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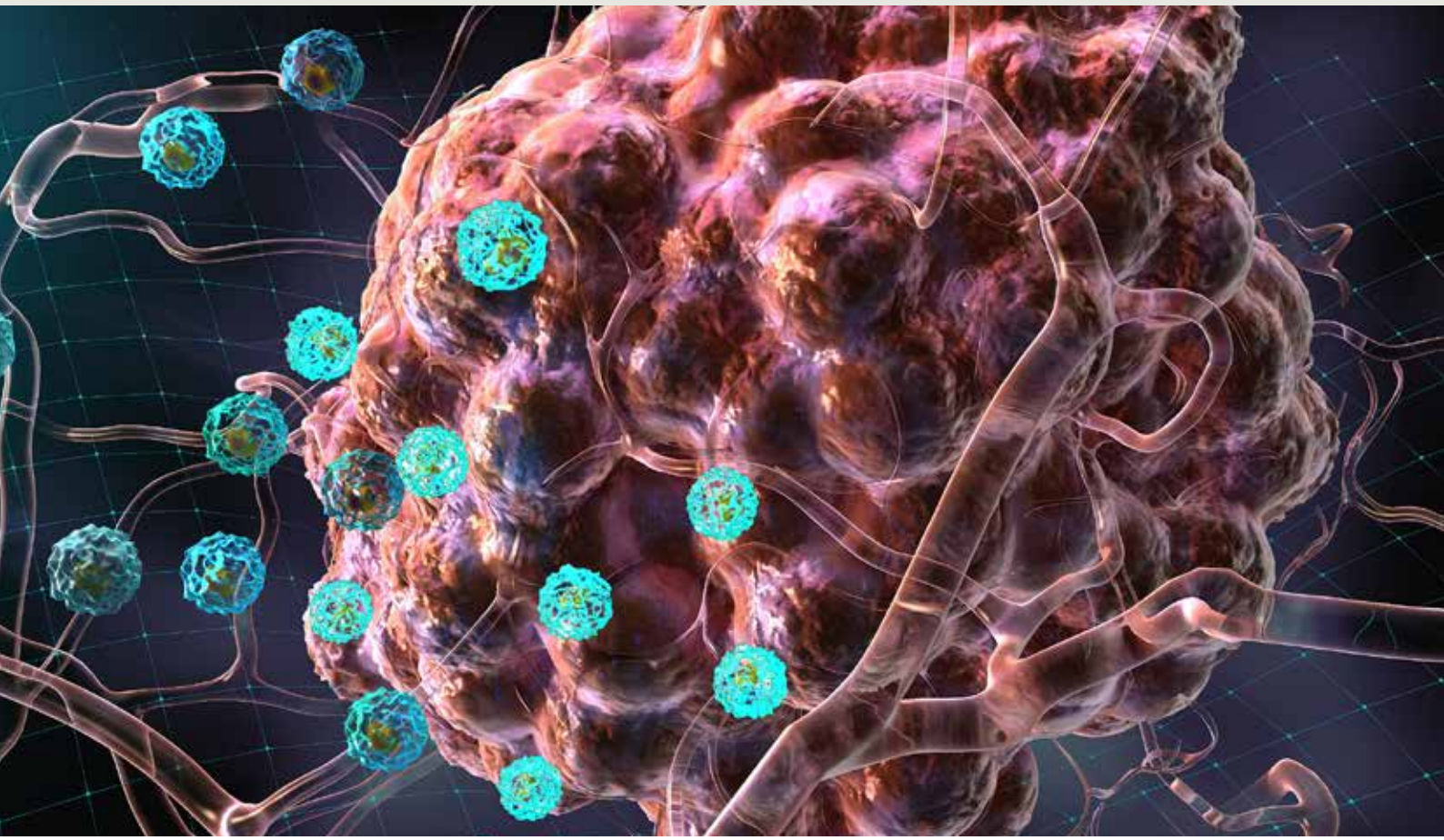

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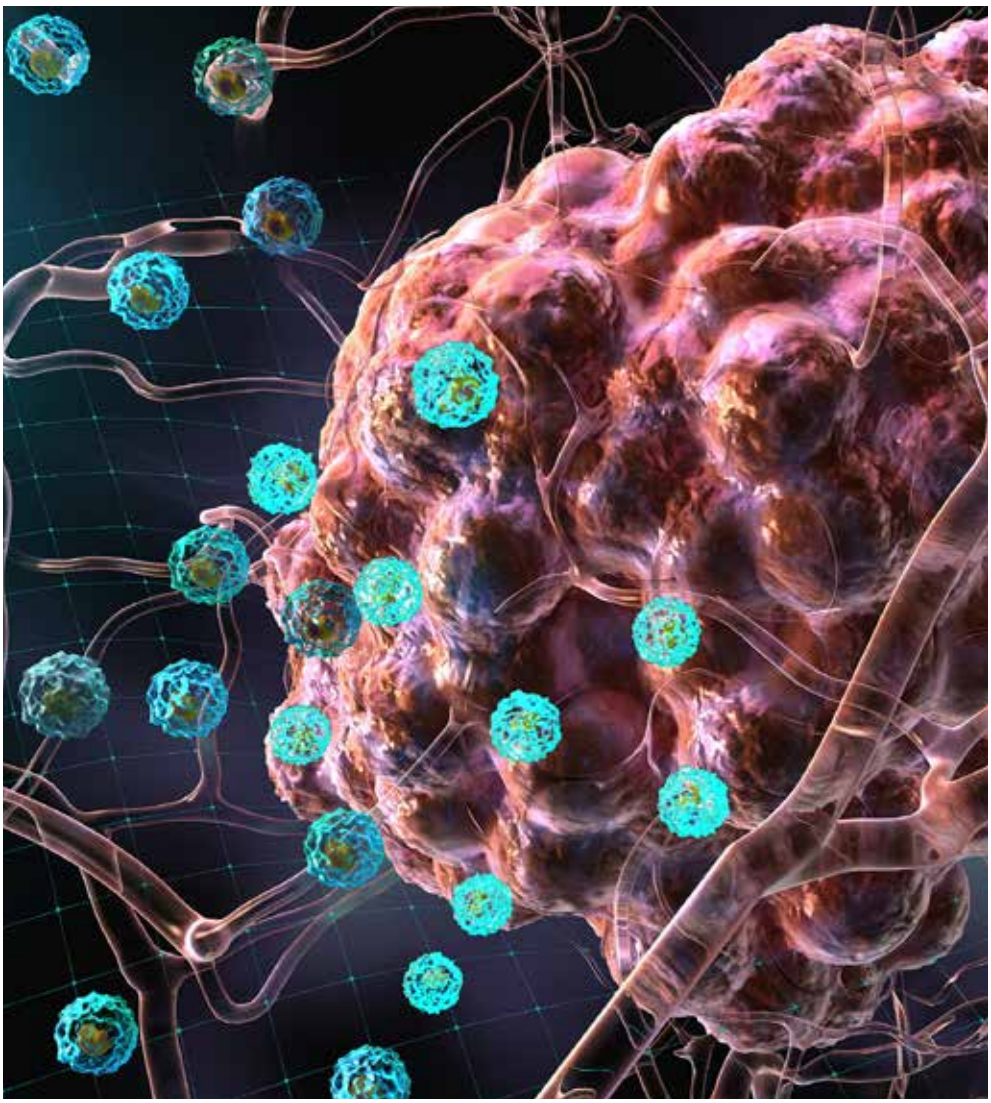


The tumour microenvironment is a busy place. As cancer cells grow and divide in an uncontrolled manner, they encounter many different types of cell, including those of the immune system. For years, researchers have been trying to pick apart these interactions to find out what's going on, and where. This information will help them to understand patients' immune response to cancer and devise ways to boost it with immunotherapies.

Traditionally, immunohistochemistry and in situ hybridization have been the tools of choice to reveal spatial gene expression in tissue sections. But the throughput of these procedures is limited to the analysis of only a few genes at a time.

More recently, single-cell sequencing technologies have uncovered previously unappreciated levels of cancer and immune cell heterogeneity. These technologies have helped define molecular profiles associated with immune cell activation and exhaustion. However, because this type of analysis demands that the tissue is dissociated and the cells segregated, their spatial context is lost.

Researchers need a way to examine individual cells in detail, without losing the information about where they are in relation to each other. By combining high-throughput imaging and sequencing technologies, it's now possible to examine the expression of the cancer transcriptome in cancer biopsy samples.

"Spatial multi-omics is the molecular profiling solution to probe both the structure and function of tumours at the level of transcriptomics, proteomics and metabolomics," says Ahmet Coskun, a bioengineer at Georgia Institute of Technology,

Atlanta. These platforms allow researchers to characterize cancer regions in whole tissue sections, informing diagnosis and helping to identify new prognostic biomarkers.

Characterizing the tumour microenvironment

The tumour microenvironment comprises a variety of resident and infiltrating host cells, secreted factors and extracellular matrix proteins, all of which have a profound influence on tumour initiation, progression, and metastasis. Many researchers are trying to unravel how the different subtypes of infiltrating immune cells are selected or recruited to tumours, and then develop therapies that stimulate the immune system to fight cancer.

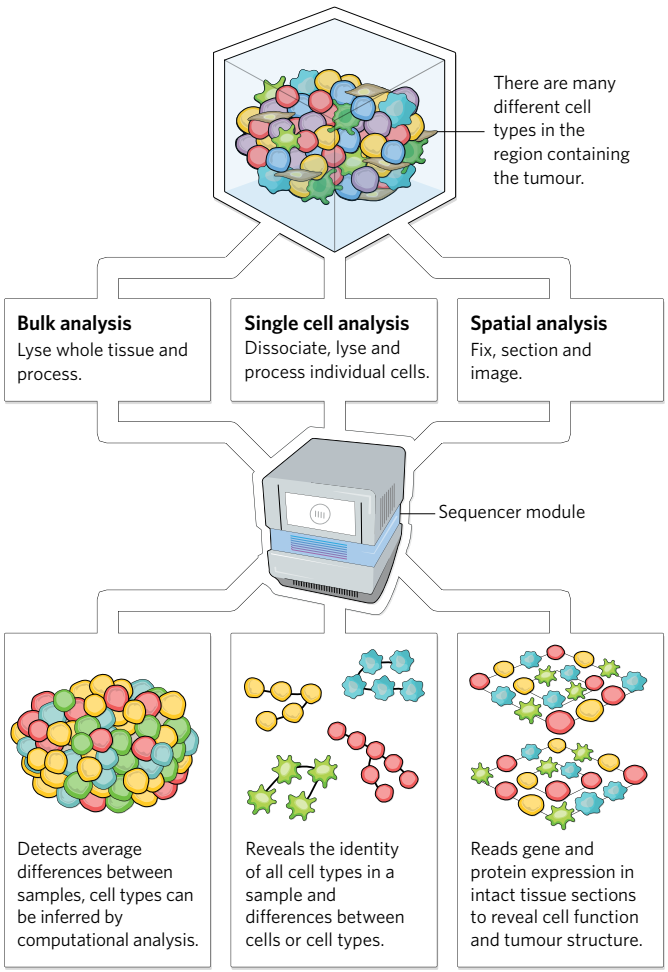
Until now, the assessment of cells in the tumour microenvironment has mainly relied on pathologists using morphology-based examination of individual tissue sections combined with immunohistochemistry to identify marker proteins and/or in situ hybridization to detect specific mRNAs. A major disadvantage of these antibody-based approaches is that they require a priori knowledge of expected cell types. To obtain unbiased, genome-wide gene expression levels from tens of thousands of cells, researchers turned to RNA sequencing — both bulk and single cell (scRNA-seq) — which can determine the cell-type composition in a sample (see 'Exploring the tumour microenvironment').

Bulk profiles contain transcripts from both cancer and non-cancer cells; the abundance of different cell populations has to be determined by computational approaches based on gene expression profiles specific for a given cell population. By contrast, scRNA-seq data provides cell-type specific expression profiles of thousands of individual cells. It can capture rare cell types or states that play key roles in immunotolerance or immune rejection, but might be missed in bulk approaches. scRNA-seq has been used to profile tumour infiltrating immune cells in many types of cancer, including skin, liver, breast, colorectal and lung cancer.

Although these characterization efforts are helping to drive the development and optimization of personalized immunotherapies, the rate of response to targeted cancer therapies remains low, with with as little as 10% of patients showing long-term responses[1] in harder to treat cancers such as glioblastoma.

EXPLORING THE TUMOUR MICROENVIRONMENT

Technological advances are improving researchers' ability to see what cell types are present, their activation state, and how they related to each other. This understanding will improve cancer detection and prediction of clinical outcome, and help optimize therapy.



Researchers hope that analysing all gene expression within the context of the cancer tissue will improve understanding of how cell interactions shape cell identity and function. "With spatial transcriptomics we are able, for the first time, to combine transcriptional profiles with location, which will reveal genes that may be important for regulating cell–cell interactions and the killing of tumour cells," says Tullia Bruno, an immunologist at the University of Pittsburgh Hillman Cancer Center.

An unbiased alternative

Patrik Ståhl and colleagues at the Karolinska Institute, the Royal Institute of Technology and the Science for Life Laboratory (SciLifeLab) in Sweden were the first to generate a spatial record of mRNA molecules in tissue sections. "We wanted to develop a high resolution, unbiased alternative to traditional histochemical stains to gain new insights into biology," he explains.

They developed a method[2] to measure the spatial distribution of transcripts by capturing and barcoding RNA in fixed brain tissue samples, performing reverse transcription followed by sequencing and computational reconstruction. Ståhl concedes the outcome wasn't by any means certain. "Not many people thought we were going to be able to capture the molecules directly where they are in the tissue," he recalls. But the team was able to verify that the transcripts indeed mapped where the cells were, using fluorescent tags.

It didn't take long for cancer

researchers to use the approach on breast and prostate cancer, and melanoma samples. "Spatial transcriptomics is an exciting new modality to characterize the complex biology of human cancers and extract maximum information from limited patient samples," says Sangeetha Reddy, a cancer researcher at UT Southwestern, Dallas.

There are now several spatial transcriptomic platforms, such as Visium Spatial Gene Expression Solution (10x Genomics) and GeoMX digital spatial profiler (Nanostring), that rely on standard next-generation sequencing (NGS) protocols to visualize and quantify gene and protein expression in tissue sections.

By helping to distinguish malignant from non-malignant cells based on their gene expression patterns, Ståhl reckons that this technology has the potential to replace the manual scoring of cancer severity by pathologists. Furthermore, the spatial transcriptomics approach might profile gene expression in situ more accurately: scRNA-seq entails isolating cells from their physical compartments, which can upregulate stress response genes[3]. "As the throughput and resolution of spatial transcriptomics improves, it could become the method of choice to characterise tissues," says Ståhl.

Dissecting immune infiltrates

Current immunotherapies aim to stimulate killer T-cells to fight cancer, but only a limited number of patients with specific types of cancer seem to benefit. Clinicians have attributed this to the insufficient and heterogeneous expression of checkpoint molecules, which regulate T-cell responses to cancer cells in the tumour microenvironment.

By using NGS technologies, researchers can analyse the genetic profile of both the tumours and the host's immune activity to identify the expression of checkpoint molecules and other biomarkers of response. Spatial genomic analyses are enabling them to zoom into the activity in the tumour microenvironment and

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gather more information about infiltrating immune cells. "With spatial transcriptomics, we can link the distribution of cells in the tumour microenvironment with genes that may regulate position or interaction with other cells," says Bruno. Comprehensive analyses of cancer-immune cell interactions prior to immunotherapy could be pivotal to therapeutic success.

In 2018, Ståhl and colleagues used spatial transcriptomics to map gene expression in prostate tumour samples[4]. They detected activation of signalling pathways related to stress, inflammation and angiogenesis in the tumour periphery, that could have significant implications for tumour initiation and progression.

More recently, they used spatial transcriptomics breast cancer datasets to train and test a machine-learning method to predict clinical outcomes[5]. "The potential of training algorithms from spatial transcriptomics data to accurately classify breast cancer regions, suggests that spatial transcriptomics could be used to support early detection and clinical decision-making" says Ståhl.

Several other studies using spatial transcriptomic platforms have highlighted previously undiscovered roles for particular types of infiltrating immune cells in cancer progression and response to therapy.

Reddy has been involved in work examining patients with melanoma. She has shown[6] that the presence of B cells in tumour compartments called tertiary lymphoid structures was associated with a favourable response to immunotherapy. By contrast, another study[7] found that recruitment and activation of neutrophils in the tumour microenvironment was associated with a poor response to immunotherapy.

These findings open the exciting possibility of modulating neutrophil or B-cell responses to complement T-cell-mediated immunotherapies. "Multiple groups, including my own, are now exploring strategies to do this," says Reddy.

Next steps in conquering the spatial dimension

The resolution of spatial transcriptomics is limited by the density of areas with the same spatial barcode, which currently stands at 50-100 µm. Developers are aiming to achieve single-cell and subcellular resolution but, as Ståhl observes, this level of detail may not always be necessary. "It's about finding the right balance between resolution, time, cost and throughput for your particular experiment."

Other improvements include combining the data from consecutive sample sections to generate genome-wide RNA-seq data from human tissue in 3D. Also, following the footsteps of single-cell sequencing, spatial genomic analysis is starting to integrate different types of -omic information to build more complete molecular profiles.

Earlier this year, a team from Seattle led by Walter Ruzzo generated spatially resolved metabolic network models in prostate cancer[8]. They found cancer-cell-specific metabolic vulnerabilities that could be targeted by small molecule compounds. Although the models are based only on transcriptomic data, which do not directly reflect metabolic activity, they highlight the usefulness of spatially resolved proteomic and metabolomic analyses.

Spatial analyses are already having an impact on basic and translational research, even if it is likely to be a few years until they enter routine clinical practice in cancer management. As end-to-end workflows for research and bioinformatics software become increasingly accessible, these analyses will become easier to perform in a clinical setting.

Results obtained so far are motivating a growing number of researchers to integrate the spatial component into analyses of tumour progression and to explore patient outcomes following cancer therapies. "These technologies are great," says Coskun. "They provide reliable gene expression profiling with spatial data at low-cost, and are compatible with existing routines in medicine." ■

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