Disrupting the Tumor's Immunosuppressive Shield: Targeting Cancer-Associated Fibroblasts (CAFs) and TGF-β to Enhance Anti-Tumor Immunity

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Immunosurveillance, the immune system's mechanism for eliminating tumor cells, plays a critical role in combating malignancies. Tumor cells, however, can modulate the immune system to induce tolerance, thereby evading immune detection. The tumor microenvironment (TME), comprising various cell types, including pericytes, endothelial cells, immune cells, adipocytes, and Cancer-Associated Fibroblasts (CAFs), contributes significantly to immune evasion, tumor growth, and metastasis [1]. CAF, the most abundant stromal cells within the TME, play a pivotal role in interacting with surrounding cells and secreting extracellular matrix (ECM) components that support tumor progression [2]. Initially believed to be passive, research has revealed that CAFs actively remodel ECM, promote angiogenesis, and facilitate cancer cell survival and proliferation through cross-communication with tumor cells [3]. The TME transitions from an anti-tumor state during early tumor development to a pro-tumor state due to cancer cells' ability to reprogram immune and stromal components. This reprogramming promotes chronic inflammation, ECM remodeling, altered pH, and immune suppression, creating an environment conducive to tumor progression. CAF-derived growth factors and cytokines, such as Transforming Growth Factor-β (TGF-β), Vascular Endothelial Growth Factor (VEGF), Interleukin-6 (IL-6), and C-X-C Motif Chemokine Ligand 12 (CXCL12), further enhance immune evasion by recruiting immunosuppressive cells and promoting angiogenesis [4]. TGF-β, a multifunctional cytokine, regulates numerous processes, including cell growth, differentiation, apoptosis, and ECM synthesis. Dysregulation of TGF-β signaling contributes to excessive proliferation, immune evasion, and metastasis. High TGF-β expression correlates with poor prognosis, increased metastasis, chemotherapy resistance, and reduced survival rates. Consequently, numerous TGF-β signaling inhibitors are under clinical investigation for cancer treatment. Targeting both cancer cells and the TME, particularly CAFs, has emerged as a promising therapeutic approach to overcome tumor progression and immune evasion.

Cancer-Associated Fibroblasts (CAF) play a critical role in regulating immunosuppressive mechanisms within the tumor microenvironment (TME). Different CAF subtypes secrete a variety of chemokines and cytokines, including TGF-β, IL-6, IL-8, IL-13, CXCL12, CXCL14, and VEGF, which collectively suppress both innate and adaptive immune responses [3]. Some CAF subpopulations even express immune checkpoint molecules such as Programmed Cell Death Ligand 1/2 (PD-L1/2), making them potential targets for immunotherapy [4]. Certain CAF subtypes also produce metabolites or metabolic enzymes, such as Indoleamine-2,3-dioxygenase (IDO), Arginase (Arg), Adenosine, and Tryptase, which help recruit and differentiate regulatory T cells (Tregs), mast cells, and Tumor-Associated Macrophages (TAMs) [1]. Moreover, CAFs contribute to extracellular matrix (ECM) remodeling by synthesizing components like collagen, fibronectin, and Matrix Metalloproteinases (MMPs), which hinder T-cell infiltration and facilitate tumor progression

[2]. CAF populations can be distinguished from normal fibroblasts by the expression of specific markers, such as α-SMA and FAP. These markers not only help identify CAFs but also correlate with their functional roles in immune modulation within the TME [5]. For example, α-SMA$^+$ CAFs, also referred to as myofibroblasts, are highly active in suppressing immune responses through ECM remodeling and other mechanisms. TAMs, which are among the most abundant innate immune cells in the TME, have a close functional association with CAFs. This interaction is particularly evident in pancreatic cancer, where α-SMA$^+$ Vimentin$^+$ Glial Fibrillary Acidic Protein$^+$ (GFAP$^+$) CAFs secrete factors such as Macrophage Colony-Stimulating Factor 1 (M-CSF), IL-6, and CCL2 to recruit monocytes and promote their differentiation into M2-polarized macrophage. Similarly, cytokines like IL-6, IL-8, TGF-β, and IL-10 secreted by α-SMA$^+$ CAFs and α-SMA$^+$ FAP$^+$ CAFs enhance monocyte recruitment and differentiation into the immunosuppressive M2 phenotype. M2 macrophages, in turn, activate CAFs, creating a feedback loop that supports tumor growth. Markers associated with CAFs, such as α-SMA, S100A4, and FAP, as well as M2 macrophage markers like CD163 and DC-SIGN, are linked to poor outcomes in patients with colorectal cancer [6]. Additionally, IL-6 secreted by α-SMA$^+$ CAFs facilitates neutrophil recruitment and activates the Janus Kinase-Signal Transducer and Activator of Transcription (STAT3) pathway along with the PD-L1 signaling cascade in neutrophils, further suppressing immune activity in hepatocellular carcinoma. Other components, such as IL-13 and Adenosine secreted by mast cells, contribute to M2 macrophage polarization and restrict CD8$^+$ T-cell infiltration into the TME. Mast cells also promote the recruitment of Myeloid-Derived Suppressor Cells (MDSCs) and Tregs, further intensifying immunosuppression within the TME [7].

Transforming Growth Factor-β (TGF-β) is a key immunosuppressive cytokine that plays a significant role in shaping immune responses within the tumor microenvironment (TME). One of its primary effects is inhibiting the activation of effector T cells by reducing calcium influx (Ca^{2+}), which disrupts the activity of important transcription factors like Nuclear Factor of Activated T Cells (NFATc), T-bet, and GATA-3—factors critical for T-cell proliferation and differentiation. In partnership with IL-2, TGF-β promotes the expression of the transcription factor FOXP3 in naïve CD4$^+$ T cells, initiating their transformation into regulatory T cells (Tregs). This process relies on the activation of Smad proteins by TGF-β, which then recruit NFAT to the FOXP3 gene promoter, resulting in FOXP3 mRNA production and driving Treg development. TGF-β also impacts dendritic cells (DCs), which serve as vital antigen-presenting cells (APCs). By suppressing the expression of Major Histocompatibility Complex Class II (MHCII), TGF-β limits the ability of DCs to present antigens effectively, weakening the immune response. Furthermore, elevated TGF-β signaling has been linked to the upregulation of Inhibitor of Differentiation 1 (Id1), which hinders the maturation of DCs into functional APCs. Instead, these cells are redirected into immature myeloid-derived suppressor cells (MDSCs), which promote an immunosuppressive environment in many tumor types [8]. Natural Killer (NK) cells, critical components of the innate immune system, are also negatively affected by TGF-β. This cytokine suppresses their development, differentiation, and activation, thereby diminishing their ability to combat tumor cells effectively [9]. TGF-β plays a crucial role in suppressing not only the differentiation of Th1 cells but also the proliferation and effector functions of T cells.

Research has shown that during the priming phase, TGF-β reduces IL-2 expression, a cytokine critical for activating and proliferating $CD4^+$ T cells. This suppression is mediated through Smad3 and Smad4, working in conjunction with the cofactor TOB1 [10]. Studies using genetically modified mice reveal that $CD4^+$ and $CD8^+$ T cells lacking TGFβR2 exhibit heightened responses to weak antigenic stimuli. These TGFβR2-deficient T cells display enhanced effector phenotypes, including increased expression of the KLRG1 receptor and elevated production of Granzyme B and IFN-γ. Additionally, TGF-β directly impairs the cytotoxic capabilities of $CD8^+$ T cells. Mechanistically, Smad proteins activated by TGF-β collaborate with the transcription factor ATF1 to repress the promoters of key cytotoxic genes, such as those encoding Granzyme B and IFN-γ [11]. Dendritic cells (DCs) are key regulators of immune responses, particularly those mediated by Th1 and Treg cells, through TGF-β signaling. As specialized antigen-presenting cells (APCs), DCs play a crucial role in countering tumor growth. However, TGF-β can diminish the ability of dendritic cells to present antigens by suppressing MHC-II gene expression, as demonstrated in vitro. In tumor models, cancer cells manipulate dendritic cells to secrete TGF-β, which facilitates the differentiation of $CD4^+$ T cells into regulatory T cells (Tregs). Within the tumor microenvironment (TME), DCs often transform into immature myeloid cells with immunosuppressive properties. This transition is driven by the upregulation of ID1 through TGF-β signaling. Tolerogenic dendritic cells also produce immunosuppressive molecules such as indoleamine-2,3-dioxygenase (IDO) and arginase, further inhibiting immune responses under the influence of TGF-β. In breast cancer and melanoma models, TGF-β signaling has been linked to enhanced immune evasion by increasing IDO expression in plasmacytoid DCs and promoting CCL22 chemokine production in myeloid DCs. These specialized dendritic cells aid in recruiting Tregs and suppressing other immune cells, which collectively bolster the tumor's ability to escape immune surveillance [12].

In conclusion the complex landscape of cancer biology, the tumor microenvironment (TME) plays a pivotal role in facilitating immune escape, with Cancer-Associated Fibroblasts (CAFs) and TGF-β signaling emerging as critical components in this process. The interplay between CAFs and TGF-β not only sustains the tumor-promoting environment but also poses significant challenges to conventional therapies. Targeting CAFs and inhibiting TGF-β signaling have shown promise in preclinical and clinical settings, offering the potential to dismantle the immunosuppressive shield of the TME and restore effective anti-tumor immune responses. By integrating CAF- and TGF-β-targeted therapies with existing immunotherapies, we can unlock new avenues for combating immune escape in cancer. Moving forward, a deeper understanding of the crosstalk between CAFs, TGF-β, and other components of the TME will be crucial in developing comprehensive strategies to enhance the efficacy of cancer treatments and improve patient outcomes.

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