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Targeting macrophages to reprogram the tumor immune microenvironment

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Immunotherapy, particularly immune checkpoint blockade (ICB) therapy, is a promising treatment modality in oncology that involves augmenting the tumor-attacking capabilities of the immune system. Several preclinical and clinical studies have underscored the transformative potential of ICBs in the context of malignancies.[1] However, the efficacy of ICB therapy is often impeded by intrinsic and acquired resistance mechanisms in solid tumors, which are commonly due to a paucity of tumor neoantigens, checkpoint targets, and the inflammatory presence of cytotoxic T lymphocytes.[2] The tumor microenvironment (TME) is profoundly immunosuppressive, which is the key reason for the limited clinical efficacy of ICB therapy. Within this milieu, tumor-associated macrophages (TAMs) are the predominant myeloid cell subset, and their high infiltration levels have been implicated in mediating resistance to ICB therapy, thereby correlating with adverse prognosis across a spectrum of cancers.[3] Recent advances reported in *Nature Cancer* have shed light on specific TAM subpopulations that are instrumental in facilitating tumor immune evasion and resistance to ICB therapy. Two recent studies have delineated the roles of Sirpα$^+$ TAMs in colorectal cancer (CRC)[4] and Siglec-9$^+$ TAMs in glioblastoma multiforme (GBM),[5] respectively. These studies suggest that targeted ablation of these key immunosuppressive subsets within solid tumors could lead to the ***re***programming of the ***t***umor ***i***mmune ***m***icro-environment, termed **ReTime**, thereby potentially augmenting the efficacy of immunotherapy. This targeted approach to modulate the TIME may offer a novel strategy to overcome the barriers posed by ICB resistance, thereby enhancing the clinical benefits of immunotherapy for patients with cancer.

Macrophages are diverse and multifunctional components of the innate immune system and play crucial roles in balancing immune responses and promoting tissue healing to maintain homeostasis.[6] Macrophages can be classified into 2 distinct phenotypes: proinflammatory (classically activated, M1) and tissue repairing (alternatively activated, M2).[7] However, the M1/M2 dichotomy is not adequate for describing their complicated roles in the TIME. With the development of single-cell RNA sequencing (scRNA-seq), recent studies have revealed that the polarization state of TAMs often exists as a continuum rather than a simple binary polarization.[8] By refining our classification of TAMs, researchers can better understand the nuances of macrophage polarization and its implications for immune regulation, tumor progression, and therapeutic intervention. The pursuit of a more nuanced taxonomy of macrophage phenotypes within the TME is not only a scientific endeavor but also a critical step toward the development of targeted immunotherapies that harness the potential of macrophages to modulate the immune response and promote a favorable microenvironment for cancer treatment.

Sirpα, an inhibitory receptor expressed on myeloid cells, interacts with its conventional ligand CD47, which is expressed on all normal cells and often highly expressed on tumor cells.[9] The CD47-Sirpα signaling pathway, also known as a "don't eat me" signal and phagocytic checkpoint, impedes phagocytosis and promotes tumor immune escape by facilitating the phosphorylation of immune receptor tyrosine inhibitory motif (ITIM) in Sirpα.[10] Despite many preclinical studies suggesting CD47 as a promising target owing to its ability to inhibit phagocytosis in tumor cells,[11] recent clinical trials have demonstrated the limited therapeutic efficacy of CD47 monoclonal antibodies in treating solid tumors.[12] This indicates that targeting CD47-Sirpα signaling pathway through CD47 blockade may not be the optimal strategy to treat solid tumors, and further investigation is required to elucidate the underlying mechanisms.

A recent and noteworthy study published in *Nature Cancer* comprehensively characterized the TME of colorectal tumors.[4] Through the analysis of scRNA-seq data of 62,288 immune cells from patients with CRC, they identified pronounced enrichment of TAMs and granulocyte-like myeloid-derived suppressor cells (gMDSCs) within the TIME. These cell populations exhibited robust immunosuppressive properties and were characterized by elevated expression of the inhibitory receptor SIRPA.[4] Utilizing CRISPR-Cas9 technology, the authors generated Sirpα-deficient (*Sirpα*$^{-/-}$) mice to dissect the role of the Sirpα inhibitory receptor in tumor progression. Compared with wild-type mice, the progression of tumors was notably restrained in both subcutaneous and spontaneous model of

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CRC in *Sirpα*$^{-/-}$ mice, and the survival rate of mice increased significantly. A similar phenomenon has been observed in liver and lung cancers. Moreover, by analyzing public clinical datasets, they found that SIRPA negatively correlated with the prognosis of patients with colon cancer, lung squamous carcinoma, or liver cancer. These findings confirmed the important role of Sirpα in tumor progression.

This work also reported intriguing findings regarding the role of Sirpα in tumor immune evasion. They demonstrated that Sirpα could facilitate this process independently of its interaction with CD47, through various approaches such as CD47 knockout in tumor cells, the use of CD47 monoclonal antibodies, or Sirpα-ED-Fc fusion proteins. Moreover, scRNA-seq analysis of intestinal tumor tissues from orthotopic colorectal models revealed that the deficiency of Sirpα reprogrammed the TIME through facilitating the differentiation of TAMs and gMDSCs into subsets with stronger antitumor activity: TAM_*Ccl8*^{hi} and gMDSC_*H2-Q10*^{hi}, which are characterized by enhanced phagocytosis, antigen presentation, inflammatory response, and chemokine activity. In vitro experiments showed that *Sirpα*$^{-/-}$ TAMs facilitated T cell recruitment via Syk/Btk-dependent Ccl8 secretion. In addition, they observed enhanced tumor suppression when combining Sirpα defect with anti-PD-L1 treatment, suggesting that targeting Sirpα may be a potential combination strategy to enhance the efficacy of ICB therapy. Collectively, Sirpα deficiency can enhance both innate and adaptive immune responses independently of CD47, and may emerge as a novel immunotherapy target that has the potential to overcome the challenge of immunotherapy resistance in solid tumors.

Another seminal study published in *Nature Cancer* highlighted the critical role of Siglec-9, a TAM receptor, in the pathogenesis of GBM.[5] GBM is the most common and aggressive type of primary brain tumor, accounting for up to 50% of all gliomas.[13] Unfortunately, despite progress in current treatments for GBM, including a combination of neurosurgery, adjuvant radiotherapy, and temozolomide (TMZ), the prognosis of patients is still extremely poor.[14] Therefore, new therapeutic strategies for the treatment of GBM are urgently required.

Siglec-9, a member of the sialic acid-binding immunoglobulin-like lectin (Siglec) family, is a receptor expressed on the surface of myeloid cells and acts as an immune checkpoint to transmit immunosuppressive signals upon binding to sialic glycoproteins on the host or cancer cell membranes.[15] TAMs with high Siglec-9 expression were found to be particularly abundant in the tumors of anti-PD-1 treatment-resistant GBM patients using scRNA-seq and spatial transcriptome analysis.[5] In particular, an increase in the infiltration of *Siglec-9*$^{+}$*SEPP1*$^{+}$ and *Siglec-9*$^{+}$*MARCO*$^{+}$TAMs was observed in non-responders to neoadjuvant PD-1 blockade compared to that in responders. These clusters are characterized by the upregulation of anti-inflammatory genes, indicative of a highly plastic and immunosuppressive phenotype. RNA velocity analysis revealed that both *Siglec-9*$^{+}$ TAM clusters originated from CD14^{+} monocytes and underwent a sequential differentiation process, with *Siglec-9*$^{+}$*MARCO*$^{+}$ TAMs potentially representing an intermediate state during the transition to *Siglec-9*$^{+}$*SEPP1*$^{+}$ TAMs.

Using an intracranial tumor model, this study demonstrated that the genetic ablation of SiglecE, the murine homolog of human Siglec-9, effectively curtailed GBM tumor growth.[5] This knockout also induced a phenotypic shift in macrophages from an immunosuppressive to a more tumoricidal subtype and significantly bolstered T cell activation and proliferation. Furthermore, the combination of *Siglece*$^{-/-}$ mice with anti-PD-1 therapy yielded an improved prognosis, highlighting the therapeutic potential of targeting Siglec-9. In summary, Siglec-9 was identified as a novel immune checkpoint in macrophages and presents a promising target for augmenting the efficacy of anti-PD-1/PD-L1 therapies in GBM treatment. This discovery offers a compelling avenue for future research and clinical development, with the potential to transform the therapeutic landscape for patients with GBM.

The elimination of cancer by T cells requires a series of steps to establish a complete Cancer-Immunity Cycle, in which each step has the potential to act as a constraining factor in generating potent anticancer immunity.[16] Within the TIME, TAMs can facilitate tumor progression through the expression of inhibitory receptors and the secretion of inhibitory cytokines and chemokines, which restrain the recruitment and function of multiple immune cell subtypes,[17] suggesting that TAMs are one of the major obstacles in the Cancer-Immunity Cycle. However, TAMs can also exert antitumor effects such as phagocytosis of tumor cells, major histocompatibility complex (MHC) class II antigen presentation, and expression of proinflammatory cytokines. Therefore, reprogramming TAMs from protumor subtypes into antitumor subtypes has the potential to elicit a durable and potent antitumor immune response. Furthermore, adopting a combination therapy approach targeting multiple steps of the Cancer-Immunity Cycle rather than relying on monotherapy with ICB,

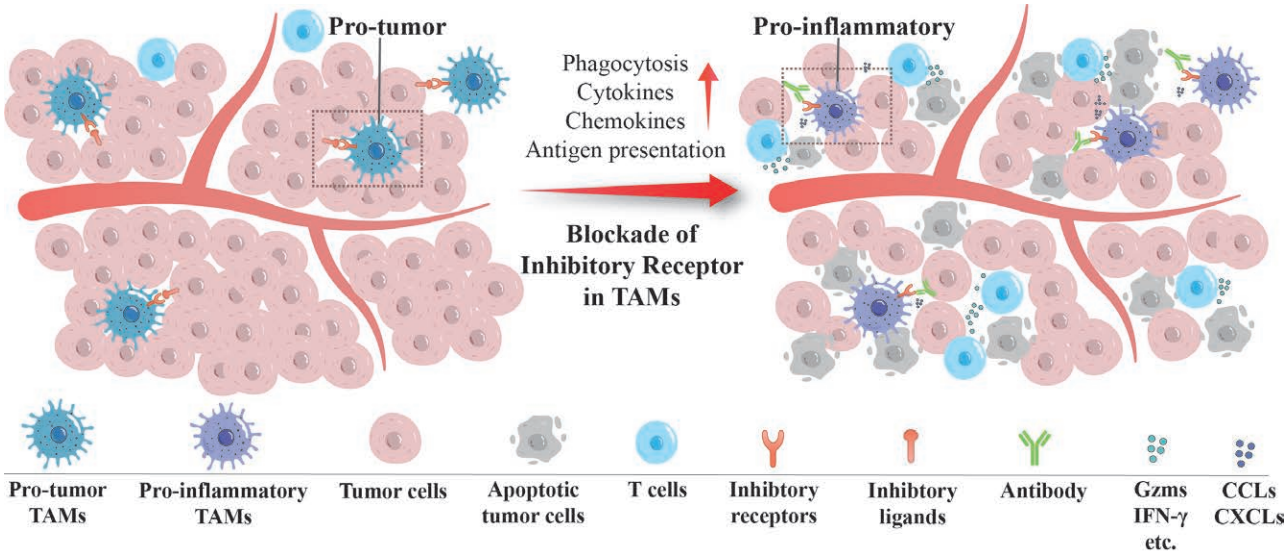

Figure 1. Targeting macrophages to reprogram the tumor immune microenvironment. CCLs = C–C chemokine ligands, CXCLs = the chemokine (C-X-C motif) ligand, Gzms = granzymes, IFN = interferon, TAM = tumor-associated macrophage.

which fails to adequately reverse the immunosuppressive state in the TIME, represents a more strategic direction for tumor treatment in the future. Strategies aimed at Re-TIME are designed to systematically enhance the Cancer-Immunity Cycle with the goal of achieving sustained tumor control. These approaches may involve modulation of the macrophage population, inhibition of immunosuppressive pathways, and stimulation of effector T-cell responses to favor antitumor activity (**Fig. 1**). In summary, a comprehensive understanding of the multifaceted roles of TAMs in tumor initiation and progression is essential for developing more effective therapeutic strategies. By elucidating the mechanisms governing the behavior of TAMs and developing interventions that can harness their potential to combat cancer, we can pave the way for novel and efficacious cancer immunotherapies that may ultimately improve patient outcomes.

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