2024 | eBook

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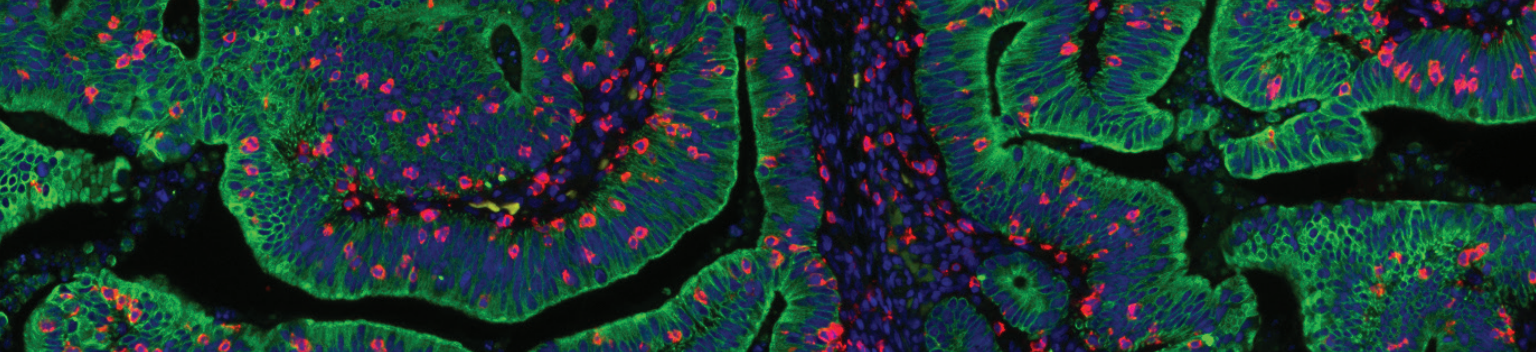

NAVIGATING THE FRONTIER OF SPATIAL BIOLOGY RESEARCH

Spatial multiomics introduces an additional layer of understanding into gene activity, cellular behavior, and tissue organization.

Embryonic development, immune responses, and a myriad of other physiological processes are dependent on the precise expression of genes in specific locations within cells and tissues. Any deviations from this spatial regulation can lead to pathological conditions.

Spatial transcriptomics aims to quantify gene transcripts in their native spatial positions in tissues, helping scientists to better unravel the mechanisms underlying cellular functions and disease processes. Today, different methods exist to capture tissues' spatial profiles by integrating techniques such as *in situ* hybridization, RNA sequencing, and microscopy. These advances have led to the development of various spatial transcriptomic technologies, each offering unique advantages for exploring gene activity and revealing cellular heterogeneity and organization across tissues.

Different pathways to spatial insights

There are two primary spatial transcriptomic methodologies: profiling-based and imaging-based. Profiling methods involve capturing transcriptomic data, while imaging-based techniques directly visualize RNA molecules' spatial distribution within individual cells.

One profiling-based spatial transcriptomic approach utilizes high throughput sequencing to construct spatial maps of gene expression. This process involves mounting the tissue onto a microarray embedded with thousands to millions of barcoded probes. These probes then capture mRNA molecules from the tissue onto distinct spots, where they undergo reverse transcription, converting into complementary DNA for subsequent sequencing. After sequencing, researchers overlay the spatial coordinates onto the transcriptomic data, generating a view of gene expression patterns across the tissue (1).

An alternative profiling-based strategy employs *in situ* hybridization probes to acquire spatial information. NanoString's GeoMx® Digital Spatial Profiler (DSP), for example, employs oligonucleotide probes that bind specifically to target RNA sequences on a tissue slide. These probes are coupled with photocleavable oligonucleotide barcodes. Upon UV light exposure to the region of interest, the barcodes detached from the probes are collected into multiwell plates, where they are then counted downstream either by next generation sequencing on an Illumina sequencer or by direct digital counting on the NanoString nCounter® Analysis System. The counts of these barcodes reflect the abundance of the corresponding mRNA, enabling researchers to spatially profile RNA expression by mapping barcode counts back to the tissue section.

In contrast, imaging-based spatial transcriptomic technologies often utilize fluorescently labeled *in situ* hybridization probes targeting specific RNA molecules. NanoString's single cell imaging platform uses automated cyclic *in situ* hybridization chemistry, which allows for multiple cycles of mRNA hybridization with fluorescent barcodes to amplify signals associated with each RNA molecule (3). The high resolution CosMx™ Spatial Molecular Imager (SMI) enables researchers to accurately visualize and determine the spatial distribution of gene expression within individual cells and even subcellular compartments (4).

Multiomics integration

The potential of these spatial transcriptomics technologies amplifies when integrated with other omics data, offering a multidimensional view of biological processes. For instance, combining spatial transcriptomic data with proteomic profiles allows researchers to link gene expression to protein abundance, shedding light on how cellular pathways and signaling networks operate within specific tissue microenvironments.

The GeoMx® DSP system, in addition to covering the whole transcriptome, can analyze the expression of over 570 proteins simultaneously on the same tissue section. This capability allows researchers to obtain a complete proteogenomic picture of cell state, signal transduction, and biological pathways. Conversely, the CosMx™ SMI platform quantifies and visualizes up to 6,000 RNAs and 64 proteins, providing multiomics insights into tissue samples at single cell and subcellular levels. With the CosMx™ SMI, researchers can more closely delineate cell types, states, and tissue microenvironment phenotypes.

These multiomic approaches open up new avenues for understanding complex diseases like cancer. By mapping the spatial distribution of various cellular elements and their molecular signatures, researchers can uncover new biomarkers, therapeutic targets, and treatment strategies. Ongoing advancements in spatial transcriptomics and multiomics will lead to a more holistic understanding of cell biology and disease pathogenesis, paving the way for future personalized diagnostics and therapeutics.

REFERENCES

1. Wang, Y. *et al*. Spatial transcriptomics: Technologies, applications and experimental considerations. *Genomics* **115**, 110671 (2023).
2. GeoMx DSP Overview. *NanoString* (2024). at <https://nanostring.com/products/geomx-digital-spatial-profiler/geomx-dsp-overview/>
3. He, S. *et al*. High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging. *Nat Biotechnol* **40**, 1794–1806 (2022).
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HOW TO CHOOSE THE RIGHT
SPATIAL BIOLOGY TECHNOLOGY

Recent advances in spatial biology enable the exploration of tissues with unparalleled depth and precision. Embarking on this exciting journey, researchers need to carefully align their choice of technology with their research needs, leveraging the capabilities of these tools to unlock valuable insights.

MULTICELLULAR SPATIAL STUDY

SINGLE CELL SPATIAL EXPLORATION

RESEARCH QUESTIONS

Are there any biomarkers or pathways in specific tissue compartments or cell populations linked to disease progression or treatment response?

What is the spatial heterogeneity of biomarker expression across samples in a given cohort?

What is the spatial heterogeneity of the expression of the whole transcriptome and high plex protein within tissue microstructures?

How do I scale up spatial multiomics for large cohort studies?

How do cells interact with each other to mediate biological processes and drive diseases?

What is the spatial arrangement of individual cells and how does their expression differ?

How can I identify biomarkers and drug targets in the subcellular context?

How can I build a spatially resolved cell atlas?

SAMPLE TYPE

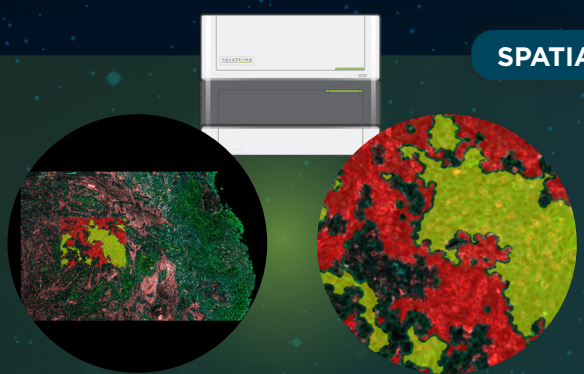
Fresh frozen tissue

Formalin-fixed paraffin-embedded tissue

Tissue microarray

Organoid

SPATIAL TECHNOLOGIES



Whole tissue section

Tissue compartment

GeoMx® Digital Spatial Profiler (DSP)

The GeoMx® DSP enables non-destructive spatial profiling of the whole transcriptome and over 570 proteins on the same slide, efficiently resolving distinct tissue compartments and cell populations. This technology allows researchers to unravel tissue heterogeneity, identify biomarkers, elucidate treatment responses, and understand disease mechanisms.

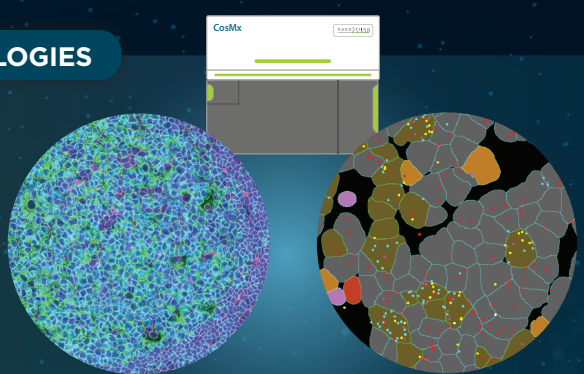
Advantages

True multiomics

Coverage of the whole transcriptome and 570+ proteins

Scalable workflow compatible with automation

Flexible tissue input and region of interest selection



Single cell

Subcellular

CosMx™ Spatial Molecular Imager (SMI)

The CosMx™ SMI quantifies and visualizes up to 6,000 RNAs and 64 proteins at cellular and subcellular resolution. Integrated with cyclic *in situ* hybridization chemistry, high resolution imaging readout, and the AtoMx™ Spatial Informatics Platform, this technology delivers deeper insights for creating cell atlases of different tissues, cellular phenotyping, identifying ligand-receptor interactions, and discovering single cell biomarkers.

Advantages

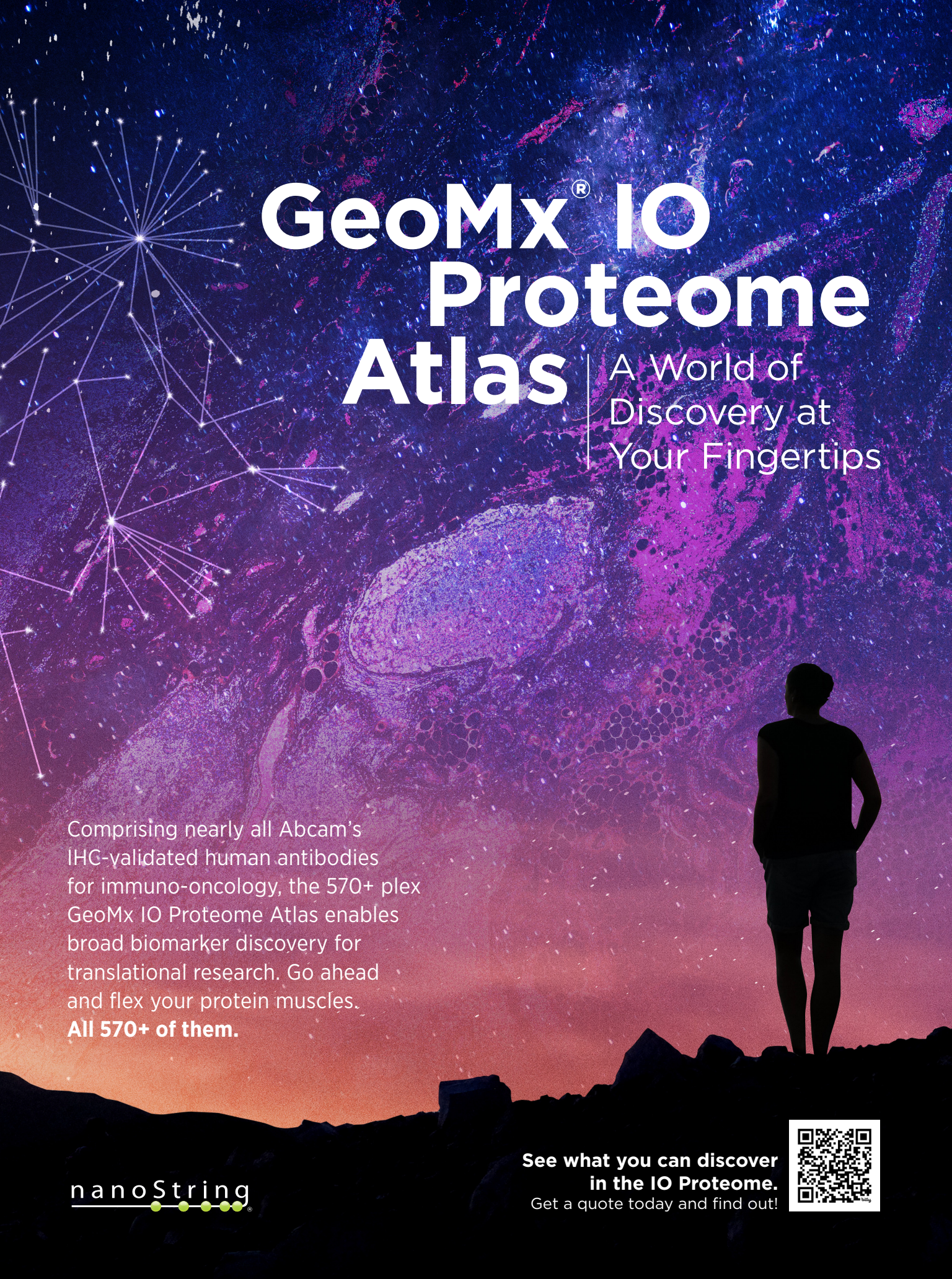
True single cell spatial biology

Gold standard cell segmentation

High plex imaging

DOUBLE THE INSIGHTS

The CosMx™ SMI complements the GeoMx® DSP by facilitating in-depth single cell investigation following whole transcriptome analysis. Conversely, the GeoMx® DSP ensures researchers do not miss key markers and allows for characterization of tissue microstructures across large cohorts. Combining these two systems allows researchers to conduct comprehensive spatial multiomics studies.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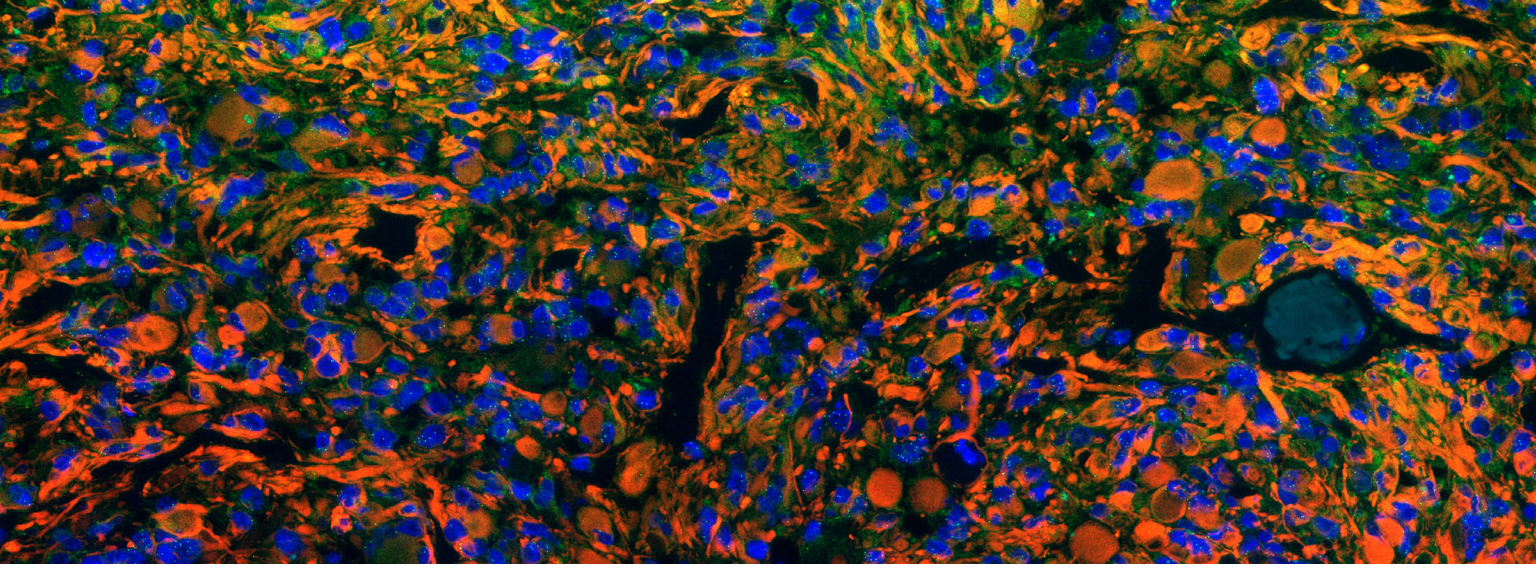

ILLUMINATING SPATIAL HETEROGENEITY IN DEADLY BRAIN TUMORS

Researchers employ cutting-edge spatial biology technologies to create the first single cell map of diverse cellular communities in gliomas.

Tumor heterogeneity is a hallmark of most advanced cancers and remains a major barrier to successful treatment. Among the most notorious examples of difficult-to-treat conditions are high-grade gliomas (HGGs), aggressive brain cancers where genetic and cellular makeup varies significantly within and between individual tumors. For decades, an inadequate understanding of the molecular complexity of gliomas has hindered the development of effective therapeutic strategies, leading to poor survival outcomes. In a recent study published in *Neuro-Oncology Advances*, a research team led by Sarah Best, a cancer researcher from the Walter and Eliza Hall Institute of Medical Research leveraged a combination of advanced spatial transcriptomic technologies to dissect the spatial heterogeneity and immune landscape of HGGs.

HGGs exhibit various subtypes based on the presence or absence of mutations in the genes encoding isocitrate dehydrogenases (IDHs), enzymes that mediate cellular metabolism. However, in glioblastoma (GBM), the most aggressive form of HGG, IDHs remain unmutated. To explore both inter- and intra-tumoral heterogeneity of HGGs, the researchers selected patient tumor samples with IDH mutations and IDH-wild type GBM samples.

They then conducted spatial whole transcriptome analyses of the samples using the GeoMx® Digital Spatial Profiler, which allowed them to map RNA transcripts and cell populations across different regions within each tumor sample. Using this approach, they uncovered significant disparities in cellular composition between IDH-mutant and IDH-wild type HGG tumors. Compared to IDH-mutant tumors, IDH wild type GBM tumors contained higher proportions of mesenchymal and progenitor tumor cells. These cell types also exhibited high spatial heterogeneity across different GBM samples.

To delve deeper into GBM heterogeneity, Best's team utilized the CosMx™ Spatial Molecular Imager, a high-plex imaging technology providing spatial transcriptomic information at single cell and subcellular resolutions. By examining multiple regions within an IDH-wild type GBM sample, they identified diverse cell types, including immune cells, normal cells, and various tumor cell subtypes such as mesenchymal, astrocytic, and progenitor cells. With CosMx™ Spatial Molecular Imaging, they precisely mapped out the spatial distribution of these cells, illustrating the cellular diversity within GBM.

The brain has a distinct immune composition within the tumor microenvironment, largely consisting of immunosuppressive microglia and macrophages that limit lymphocyte infiltration. When the researchers spatially mapped the immune landscapes of GBMs, they discovered significantly different immune profiles in neighboring regions within the same tumor. For instance, regions rich in mesenchymal cells showed increased lymphocyte populations, while microglia coexisted with astrocytic cells. These findings suggest close spatial associations between tumor cell types and immune infiltrate distribution.

To investigate whether consistent patterns of tumor architecture and immune infiltration exist in GBM, the researchers then examined spatial transcriptomics data from a cohort of 16 GBM tumors. They identified two main tumor niches: a brain-intrinsic niche comprised of astrocytic and oligodendrocytic tumor cells associated with the brain resident microglia, and a brain-extrinsic niche containing mesenchymal patches infiltrated by monocytes and other immune cells like neutrophils, T cells, monocytes, and macrophages clustered near blood vessels.

Combining different spatial transcriptomics tools like GeoMx and CosMx allowed Best's team to elucidate transcriptional heterogeneity in gliomas comprehensively while capturing single cell details. By elucidating the spatial organization of tumor cells and their immune microenvironments, the study lays the groundwork for developing targeted therapeutic strategies that disrupt tumor-immune interactions and improve treatment outcomes for patients with these deadly brain tumors.

REFERENCE

Moffet, J. J. D. *et al*. Spatial architecture of high-grade glioma reveals tumor heterogeneity within distinct domains. *Neuro-Oncology Advances* **5**, vdad142 (2023).

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CosMx™ 6K Discovery Panel

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