

SPATIAL OMICS DRIVEN PRECISION DRUG DELIVERY IN CANCER

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ABSTRACT

The developing method in biology called "spatial omics" investigates the exact spatial positions of molecules in cells and tissues, such as proteins, metabolites, DNA, and RNA. The spatial structure of cells is frequently ignored by conventional omics approaches that describe the molecular composition. By fusing genetic analysis with tissue architecture, spatial omics get beyond this restriction. This enables researchers to observe the molecules' locations, times, and interactions with nearby cells. Spatial proteomics, genomics, and metabolomics techniques that concentrate on the distribution of proteins, DNA, and metabolites in tissues make up spatial omics. Spatial omics encompasses techniques like spatial proteomics, genomics, and metabolomics with a focus on the distribution of proteins, DNA, and metabolites in tissues. Tissue preparation, molecular capture or detection, imaging or sequencing, and computer analysis are all necessary to produce spatial maps. Applications include the study of pathology, immunology, developmental biology, cancer, and neurology. They aid in differentiating between distinct cell types and understanding the expression of genes in diverse brain regions. Two of the main advantages are the preservation of tissue architecture and the study of cellular connections; the disadvantages include high cost; complex analyse and sensitivity issues.

Comparison with Conventional Dosage Form

Traditional methods search for recognized indicators (e.g., individual proteins, single genes, or fundamental physiological features like color and weight loss). The entire collection of DNA, RNA, proteins, or metabolites is examined in an untargeted, global way using omics techniques (genomics, transcriptomics, proteomics, and metabolomics). Omics techniques.^[1]

Resolution and Depth: Traditional approaches can produce averaged or limited perspectives which might overlook rare variations or complex systemic changes, Omics techniques map out intricate biochemical pathways and out intricate biochemical pathways and regulatory networks, showing subtle shifts in cellular function or complex environmental interactions.

Data Complexity: Traditional tests generate basic low dimensional data, which is easy to interpret Omics procedures produce enormous datasets which require sophisticated bioinformatics, highthroughput sequencing or mass spectrometry and extensive processing power.^[2]

KEYWORDS: Spatial Omics, Spatial Proteomics, Genomics, Metabolomics, Tissue Architecture.

INTRODUCTION

Spatial omics is a broad term that refers to spatially resolved molecular technologies designed for the analysis of biological molecules in their native location

within a tissue. These technologies are unique in their ability to maintain the spatial context.^[3]

The convergence of developments in computational analysis, imaging, and molecular biology is reflected in its history. early foundations (1980s-1990s): by allowing the visualization of particular proteins and nucleic acids within tissue sections while maintaining spatial context, methods like in site hybridization (ISH) and immunohistochemistry (IHC) set the foundation. developments in molecular profiling and imaging (2000s) spatial resolution enhanced by the development of high- resolution microscopy and fluorescence methods. When microarray and next generation sequencing (NGS) technologies first appeared on they were devoid of spatial information. In the 2010s, spatial transcriptomics emerged: spatial transcriptomics techniques, which combine RNA sequencing and spatial barcoding to map gene expression in tissues while preserving spatial data, were developed around 2016. Among the notable technologies were MERFISH, 10x genomics and slide -

seq. multiomics expansion (late 2010s-2020):in order to enable through molecular mapping of tissues, researcher ex standard spatial omics beyond transcriptomics to include proteomics, epigenomics, and metabolomics.^[4]

The spatial omics was developed by (Joakim Lundeberg) co – developed the first widely adopted spatial transcriptomics (ST) method (2016), enabling genome-wide RNA mapping in tissue. (Patrik Stahl) first author of the landmark 2016 science paper introducing spatial transcriptomics. (Jonas Frisen) co – led the development and biological application of early spatial transcriptomics methods. (Xiaowei Zhuang) invented MERFISH, a high- resolution imaging-based spatial transcriptomics technology. (Long Cai) developed seqFISH and seqFISH+, enabling imaging of thousands of RNA molecules in cells.^[5]

Table 1: Different cancer drugs and how they work in cancer cells using spatial omics.

CATEGORY	DRUGS USING SPATIAL OMICS
Chemotherapy	Doxorubicin and paclitaxel effects on tumor microenvironment and immune infiltration
Targeted therapies	Imatinib (a kinase inhibitor) in chronic myeloid leukemia tissues. Erlotinib in lung cancer tissues.
Immunotherapies	Pembrolizumab (anti-PD-1) to study immune checkpoint blockade effects.
Small molecular inhibitors	BRAF inhibitors in melanoma. PARP inhibitors in ovarian and breast cancer.

HOW SPATIAL OMICS IS USED IN DRUG RESEARCH

The application of spatial omics in medical research^[6]

DRUG EFFECT MAPPING: Spatial omics show how medications alter the distribution of metabolites, protein levels, or gene expression in certain tissue regions.

MECHANISMS INSIGHT: It illustrates how cells react to medications in various microenvironments, such as surrounding tissues versus tumors.

BIOMARKER DISCOVERY: Finding geographical biomarkers that indicate medication response or resistance.

DRUG TARGET VALIDATION: Verifies the function and placement of drug targets in tissues.

CANCER SPATIAL OMICS

Because of the interactions between the tumor cells and the tumor microenvironment, cancer is an extremely diverse illness. So that single cell omics have provided critical insights into the genetics, epigenetic, and transcription programs. the emergency spatial omics technologies including spatial transcriptomics, spatial proteomics, and multiplexed molecular imaging. Genomic alterations and tumor associated microbiomes, offering and preceded

opportunities to dissect the coordinated organization and dynamic interactions of cells within intact tissues. This field is now entering a new phase focused on improving accessibility, advancing integrative computational frameworks, and expanding clinically oriented applications. As a result, these systems make it possible to characterize transcriptome and proteome landscapes in situ with great resolution.

There are many different types of cancer.

BREAST CANCER

A variety of internal and external factors can contribute to the development and occurrence of breast cancer. A physiological link between cancer and inflammation 1863 He thought that persistent inflammatory foci were the source of cancer. We now know that inflammatory cells play a significant role in the development of tumors. Leukocyte infiltration, cytokine release, and tumor necrosis are only a few of the pro-tumor impacts of inflammatory cells. chemokines such CCL2 and CXCL8, neoangiogenesis, active tissue remodeling, and TNF or IL-1. Macrophages linked to cancer are crucial in controlling the connection between inflammation and cancer.^[7]

GASTRIC CANCER

Gastric cancer is the most common type of stomach cancer. Neuroendocrine tumors, lymphomas, sarcomas, and gastrointestinal stromal tumors come next. Gastric adenocarcinomas (GC), which have a range of characteristics, account for the majority of this varied illness. The two primary GC depending on topographic locations are the cardia and non-cardia GC. Alcohol consumption and smoking are strongly associated with a higher risk of stomach cancer. Either the proximal stomach (cardia) or the distal stomach (non-cardia) can have gastric cancer. Surgery, chemotherapy, radiation treatment, targeted therapy, and immunotherapy are typically used to treat stomach cancer. The main treatment is surgical resection; radiation therapy and adjuvant or neoadjuvant chemotherapy are additional alternatives.^[8]

PANCREATIC CANCER

Low survival rates, resistance to most treatments, and delayed detection are common characteristics of pancreatic cancer. As the population ages and obesity and pancreatitis, two risk factors for pancreatic cancer, rise, so does the prevalence of pancreatic cancer. 68% of people with type 2 diabetes have metabolic dysfunction-associated steatotic liver disease (MASLD), the most common chronic liver condition worldwide. Nevertheless, there is no data on pancreatic inflammation and fat infiltration in MASLD patients. Cigarette smoking, diabetes mellitus, body mass index, alcohol, pancreatitis, allergies, and microbiota function are risk factors for pancreatic cancer.^[9]

ORAL CANCER

The most prevalent type of cancer in the head and neck is cancer of the paranasal sinuses, larynx, nasal cavity, pharynx, orbital cavity, and lips. Cancers of the mouth, head, and neck are closely linked to squamous cell carcinoma (SCC). A human papillomavirus infection that causes mouth cancer is known as an oral papillomavirus infection (HPV infection). Medicine has been aware of and documented mouth cancer and its symptoms and signs since ancient times, from the primitive descriptions of oral neoplasms by various prominent physicians and surgeons of ancient past civilizations to the current and developing methods for treating them. Men in their sixth and seventh decades of life are more likely to develop oral cancer, and they are also more likely to smoke and consume large amounts of alcohol. Over the past few decades, the incidence of oral cancer in younger patients (those under 45) has risen from 3% to 5% in the 1970s and 1980s to about 10% currently.^[10]

PROSTATE CANCER

The prostate gland, which surrounds the urethra and is situated behind the bladder, is an auxiliary organ of male reproduction. The primary function of the prostate gland is to supplement semen with the

secretions required to produce ejaculate and maintain sperm viability. The most prevalent kind of tumor appears in the middle to late stages of life and is located in prostate gland cells. Men of any race or ethnicity are susceptible to prostate cancer. Males in lower socioeconomic levels have a higher death rate due to this disease's delayed detection. The significance of hereditary prostate cancer has been validated by twin and epidemiological research. Multiparametric magnetic resonance imaging (mpMRI) and serum prostate-specific antigen (PSA) have decreased the necessity for multimodal early detection. With its lower risk of postprocedural sepsis, multiparametric magnetic resonance imaging (mpMRI) and serum prostate-specific antigen (PSA) diminish the need for transperoneal biopsies. In order to prevent dangers such radiation cystitis and proctitis, erectile dysfunction, and incontinence, active surveillance is now a therapy option for localized disease. In addition to preventing needless local procedures, PSMA positron emission tomography/computed tomography (PET/CT) enables more precise staging.^[11]

GALL BLADDER CANCER

GALL bladder cancer (GBC) is a global issue that is more common in regions of the world where cholelithiasis is prevalent. GBC has a significant death rate and is typically identified at an advanced stage. In addition to intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), and perihilar cholangiocarcinoma (pCCA or Klatskin), gallbladder cancer (GBC) is a subset of biliary system malignancies. A general practitioner will encounter a wide range of illnesses in contemporary medicine that could impair the gall bladder's capacity to function normally. People everywhere, particularly those at high risk for GBC, are in dire need of reliable, practical cancer prevention knowledge in a world of conflicting and varied health information. The incidence of GBC can be reduced and the prognosis for those who have it improved by public health initiatives that educate the public about risk factors for the condition, such as obesity, gallstones, chronic gallbladder inflammation, and variables that raise mortality rates, like consuming red meat.^[12]

COLORECTAL CANCER

The second most deadly and third most prevalent type of cancer worldwide is colorectal cancer (CRC). Colon and/or rectal cancer are included in CRC. One of the worst cancers is colon cancer. The liver, lungs, ovaries, and other gastrointestinal organs may be affected. Because of anatomical issues and the possibility of local recurrence, treating rectal cancer is more challenging than treating colon cancer. Men had 25% higher incidence and mortality rates than women, with 5- and 10-year survival rates of 65% and 58%, respectively. Pacific Islanders and Asian Americans have the lowest rates of colorectal cancer (CRC) incidence and mortality, while non-Hispanic

Blacks have the highest rates. Carcinoembryonic antigen (CEA) is currently the most widely utilized CRC serum biomarker. The protein CEA is produced by both developing fetuses and cancer cells. While CEA levels are typically modest in healthy people, patients with colorectal cancer (CRC) may have higher amounts. Because CEA can be elevated in individuals with other illnesses, such as colon inflammation, it is not a reliable indicator for colorectal cancer.^[13]

THYROID CANCER

The most prevalent endocrine cancer is thyroid cancer. The parafollicular or follicular epithelial cells are the ones that develop into malignancy in thyroid cancer. There are four histological kinds of thyroid tumors that originate from follicular cells: Anaplastic thyroid cancer (ATC, <2%), follicular thyroid cancer (FTC10-15%), poorly differentiated thyroid cancer (PDTc, <2%), and papillary thyroid cancer (PTC80-85%). The diagnosis and treatment of individuals with follicular cell-derived thyroid cancer have changed over the past few decades due to better patient classification that better correlates with clinical outcomes, as well as developments in laboratory, molecular, imaging, and knowledge technology. The cornerstones of treatment are thyroid surgery, radioactive iodine therapy, and TSH suppression. This increase in knowledge has made it possible to improve the ability to optimize treatments for patients with persistent and/or progressive disease, better define treatment options for patients with more aggressive disease, and escalate therapy for those with low-risk, well-differentiated thyroid cancer.^[14]

OVARIAN CANCER

The diagnosis of ovarian cancer is rather uncommon. With a lifetime incidence of roughly 3.1%, uterine cancer is the most gynecologic cancer in the US.^[15]

OMICSPATIAL PRINCIPLES

- Retention of spatial information: Cells, proteins, RNA, and other macromolecules are kept in their original positions in tissue samples. Molecular identification in the tissue, specific biomolecules such as proteins, RNA, DNA, and metabolites are found and recognized.^[16]
- Position in space: Researchers map the compounds they have discovered to their exact or approximate position within the tissue in order to observe how the molecules are distributed.
- High throughput analysis: Thousands of genes, proteins, or other molecular components can be examined in parallel across several cells thanks to spatial omics.^[17]
- Single-cell or subcellular resolution: Using sophisticated spatial omics approaches, molecular alterations can be detected at the single-cell or even subcellular level.
- Integration of multi-omics data layers: To gain a

thorough understanding of tissue biology, a variety of molecular data types, including transcriptomics, proteomics, genomics, and metabolomics, can be merged.

- Computational analysis: To display, process, and interpret spatial molecular data, bioinformatics tools and imaging techniques are employed.^[18]
- Identification of tissue architecture: Cellular heterogeneity, tissue architecture, cell-cell interactions, and disease-related alterations can all be seen using spatial omics.

SPATIAL OMICS TECHNOLOGIES' BENEFITS IN CANCER

Single cell and subcellular resolution

It offers information on metabolites, proteins, and gene expression at the single cell or even subcellular level in tumor tissues. Integration of several omics the integration of spatiotemporal transcriptomics, proteomics, and epigenomics reveals a thorough molecular landscape of cancer cells.

High multiplexing and throughput

Sophisticated platforms can profile thousands of genes or profiles, boosting throughput and data richness.

Understanding the tumor microenvironment

To better understand tumor growth and treatment resistance, spatial omics can map in detail immune cell infiltration, stromal interactions, and cell signaling.

Clinical applications

By revealing spatial molecular fingerprints linked to prognosis and treatment response, these technologies aid in the development of target medicines, patient stratification, and biomarker discovery.^[19]

ADVANTAGES OF SPATIAL OMICS TECHNOLOGY ON CANCER

- Single cell and subcellular resolution: It provides gene expressions, proteins and metabolites at single cell or even subcellular levels in tumor tissues.
- Multi – omics integration: Spatial transcriptomics proteomics and epigenomics integration reveals understanding molecular landscape of cancer cells.
- High throughput and multiplexing: Modern technologies allow profiling thousands of genes or profiles and increase data richness and throughput.^[20]
- Insights into tumor microenvironment: Spatial omics can provide accurate mapping of immune cell infiltration, stromal interactions and cell signaling that can help in understanding tumor growth and therapy resistance.
- Clinical applications: These technologies help to uncover spatial molecular fingerprints associated with prognosis and treatment response, hence facilitating the discovery of biomarkers, patient

stratification, and target therapy development.

- Concept spatial omics technologies have rapidly emerged. Meanwhile, growing demand for large pannels high accuracy, enhanced resolution, and multi model capabilities have accelerated the transition of these technologies.
- Commercialiation has increased practical workflows, analysis tools, enhanced cost effectiveness standardized data structures have been growing demand increases larger panel.^[21]

RECENT ADVANCES IN SPATIAL OMICS TECHNOLOGY

- The discipline has undergone a spatial revolution, and there are now more and more proof-of-concept spatial omics technologies.
- The transition of these technologies has been expedited by the increased demand for huge panels, high precision, improved resolution, and multi-model capabilities.
- Larger panels are becoming more and more in demand as a result of commercialization, which has boosted useful processes, analysis tools, cost effectiveness, and standardized data forms.^[22]

AN OVERVIEW OF SPATIAL OMICS TECHNOLOGIES

SPATIAL TRANSCRIPTOMICS

There are now a number of techniques for capturing transcripts that preserve spatial information or for in situ hybridization. These 15–20 tactics are frequently classified as already reviewed. methods based on sequencing and imaging. Here, we concentrate on cutting-edge innovations that have been commercialized. describing how the two main types' subdivisions differ from one another. We differentiate between cyclic in situ and cyclic combinatorial decoding sequencing in terms of imagingbased methods, which primarily differ in how the target identification is read out (base calls versus fluorescent codes).to methods based on sequences. We differentiate between tissue-on-array capture. The fundamental concept of in situ-based technology is barcode-to-cell (in-tissue) and spatial barcode sequencing barcoding, which differ in how spatial transcripts 20 and 21 associate with coordinates decoding in cycles.^[23] The fundamental concept of in situ-based technology is to directly view transcripts in cycles of decoding using customized fluorescence. For the detection of single transcripts, fluorescence in situ hybridization (FISH) and its variations2–25 combine RNA-probe-based targeting with high-resolution imaging. Multiplexing is made possible by recurrent hybridization cycles, but panel size is still constrained by practical repeat number restrictions. Therefore, cyclic in situ sequencing or cyclic combinatorial decoding are used to attain increased multiplexity. Cyclic decode techniques hybridize target probe sets to decode data through repeated cycles. thousands of genes and analyze the results using iterative cycles.

The way that various technologies encode (such as MERFISHstyle hamming codes28), delete or amplify fluorophores between cycles, and design their probes (such as scaffold-based single amplification (RCA) in xenium27) varies. Notable commercial platforms are Xenium Institute (10x genomics) and CosMx (Bruker). CosMx whole transcriptome (WXT) paneks employing their most recent platforms include 5000, 6000, and 18000 genes, respectively. Near-wholetranscriptome profiling, separate panels of up to 300 genes, and bespoke RNA panels of up to 50 genes are all possible with 1120 COSmx.^[24] Additionally, xenium offers electrospray ionization (DESI) and great versatility, allowing for add-on panels with up to 100 targets. comprising probes to microbial sequences, single nucleotide variants, isoforms, and T and B cell receptor (TCR/BCR) regions, as well as independent custom panels of up to 480 genes, such as AtoMx for CosM xenium prime 5K, CosMx 6K, and Xenium Explorer for Xenium. A novel and effective method for examining gene expression in the geographic context of tissues is spatial transcriptomics. By shedding light on how cellular connections and functions are arranged in their natural habitat, it closes the gap between classical transcriptomics and histology. By enabling precise mapping of cellular heterogeneity, tissue architecture, and disease causes, this method is revolutionizing biology and medical research. Spatial transcriptomics will continue to enhance our comprehension of intricate biological systems and diagnoses as technology develops.^[25]

PATIAL PROTEOMICS

The study of the locations and distribution of proteins within cells or tissues is known as spatial proteomics. This aids in our comprehension of how proteins function and interact with one another within the context of the cellular architecture. It combines geographical data with proteomics to determine the location and arrangement of proteins within biological systems. Strong single-cell resolution with preserved tissue morphology and direct use of canonical protein markers for accurate cell and structure typing (e.g., CD31 for labelling vascular endothelium or PNA^d for identifying high endothelial venules, where no single transcript equivalently defines the phenotypes) are two distinct advantages of spatial proteomics over spatial transcriptomics. There are still issues with panel standardization and quantitative calibration. currently, the majority of commercialized platforms can detect between 20 and 60 proteins, with very few being able to detect more than 100plex. the availability of highly specific antibodies, possible cross -reactivity in complex panels, and spectrum or cycle-numberconstraints intrinsic to fluorophore-based detection are the main causes of this limited multiplexity.^[26] The phenocycler-fusion 2.0 system (previously CODEX 46; Akoya biosciences) and its phenocode discovery IO60 human protein panek (60-plex) are examples of representative platforms. CosMx

offers approved protein tests up to 68-plex, much like the CODEX assay system: xenium offers, singular genomics G4X presently enables 17-plex spatial proteomics in addition to RNA to a single run, and optional in-line protein expression via protein subpanels (above 20-plex in simultaneous RNA+protein runs). use in metal-conjugated antibodies with laser ablation and mass spectrometry, the imaging mass cytometry (IMC) 47 platforms (standard bio tools) allows for the detection of more than forty markers. using common label-free antibodies, the COMET platform allows for quick and versatile mIF panels upto above -plex. the detecting platforms used for by these platform vary. For instents, phenocycler, cosMX and singular G4X achieve spatially resolved, multiplexed protein detection by combining oligo nucleotide barcoded antibodies with cyclic fluorophore focused imaging. highly specific antibody sets that are compatable with co-hybridization are needed for these methods. the COMET plate form, on the other hand minimizes potential cross reactivity and streic hindrance by applying only two label free primary antibodies at a time with iterative staining and elusion. IMC is mass spectrometry based techniques that avoids spectrum overlap by using antibodies linked to metal isotopes to detect proteins using laser ablation and time of flight mass spectrometry compared to optical imaging based plateforms, it operates at a some what lower spatial resolution (about 1micrometer) while avoiding spectrum overlap.^[27]

SPATIAL PROTEOMICS

Metabolomics is the study of spatial distribution of metabolites with in biological tissues, combining metabolite profile with spatial information to understand biochemical process in their precise location. metabolomics in space tiny molecules, including as lipids, glycans, metabolics intermediates, and tiny peptides, are directly mapped onto tissue sections using spatial metabolomics. This is usually accomplished by laser ablation and surface ionization methods, frequently using unique ionization procedures adapted to the chemical characteristics of particular analytes one of the most well-know and often utilized mass spectrometry imaging (MSI) modalities in spatial metabolomics imaging is MatrixAssisted Laser Desorption /ionization mass spectrometry (MALDI-IMS). it was obtained spatially resolved mass spectra from intact tissues sections and enables both untargated and targated detection of variety of metabolites by apply a chemical matrix to aid ionization spatial resolution of 10 – 50 micrometers are usually attained. desorption secondary ion mass spectrum (SIMS) are two other cutting edge MSI techniques that provide complimentary capabilities. DESI is apporopriate for quick clinical applications since it requires little sample preparation and uses a charged solvent spray for ambient ionization. on other hand provides submicron spatial resolution but more restricted molecular coverage by sputtering and

ionizing molecules from the tissue surface using a concentrated ion beam. recent developments are increasing sensitivity and broadening metabolite coverage in spatial metabolomics, offering unique oppurtunities to investigate metabolic gradients and their clinical relevance.

SPATIAL GENOMICS

Spatial genomics that refers to methods that detect and localize genomic elements-such as DNA sequences, mutations, chromatin organization or structural variations -with in intact tissue sample or cellular environments. It aids in comprehending how chromatin stakes and genetic variants affect tissue architecture and cellular function in their native spatial context, which is lost in conventional bulk or single cell sequencing. In situ hybridization (ISH); certain DNA sequences with in cells are the target of probes. fluorecence in-situ hybridiazation (FISH); this technique uses fluorecence tags to mark particular DNA sections so that their spatial distribution may be seen. direct DNA sequencing on tissue slices is knowas in situ sequencing. imaging basic techniques: in situ detection of chromatin accessibility or structure. New methods combine genomic and geographical information to map tissues genomes at high resolution.^[28]

SPATIAL EPIGENOMICS

Spatial epigenomics involves mapping epigenetic features -such as DNA methylation, histone modification and chromatin acceptability directly with in intact tissue section or cells, preserving their precise spatial position. The goal is to assist comprehend cellular idntity, development and disease process and geographical context by revealing how epigenetic regulation various across different tissue location or cell types. there are several methods like in-situ techniques; use probes or antibodies to find particular epigenetic markers in tissues, chromatin accessibility is mapped geographically using spatial ATAC-sequence. imaging basing methods; see the pattern of DNA methylation or histone changes in tissues. integration with spatial transcriptomics to establish spatial correlation between gene expreesion and epigenetic states.^[29]

EVOLUTION OF SPATIAL OMICS TECHNOLOGY

Analyzing RNA to deduce broad tissue attributes has been made possible by conventional genomic techniques like bulk RNA sequencing. Nevertheless, the spatial resolution constraints of these methods restrict their capacity to pinpoint the origin of certain transcripts in the tissue architecture and prevent an understanding of cellular interactions in situ. The recent advancement of single cell transcriptomics, which makes it possible to analyze transcriptomes at the single cell level, is a significant stride in this strategy. This improved the categorization of cell kinds and states and provided fresh perspectives on intercellular

dynamics. Technologies for spatial transcriptomics were created to bridge this gap. Transcriptomic study of different tissue locations was made possible by early techniques like laser capture microdissection. Similar to this, single-molecule FISH-like chemistries have been used by various high multiplexing in situ hybridization (ISH) and in situ sequencing (ISS) methods to subcellularly profile hundreds to thousands of genes within tissue.^[30]

APPLICATIONS OF SPATIAL OMICS TECHNOLOGY

- The in-depth investigation of cellular functions is the most promising use of spatial omics
- The tumor microenvironment (TME) in cancer can be mapped using spatial omics at the single-cell and subcellular levels, displaying the spatial distribution of biomarker expression and interactions of different cell types, such as fibroblasts, endothelial cells, immune cells, and cancer cells.
- Finding potential treatment targets and comprehending how the TME promotes tumor development, metastasis, and immune evasion are crucial.
- Cellular composition and spatial location can be ascertained by creating high-resolution maps of the TME by combining spatial omics with single-cell transcriptomics.
- The spatial structure and interaction of immune and tumor cells in the PCNSL TME, particularly the location-dependent immune suppression and signaling patterns that are undetectable by traditional single-cell or bulk techniques, were uniquely described using spatial omics.
- By identifying distinct tumor subpopulations, such as "defenders," "attackers," and "aggressors," the study showed how immune cell infiltration affects tumor behavior and offered information on targeted therapies based on TME features.
- By revealing the interactions of various CAF clusters with immune and cancer cells, this combined single-cell and spatial omics approach advanced our understanding of the TME organization and offered potential treatment strategies that target FAP⁺ CAFs to increase the effectiveness of immunotherapy.
- Tumor cells were frequently seen in close proximity to immunosuppressive myCAF clusters, immunosuppressive macrophages (TREM2⁺ TAM), and regulatory lymphoid cells (FOXP3⁺ Tregs, NKG2A⁺ NKregs).
- Researchers can better understand cancer behavior and treatment response by using spatial information to comprehend not only the function of individual cells but also where and how they interact with one another.
- With their high-resolution insights into tumor architecture and cellular interactions, spatial omics technologies are transforming cancer

research.^[31,32,33]

TUMOR MICROENVIRONMENT AND SPATIAL HETEROGENICITY: TUMOR MICROENVIRONMENT (TME)

The intricate ecosystem around a tumor, which includes different cell types, signaling chemicals, blood arteries, extracellular matrix, and immune cells, is referred to as the tumor microenvironment (TME). Tumor growth, metastasis, and response to therapy all depend on it. Additionally, it changes the TME so that later nanomedicines must undergo anti-angiogenic, cytotoxic, and radiation treatments.^[34]

Heterogeneity in space

Because of the geographic heterogeneity within the tumor microenvironment, these components and their interactions vary in various parts of the same tumor. Tumor behavior, responsiveness to treatment, and disease progression are all impacted by this variability. Cancer cells, stromal cells, immune cells, and extracellular matrix are all part of the TME. The non-homogeneous distribution of chemicals and cells is the cause of spatial heterogeneity. Certain tumor sites may have distinct metabolic profiles, fluctuate immune cell infiltration, or be more hypoxic. Understanding regional heterogeneity and its effects on tumor aggressiveness, metastatic potential, and treatment resistance is necessary to improve therapy efficacy.^[35]

Importance

- Controls the propensity for tumor growth and metastasis.
- Impacts the effectiveness of targeted medicines and immunotherapies.
- To describe TME heterogeneity, sophisticated imaging and molecular profiling methods are used.
- TME spatial heterogeneity can be investigated using multiplex immunohistochemistry, spatial transcriptomics, and imaging mass cytometry.
- This heterogeneity must be understood in order to generate individualized cancer treatments.

AN INDIVIDUALIZED CANCER THERAPY DIAGNOSTIC TOOL

Numerous well-established biomarker paradigms imply the capacity to convert intricate molecular, immunological, and geographic features into clinical assays that can be used. An FDA-approved companion diagnostic test for determining PD-L1 protein expression in FFPE tumor tissue, the PD-L1 IHC 22C3 pharmDx assay (Agilent/Dako) is used to identify patients who might benefit from immune checkpoint inhibitor therapy across a variety of tumor types. The Combined Positive grade (CPS), which integrates PD-L1 labeling in tumor cells, lymphocytes, and macrophages into a single clinically useful scoring metric, is used to grade PD-L1 expression in specific indications. Likewise, Immunoscore shows that the

geographical distribution of immune cells, and the density of CD3+ and CD8+ T-cells at the center and invasive margin of the tumor can be translated into a clinically relevant immunological score. On Distribution can be translated into a therapeutically relevant immune score, especially CD3+ and CD8+ T-cell density in the tumor center and invasive margin. An example of multigene expression data reduced to a quantitative tool for treatment decisions in breast cancer is the Oncotype DX® 21-gene Recurrence Score, validated in the TAILORx study. These precedents collectively support the hypothesis that complex information at the molecular, cellular, and geographical level can be reduced into standardized, clinically interpretable assays. A similar companion diagnostic for omics-guided nanomedicine would need to transform high-dimensional spatial omics signatures into a format that is equally interpretable and deployable in order to stratify patients based on delivery-relevant TME states. This translational challenge is the driving force behind the proposed tiered pathway.^[36,37]

COMPANION DIAGNOSTIC TESTS USING SPATIAL OMICS SIGNATURES

Tier1

This high-priority, clinically validated spatial omics companion diagnostic uses spatial omics technology to examine gene or protein expression in tissue samples within a spatial context. To assist in customizing treatment choices, it offers a thorough molecular and geographical picture of a patient's illness, typically cancer.^[38]

Tier2

A test that employs spatial omics technologies to examine gene or protein expression in tissue samples is known as a spatial omics companion diagnostic. In contrast to Tier 1, this test has a modest level of clinical validation and priority. It is frequently employed in research or developing clinical settings and offers insightful molecular and geographical information to support individualized treatment decisions.^[39]

Tier3

Compared to Tier 1 and Tier 2, the spatial omics companion diagnostic test has less clinical validation and priority. It uses spatial omics technology to assess gene or protein expression inside tissue samples. It is mostly utilized in exploratory research to find possible treatment targets and investigate disease causes.^[40]

DRUG DELIVERY SYSTEMS UTILIZED IN CANCER

Employing nanoparticles for drug administration

This approach enhances the concentration of medication that gathers in tumors by directing the treatments specifically to cancer cells using nanoparticles, which can enclose anticancer drugs

(ranging in size from 1 to 100nm) to boost their solubility, stability, and targeting capability. The nanoparticles used include dendrimers, metallic nanoparticles (like gold), and polymeric nanoparticles. Due to the leaky blood vessels and the enhanced permeability and retention (EPR) effect, nanoparticles can accumulate more effectively in tumor tissues.^[41]

Liposomes

- Liposomes are round vesicles that improve the stability and targeting of pharmaceuticals; their phospholipid bilayers allow them to transport both water-soluble and fat-soluble drugs. They aid in minimizing systemic toxicity by delivering medications specifically to tumor cells. For instance, Doxil (liposomal doxorubicin) is utilized in the treatment of Kaposi's sarcoma and ovarian cancer.
- Polymeric micelles are nanocarriers that improve the solubility and delivery of hydrophobic medications.
- Antibody-drug conjugates (ADCs) connect cytotoxic drugs to monoclonal antibodies targeting tumor-associated antigens; the antibody binds to cancer cells, delivering the drug directly and minimizing harm to healthy cells.^[42]
- Implantable drug delivery devices allow for a controlled and localized release of medications at the tumor site. These medical devices are inserted into the body and facilitate the slow and continuous delivery of medication over time. The types include osmotic pumps, polymeric matrix implants, and Programmable electronic microchips provide targeted treatment, reduce side effects and eliminate the need for taking pills every day. Osmotic pumps work by using the absorption of body fluids through a semi-permeable membrane to drive the medication out at a controlled and steady rate via a small hole. Polymeric implants are solid or gel-like in design and consist of drugs embedded in a plastic or biodegradable matrix.

Hydrogel system

Hydro gel system provide a controlled drug release at the tumor site. These systems consist of intricate 3D polymer networks that are water-swollen and utilized in cancer treatment for targeted, prolonged, and stimuli-responsive drug delivery. Key varieties include stimuli-responsive hydrogels, injectable nanocomposite gels, and local depot systems. Hydrogel systems are sophisticated 3D polymer structures imbued with water, designed for targeted, sustained, and stimuli-responsive distribution in cancer therapy. The primary categories consist of stimuli-responsive hydrogels, injectable nanocomposite gels, and local depot systems.^[43]

CHALLENGES/PROBLEMS IN SPATIAL OMICS

- Some major problems exist in spatial omics, such

as high levels of data complexity, limitations of resolution and data analysis.

- It makes the comparison of results across the experiments very tough.
- Limitations of resolution, both technically and experimentally.
- There are several spots that include more than one cell.^[44]

INTEGRATION OF MACHINE LEARNING IN SPATIAL

There exist a number of popular platforms for carrying out spatial transcriptomics (ST) research. Some of the popular platforms that utilize image-based ST techniques include Xenium by 10X Genomics, which comprises in situ hybridization (ISH) and in situ sequencing (ISS) techniques and operates on the principle of CARTANA. ISH forms the basis of MERSCOPE and CosMx Spatial Molecular Imager by NanoString. Some of the array-based ST platforms include Visium by 10X Genomics. In addition, there exists another low-resolution platform of Biomarker and Slide-seq STomics BMKMANU S1000 by BGI. Instead of microarray, GeoMx Digital Spatial Profiler uses microdissection technique While there are many techniques and technology, spatial proteomics seems to be lagging behind the spatial transcriptomics. Highly multiplexed identification of the protein can be achieved using the CO technology through which up to sixty proteins can be displayed in one area. In order to achieve high dimensionality in analyzing the proteins, the Hyperion Imaging System uses mass cytometry combined with imaging technique. Multiplexed ion beam imaging (MIBI) uses the secondary ion mass spectrometry with metal-tagged antibodies for multiplexed detection and cellular spatial resolution.

Cyte Finder uses multi-parameter protein detection High-content imaging is done through. Matrixassisted laser desorption/ionization (MALDI) mass spectrometry (MS) is used in Bruker IMS (Imaging Mass Spectrometry) platforms. the cutting-edge technique of spatial proteomics involves imaging where the spatial distribution of proteins in tissue slices is done with high spatial resolution. This enables the identification and spatial localization of different biomolecules in an accurate and highly resolving manner using the Fourier transform ion cyclotron resonance (FTICR) mass spectrometer coupled with MALDI for ionization of the samples. Protein expression quantification in spatially defined sections is also enabled by GeoMxDSP. These platforms are important in providing critical information that helps in understanding the roles played by proteins and RNAs in tissues and cells.^[45]

ARTIFICIAL INTELLIGENCE -DRIVEN PATHOLOGICAL DIAGNOSIS

In recent years, there has been an explosion of fundamental and translational research programs focused on developing novel processes powered by AI and ML approaches. The increasing availability of whole slide image (WSI) databases and the rapid improvement in computational pathology performance have spurred efforts to find and quantify additional factors beyond the traditional histology parameters of nuclear and cellular morphology. In recent. Quantification of immune cell infiltration Analysis of the stromal presence of cancer-associated fibroblasts or extracellular matrix components, may give clues to tumour invasiveness and metastatic potential. Assessment of glandular architecture and its disruption may be useful in grading and staging, particularly of prostate cancer. In the studies reviewed we found urological specific features used such as cribriform glandular pattern in prostate cancer and glomerular structure with tubular morphology for kidney cancer. Machine learning can assist in identifying and quantifying such important characteristics. Moreover, scientists now go beyond simple cancer diagnosis to incorporate these features in model training to create more comprehensive and predictive models for purposes like therapy stratification and survival predict.^[46] This is a paradigm shift in the profession – using big data from pathology slide images and machine learning to gain new insights and improve patient outcomes. Prostate cancer has been the most investigated of all the cancers arising from the urological system. Motivation: The motivation of this study comes from the high incidence rates of prostate cancer and its substantial contribution to cancer-related morbidity and mortality. Huang et al. trained their model with randomly selected tiles from H&E WSIs from The Cancer Genome Atlas (TCGA) Prostate Adenocarcinoma (PRAD) dataset. The convolutional neural network (CNN) model was used to extract cell-specific information (nuclear detail, glandular environment, and different TME components such as immune cells and stroma) to determine patterns indicative of early recurrence. Another study trained a deep learning (DL) model to predict TP53 mutation status from WSIs using the TCGA-PRAD dataset. Similar efforts have also been made for kidney and bladder cancer. employed CNN to extract TME features from TCGA bladder urothelial cancer (BLCA) WSI data to cluster the images using the K-means approach.^[7] The clusters were related to immune scores and prognosis, which showed differences in immune checkpoint inhibitor reactivity. The authors tried to quantify the TMI characteristics using higher the AI score, the better the therapeutic response to immunotherapy.^[47]

DECODING FUNCTIONAL NICHES IN THE TUMOUR MICROENVIRONMENT

The TME is structured in cellular niches that are geographically unique with important biological and therapeutic implications. However, we still do not understand well the spatial architecture and functional dynamics of these networks. Spatial profiling tools currently provide a powerful approach to identify recurrent and context-specific niches. Research today has identified a variety of functional niches with unique cellular organization, communication networks and molecular markers because of this development.^[48]

TERTIARY LYMPHOID STRUCTURE (TLSs)

TLSs are well defined structures in space that coordinate local B cell mediated anti-tumour immunity and are associated with favourable clinical outcomes. Spatial profiling now allows detailed characterisation of the TLS distribution, developmental stages and functional roles. TLSs are sites of *in situ* B cell maturation toward plasma cells in renal cell carcinoma (RCC). IgG deposition on tumour cells in patients treated with immune checkpoint inhibitors (ICIs) is related to malignant cell death, increased response rates and longer survival. These plasma cells migrate along CXCL12+ fibroblastic tracks and disseminate.^[49]

147 In addition to RCC, geographic studies of malignancies, including the tumour core of gastric cancer enriched in CD8+ T lymphocytes, particularly PD-1 but not TIM-3 expressing ones, a pattern associated with improved survival, also investigated the TME characteristics associated with TLS and their clinical impact. CXCL13+ CAFs and CXCL13+ CD8+ T cells co-localize within TLSs in nasopharyngeal cancer with B cell aggregates. CXCL13+ CAFs are involved in the adhesion of B cells and antibody production, and plasma cell-derived antibodies induce apoptosis of EBV-positive malignant cells. TLS areas in breast cancer are composed of several cell types including two fibroblast subsets (CCL21+ and APOD+) that attract T and B cells through the CXCL12–CXCR4 axis. More recently, studies have also investigated the mechanisms that control TLS development and maturation. Cancer-educated mesenchymal stem cells may inhibit TLS production and activity in high-grade serous ovarian cancer, with region-dependent effects. Pathways for TLS maturation have been developed in hepatocellular cancer, splitting immature TLSs into subgroups that conform or deviate from. We found that TLS maturation was inhibited by tryptophan metabolites produced by tumours. To identify variables that impair TLS maturation or induce TLS dysfunction, systematic efforts are required to monitor TLS cellular dynamics, maturation trajectories, and functional states over time.

VASCULAR STEM CELL NICHES

Tumour blood veins are home to perivascular niches, which are essential hubs for coordinating local immune responses. These niches enable interactions between dendritic cells (DC) and T cells for antigen presentation and local T cell priming, hence enabling effective anti-tumour immunity. DC-T cell clusters are often dispersed with endothelial and stromal cells, resulting in a layered architecture that influences the local immunological milieu, whereas macrophages and regulatory populations are more commonly found in the periphery of these niches. Chemokine gradients, such as CCL19/21–CCR7, facilitate the recruitment of immune cells that are both effector and immunosuppressive. The ratio of effector cell recruitment to suppressive cell accumulation ultimately determines whether the niche fosters immunological exclusion or anti-tumoral immunity. Spatial profiling has enabled high-resolution mapping of vascular-stromal-immune interaction in these environments. One of the most common features of perivascular niches in recurrent glioblastoma (GBM) tumours was found to be a collagen-rich extracellular matrix rim. These niches were rich in immunosuppressive macrophages, glia, and collagen-producing stromal cells, indicating that treatment had altered the perivascular environment.^[50]

MEMORY- LIKE CD8+ T CELLS

Stem-like CD8+ T cells, which are identified by the high expression of TCF1 and CD28 and the absence of terminal exhaustion markers (like TIM-3), create specialised niches near antigen-presenting cells (APCs). Within these niches, stem-like CD8+ T cells serve as a self-renewing reservoir, maintaining a stem-like subset while retaining proliferative ability and asymmetric division to replenish the effector pool. This process, which is crucial for preserving anti-tumour immunity, is supported by epigenetic reprogramming, CXCR6 upregulation, crosstalk with CDCs via CXCL9, IL-15, and CCR7-binding chemokines (CCL19 and CCL21), as well as other co-stimulatory and cytokine inputs that preserve the stem-like pool and encourage effector overexpression of CXCR6, CXCL9, IL-15, CCR7-binding chemokines (CCL19 and CCL21), and other co-stimulatory and cytokine inputs that promote effector differentiation and maintain the stem-like pool. These ecosystems are closely linked to clinical responses to immunological checkpoint blockade (ICB). Their absence may enable tumour immune evasion. Preclinical research has shown that DC activation with FLT3L or CD40 can expand these niches, indicating a possible strategy to supplement ICB. High-resolution data on the cellular architecture and communication networks governing the growth, upkeep, and therapeutic remodelling of these niches are provided by spatial multimodal analyses, indicating their potential as patient biomarkers.^[51]

FIBROBLAST NICHES CONNECTED WITH CANCER

Cancer-associated fibroblasts (CAFs) have a wide range of cellular origins, phenotypic states, and roles. The flexibility and situational dependency of CAF biology make it more challenging to study. Singlecell and spatial evaluations reveal that CAFs offer distinct structural and functional niches that influence the immune milieu and therapeutic responses. In many malignancies, CAFs produce matrix that forms thick, collagen-rich barriers that restrict T cell penetration and interaction with tumour cells, leading to immune exclusion. Inflammatory CAFs release immunomodulatory cytokines and chemokines that affect immune cell recruitment, polarisation, and function, such as CXCL12 (SDF-1), IL-6, and TGF- β . By conveying antigens to T cells, antigen-presenting CAFs can boost anticancer immune responses. The bulk of geographical studies have first identified the features of CAFs via marker expression; this approach may be limited by the availability of antibodies or gene coverage. In a recent pan-cancer spatial multi-omics study, Lie *et al.* identified four conserved CAF organisational patterns (s1-s4 CAFs) across malignancies using a novel method based on neighbouring cell composition. Inflammatory CAFs release immunomodulatory cytokines and chemokines that affect immune cell recruitment, polarisation, and function, including CXCL12 (SDF-1), IL-6, and TGF- β . By transferring antigens to T cells, antigen-presenting CAFs can induce anticancer immune responses. 167,000 Most spatial studies have first identified the features of CAFs using marker expression, which may be limited by gene coverage or antibody availability. Using a novel method based on neighbouring cell composition, Liu *et al.* discovered four conserved CAF organisational patterns (s1-s4 CAFs) across tumours in a recent pancancer spatial multi-omics study.^[52]

TUMOUR INVASION FRONT

The tumour invasive front, a dynamic interface of cancerous cells with stromal and immune components, is an essential component of cancer genesis and spread. Spatial profiling revealed unique cellular compositions and signalling gradients in this region. This region is often linked to an immunosuppressive microenvironment that reduces anti-tumour immunity and is abundant in neutrophils, regulatory T cells, and tumour-associated macrophages (TAMs). Tumour cells are often converted to a cell state that promotes stemness, hypoxia, or treatment-resistant phenotypes near the invasive front. These innovative phenotypic alterations are associated with a changed immunological environment, indicating flexibility to avoid immune surveillance. based on spatial analyses. Furthermore, malignant cells close to the leading edge often exhibit enhanced invasive and migratory programs, downregulate antigen presentation, and overexpress immune checkpoint molecules—features

associated with treatment resistance and poor prognosis in solid tumours.^[53]

IMMUNE-RESTRICTED NICHES

Immune-excluded niches are areas of the tumour where lymphocytes, especially cytotoxic T cells, are confined to the stroma or periphery but do not penetrate the parenchyma. These niches are associated with poor responses to ICB. According to geographical analyses, these niches are often packed with immunosuppressive myeloid cells and surrounded by robust stromal barriers, creating a milieu that is harmful to lymphocyte trafficking. Similar trends are seen in several types of cancer. thick extracellular matrix barriers created by CAFs; chemokine gradients that reject or misdirect lymphocytes, including the CXCL12-CXCR4 axis; immunosuppressive cytokine signalling, especially TGF- β ; and inhibitory connections that reinforce immunological exclusion, like CD47-THBS1. It has been shown that addressing these elements.^[54]

HYPOXIC MICROENVIRONMENT

Hypoxic niches are biologically distinct microenvironments where reduced oxygen availability causes tissue architectural reconfiguration and phenotypic changes in stromal and malignant cells. In these environments, immunosuppressive TME subsets and aggressive tumour cells are more common.

Through spatial profiling in GBM, ordered, layered structures around hypoxic niches were found, indicating adaptation to oxygen gradients across tissue zones. TAMs with proinflammatory and immunosuppressive traits are preferentially localised in hypoxic environments, whereas microglia are normally found in normoxic settings. The induction of SPP1+ TAMs, which co-localize with CAFs to promote immunological exclusion, is linked to hypoxia in HCC. Furthermore, in latent tumour cells, hypoxia promotes the activation of stemness and chemoresistance pathways. There is growing evidence that neutrophil reprogramming is induced by hypoxic environments.

NEURAL-IMMUNE CROSSTALK

Neural elements are increasingly acknowledged as active components within the TME that control local immunity, including the release of neurotransmitters, neuropeptides, and axonglia signalling. Spatial profiling has identified specialised neuroimmune niches where brain signals influence immune function and have context-dependent effects ranging from immune suppression to enhancing anti-tumor immune responses. In GBM, myelin and lipid debris damage may activate TREM2+ TAMs and change their activation states. According to a recent geographic investigation in PDAC, non-invaded nerves are often associated with lymphoid aggregates/TLS, but invaded nerves are surrounded by NLRP3+ TAMs and myCAFs. These findings suggest

different neuro-immune interactions that may affect both neural invasion and immunological landscapes. Spatial multi-omics in three dimensions may offer a thorough understanding of neuroimmune interactions in situ, their spatial organization, their relevance to functional outcomes, and the possibility of the neuroimmune interface as a therapeutic axis.^[55]

MICROBIOME-RELATED MICROENVIRONMENT

Spatial multi-omics enables high-resolution mapping and quantification of microbiota species and their interactions with tumour and TME cells, revealing how they impact cancer cell phenotypes and metabolism, alter the surrounding microenvironment and anti-tumour immunity, and affect cancer progression and treatment responses. Another study found a higher bacterial burden in tumour cells compared to the TME or adjacent normal tissue using microbial spike-in probes by Geo DSP in lung cancer tissues. Oncogenic pathways including β -Catenin signalling were linked to the bacterial load within the tumour.

SPATIAL OMICS AND INFECTIOUS DISEASES

Prior to the discovery of spatial transcriptomics, gene expression studies of infectious diseases were conducted using bulk or dissociated approaches. In order to compare infected and uninfected tissues, microarrays and bulk RNA-sequences were widely used in the early 2000s. These studies discovered many genes that were differentially expressed, but they were unable to identify the cells in the tissue that produced them. Then, single-cell RNA-sequences (SCRNA-sequences) revolutionised immunology by recording cell types and states in infection. However, each cell's location inside the organ is lost since SCRNA-sequences necessitates tissue separation. A few transitional methods helped to partially close this gap. Laser capture microdissection (LCM) allowed specific sections to be isolated for targeted sequencing, despite the method's time-consuming nature and low throughput. RNA scope and HCR-FISH provided spatial information on a few genes at single-molecule resolution, but each experiment was limited to a small number of transcripts. However, spatial transcriptomics extends current techniques by simultaneously profiling thousands of genes in situ. In fact, ST may be seen as the latest stage of a technological progression from bulk to single-cell to spatial, each of which adds more detail to our understanding of the biology of pandemic and endemic illnesses while also revealing new kinds of information about the locations and mechanisms of immune responses. For instance, in acute illnesses such as COVID-19, spatial profiling of the lung, such as COVID-19, has shown "hotspots" of viral RNA and immune activation as well as stage-dependent alterations in immune cell composition. Spatial profiling of chronic illnesses like

tuberculosis (TB) can also be used to define the cellular architecture of granulomas.^[56]

Cardiovascular disease

Recent developments in histology technologies have improved our understanding of the mechanisms and cells involved in CVDs. In addition to revealing gene regulatory networks or new potential biomarkers unique to disease and cell type, single-cell RNA sequencing (SCRNA-sequences) can assess cell-cell and receptor-ligand interactions. It also enables the analysis of cell trajectories based on the transcriptome of each individual cell, which aids in elucidating the changes in cell states that occur during the maturation and differentiation of progenitor or stem cells. However, SCRNA-sequences lack of spatial resolution makes it unreliable for understanding cell-cell interactions, cellular microenvironments, and spatial changes in cells and chemicals in CVDs.

In this sense, spatial transcriptomics (ST) enables 3-dimensional, nonhomogeneous, and precise analysis. ST integrates spatial information into SCRNA-sequences data to improve our understanding of the niches of local tissues that make up the cellular microenvironment and facilitate cell-cell interactions, in addition to clarifying the genes and proteins dependent on this spatial environment in three-dimensional tissue structures. Consequently, it can offer a clear explanation of the molecular mechanisms behind cardiac injury and remodelling. In light of this, this review offers a comprehensive overview of single-cell and ST technologies as well as recent studies on these topics in connection to CVD. This research could be used as a basis for examining and clarifying.^[57]

DATA ANALYSIS TOOLS IN SPATIAL OMICS:

Discussing some of the technologies used to collect geographic omics data, we now concentrate on the tools for assessing such data. Spatial omics technologies generate large datasets of tens to thousands of genes and proteins from several samples, which are often subject to biological and technical variability. The extraction of significant results is made possible by strong analytical procedures. NGS-based spatial barcoding techniques and imaging-based approaches are the two general groups into which this process can be separated. Imaging-based methods offer subcellular resolution, but in order to generate a cell-by-feature matrix, they require complex preprocessing steps such as signal decoding, segmentation, and stitching. Even though deep learning and AI have improved segmentation, problems like low marker specificity persist. Conversely, NGS-based methods relate sequencing reads to spatial coordinates and assign biomolecule IDs using genomics-like processes. This process can be broadly classified into two types of spatial barcoding strategies: imaging-based and NGS-based. Sub-cellular resolution is possible using

imaging-based methods, but they require complex preprocessing steps like stitching, segmentation, and signal decoding to produce a cell-by-feature matrix. Even while segmentation has improved because to AI and deep learning, low marker specificity remains an issue. On the other hand, NGS-based methods connect sequencing reads to biomolecule IDs and spatial locations using genomics-like processes. This technique establishes single-cell or subcellular areas so that the molecular profiles. In order to attribute molecular profiles and spatial coordinates to particular cells, this technique establishes subcellular or single-cell areas. Segmentation methods must be adapted to particular cell types and tissue architectures in order to prevent molecular signal misassignment. By supplying reliable input data for subsequent computational phases, appropriate segmentation guarantees the quality of downstream analysis.^[58]

REGENERATIVE MEDICINE FOR SPATIAL TRANSCRIPTOMICS

One of the ultimate goals of regenerative medicine is to replace damaged tissues with natural growth and repair mechanisms, which are essentially absent in the mature human body. However, some smaller vertebrates, such as salamanders and zebrafish, have the remarkable capacity to both physically and functionally restore a variety of damaged body parts. Biologists who are dedicated to comprehending the molecular principles behind programmed regeneration and how fundamental scientific discoveries could influence the practice of regenerative medicine have long been interested in these animals because to their innate skills. The gap between the enormous complexity of regenerative responses and the lowthroughput readout of the canonical methods has hindered the systematic elaboration of the mechanism by scientists, whereas early efforts were concentrated on one or a few molecules/pathways/cell types, extensively substantiating our understanding in this field. To address this bottleneck, single-cell RNA sequencing technologies (SCRNA-sequences) have recently been introduced. They can capture the molecular traits and dynamics of every known cell type and make it possible to identify novel or transitory cell states. Using SCRNA-sequences, researchers found a critical cell state transition in axolotl limb regeneration from connective tissue to blastema cells. This alteration is necessary for the eventual rebuilding of lost tissue, possibly via the mechanism of the epithelial-mesenchymal transition.^[59]

Recent developments in histology technologies have improved our understanding of the mechanisms and cells involved in CVDs. In addition to revealing gene regulatory networks or new potential biomarkers unique to disease and cell type, single-cell RNA sequencing (scRNA-seq) can assess cell-cell and receptor-ligand interactions. It also enables the analysis of cell trajectories based on the transcriptome

of each individual cell, which aids in elucidating the changes in cell states that occur during the maturation and differentiation of progenitor or stem cells. However, scRNA-seq's lack of spatial resolution makes it unreliable for understanding cell-cell interactions, cellular microenvironments, and spatial changes in cells and chemicals in CVDs.^[60]

ADVANTAGES OF SPATIAL OMICS

- **Preserves Cellular Context:** Maintains cells in their original spatial arrangement, unlike singlecell sequencing, which requires tissue dissociation.
- **Molecular GPS Mapping:** Identifies the precise location of active genes and proteins within tissue sections.
- **High Resolution:** Provides single-cell or even subcellular resolution, helping identify rare cell populations and structural changes.
- **Unbiased Profiling:** Reveals hidden cellular heterogeneity and complex tissue dynamics during development and disease.
- **Multidimensional & Multi-Omics Analysis:** Combines transcriptomics (what genes are being expressed) and proteomics (what proteins are present/active) to provide a comprehensive view of tissue biology.
- **Detailed Microenvironment Profiling:** Enables in-depth characterization of complex biological environments such as the tumor microenvironment.
- **Histological Integration:** Molecular information can be overlaid with conventional histological images, including H&E staining, linking molecular changes with tissue morphology.
- **Maintains Tissue Architecture Preserves in-situ context:** Maintains the original 2D and 3D spatial interactions between cells. Avoids tissue dissociation: Unlike conventional single-cell RNA sequencing, spatial omics does not require extensive enzymatic digestion of tissues. Preserves tissue morphology: Connects molecular/genomic information with microscopic tissue structure.
- **Enhances Disease Pathology Helps study diseases such as cancer and immune disorders by identifying:** Immune-cell infiltration Tumor microenvironment Treatment resistance Diseaseassociated cellular changes.
- **Enables Before-and-After Treatment Comparison** Spatial omics allows researchers to compare tissue before and after treatment. This helps determine: How tissue architecture changes How immune cells infiltrate tumors Which molecular pathways are activated How cells respond to treatment How treatment-resistant regions develop.

FUTURE PERSPECTIVES AND CHALLENGES

From Static Images to AI Intelligence

- AI and machine learning will make spatial omics

platforms more intelligent.

- Spatial foundation models and AI-based analysis can convert complex tissue images into meaningful biological insights.
- This may reduce the need for expensive and time-consuming experiments.^[61]

Subcellular Resolution

- Future technologies will go beyond whole-cell mapping to study subcellular structures.
- They can track mRNA movement, cell polarization, and subcellular organization with greater precision.^[62]

3D Tissue Reconstruction

- Combining serial tissue sections with advanced imaging can create detailed 3D maps of organs and tumors.
- This provides a better understanding of how cells are organized in three dimensions.^[63]

Spatial Pathology

- Combining molecular maps with traditional histopathology can improve:
- Disease diagnosis
- Patient classification
- Diagnostic accuracy and reproducibility^[64]

Understanding Therapeutic Resistance

- Spatial omics can identify cell-to-cell communication within the tumor microenvironment.
- This may help discover new therapeutic targets to overcome immunotherapy resistance.^[65]

Expanding Beyond Cancer

- Applications are rapidly expanding into chronic inflammatory and neurological diseases.
- Spatial omics may help map cellular networks involved in diseases such as Parkinson's disease and Alzheimer's disease.^[66]

LIMITATIONS OF SPATIAL OMICS

Trade-off Between Molecular Coverage, Sensitivity and Spatial Resolution

- Spatial omics technologies often face a trade-off between molecular coverage, detection sensitivity, and spatial resolution.
- A method with very high spatial resolution may detect fewer molecules, while methods with broader molecular coverage may have lower spatial resolution.
- Therefore, it is difficult to achieve high resolution, high sensitivity, and comprehensive molecular profiling at the same time.

RNA Diffusion and Mislocalization

- During tissue permeabilization, RNA molecules can move away from their original position.
- This process, known as RNA diffusion, may cause transcripts to be detected in the wrong

location.

- As a result, the exact spatial boundaries of cells may become blurred, reducing the accuracy of spatial information.

Microscopy Bottlenecks

- Imaging-based spatial omics methods require repeated cycles of staining, washing, imaging and signal detection.
- These procedures can make the overall process slow and technically demanding.
- Large tissue sections may also have a limited field of view, requiring multiple images to be combined.
- Image stitching and co-registration can introduce additional alignment errors.

Errors in Cell Segmentation

- Correctly identifying the boundaries of individual cells is a major challenge.
- Cells may have irregular shapes, overlap with neighbouring cells, or have poorly defined boundaries.
- If segmentation is inaccurate, RNA transcripts may be assigned to the wrong cell.
- This can ultimately affect cell-type identification and downstream biological analysis.

Sample Damage

- Tissue sections, especially fragile or poorly preserved samples, can be damaged during repeated staining, washing, fluid exchange and imaging cycles.
- Physical damage or degradation of the tissue may reduce the quality of molecular and morphological information.
- Therefore, careful sample preparation and handling are essential.

Computational and Analytical Challenges

- Spatial omics produces large, complex and multidimensional datasets.
- Processing these datasets requires advanced computational methods and considerable storage and computing resources.
- Uncertainty in spatial localization, segmentation and molecular detection can make data interpretation difficult.
- Advanced bioinformatics and AI-based approaches are increasingly being used to overcome these challenges.

Data Heterogeneity and Integration

- Different spatial omics platforms may generate data with different formats, resolutions, coordinate systems and molecular measurements.
- Combining data from multiple platforms or modalities can therefore lead to batch effects and alignment errors.
- Integrating spatial transcriptomics, proteomics and

histological information into a single biological picture remains challenging.

Lack of Standardization

- Spatial omics is a rapidly developing field, and there is still a lack of universally accepted standard protocols and benchmarking methods.
- Different laboratories may use different sample preparation methods, instruments and computational pipelines.
- The absence of common data formats, standardized analysis pipelines and consensus performance measures can make results difficult to compare and reproduce.
- Developing common standards will be important for the clinical and research applications of spatial omics.

CONCLUSION

Spatial omics is an advanced approach that combines molecular information with the spatial organization of tissues, allowing researchers to understand where genes and proteins are expressed.

Conventional bulk omics gives an overall average signal from many cells, like a “smoothie,” while single-cell omics identifies individual cell types like a “separated fruit bowl” but may lose their original spatial locations. Spatial omics overcome this limitation by preserving both molecular information and the location of cells within the tissue. It helps researchers understand how cells are arranged, interact, and communicate with each other in their natural environment. This approach provides valuable insights into tissue organization, disease progression, tumor microenvironments, immune responses, and cellular interactions. Therefore, spatial omics serves as an important bridge between molecular profiling and tissue architecture, providing a more complete understanding of biological processes and supporting future applications in biomedical research, diagnosis, and personalized medicine.

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