /Biological Functions	MSI Technique	Biological/Clinical Implications	Reference
Esophageal Squamous Cell Carcinoma (ESCC)	ESCC vs. Matched Adjacent Normal	Amino acids (Glutamine, Glutamate), Fatty acids (FA 16:0, FA 18:1), Choline metabolites (Choline, Phosphocholine, GPC), Phospholipids (PCs, PEs, PIs), Nucleosides (Inosine)	Amino Acid Metabolism (Glutaminolysis), Lipid Metabolism (Fatty Acid Metabolism, *De Novo* Phospholipid Synthesis), Energy Production, Biosynthesis	MALDI-MSI	Metabolic Heterogeneity Linked to Proliferative States; Dysregulation of Key Metabolic Enzymes (GLS, FASN, CHKA, cPLA2)	Zang et al.[49]
Renal cell carcinoma	Clear Cell Renal Cell Carcinoma vs. Matched Adjacent Normal Tissues	Glucose, Arachidonic Acid, Lactate, Glutamate, Succinate, Oleic Acid, Glycerophospholipids (e.g., PG 34:1, PI 38:4)	TCA Cycle, Aerobic Glycolysis (Warburg Effect), Fatty Acid Metabolism, Phospholipid Metabolism	DESI-MSI	Proof-of-Concept for Rapid Intraoperative Margin Assessment; Metabolic Rewiring Driven by VHL/HIF Pathway; Combined Model (Metabolites + Glucose/ Arachidonic Acid Ratio) Achieved 85.3% Accuracy	Vijayalakshmi et al.[50]
Brain cancer	Glioblastoma (GBM) vs. Normal Cortex	Cardiolipins (CLs), specifically Long-chain Polyunsaturated CL species (e.g., CL(78:12), CL(76:10)) and Short-chain CL species (e.g., CL(72:6))	Mitochondrial Energy Metabolism (Oxidative Phosphorylation, Electron Transport Chain Function); Mitochondrial Membrane Architecture and Dynamics; Apoptotic Signaling	DESI-MSI	Aberrant Mitochondrial Lipid Composition Indicative of Metabolic Dysfunction; Correlation with Tumor Grade and Intra-tumoral Heterogeneity; Potential as a Spatial Biomarker for Metabolic Alterations in Glioma	Krieger et al.[51]
	Pediatric Brain Tumors vs. Matched Adjacent Normal	Phosphatidylcholines (e.g., PC 34:1), Phosphatidylinositols (e.g., PI 38:4), Phosphatidylserines (e.g., PS 40:6)	Membrane Phospholipid Metabolism, Cell Proliferation, Apoptosis Resistance	DESI-MSI	Intraoperative Discrimination of Tumor from Non-tumor Tissue with High Accuracy (94–96%); Potential for Real-time Surgical Margin Assessment	Seidinger et al.[52]
Breast Cancer	Invasive Ductal Carcinoma, Ductal Carcinoma *In Situ* vs. Various Cell Types in TME	Phospholipids (PIs, PEs, PS, LysoPA), Fatty Acids (C18:0, C20:4, etc.), Glucose Metabolism-Related Metabolites (Hexose Phosphate, etc.)	PI3K/AKT Signaling Pathway, Lipid Metabolic Reprogramming, Inflammatory Response	SIMS	Reveals Metabolic Heterogeneity at Single-Cell Resolution; Elucidates Metabolic Basis of Cell–Cell Interactions; Provides Novel Insights into Tumor Immunometabolism and Precision Therapy	Tian et al.[53]

(*Continued on next page*)

Table 1. *Continued*

Tumor Type	Tumor Subtype/ Comparative Tissue	Key Dysregulated Metabolites	Implicated Pathways /Biological Functions	MSI Technique	Biological/Clinical Implications	Reference
Lung Adenocarcinoma(LUAD)	LUAD vs. Lung Squamous Cell Carcinoma vs. Normal Adjacent Tissue	Glycogen (specifically long-chain polymers CL6-CL9, phosphorylated)	Glycogen Metabolism, Central Carbon Metabolism (Glycolysis, TCA Cycle)	MALDI-MSI	Glycogen accumulation drives progression and poor prognosis, promotes tumorigenesis when elevated, and is suppressed by inhibiting synthesis	Clarke et al.[54]
Thyroid Cancer	Differentiated Thyroid Carcinomas vs. Benign Follicular Adenoma (FA)	Amino Acids (e.g., Glutamine, Histidine); Dipeptides (e.g., Glutamylvaline); Acylcarnitines (e.g., Butyrylcarnitine); Fatty Acids; Nucleotides; Eicosanoids	Amino Acid Metabolism, Lipid Biosynthesis and Signaling, Eicosanoid-Inflammatory Pathways	AFADESI-MSI.	Metabolic Heterogeneity Visualization; Diagnostic Model for Benign-Malignant Discrimination; Potential Intraoperative Molecular Pathological Biomarkers	Huang et al.[55]
Gastric Cancer	Tumor, Tumor-Gland, Normal Epithelium, Intestinal Metaplasia, Lymphoid Follicle, Muscularis Mucosa, Peritumoral Muscularis	Arginine, Proline, Polyamines (Spermine, Spermidine); Glutamine, Glutamate; Fatty Acids (e.g., FA-16:0, FA-20:4); Phospholipids (PC, PE, PI)	Arginine/Proline and Polyamine Metabolism; *De Novo* Lipogenesis and Phospholipid Synthesis; Immunometabolic Reprogramming	MALDI-MSI	Profound Metabolic Heterogeneity; A Remodeled Immunometabolic Interface; Metabolic Crosstalk Driving Tumor Progression	Sun et al.[56]
	Gastric Signet Ring Cell Carcinoma (GSRC) vs. Poorly/Moderately Differentiated Adenocarcinoma vs. Normal Tissue Adenocarcinoma, Adjacent Normal Tissue	Phosphatidylethanolamine N-methyl (PE-NMe), Phosphatidylethanolamine (PE), Sphingomyelin, Diacylglycerol (DG), Phosphatidic Acid (PA), Phosphatidylcholine (PC)	Glycerophospholipid and Glycerolipid Metabolism; Sphingolipid Metabolism; mTOR/PKC Signaling in Cell Survival & Proliferation	DESI-MSI	Reveals lipid biomarkers for GSRC diagnosis and suggests novel targets for lipid metabolism-based therapy	Shi et al.[57]
Liver cancer	Hepatocellular Carcinoma vs. Liver Cirrhosis (LC) vs. Matched Adjacent Normal Tissue	Amino Acids (e.g., Arginine), Fatty Acids/Phospholipids (e.g., Phosphatidylcholines), Carbamoyl Phosphate and other key molecules (Taurine, Histamines. etc.)	Amino Acid and Urea Cycle Metabolism (Arginine/ Proline); Glycerophospholipid and Choline Metabolism	AFADESI-MSI	Metabolic Reprogramming in Tumor Stroma; Metabolic Heterogeneity Across Sub-regions; Identification of Potential Biomarkers for Early Detection and Disease Progression	He et al.[58]
	Hepatocellular carcinoma(HCC) tumor tissue vs. Adjacent non-tumor tissue	Arginine and its metabolites (Spermine, Spermidine); Choline metabolic pathway (e.g., Choline); Phospholipids and Fatty Acids	Polyamine Metabolism; Choline and Phospholipid Metabolism; Fatty Acid Metabolism and β-oxidation	MALDI-MSI	Spatially Resolved Metabolic Heterogeneity Increases with Tumor Progression; Identification of Potential Metabolic Vulnerabilities for HCC Therapy	Ma et al.[59]

diversity in cell types and the complexity of their interactions present significant challenges for targeted therapies. The metabolic compositions of tumor and stromal cells contain a wealth of biological information. Serving as a powerful complement to conventional histopathology, MSI enables precise delineation and visualization of tumor boundaries based on characteristic metabolic signatures. For example, the death rate associated with gastric cancer continues to be notably high, and current therapeutic approaches show considerable variation from one patient to another.[65] By comparing the metabolic profiles of the tumor and stromal regions, Wang et al. identified a tumor-specific subtype, T1 ($HER2^{+}MIB^{+}CD3^{+}$), which was associated with an improved response to trastuzumab therapy in an independent validation cohort. This subtype exhibits marked upregulation of nucleotide metabolic pathways and accumulation of metabolites involved in DNA biosynthesis, suggesting a metabolic foundation that supports enhanced cell proliferation. These findings imply that metabolic profiling could predict sensitivity to targeted therapies, such as trastuzumab, potentially through mechanisms linked to DNA metabolism, offering a novel approach for stratifying patient treatment.[66] Non-clear cell renal cell carcinoma (nccRCC) is a highly heterogeneous malignant tumor. This heterogeneity presents a significant diagnostic and therapeutic challenge, particularly in distinguishing between morphologically similar subtypes such as chromophobe RCC (chRCC) and renal oncocytoma (RO).[67] The application of MALDI-MSI imaging by Kriegsmann et al. yielded a high discriminatory accuracy of 89% for chRCC and RO.[68] These investigations demonstrate that SM enables a more detailed classification of cancer cell types, reveals connections between distinct metabolic profiles and how tumors respond to treatments, and significantly advances tailored cancer therapies.

Spatial proteomics: Mapping and identifying unique biomarkers

As the main functional molecules in biological systems, proteins mediate key cancer hallmarks, including proliferation, immune evasion, and metastasis. However, conventional proteomic methods, such as bulk or single-cell mass spectrometry, fail to capture the spatial context of protein distribution within their tissue architecture. SP addresses this gap by enabling *in situ*, high-plex analysis of protein distribution, abundance, and interactions with high resolution and specificity, thereby mapping the functional landscape of the TME while preserving spatial context.

Technological approaches for spatial proteomics

Current SP technologies can be broadly categorized into two main groups: targeted antibody-based methods and untargeted MSI-based methods.[69,70] These platforms differ in multiplexing capacity, detection principle, spatial resolution, and suitability for discovery versus validation studies. Antibody-based platforms rely on antibody panels to detect predefined protein targets. For instance, CODEX achieves high multiplexing through DNA-barcoded antibodies and iterative staining, while MIBI-TOF and IMC use metal-tagged antibodies with mass spectrometry detection[71,72]; both approaches enable the detection of over 40 protein markers. In contrast, multiplex immunofluorescence (mIHC) offers compatibility with standard pathology workflows.[73] Digital spatial profiling (DSP) uses photocleavable DNA-barcoded antibodies for region-of-interest-based analysis, which offers greater flexibility and multiplexing capacity.[74]

Complementary to these targeted approaches, MSI-based methods, such as MALDI-MSI, offer label-free SP, enabling simultaneous detection of hundreds of peptides and proteins without the need for predefined antibody panels. This makes MSI particularly suitable for discovery-driven studies aimed at identifying novel biomarkers. Although MSI generally exhibits lower throughput for targeted protein quantification compared with antibody-based platforms, the two approaches are often complementary: MSI excels at unbiased discovery, whereas antibody-based platforms enable high-plex targeted validation and spatial phenotyping.[75]

Spatial proteomics deciphering the functional architecture of the tumor microenvironment

By preserving spatial context, SP reveals that biomarkers are often cell-type or compartment-specific. As illustrated by the examples later in discussion, this contextual specificity operates at multiple levels of spatial organization—ranging from tissue compartments to individual cell types to subcellular niches— offering progressively finer resolution into the functional architecture of the TME.

At the tissue compartment level, Fan et al. resolved high-grade serous ovarian cancer into tumor epithelium and stroma, validating compartment-specific candidates TFRC (tumor) and PDLIM3 (stroma) as predictors of chemotherapy response—demonstrating that biomarker significance is spatially constrained even between adjacent regions.[76] Advancing to cell-type resolution, Yang et al. interrogated mesenchymal stem cells (MSCs) within the lung TME and identified SFPQ as specifically overexpressed in cancer-associated MSCs, where it drives stemness and chemoresistance via CD44v6 regulation.[77] At the subcellular level, Solis-Fernandez et al. mapped protein redistribution during colorectal cancer metastasis, revealing that BAIAP2 shifts from cytoplasm to membrane in metastatic cells, where its membrane-specific expression—but not total abundance—correlates with poor prognosis. Similarly, nuclear accumulation of MUC5AC, obscured in whole-cell analysis, was linked to adverse survival.[78] By preserving spatial architecture at each level of resolution, SP transforms biomarker discovery from a static molecular inventory into a functionally anchored map of the TME.

The potential of spatial proteomics technology in the clinical detection of biomarkers

Building on the spatial context established above, SP has advanced toward clinical translation, accelerated by its compatibility with routine pathology specimens and established antibody-based workflows.[79] By enabling multi-region, multi-target *in situ* profiling directly from patient biopsy samples,[80] SP allows detailed interrogation of marker expression and spatial distribution within the TME. In HER2-positive breast cancer, SP tracks dynamic changes in tumors and their surrounding immune environments during neoadjuvant treatment, identifying that specific changes in CD45 expression could predict pathological complete response—offering a strategy for treatment tailoring.[81]

Beyond capturing dynamic changes during therapy, SP can also resolve conflicting clinical implications of the same molecular marker by pinpointing its cellular origin. Yan et al. combined GeoMx DSP with mIHC in early-stage non-small cell lung cancer,

revealing that elevated CD44 expression specifically in lymphocytes was associated with resistance to stereotactic body radiotherapy but a favorable response to surgical resection, whereas patients with low CD44 expression in the same compartment exhibited the opposite pattern. These findings demonstrate that the predictive value of CD44 emerges only when its cellular context is considered.[82] Although SP has already demonstrated substantial potential in biomarker discovery and clinical decision-making, capturing the full complexity of therapeutic responses will likely require integration with complementary spatial modalities to generate a comprehensive multi-layered view of tumor biology.

INTEGRATED SPATIAL MULTI-OMICS FOR SYSTEMATIC AND CROSS-DISCIPLINARY CANCER RESEARCH

The development and progression of cancer arise from intricate, multilayered interactions involving gene expression patterns, protein functions, and metabolic processes. Spatial multi-omics technology offers a powerful approach that combines single-cell sequencing data to simultaneously capture multi-dimensional molecular details directly within tissue samples. This integration allowed for cross-omics validation and complementarity, ultimately revealing spatiotemporal synergy across these molecular dimensions (Figure 2A). This method greatly improves the ability to map cellular communication networks and interpret spatially organized information within the TME, offering fresh insights into the broader regulatory mechanisms of cancer. As this technology continues to advance and become more widely adopted, it has the potential to open new avenues for precision and personalized treatment strategies.

Spatial multi-omics reveal the spatial drivers of tumor evolution

Understanding how tumors begin and develop forms an essential scientific basis for preventing and curing cancer. Such knowledge provides the foundation for early prevention and precise diagnosis while opening avenues for novel therapies to improve patient outcomes. Recent advances in spatial multi-omics now enable the precise characterization of tumor cell types within their native 3D architecture, which helps uncover the complex molecular pathways and interactions within the TME that contribute to the onset and progression of the disease.

Spatial multi-omics reveals spatiotemporal dynamics and immune escape mechanisms in the tumor microenvironment

To survive and proliferate, tumor cells evade immune surveillance by fostering a local immunosuppressive microenvironment. This process, which is driven by complex metabolic reprogramming and dysregulated signaling pathways, presents major challenges for immunological research and targeted therapy development.[84] Spatial multi-omics enables the precise delineation and validation of functional domains, such as immune-activated and immunosuppressive regions, within the tumor immune microenvironment from multiple molecular dimensions. Through integrated analysis, it further allows for the reconstruction of spatial interaction networks, revealing how topological structures regulate immune function, and provides novel perspectives for overcoming resistance to immunotherapy (Figure 2B).

For instance, Yousuf et al. integrated scRNA-seq with ST and SP to systematically characterize the spatial interactions among cells in pancreatic ductal adenocarcinoma. Their study was the first to identify the immunosuppressive role of $CD4^{+}CD52^{+}$T cells and revealed that natural killer T (NKT) cells located in the tumor boundary zone can adopt a regulatory phenotype expressing IL-4 and IL-13. These findings demonstrate that the TME drives the functional reprogramming of immune cells through spatially dependent mechanisms.[85] Sun et al. integrated SM, spatial lipidomics, and ST data for gastric cancer, revealing that the tumor-normal boundary region was enriched with B cells and Th2-like T cells. Concurrently, metabolic heterogeneity was observed, including glutamine depletion due to competitive consumption and accumulation of lipids and cholesterol, which collectively contribute to immunosuppression and tumor progression.[56] Furthermore, Zhi et al. integrated ST and SM to demonstrate that OSCC tumor cells acquire a fibroblast-like phenotype through partial EMT. These cells drive immune cells toward exhausted or regulatory states via spatially constrained mechanisms, such as polyamine metabolic reprogramming, thereby promoting immune evasion. This study also identified the distinct spatial clustering of B and T/NK cells within tertiary lymphoid structures, underscoring how the microenvironmental topology regulates immune responses.[86] Therefore, spatial multi-omics can systematically reveal the spatiotemporal dynamics of the tumor immune microenvironment at molecular, cellular, and regional scales. This approach provides crucial technical support for elucidating the mechanisms of immune escape and developing new microenvironment-targeting strategies.

Spatial multi-omics elucidates microenvironmental remodeling and niche formation at the tumor-host interface

The boundary region where a tumor meets the surrounding healthy tissue, known as the tumor-host interface, is a highly active and constantly changing area.[87] This zone features a diverse mix of cell types and intricate molecular communications that play a crucial role in influencing how the tumor grows and spreads. Spatial omics technologies allow for the precise capture of molecular data along with their spatial context within tissues, gradually uncovering the disease-related mechanisms at this critical interface (Figure 2C). For instance, Wu et al. integrated ST with the Cottrazm algorithm to delineate the interface between breast tumor tissue and the surrounding stromal compartment, pinpointing genes that exhibit distinct expression patterns in specific regions. They used SP to perform multiplex fluorescence validation, which revealed an enrichment of M2-type macrophages and functionally exhausted T cells at the invasive edge of breast cancer. This accumulation contributes to the formation of an immunosuppressive TME. These results imply that the crosstalk between CAFs and M2-type macrophages represents a promising target for therapeutic intervention.[88] Nirmal et al. integrated SP and ST technologies to identify an ‘‘immunosuppressive microsheath’’ composed of $PD\text{-}L1^{+}$myeloid cells and $PD\text{-}1^{+}$T cells at the melanoma invasion front. This structure was accompanied by localized

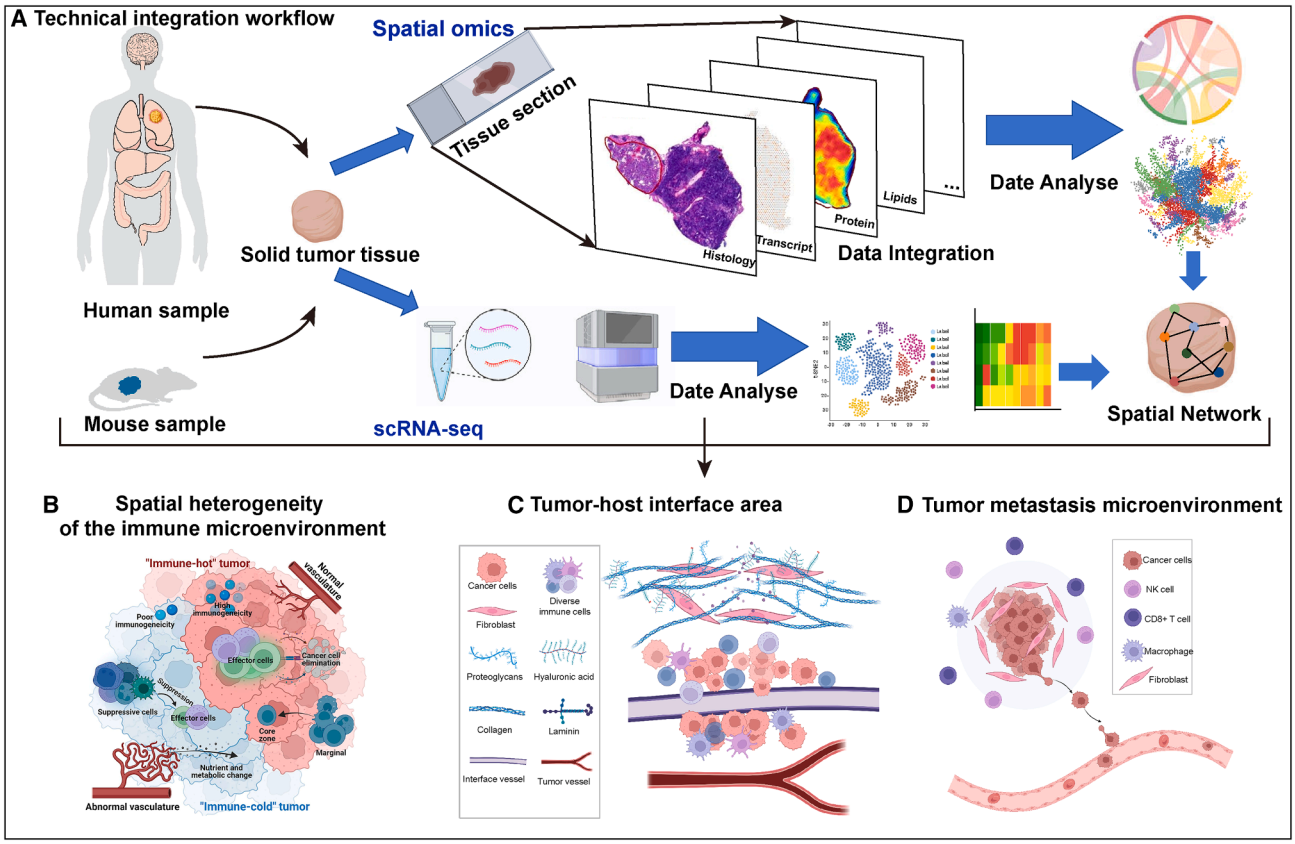

Figure 2. Spatial multi-omics integration analysis unveils coordinated regulatory patterns in the tumor microenvironment

(A) Technical integration workflow: This approach involves the precise spatial alignment of diverse multi-omics datasets—including transcriptomics, proteomics, and metabolomics—alongside corresponding histological imaging derived from identical tissue samples. The analysis is further enhanced through the incorporation of the complementary single-cell sequencing data to provide a comprehensive molecular and cellular perspective.

(B) Tumor–host interface area: Focused examination of the dynamic ecological niche at the tumor invasive front highlights both the physical and functional interactions occurring between malignant cells and surrounding host tissues. Adapted from Springer Nature,[83] published under a Creative Commons Attribution 4.0 International License.

(C) Spatial heterogeneity of the immune microenvironment: Detailed spatial mapping of immune cell populations reveals their localized distribution patterns, functional phenotypes, and the molecular mechanisms underlying immune evasion strategies employed by cancer cells.

(D) Tumor metastasis microenvironment: Systematic spatial analysis of cellular interactions identifies a complex regulatory network that governs the initiation and progression of metastatic processes. Collectively, this integrated approach deciphers the multi-dimensional and systemic regulatory mechanisms of cancer.

IFNγ-JAK-STAT signaling activation and MHC-II upregulation. Further spatial modeling analysis revealed the co-evolution of immune suppression and tumor progression in space.[89]

ECM remodeling is a dynamic process occurring at the tumor-host interface and is cooperatively mediated by tumor and stromal cells.[90] Spatial multi-omics can simultaneously analyze the multi-dimensional molecular characteristics of tumor cells, stromal cells, and the ECM in this region and precisely depict the key signaling and spatial interaction networks that drive ECM remodeling (Figure 2C). For instance, by integrating NanoString GeoMx (ST) with CODEX (SP) in early signet-ring cell (SRC) lesions associated with *CDH1* mutations, Gallanis et al. demonstrated that ECM remodeling is cooperatively driven by tumor and stromal cells at the tumor-host interface. Their spatial and scRNA-seq data showed significant upregulation of ECM components (e.g., *COL3A1*) and remodeling genes in SRC lesions, along with marked enrichment of pathways related to ECM degradation, integrin interactions, and proteoglycans. These changes form a spatially coordinated network involving fibroblasts and pericytes, collectively promoting ECM architectural reorganization.[91] By integrating ST and SM, Wu et al. identified specific ligand-receptor interactions such as ADGRE5-CD55 and COL1A1-CD44 among cancer cells, macrophages, and CAFs in the hypermetabolic regions of pancreatic cancer. These interactions are closely associated with ECM-receptor interactions and focal adhesion pathways and coincide with the regional enrichment of metabolites, including polyamines and phospholipids, collectively driving the microenvironmental basis for ECM remodeling and metabolism.[92] Therefore, spatial-omics deciphers the spatial heterogeneity and regulatory networks of ECM remodeling, paving the way for innovative strategies to target tumor malignancy.

Furthermore, CAFs serve as the primary source of ECM components and various secreted factors and play a pivotal role in

the invasive front.[93,94] Spatial multi-omics adds a novel spatial dimension for characterizing the cellular origins, functional subtypes, and microenvironmental interactions of CAFs. For instance, in primary liver cancer (PLC), Jing et al. identified a novel CAF subpopulation, termed F5-CAFs, by integrating ST with proteomics. This F5-CAF subset was spatially enriched at the tumor-normal interface and was associated with unfavorable patient prognosis. Subsequent functional validation demonstrated that F5-CAFs directly promoted tumor cell proliferation and stemness. These findings highlight the potential of targeting CAF-driven mechanisms as a therapeutic strategy against PLC.[95] In a study of colorectal cancer liver metastasis, Gao et al. integrated ST and SM to identify a distinct subtype of CAFs with high *SPP1* expression. These *SPP1*$^{+}$ CAFs were found in spatial proximity, interacted with tumor cells via the SPP1-CD44 axis, and were linked to the accumulation of specific metabolites, collectively fostering an immunosuppressive and pro-metastatic microenvironment.[96] These studies revealed the functional specialization, interaction mechanisms, and clinical relevance of CAF subtypes spatially, highlighting the pivotal role of spatial multi-omics in deciphering CAF heterogeneity and its crosstalk with the TME.

Spatial multi-omics uncovers the spatial-specific cell communication network driving tumor metastasis

Moreover, spatial multi-omics has enabled the establishment of a multilevel interaction framework through the inference and validation of spatial proximity and cell-to-cell interaction information, thereby elucidating the core mechanisms of metastatic dissemination (Figure 2D). For instance, Zhou et al. integrated ST and SP to study gallbladder cancer and revealed that the malignant cell subtype GM16 co-localized with *AREG*$^{+}$T cells, B cells, dendritic cells, and macrophages within a metastasis-promoting niche. Within this spatial context, immunosuppressive interactions, such as the ANXA1–FPR1 axis between dendritic cells and macrophages, were delineated. Furthermore, this cellular network promoted neutrophil recruitment via the AREG-EGFR-pERK-EGR1-CXCL5 signaling axis. This process facilitates tumor metastasis and contributes to resistance to immunotherapy.[97] Qiu et al. used ST to reveal significant spatial colocalization between *GREM1*$^{+}$fibroblasts and *SPP1*$^{+}$macrophages in gastric cancer. These cells form a close communication network via the SPP1-ITGAV/ITGB5 and collagen signaling pathways. This interaction is strongly associated with key pro-metastatic pathways, including EMT and ECM remodeling. Furthermore, high co-infiltration of both cell types significantly correlated with poor patient prognosis and therapy resistance.[98]

These interactions often extend beyond simple bidirectional communication to form complex self-reinforcing functional niches. A study on cervical cancer using scRNA-seq, ST, and SP clearly delineated a system comprising a metastatic niche of Epi0_*AGR2* epithelial cells, neutrophils, and CAFs. These cells are co-localized in space and engage in a positive feedback loop mediated by TGF-β, CXCL, and OSM/OSMR. This reciprocal activation among distinct cell types enhances EMT and the metastatic properties of epithelial cells.[99] Ong et al. employed ST and SP to identify a critical stromal cluster (SC2) niche in colorectal cancer peritoneal metastasis formed by co-localized CAFs and M2 macrophages. Within this niche, signaling pathways such as TGF-β and IL-6/JAK/STAT3 converge on the central molecule *SERPINE1* (*PAI-1*). *SERPINE1* functions as a hub that coordinates fibrotic remodeling, EMT induction, and immunosuppression, thereby creating a stable, self-reinforcing functional unit that drives metastasis.[100] This method, based on spatial adjacency, can be used to infer cellular interactions and decipher the spatially specific communication network that drives tumor metastasis. Thus, it provides a powerful analytical framework and novel research paradigm for comprehensively investigating these networks.

Spatial multi-omics enables personalized and precision oncology

The spatiotemporal heterogeneity of tumors, coupled with drug resistance and interindividual variation, drives the shift toward precision and personalized medicine.[101] Spatial multi-omics provides a pivotal approach to these challenges by enabling the *in situ* analysis of molecular expression and spatial architecture within tumors. In highly heterogeneous and genomically unstable cancers such as GBM, which harbor complex therapeutic barriers,[102] this technology systematically maps the spatial heterogeneity of the TME. It identifies the features governing drug delivery and treatment response, directly informing the development of personalized chemotherapy, radiotherapy, and immunotherapy.

Yu et al. developed an integrated multi-omics workflow for the analysis of GBM stereotactic biopsy samples, incorporating scRNA-seq, ST, SM, SP, and phosphoproteomics. This approach facilitates the systematic characterization of tumor-cell interactions and the TME, enabling the prediction of treatment responses. Their work established a technical foundation for a ''biopsy-based personalized multi-omics platform'' advancing the clinical translation of precision subtyping and therapy monitoring.[103] Song et al. used a DSP approach to analyze 18 tumor samples taken from patients with advanced non-small cell lung cancer who had been treated with the bispecific antibody KN046.[104] Their findings revealed that tumor regions were enriched with epithelial and proliferation/metabolism-associated markers, whereas stromal regions exhibited high expression of immune-related molecules. Further analysis revealed that the treatment efficacy was closely linked to the modulation of Treg-related molecules within the stromal compartment. This provides valuable information for predicting responses to immunotherapy, identifying patients who are most likely to benefit, and guiding the development of combination therapeutic strategies.[105]

Furthermore, spatial multi-omics offers a new perspective on the differences between primary and metastatic lesions and provides valuable insights for developing more targeted therapeutic strategies. Gao et al. applied this approach to paired primary breast cancer and lung metastases and revealed a distinct spatial cellular hub composed of HLA-DR^{+} epithelial cells and adjacent endothelial cells in triple-negative breast cancer. This hub is likely a core element of the immunosuppressive microenvironment. Subsequent investigations suggested that anti-angiogenic therapy could dismantle this architectural organization, freeing up exhausted T cells and creating an opportunity for immune checkpoint inhibitors to restore T cell function. These insights establish a rationale for personalized combo

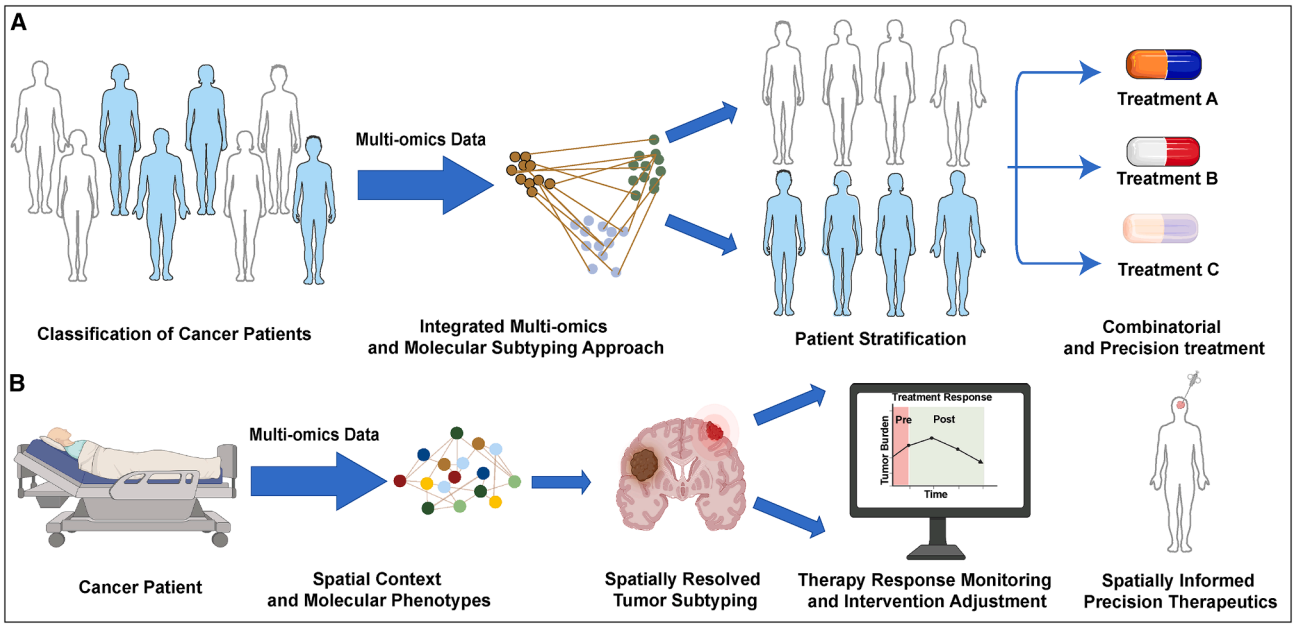

Figure 3. Spatial multi-omics advances the clinical workflow of precision oncology

(A) Through the integration of spatial and molecular data, it facilitates accurate tumor classification and patient grouping, thereby guiding the choice of tailored combination treatments.

(B) In addition, by tracking therapeutic responses and directing modifications to interventions, it develops a structured approach for spatially guided precision medicine, ultimately enhancing patient care and outcomes.

therapies that integrate anti-angiogenic agents with immunotherapy.[106] In summary, spatial multi-omics is critical for monitoring treatment efficacy, guiding biopsies, identifying targets, and elucidating resistance mechanisms as it reveals the molecular landscape and spatial architecture of tumors. This provides multi-dimensional scientific evidence needed to advance precision cancer therapy (Figure 3).

PROSPECTS AND CHALLENGES IN THE APPLICATION OF SPATIAL MULTI-OMICS

Taken together, the multi-dimensional integration of spatial multi-omics technologies provides an unprecedented molecular landscape and spatial architecture of tumors and clearly reveals dynamic intercellular communication networks, key regions of metabolic reprogramming, and the spatial distribution patterns of biomarkers. This provides a powerful tool for understanding interactions within tumor heterogeneity, thereby laying a methodological foundation for precise and personalized cancer therapeutic strategies.

Although spatial multi-omics technologies have advanced rapidly, several challenges still hinder their practical application. First, simultaneously mapping the spatial distribution and expression levels of a set of biologically related molecules requires multimodal profiling of a single tissue section. However, the intrinsic characteristics of current technologies often preclude this approach. For example, the destructive nature of sequencing-based ST makes repeat *in situ* analyses of the same section infeasible.[107] Consequently, using consecutive sections for complementary analysis has emerged as a viable strategy. For instance, in investigating spatiotemporal metabolic remodeling in gastric cancer, Sun et al. performed SM (AFADESI-MSI), spatial lipidomics (MALDI-MSI), and ST (10× Visium) on consecutive tissue sections. It is critical to spatially align these multi-omics datasets through high-precision image registration.[56] Future efforts should focus on developing genuine *in situ* and non-destructive multi-omics technologies alongside methods for sequential multimodal profiling of a tissue section, which will help overcome the fundamental constraint of sample compatibility.

Second, beyond challenges in sample preparation, substantial analytical difficulties arise when integrating multi-omics data. These challenges mainly include cross-modality batch effects caused by technical differences across platforms (e.g., sensitivity, resolution, and coverage); alignment errors when registering images or spatial coordinates from distinct technologies, and biases in cell-type assignment resulting from resolution mismatches between spatial and single-cell datasets. Notably, it is important to distinguish between spatial multi-omics and spatially resolved single-cell multi-omics. While spatial multi-omics typically integrates multiple molecular layers measured across spatial regions or spots, spatially resolved single-cell multi-omics aims to capture multiple omics modalities at true single-cell resolution within intact tissue. The latter provides higher cellular precision but also introduces additional analytical challenges, including increased data sparsity, higher dimensionality, and more complex cross-modality alignment. In addition, the high dimensionality and resolution inherent in spatial multi-omics data increase the computational complexity of integrative analyses, such as cell-type deconvolution and gene set enrichment.[33,108] This challenge is exacerbated by technical disparities in resolution, data structure, and

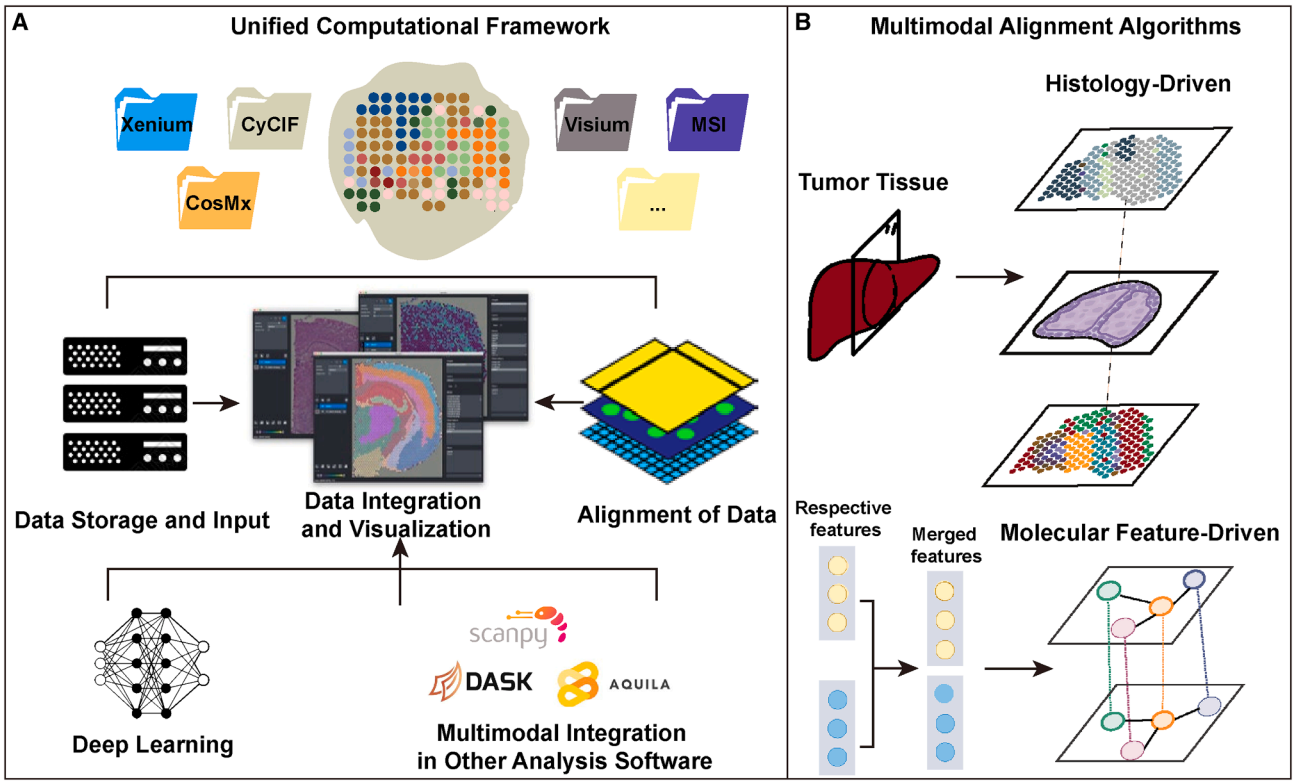

Figure 4. Approaches for integrating spatial multi-omics data

The process of spatial multi-omics integration relies on two essential elements: (A) unified workflows that standardize and consolidate various data modalities within a shared platform for data storage, processing, and visualization; and (B) spatial alignment techniques, such as image registration methods and molecular feature-based computational algorithms, which facilitate accurate data congruence and enable comprehensive analytical insights.

dimensionality reduction across platforms, which directly contribute to cross-modality batch effects. Therefore, the focus of current research is on developing standardized computational and data integration frameworks (Figure 4A). For example, the SpatialData framework aims to build a universal infrastructure for multimodal data. Establishing unified standards and interfaces provides streamlined solutions for storing, processing, and annotating spatial data, effectively reducing biases arising from technical variations in sensitivity, resolution, or coverage,[109] thereby mitigating cross-modality batch effects. In contrast, Squidpy offers a ready-to-use analytical ecosystem. With its AnnData-based structure and built-in tools for spatial statistics and image analysis, it provides a scalable foundation for the quantitative interpretation of high-resolution data.[110]

In addition, it is crucial to develop diverse spatial alignment and integration algorithms to address alignment errors. One key approach involves registration strategies driven by histological images (Figure 4B). For instance, STaCker employs a combination of histological imaging and gene-expression-derived contours to facilitate the nonlinear elastic alignment of spatial transcriptomic slices into a unified coordinate framework. Although this approach successfully maintains the overarching tissue structure and enables cross-platform integration, its performance is contingent on high-quality image inputs and is limited in achieving single-cell resolution or correcting severe morphological distortions,[111] which can introduce alignment errors. In recent years, computational strategies have emerged that align tissues based on molecular features rather than imaging data (Figure 4B). For example, SLAT leverages a graph-based framework and adversarial learning to excel in aligning heterogeneous sections across technologies and modalities. It thus overcomes the key shortcomings of image-driven methods (e.g., STaCker) at the cellular level, in non-rigid alignment, and multimodal integration,[112] explicitly minimizing alignment errors through molecular feature-based alignment. SpatialGlue uses a dual-level attention mechanism (intra- and inter-modal) to adaptively fuse diverse omics data with their spatial contexts. This design ensures broad compatibility across platforms and tissue types, and provides a powerful and interpretable computational framework for spatial multi-omics.[113] Furthermore, to address biases in cell-type assignment, integration with single-cell reference data via tools such as SPOTlight and Cell2location (discussed in section computational integration and investigation into functional analysis) remains essential for refining spatial mapping accuracy. By overcoming these challenges, spatial multi-omics is expected to evolve from a specialized tool into a widely accessible platform, thereby accelerating biomedical discovery and advancing precision oncology.

Despite promising advances achieved by other omics technologies in personalized and precision oncology, the clinical

translation of spatial multi-omics still faces substantial practical barriers. High costs and prolonged turnaround times remain major hurdles, such as Stereo-seq, which requires sequencing depths up to 150 GB/cm2, with per-sample costs exceeding $700.[114] As a mainstay of clinical samples, FFPE tissues are especially vulnerable to RNA degradation and cross-linking artifacts, compromising detection efficiency and reproducibility across platforms.[115] Beyond these technical constraints, the broader clinical adoption of spatial multi-omics will further depend on advances in protocol standardization, regulatory alignment, and reimbursement frameworks.[116]

Addressing these issues will require sustained collaboration among researchers, clinicians, industry, and regulators. AI integration offers a promising strategy to facilitate this process. For example, the GUIDE pipeline for IDH-mutant astrocytoma and the TIMES scoring system for hepatocellular carcinoma can automate image analysis,[117,118] integrate multimodal data, and discover spatially informed biomarkers clinical factors. However, realizing the full potential of these approaches requires large-scale, multi-center validation. Realizing this potential will require large-scale, multi-center validation. Recent initiatives, including a multi-center glioma study and the stClinic framework,[119,120] have validated clinically relevant spatial niches and multi-omic features across independent cohorts. These efforts will help establish large clinical reference atlases, which are essential for benchmarking new datasets, reducing batch effects, and ultimately facilitating the translation of spatial omics into precision oncology.

CONCLUSIONS

Spatial multi-omics provides an exceptionally detailed view of the TME while preserving its original spatial architecture through the comprehensive integration of transcriptomic, metabolomic, and proteomic datasets, alongside other multi-dimensional information. This advanced approach allows the simultaneous examination of interconnected changes in gene activity, metabolic pathways, and protein signaling across distinct cellular zones. Moreover, it enables the precise identification and mapping of critical biological characteristics, including tumor diversity, interactions between immune cells and the surrounding stroma, and adaptations in cellular metabolism. Although the field faces hurdles such as complex sample preparation, the need for consistent data formats, and the high costs associated with translating findings into clinical practice, spatial multi-omics presents distinctive benefits. These include the ability to identify biomarkers specific to certain regions, trace the routes of cancer metastasis, and thoroughly analyze the immune and metabolic landscape of the tumor. As technology continues to evolve and improve, it is expected to play a pivotal role in advancing precision oncology by incorporating insights from spatial biology. This progress will inform and enhance strategies in immunotherapy, the development of targeted therapies, and the formulation of personalized treatment plans.

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AUTHOR CONTRIBUTIONS

W.C.: conceptualization, investigation, writing—original draft, and writing—review and editing; W.Z.: conceptualization and supervision; K.L. and P.H.: conceptualization, supervision, and writing-reviewing and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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