Voices

Innate immune cells in the tumor microenvironment

The tumor immune microenvironment (TIME) is a complex ecosystem that contains adaptive and innate immune cells that have tumor-promoting and anti-tumor effects. There is still much to learn about the diversity, plasticity, and functions of innate immune cells in the TIME and their roles in determining the response to immunotherapies. Experts discuss recent advances in our understanding of their biology in cancer as well as outstanding questions and potential therapeutic avenues.

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Innate lymphocytes in cancer

Innate lymphocytes that lack antigen-specific receptors constitute heterogeneous populations of lymphoid lineage cells that differ in terms of effector functions and residency properties. Conventional natural killer (cNK) cells recirculate in blood and can directly kill target cells through the release of granzymes and perforin. Innate lymphoid cells (ILCs) reside in peripheral tissues, produce an array of inflammatory cytokines, and are generally considered noncytotoxic. While a role for cNK cells in eliminating cancer cells disseminated to the circulation has been well documented, whether and how innate lymphocytes in tumor tissues suppress cancer progression is incompletely understood. In genetic models of murine epithelial cancers, tumor-resident innate lymphocytes express granzymes and kill cancer cells in a perforin-dependent manner. Whether these cytotoxic innate lymphocytes are differentiated along the ILC lineage or are converted from cNK cells remains to be determined. Tumor-associated signals that promote their expansion, tissue retention, and cytolytic activity are also largely unexplored. In addition, whether tissue-resident cytotoxic innate lymphocytes suppress colonization of cancer cells at sites of metastasis is an open question. So is defining oncogenic events that enable cancer cell evasion from innate lymphocyte-mediated cancer surveillance. Tissue-resident innate lymphocytes are also present in human solid tumors. Whether they are differentiated and regulated similarly to their murine counterpart needs further study. In-depth understanding of the tumor-elicited innate lymphocyte response will facilitate its targeting for cancer immunotherapy.

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NK cell cancer immunotherapy

Natural killer (NK) cells are innate lymphocytes that use the perforin/granzyme system to kill tumor cells without prior immunization. They also produce inflammatory cytokines IFNγ, TNFα, and various chemokines, which recruit other immune responses. They express activating receptors including NKG2D and natural cytotoxicity receptors that recognize stress-induced ligands expressed by most tumors. They also express MHC I-specific inhibitory receptors (KIR and NKG2A) and hence preferentially kill MHC I-deficient tumor cells, which can arise spontaneously or in response to checkpoint immunotherapy. Cellular NK-based immunotherapies under investigation include reinfusing patient NK cells that are expanded *ex vivo* in IL-12/IL-18/IL-15 cytokines. Trials are underway with NK-CARs, in some cases allogeneic "off-the shelf" products developed from cell lines, expressing chimeric antigen receptors targeting specific tumor antigens. *In vivo* approaches to mobilize endogenous NK cells under investigation in our lab and elsewhere include STING agonists, which induce robust NK cell responses against MHC I-deficient tumors, and cytokines, including native and super-agonist forms of IL-2, IL-15, IL-12, and IL-18, which may prevent or reverse NK cell "exhaustion." Bi- or tri-specific "NK cell engager" antibodies that bridge NK activating receptors to tumor antigens are being intensively studied. Finally, checkpoint blockade antibodies can enhance antitumor NK activity, including anti-TIGIT and anti-PD-1, and anti-NKG2A and anti-KIR antibodies that block MHC I-specific inhibitory receptors. Increasingly, NK cells are recognized as exciting next-generation therapeutic targets.

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Manipulating macrophage versatility

Mapping the uncharted territories of innate immune cell landscapes using next-generation single-cell sequencing and spatial profiling has revealed the exquisite heterogeneity of myeloid populations in the tumor microenvironment. This ever-expanding knowledge increased the appreciation of tumor-associated macrophages' (TAMs') multifaceted roles in hampering anti-tumor immunity and fueling cancer progression, thus holding therapeutic promises to alleviate TAMs immunosuppressive attributes. However, as we gained more knowledge into these cells' origin, function, and plasticity, "conventional" pan-TAM targeting approaches show limited efficacy in the clinic, underlying the need for more refined subset rewiring forsaking depletion strategies.

We now appreciate that TAM features are sculpted in an organ-, tumor-stage-, and cancer cell genetics-dependent manner. While genetically stable, TAM long-lived phenotype and adaptability to metabolic, genetic, or niche hijacking signals represent ruthless bottlenecks in efficient anti-tumor rewiring. Overcoming these challenges requires innovative approaches utilizing the wealth of information gained on these cells to harness them properly and in a timely manner. For instance, advances based on nanoparticle drug cargos designed to target specific TAM subsets will present the advantage of combinatorial and sequential targeting with non-invasive imaging validation. Hence, leveraging novel and integrated translational insights from murine models, *ex vivo* patient sample cultures, and *in vitro* functional assays need to be harmonized with rational clinical studies accounting for TAM dynamic heterogeneity. Looking forward, it will be in our reach to complement the current T cell-centric therapies to benefit broader patient populations.

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The power of neutrophils

The presence of neutrophils within tumors is associated with poor clinical prognosis in a wide range of cancers. Indeed, neutrophils can promote tumor growth by, for example, promoting tumor vascularization or suppressing anti-tumor immunity. Other devastating effects of neutrophils include their ability to awaken dormant cancer cells, protect circulating cancer cells, and facilitate metastasis. Conversely, there is evidence of neutrophils that oppose tumor progression. In some cases, neutrophils may exhibit direct cytotoxicity against cancer cells or activate other cells with anti-tumor functions.

Currently, our ability to distinguish "good" from "bad" neutrophils in cancer remains limited. However, the advent of single-cell "omics" approaches has revealed the complexity of tumor-associated neutrophils at unprecedented resolution. Recent studies have shown that these cells form a continuum of states that have divergent phenotypes. The description of the phenotypic heterogeneity of neutrophils opens doors to interrogate the functions of these newly identified cell states. Many of them are conserved between humans and mice, indicating that mouse models may be useful for studying the roles of specific neutrophil states present in human cancers.

Ultimately, it will be critical to understand what dictates the emergence of specific subtypes of neutrophils, and whether these cells exhibit distinct functions and can be selectively manipulated. Answers to these questions should help us to better understand the basis of discrepant conclusions surrounding "good" versus "bad" neutrophils in cancer and may reveal strategies to exploit these cells for therapeutic purposes.

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MDSCs: major drivers in cancer

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of monocytic and polymorphonuclear immature myeloid cells that develop under inflammation-driven emergency myelopoiesis. In most individuals with cancer, the expansion of MDSCs limits the development of protective anti-tumor immunity, promotes tumor cell growth and metastasis, and restricts the effectiveness of cancer immunotherapy. Detrimental immunoregulatory and tumor-promoting actions of MDSCs are amplified upon tumor infiltration and triggered by a multitude of surface, intracellular, or excreted proteins and metabolites. Despite their relevance, there are no effective therapies to fully overcome the activity of MDSCs in cancer. Previous therapeutic approaches based on antibodies or small-molecule inhibitors aimed to deplete MDSCs or target their development, mobilization to tumors, or immunoinhibitory mediators. However, these strategies have been only partially effective and limited by the heterogeneous nature of MDSCs, the lack of MDSC-specific markers, MDSC rebounds after therapy, and induction of compensatory events. Central programs governing MDSC function are currently being elucidated, which provides a new therapeutic option to functionally reprogram MDSCs in tumors. It has been interesting to observe that overcoming key cellular stress mediators or preventing metabolic polarization of MDSCs in tumors switched MDSCs into cells that prime anti-tumor T cell immunity or directly kill cancer cells. Although this therapeutic strategy remains preliminary, it could set the foundation for means to efficiently block MDSCs in tumors, thereby enhancing the effects of radio, chemo, and immune therapies.

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Myeloid cell therapy: a new era

Tumor and metastatic microenvironments are composed of multiple interacting cell types, with tumor cells often in the minority. There is growing appreciation for the diverse and functional impact that non-tumor cells have on cancer progression. Detailed single-cell maps of tumors show that myeloid cells represent an abundant component of the tumor microenvironment, and these cells predominate at sites of metastatic initiation known as pre-metastatic niches. Myeloid cells are part of the innate immune system that can efficiently home to tumor and metastatic sites and communicate with other immune and non-immune cells to orchestrate immune responses. Myeloid cells in the pre-metastatic niche are enriched in gene expression pathways, activating immune suppression and negatively regulating T cell responses. On the other hand, myeloid cells play a pivotal role in the phagocytosis and killing of tumor cells, as well as activating adaptive immunity through antigen presentation and co-stimulation. These apparently conflicting roles make targeting this cell population a challenge. Ideally, immune-suppressive myeloid cells would be targeted and T cell-activating myeloid cells would be spared; however, the plasticity and context-dependent functions of these cells make this approach complex.

To leverage their homing properties and harness their immune-modulating potential, myeloid cells engineered to express IL-12, a potent antitumor cytokine, and foster interaction between the innate and adaptive arms of immunity. IL-12 drives antigen presentation, T helper cell differentiation and T and NK cell proliferation, IFNγ production, and cytotoxic function. Myeloid cell therapy can be adapted to deliver cytokines, chemokines, or decoy receptors into the tissue to locally modulate immune responses. This approach to harness innate cells has the potential to rebalance altered microenvironments and usher in a new era of cell therapies.

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Eosinophils

Eosinophils have potent capabilities to impact local immunity and remodeling during homeostasis and disease. Emerging data highlight that eosinophils infiltrate multiple tumors, where they display pleotropic and even opposing roles (i.e., pro- versus anti-tumorigenic activities). Yet, several key questions regarding their function await to be addressed.

The environmental triggers that induce eosinophil recruitment and survival in distinct tumor microenvironments are still unclear. Furthermore, limited knowledge exists regarding the signals, which direct the phenotypes of eosinophils and whether eosinophils display phenotypic heterogenicity and/or plasticity in the TME. Addressing these questions may be technically challenging, since isolation of high-quality or high-quantity RNA from eosinophils is difficult due to the relative abundance of RNases in their intracellular granules. Thus, eosinophils are "missing" from most single-cell RNA-seq analyses, and even bulk RNA-seq is challenging when working with these cells.

Furthermore, recent data demonstrate an important crosstalk between eosinophils and T cells, where activated eosinophils induce the migration of CD8$^+$ T cells into the TME. This crosstalk has been also suggested in patients treated with immune checkpoint inhibitors, since increased eosinophilia was associated with responsiveness to therapy. The interactions between eosinophils and additional cells in the TME should be characterized with emphasis on T cells, macrophages, NK cells, and fibroblasts. Better understanding the molecular pathways regulating eosinophil activities in the TME may provide new directions for eosinophil-targeted therapies in cancer.
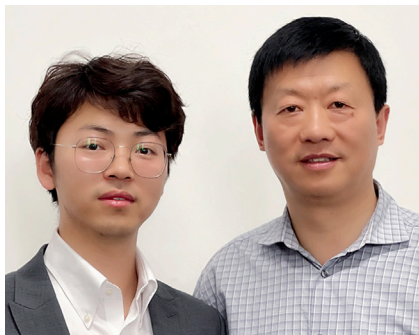
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Mast cell diversity in focus

Mast cells are one of the innate immune cell types that infiltrate tumors with variable abundances. Their overall impact in tumor progression remains elusive due to contradictory reports on association between mast cell infiltration and cancer prognosis. In fact, mast cells are like a double-edged sword in tumor progression, playing both tumor-promoting and anti-tumor roles. Our recent pan-cancer analysis of tumor-infiltrating mast cells observed both mutually exclusive and co-expression patterns of tumor-promoting and anti-tumor signals at single-cell resolution and highlighted that the ratio of TNF^+ to $VEGFA^+$ mast cells could represent their overall activation state. Future research could focus on dissecting tumor-intrinsic factors that mediate the diverse activation states of mast cells in various cancer types. The transcriptional regulators of their tumor-promoting and anti-tumor signals should be investigated to reveal the intracellular switches of mast-cell-orchestrated immune response. The location of tumor-infiltrating mast cells could also directly affect their surrounding stimuli, or extracellular factors that mediate their complex molecular phenotypes. The spatially resolved transcriptomics provides an approach to systematically map cellular positional context and can be utilized to define mast cells in diverse niches, which appear to determine their crosstalk with various cells (e.g., immune cells, endothelial cells, fibroblasts) in the tumor microenvironment. Overall, the in-depth understanding of the functional mechanism of tumor-infiltrating mast cells could unearth novel therapeutic targets for mast-cell-based immunotherapy.

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Dendritic cell vaccines: are we there yet?

Dendritic cells (DCs), "nature's adjuvant," are antigen-presenting cells essential for priming anti-tumor immunity. They formed the basis of the first FDA-approved antigen-specific therapeutic tumor vaccine in patients with castration-resistant advanced prostate cancer. Despite multiple attempts to harness and improve their adjuvant activity, no other DC-based cancer vaccine has been yet approved. Encouragingly, advances in the field indicate that DC platforms can be empowered to eventually induce clinically significant anti-tumor activity in humans. We have a much better understanding of human DC subsets, appreciating that they come in various flavors (conventional DC subsets: cDC1, cDC2, and cDC3 and plasmacytoid DC) with unique characteristics. Traditionally, we relied on using monocyte-derived DC versus primary DC in vaccine trials (which are immunogenic *in vivo*), but as we can now generate millions of each DC subset, one could methodically test the immunogenicity of each subset side by side. This innovation will enable enhancement of DC activity through approaches to improve antigen presentation and blockade of checkpoint molecules, or immune-suppressive mediators or cytokines, and can be combined with conventional approved immunotherapy (such as checkpoint inhibitors). Finally, the use of "DC-targeting vaccines" to direct antigen to DC receptors (e.g., DEC-205) has shown promise in the clinic, and the application of more-specific targeting approaches to deliver antigen to the major cross-presenting DCs (cDC1) via CLEAC9A or XCR1 are under consideration. These, together with systemic mobilizers of DCs (FLT3-L), promise to improve the induction of more potent anti-tumor immunity.