CHARTING THE TUMOR MICROENVIRONMENT:

NAVIGATING COMPLEX SYSTEM INTERPLAY

The tumor microenvironment is a complex network of cancer, immune, vascular, and stromal cells, generally characterized by extracellular-matrix (ECM) remodeling, immune suppression, and hypoxia due to poor vascularization.[1] These combine to create favorable conditions for cancer-cell survival, proliferation, and motility, resulting in tumor growth, invasion, and metastasis.[1] Understanding the components of the tumor microenvironment and their interplay will be essential to better targeting tumor growth and metastasis in the laboratory and clinic.

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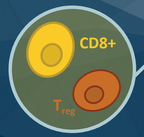
DENDRITIC CELLS AND MACROPHAGES

Dendritic Cells (DCs) (Markers: CD303, CD1c, CD141, CD14^{10,15}) are required for CD8+ T-cell activation as antigen-presenting cells.[3] Hypoxia induces DC suppression of T-cell activity via PD-L1 production.[4]

Tumor-associated Macrophages (TAMs) (Markers: CD68, CD163, CD204^{11}) are immunosuppressive cells, associated with poorer prognoses, that promote ECM remodelling and cancer-cell escape.[4-6]

FIBROBLASTS AND THE EXTRACELLULAR MATRIX

ECM structure influences cancer progression. Hypoxia promotes the recruitment and activation of cancer-associated fibroblasts (Markers: α-SMA, FAP^{12}), increasing collagen deposition, which has been linked to mortality.[1] The microenvironment promotes ECM remodelling by stimulating matrix metalloproteinase secretion, facilitating cancer-cell proliferation and metastasis.[1,7]

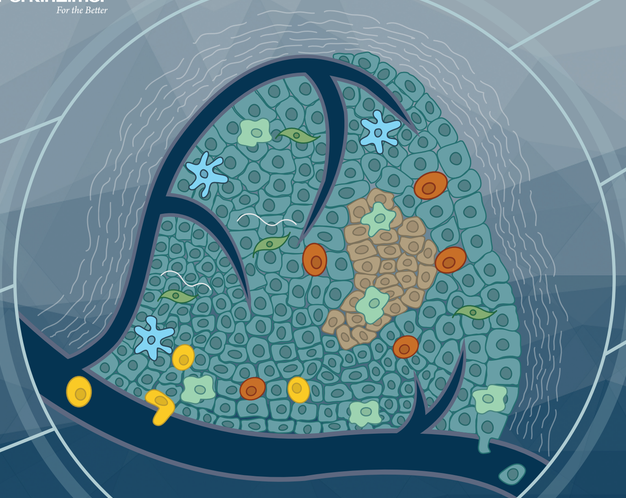


T CELLS AND CHECKPOINT INHIBITORS

CD8+ cytotoxic-T cells are the primary effectors of tumor-cell death, restricting metastasis.[3] Evading these T cells is critical to net tumor growth.[3]

Hypoxia induces CD4+ regulatory T cell (T$_{reg}$)-mediated CD8+ T-cell deactivation, resulting in CD8+ T-cell anergy and increased T$_{reg}$ counts.[2,3] T-cell entry is physically impeded by the immunosuppressive tumor microenvironment, increased stroma density, and decreased endothelial adhesion protein expression.[2,3]

Tumor cells and T$_{reg}$ cells express "checkpoint proteins" such as PD-1 and CTLA-4, which bind to CD8+ T cells, inactivating them. Specialized molecules called "checkpoint inhibitors" have been developed to prevent this interaction, thus reducing tumor-cell evasion and augmenting the CD8+ T cell response.[17,18] T$_{reg}$ depletion, immunosuppressive-pathway downregulation, and T-cell stimulation also represent therapeutic options. A simultaneous multi-method approach yields optimal results.[3]



HYPOXIA

Tumor hypoxia is caused by intercapillary distances exceeding O_2 diffusion range,[1] and can be marked by transcription-factor upregulation (e.g., HIF-1α[8]) or detected chemically using engineered probes.[15] Hypoxia stimulates ECM remodelling and fibrosis,[1] while hampering cell-mediated immunity[1] by promoting immunosuppressive phenotypes[4] and conferring increased cancer-cell resistance to effector-cell-mediated killing.[3]

THE VASCULATURE

Angiogenesis facilitates tumor growth and is stimulated by tumor cells and hypoxia. Tumor-stimulated angiogenic factors (e.g., VEGF, TGF-β, PDGF, endothelin[1,2,14]) can also limit immune-cell entry by downregulating endothelial-adhesion protein expression.[2] Endothelial cells also deactivate CD8+ T cells through PD-L1 and Fas ligand signaling.[2,8]

TUMOR INVASION AND METASTASIS

Tumor cells, either individually or collectively, invade the stroma and intravasate into the circulatory system.[9] They extravasate and initiate tumorigenesis at a different location. This process is termed "metastasis" and causes 90% of cancer-attributed deaths.[1]

The metastatic cascade exposes cancer cells to immune-system detection.[4,6] The tumor microenvironment counters this by hindering immunosurveillance,[9] altering ECM topography,[1] promoting angiogenesis, and recruiting TAMs.[4] These mechanisms facilitate cancer-cell evasion, motility, and escape.[1]

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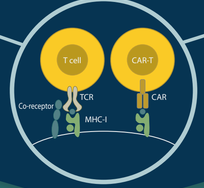
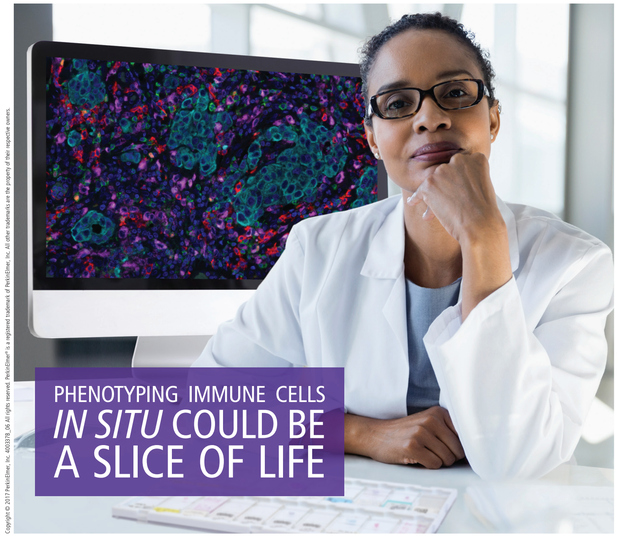
CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS

CAR-T cells express synthetic T-cell receptors (TCRs), facilitating selective targeting of tumor-surface antigens. CAR-T cells have been successful in treating hematological cancers, but – to date – present less efficacy in solid tumors.[13] The tumor microenvironment limits CAR-T cells' therapeutic efficiency by downregulating T-cell trafficking and inducing dysfunction via immunosuppression mechanisms. Simultaneous cotherapy to alleviate these impediments using cytokines, chemokines, and/or antibodies is required for optimal therapeutic efficacy in solid tumors.[13]



QUANTITATING IMMUNE-CANCER INTERACTIONS

Immunohistochemistry is the conventional avenue for investigating the presence of various cell types, functional states, and protein expressions within tumor tissue. While quite effective for detecting one protein or one cell type at a time, the technique is limited in its capability to reveal specific cell-to-cell interactions occurring within the tumor microenvironment, especially interactions between immune cells and tumor cells. Flow cytometry of disaggregated tissues is often used when multiple proteins are needed to identify multiple cell types, but all spatial information is lost, thus important cellular arrangements and interactions cannot be assessed. Multispectral imaging coupled with multiplexed immunohistochemistry allows for the analysis of multiple protein expression signals within a single tissue section.[19,20] This opens up the exploration of specific cell-to-cell level mechanisms driving immune system-tumor interactions, and can be used as the basis for confirming drug method-of-action, for identifying new mechanisms to target, and potentially for future predictive tests in immuno-oncology.



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PerkinElmer, Inc. is a global leader focused on supporting the needs of precision oncology drug discovery research. Our full solutions, including reagents, instrumentation and software, for in vitro, ex vivo and in vivo models, enable cancer researchers to make more targeted discoveries, faster.

CHARTING THE TUMOR MICROENVIRONMENT:
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PerkinElmer's complete Phenoptics™ workflow solution for quantitative pathology research includes multiplex immunohistochemistry staining solutions, multispectral imaging systems, and advanced image-analysis software.

Recently, PerkinElmer launched the Vectra® Polaris™ Automated Quantitative Pathology Imaging System. This new multi-modal tissue imaging system enables immuno-oncology researchers to gain a deeper level of understanding of disease mechanisms related to new cancer immunotherapy approaches.

The Vectra Polaris system integrates high-throughput, seven-color multispectral imaging with whole-slide scanning in a simplified digital pathology workflow to support the quantification and analysis of tissue sections that are stained with multiple immunohistochemical stains. This helps scientists assess biomarkers that probe deeper into the understanding of the tumor microenvironment, by detecting multiple cell types, functional states, as well as spatial distributions.

"From basic research to clinical research studies, scientists continue to seek advanced imaging technologies to better analyze and understand disease mechanisms," said Jim Corbett, Executive Vice President and President, Discovery & Analytical Solutions, PerkinElmer. "The Vectra Polaris system is an innovative solution that helps further the exploration of new cancer immunotherapy approaches to help unlock the promise of precision medicine."

"PerkinElmer's multiplex IHC platform has addressed a critical need in immuno-oncology research to reveal the cell-level biology occurring in the tumor and its microenvironment that drives disease progression and response to immunotherapy," said Dr. Bernard A. Fox, PhD, Chief, Laboratory of Molecular and Tumor Immunology, Robert W. Franz Cancer Research Center in the Earle A. Chiles Research Institute at Providence Cancer Center (Oregon). "The development of the Vectra Polaris system has come at the right time, to support the transition from an exploratory research tool to a high throughput rugged high speed slide analysis research system that overlays PerkinElmer's unique multispectral technology on to a digital pathology workflow. I believe the Vectra technology will become the standard for tissue biomarker studies in immuno-oncology research and form the basis for tailoring cancer therapies of the future."

For more information on Vectra Polaris and our complete cancer research solutions, please visit **www.perkinelmer.com/AACR**

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