SPOT LIGHT

Discovering a chemokine's purpose in fighting tumors

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STANDARD BIOTOOLS™

RNA and protein co-detection with Imaging Mass Cytometry in metastatic melanoma

Whether a tumor is "hot" or "cold" plays a significant role in a patient's response to immunotherapy. An inflamed "hot" tumor is more likely to benefit from immune checkpoint inhibition, while a "cold" tumor contains low levels of immune infiltration and is associated with low success.

The complex interplay of tumor and immune cells within the tumor microenvironment creates these circumstances, which are unique to each individual and impact tumor control and antitumor response. Without known factors that can determine response to immunotherapy, this complexity confounds patient outcome.

Chemokines as a therapeutic target

Immune cell trafficking and recruitment into tumors is mediated by chemokines, a class of small soluble factors that attract cells via receptor interaction. Chemokine detection is fairly difficult, so their prevalence and distribution are largely unknown.

Daniel Schulz, PhD, Senior Scientist at the University of Zurich, and colleagues recently investigated how chemokines direct immune cell activity in tumors. A majority of studies focus on cell types, behaviors and location in the spatial landscape. Schulz wanted to know how these cells get there in the first place, thus determining the underlying molecular mechanisms of cell behavior and further allowing therapeutic development that effectively enhances antitumor activity.

RNA and protein co-detection development and validation

Since they are secreted, chemokines are tough to associate with a cell of interest. In order to map chemokines in relation to cell behavior, Schulz's work has revolved around the ability to detect chemokines through their RNA and simultaneously use proteins to detect cell types.

The 12-plex RNAscope™ system by Advanced Cell Diagnostics enables the study of multiple chemokines through high-plex imaging. Detecting both RNA and proteins on the same image provides an abundance of data about what interactions are occurring and where.

RNA present in formalin-fixed, paraffin-embedded tissue can be detected by hybridizing pairs of probes to the RNA of of interest, amplifying these probes and imaging them. Once the pairs are bound to their targets, they are amplified in sequential hybridization steps, followed by the hybridization of fluorescently labeled oligonucleotides, in total called amplification trees. Because of these large amplification structures, it is possible to detect single molecules with strong signal amplification, high specificity and multiplexing capability.

This approach can be modified for use with Imaging Mass Cytometry™ (IMC™) by exchanging the fluorophore-tagged oligos with metal-tagged oligos to detect RNA, followed by standard staining with metal-tagged antibodies to detect proteins. The method leverages the sensitivity of IMC, even for medium- and low-expressed genes,

due to a lack of background staining issues. The 12-plex system Schulz designed included one RNA channel to detect a negative control probe (DapB) and 11 channels to detect mRNA encoding chemokines known to attract T cells, monocytes, macrophages and dendritic cells (CXCL9, CXCL10, CXCL12, CXCL13, CCL2, CCL4, CCL18, CCL19 and CCL22), B cells (CXCL13) and neutrophils (CXCL8).

"That's the beauty and strength of this approach. You're not dependent on the antibody and the antibody being available. Theoretically, it's unlimited – you can detect what you want."

Mechanism of immune cell infiltration in melanoma

The team applied the approach to tissue microarrays (TMAs) built from a melanoma cohort of 69 patients that highlighted tumors with different levels of inflammation in metastatic sites from stages III to IV.

An RNA and protein panel was designed combining the prepared RNA probes and antibodies with 21 proteins. A second protein panel targeted 39 markers of tumor cells (including SOX9, SOX10, MITF, Ki-67, S100A1, p75, β-catenin, H3K27me3, pERK, pS6 and PD-L1) and immune cell phenotypes and states (including CD303, CD20, granzyme B, PD-1 and TCF7), enabling the analysis of chemokine expression as well as tumor and immune phenotypes in hot and cold tumors. The team used cytomapper and imcRtools, two R/Bioconductor packages, to analyze imaging data.

"From our experience, the signals are fairly stable across measurements for TMAs because you have all your samples that have been processed in one batch and stained with the same antibodies in the exact same concentrations for the exact same time. So that really minimizes your experimental variation," Schulz explains. The team observed that tumors with no immune infiltration did not have chemokine expression, exhibiting low levels of antigens and inflammation. Infiltrated tumors were characterized by expression of multiple chemokines, primarily CXCL9 and CXCL10 localized in patches associated with dysfunctional T cells expressing CXCL13. B cell patches were also enriched with TCF7+ naive-like T cells, a self-renewing cell type predictive of immunotherapy response.

CXCL13 is the most important chemokine for B cell recruitment

CD8+ T cells are known mediators of antitumor immunity. CXCL9 and CXCL10 can recruit CD8+ T cells into inflamed tumor tissues, creating hotspots of inflammation. Once CD8+ T cells infiltrate tumors, persistent antigen exposure can cause prolonged stimulation that leads to dysfunction and exhaustion. It is this exhaustion that can be reversed by immunotherapy.

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The team was able to visualize that T cells start expressing CXCL13, likely after exposure to a cognate antigen, to recruit B cells to support them as they get exhausted and keep an antitumor reaction going. These CXCL13-expressing T cells also recruit B cells to facilitate tertiary lymphoid structure formation, a presence associated with improved clinical outcomes.

"Combining this data from the detection of chemokines, or of RNA in general, with detecting cell types in the tissues provides so much information," Schulz says. "I think the field is wide open to take any target you want and investigate it. That's the beauty and strength of this approach. You're not dependent on the antibody and the antibody being available. Theoretically, it's unlimited – you can detect what you want."

Armed with tools like RNAscope and IMC, this type of work provides a thorough examination of the dynamic networks of cells and other factors that influence their behavior, shedding light on specific targets that could lead to the development of more effective therapies.

References

Read the publication to get a detailed description of numerous chemokines in the tumor microenvironment and how they influence antitumor immunity: science.org/doi/10.1126/sciimmunol.abk1692

Watch Schulz's Scientist in the Spotlight talk, which highlights the strength of targeted RNA and protein co-detection to analyze tumor immune microenvironments based on chemokine expression: videos.sis.standardbio.com/categories/imaging-mass-cytometry

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