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REVIEW

Blocking TGF-β Signaling To Enhance The Efficacy Of Immune Checkpoint Inhibitor

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Abstract: During malignant transformation, a growing body of mutations accumulate in cancer cells which not only drive cancer progression but also endow cancer cells with high immunogenicity. However, because one or multiple steps in cancer-immunity cycle are impaired, anti-cancer immune response is too weak to effectively clear cancer cells. Therefore, how to restore robust immune response to malignant cells is a hot research topic in cancer therapeutics field. In the last decade, based on the deeper understanding of cancer immunity, great signs of progress have been made in cancer immunotherapies especially immune checkpoint inhibitors (ICIs). ICIs could block negative immune co-stimulatory pathways and reactivate tumor-infiltrating lymphocytes (TILs) from exhausted status. ICIs exhibit potent anti-cancer effect and have been approved for the treatment of numerous cancer types. Parallel with durable and effective tumor control, the actual response rate of ICIs is unsatisfactory. Although a subset of patients benefit from ICIs treatment, a large proportion of patients show primary or acquired resistance. Previously intensive studies indicated that the efficacy of ICIs was determined by a series of factors including tumor mutation burden, programmed death ligand-1 (PD-L1) expression, and TILs status. Recently, it was reported that transforming growth factor-beta (TGF-β) signaling pathway participated in cancer immune escape and ICI resistance. Concurrent TGF-β blockade might be a feasible strategy to enhance the efficacy of immunotherapy and relieve ICI resistance. In this mini-review, we summarized the latest understanding of TGF-β signaling pathway and cancer immunity. Besides, we highlighted the synergistic effect of TGF-β blockade and ICIs.

Keywords: immunotherapy, immune checkpoint inhibitor, PD-1, PD-L1, TGF-β, tumor immune microenvironment, tumor infiltrating lymphocyte

Introduction

Host immunity could recognize and clear non-self immunogenic materials. Theoretically, neoantigens or tumor-associated antigens generated during oncogenesis could initiate anti-cancer immune attack. The robust anti-cancer immune response is usually described as cancer-immunity cycle model.[1] Firstly, cancer cells-derived neoantigens or tumor-associated antigens are captured by dendritic cells (DCs). After antigen processing, DCs present cancer antigens with major histocompatibility complex (MHC) molecules to naïve T cells in peripheral lymphoid organs. Following the priming and activation, T cells could specifically recognize cancer antigens. Then, primed T cells traffic and infiltrate into tumor beds. Tumor-infiltrating lymphocytes (TILs) could directly eliminate tumor cells which further release more tumor antigens and upregulate the magnitude of anti-cancer immune response.[1] However, this series of stepwise procedures tend to be

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interrupted by several factors such as downregulated MHC on tumor cells,[2] immune editing,[3] as well as increased immune checkpoints.[4,5] As a result, malignant cells escape from immune attack and eventually develop into visible tumor mass.

Cancer immunotherapy is aiming to launch a self-sustaining cancer-immunity cycle which could self-amplify and self-propagate with minimized treatment-related auto-inflammation.[6] Immune checkpoints such as programmed death 1 (PD-1),[7] cytotoxic T lymphocyte antigen 4 (CTLA-4),[8] lymphocyte activation gene 3 (LAG-3),[9] as well as T-cell immunoglobulin and mucin-domain containing-3 (TIM-3)[10] are vital factors maintaining pro-tumor immune microenvironment, which are also regarded as ideal targets for cancer immunotherapy. However, anti-cancer immune response is a cyclic and stepwise process.[11] The actual effect of anti-cancer immune elimination is determined by upstream immune editing (depletion of cancer cell sub-clones with T cell targets), downstream immunosuppressive tumor microenvironment including antigenic modulation and immune inhibitory cytokines especially transforming growth factor-beta (TGF-β) in tumor beds.[12–14] It is generally believed that the upregulated immune checkpoints on cancer cells are rate-limiting steps in cancer-immunity cycle.[1,15] Nevertheless, the frequent ICIs resistance indicate that PD-1- or CTLA-4-targeted monotherapy could not completely counteract immunosuppression in the tumor microenvironment.[16] A comprehensive framework containing multiple factors would be meaningful to remove adverse factors and amplify the whole anti-cancer immunity.[17]

TGF-β is a versatile molecule which could bi-directionally regulate the initiation and progression of cancer.[18–20] Besides, TGF-β has a multifaceted influence on tumor immune microenvironment.[21] Increasing evidence suggests that the excessive secretion of TGF-β in tumor closely relates to increased pro-tumor immune elements, restrained tumor-killing effect of TIL, as well as limited infiltration of immune effector cells.[22–24] TGF-β might be an evaluable target for cancer treatment and the dual-blockade of TGF-β/immune checkpoints would have a synergistic effect.

Immune Checkpoints In Tumor Microenvironment

T cell activation is a complex process containing two signals.[25] The first activation signal is the specific binding of antigenic peptide-MHC complex on antigen presentation cell (APC) and T cell receptor (TCR) on naïve T cell.[25] The second activation signal is also known as co-stimulatory signal which refers to the interaction between co-stimulatory molecules on APC and corresponding receptors on T cell.[26] Simultaneous stimulations from first and second signals are the prerequisite of optimal T cell activation. In the absence of co-stimulatory, T cells are prone to be unresponsive to antigenic materials (anergic T cells).[26] Besides, some negative co-stimulatory (also termed as co-inhibitory) signals participate in T cell activation as well. Under the physiology condition, co-inhibitory signals maintain peripheral tolerance and prevent anti-immune diseases via counterbalancing co-stimulatory signals.[27,28] In tumor immune microenvironment, upregulated immune checkpoints blunt effector T cells and protect cancer cells from immune killing.[29] Among all immune checkpoint pathways, PD-1/PD-L1 and CTLA-4/B7 (CD80/CD86) pathways are most well-studied. Multiple agents targeting PD-1, PD-L1, and CTLA-4 have been applied in clinic.[30–32]

PD-1-PD-L1 Signaling Pathway

PD-1 molecule consists of an extracellular IgV-like domain, a transmembrane domain, and an intracellular tail. The cytoplasmic tail of PD-1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) which are the core structures for immune inhibitory function.[33] PD-1 is widely expressed on multiple immune cells such as activated T cells, DCs, and natural killer cells (NKs).[34–36] The expression pattern of PD-L1 is different from PD-1. PD-L1 is constitutively expressed on immune cells including B cells, T cells, macrophages, DCs, and mesenchymal stem cells.[37] Besides, the expression of PD-L1 could be transiently induced by a panel of cytokines especially interferon-gamma (IFN-γ).[38,39] Apart from immune cells, a broad range of non-immune cells express PD-L1 as well.[35] The upregulation of PD-L1 in tumor cells could be attributed to two factors. Firstly, some oncogenic pathways contribute to PD-L1 overexpression.[40] Moreover, locally pre-existed inflammation leads to PD-L1 upregulation as a feedback termed as adaptive immune resistance.[41]

PD-1 molecule could transduce the signal of PD-L1 when it is cross-linked with TCR. After the stimulation from TCR, the binding of PD-1 and PD-L1 activates this immune inhibitory pathway.[42] The tyrosine residues of ITIM and ITSM are phosphorylated and recruit SHP1/2, which could counteract the TCR/CD3- or CD28-mediated phosphorylation.[43] PD-1/PD-L1 signaling not only blocks TCR/CD3 pathway by dephosphorylating the core components of TCR

downstream signaling such as Zap-70 and Lck but also inhibits PI3K-Akt and Ras-MEK-ERK pathways in T cells.[44] The suppressed PI3K and MAPK pathways result in reduced glycolysis/amino acid metabolism and increased fatty acid oxidation. These metabolic changes of T cells propel the differentiation of T cells towards regulatory T cells (Tregs) and exhausted T cells but hamper the differentiation towards effector T cells and memory T cells.[42] Besides T cell differentiation, PD-1 signaling could interrupt cell cycle process of T cells via inhibiting PI3K and MAPK pathways.[42]

CTLA-4-B7 Signaling Pathway

Similarly to PD-1, CTLA-4 molecule contains an extracellular IgV-like domain, a transmembrane domain, and an intracellular tail as well.[45] CTLA-4 is upregulated on activated T cells but rarely expressed on naïve T cells.[46] Moreover, CTLA-4 is constitutively expressed on Tregs.[47] Due to the similar molecular structure as well as higher affinity and avidity, CTLA-4 could competitively antagonize the binding between CD28 and B7.[48] CTLA-4 inhibits T cell activation by multiple manners. Firstly, CTLA-4 could recruit phosphatases such as PP2A to reverse TCR/CD3 mediated phosphorylation of downstream proteins.[49] Secondly, CTLA-4 downregulates the transcription of IL-2 which is the core cytokine for T cells activation and proliferation.[49] Besides, CTLA-4 induces the abundance of B7 molecules on T cells by transendocytosis.[47] Moreover, CTLA-4-B7 signaling pathway could induce the generation of several immune inhibitory components such as indoleamine-2, 3-dioxygenase (IDO) and TGF-β.[50,51]

TGF-β Signaling Pathway And Cancer

Multiple cancers possess a TGF-β-enriched tumor microenvironment. Numerous components of tumor microenvironment including cancer cells, fibroblasts, macrophages, and platelets could secret TGF-β.[52]

The Structure Of TGF-β

The TGF-β family contains three members: TGF-β1, β2, and β3. All of the three cytokines are synthesized as precursors consisting of a signal peptide, a latency-associated peptide (LAP), a C-terminal fragment.[53] Under the guidance of signal peptide, the TGF-β precursor is translocated to endoplasmic reticulum which is further assembled to a dimer by inter-chain disulfide bonds.[54] After furin-mediated cleavage, the disulfide-linked C-terminal fragment is non-covalently associated with the disulfide-linked N-terminal LAP, which eventually form the small latent complex.[54] The LAP domain folds around C-terminal fragment (mature TGF-β), blocking access of TGF-β to corresponding receptor.[54] The active TGF-β homodimer is released from small latent complex by (1) extracellular protease cleavage; (2) in the assistance of latent TGF-β binding protein, separating active TGF-β by cell contraction-derived and integrin-mediated tension; (3) with the help of GARP on the cells such as Tregs or macrophages, releasing active TGF-β by cell contraction-derived and integrin-mediated tension.[52,55] All the three TGF-β isoforms are highly homologous (71–79% sequence identity in C-terminal fragment TGF-β regions) and have the similar functions in vitro.[54] In vivo studies showed that the predominantly immunity-related isoform was TGF-β1 and TGF-β1 deficiency led to embryonic lethal or severe multi-organ inflammation.[56]

TGF-β Signaling Pathway

TGF-β signal is transduced by TGF-β receptor complex which consists of a TGF-βI receptor homodimer (TGF-βRI) and a TGF-βII receptor homodimer (TGF-βRII).[57] Firstly, extracellular TGF-β binds to TGF-βRII homodimer which further complex with TGF-βRI homodimer.[57] Following TGF-β engagement, TGF-βRII homodimer phosphorylates the intracellular domain of TGF-βRI.[57] Notably, the transduction of TGF-β2 is usually with the assistance of co-receptor β-glycan (also termed as TGF-βRIII).[57] The engagement of TGF-β receptor complex recruits receptor Smad (R-Smad) molecules Smad2 and 3 to the intracellular domain of TGF-βRI.[58] Subsequently, Smad2 and 3 are phosphorylated which then form a trimeric complex with Smad4.[58] The trimeric Smad complex could translocate to nuclear and regulate gene expression.[58] Besides, phosphorylated Smad 2 and 3 could also form a trimeric complex with TIF1γ to regulate the expression of targeting genes.[54] Moreover, it was reported that some other members of Smad family including Smad 1 and 5 might participate in TGF-β signaling pathway in partial cells such as Th17 cells.[59] Apart from classic Smad pathway, TGF-β signal could also be transduced by some Smad-independent pathways such as PI3K, MAPK, and Rho GTPase pathways.[60–62] It has been well-established that the exact downstream signaling pathway of TGF-β signal is context-dependent.[54]

TGF-β Pathway And Cancer Progression

The role of TGF-β pathway is bi-directional for cancer.[20] For pre-malignant cells, TGF-β acts as a tumor suppressor via suppressing cell proliferation and promoting cell apoptosis.[63] However, for advanced cancer, TGF-β

promotes cancer metastasis and induces pro-tumor immune microenvironment.[63] In the tumor microenvironment, by paracrine and autocrine ways, overexpressed TGF-β could regulate the functions of cancer cells and stromal cells.[64] The increased TGF-β is contributed by cancer cells especially cancer stem cells, Tregs, Bregs, tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), as well as myeloid-derived suppressor cells (MDSCs).[64] Notably, Stanford et al found that in postpartum breast cancers, dying cancer cells could be engulfed by neighboring macrophages (the process is also known as efferocytosis) via receptor tyrosine kinase MerTK.[65] As a result, this efferocytosis enhanced the transcription of multiple wound-healing cytokines including TGF-β.[65] So far, the mechanism by which the production of TGF-β is increased in CAFs is not clear yet. Previous studies indicated multiple components in the tumor microenvironment such as bone marrow-derived mesenchymal stem cells participated in the transformation of normal fibroblasts to CAFs and enhanced the secretion of TGF-β of CAFs.[66] Besides, some cancer-specific miRNA expression pattern elevated the TGF-β generation in CAFs as well. Tanaka et al reported that in esophageal cancers, increased extracellular miR-27 was an unfavorable predictive factor for prognosis. After miR-27a/b transfection, normal fibroblasts exhibited more CAFs-associated markers including increased expression of α-smooth muscle actin and TGF-β.[67]

As a growth-inhibitory cytokine, TGF-β could effectively suppress cell proliferation by increasing the activity of cyclin-dependent kinase inhibitors such as p15INK4, p21CIP1, and p27KIP1.[68–70] Simultaneously, TGF-β could downregulate the expression of Myc.[71] Besides, TGF-β inhibits tumorigenic inflammation and maintains immune homeostasis.[63] Therefore, TGF-β exhibits tumor-suppressing function during the early stage of carcinogenesis.[52] However, persistent TGF-β exposure introduces selective pressure and a subset of cancer cells could overcome TGF-β-related tumor-suppressing effect and eventually develop to advanced tumor.[63] Actually, the transformation of TGF-β from cancer promotor to cancer suppressor occurs by two approaches.[63] Firstly, partial cancer cells acquire mutations in TGF-β signaling pathway and abrogate TGF-β-mediated tumor-suppressing effect.[52] In the same time, TGF-β signaling in stromal cells promotes cancer progression by inhibiting immune surveillance and promoting the secretion of some carcinogenic cytokines such as IL-11.[63] Besides, oncogenes or tumor suppressors interact with TGF-β signaling and switch TGF-β function.[72] For some cancer cells with intact TGF-β signaling pathway, some oncogenic pathways could counteract the pro-apoptosis effect of TGF-β.[52] As a result, cancer cells undergo non-lethal epithelial–mesenchymal transition (EMT) and obtain the increased capabilities of migration and distant colonization.[52]

Notably, overexpressed TGF-β in the tumor microenvironment is highly related with hypoxia. Stephen et al found that breathing supplementary oxygen could effectively relieve regional hypoxia and decrease TGF-β abundance in tumors.[73] This treatment using supplementary oxygen convert immunosuppressive and TGF-β-enriched tumor microenvironment to normal microenvironment, which is a promising adjuvant strategy to restore robust anti-cancer immune response.[74]

The Effect Of TGF-β Signaling Pathway On Immunity

TGF-β signaling pathway has a substantial influence on various immune cells which not only participates in immune cell differentiation but also regulates the activity of immune components (Figure 1).[75] Generally, TGF-β acts as an inflammation-inhibitory factor.

Th1 Cell

Th1 cell is a vital player in anti-cancer immunity and the differentiation of Th1 cells needs APC-derived cytokine IL-12.[76] IL-12 could induce Th1 cell to generate abundant IFN-γ and the secretion of IFN-γ could be self-propagated as a positive feedback loop.[77] Besides, IL-12 upregulates the expression of key transcription factor T-bet which is the other determinant for Th1 cell differentiation.[76] TGF-β could effectively hamper Th1 cell differentiation. Firstly, TGF-β reduces the expression of the receptor of IL-12 on Th0 cells and downregulates the sensitivity of Th0 cells to IL-12.[78] Secondly, TGF-β downregulates the level of T-bet in Th1 cells.[79] Thirdly, TGF-β inhibits the production of IL-12 of NK.[80] Although most previous studies showed that TGF-β suppressed the Th1 differentiation, some studies also indicated that TGF-β could also induce Th1 cell differentiation in some certain background such as in the presence of IFN-γ or IL-4.[81] However, the significance of TGF-β-mediated Th1 cell differentiation under certain condition needs further exploration.

$CD8^+$ T Cell

In addition to Th1 cell differentiation, TGF-β could inhibit T cell proliferation by Smad signaling pathway. Previous

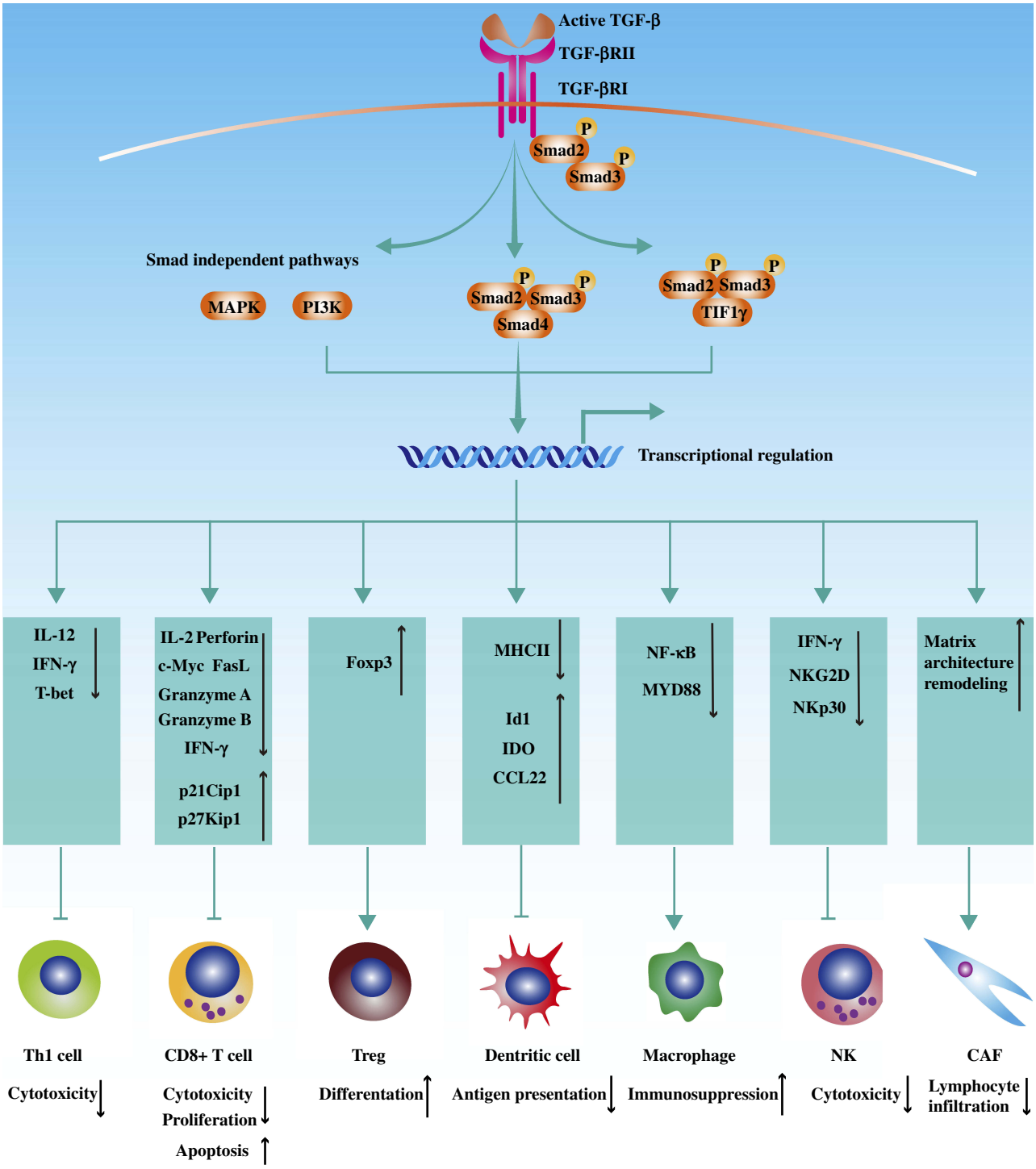

Figure 1 TGF-β signaling pathway and its role in tumor immune microenvironment. TGF-β signal is transduced by TGF-β receptor complex which consists of TGF-βI receptors and TGF-βII receptors (TGF-βRI and TGF-βRII). Firstly, extracellular TGF-β binds to TGF-βRII homodimer which further complex with TGF-βRI homodimer. Following TGF-β engagement, TGF-βRII homodimer phosphorylates the intracellular domain of TGF-βRI. The engagement of TGF-β receptor complex recruits receptor Smad (R-Smad) molecules Smad2 and 3 to the intracellular domain of TGF-βRI. Subsequently, Smad2 and 3 are phosphorylated which then form a trimeric complex with Smad4. The trimeric Smad complex could translocate to nuclear and regulate gene expression. Besides, phosphorylated Smad2 and 3 could also form a trimeric complex with TIF1γ to regulate the expression of targeting genes. Apart from classic Smad pathway, TGF-β signal could also be transduced by some Smad-independent pathways such as PI3K, MAPK, and Rho GTPase pathways. TGF-β signaling pathway has a substantial influence on various immune cells including downregulating the cytotoxicity of effector T cells and NKs, promoting the apoptosis of effector T cells, inducing the differentiation towards Tregs, hampering the antigens presentation of DCs.

Abbreviations: NK, natural killer cell; CAF, cancer-associated fibroblast; MHC, major histocompatibility complex; IDO, indoleamine-2, 3-dioxygenase; Id1, inhibitor of differentiation 1; Treg, regulatory T cell.

studies showed TGF-β suppressed $CD4^+$ and $CD8^+$ T cell proliferation via downregulating the transcription of IL-2 and c-Myc, as well as upregulating p21Cip1 and p27Kip1.[82–84] Besides, TGF-β directly inhibits the cytotoxic activity of $CD8^+$ T cells.[85] The undermined lytic function is related to TGF-β-Smad signaling-mediated downregulation of granzyme A, B, perforin, Fas ligand, and IFN-γ.[14]

Treg

$CD4^+$ $CD25^+$ $Foxp3^+$ Treg cell is a vital subpopulation of T cells which maintains immune homeostasis and participates in cancer immune evasion.[86] TGF-β could suppress immune response by regulating Treg cells. The results of mouse experiments indicated that mice lacking TGF-β1 or harboring TGF-βRII deficiency in T cells had decreased $Foxp3^+$ Treg cells in peripheral blood.[87,88] In the presence of IL-2, TGF-β promotes the differentiation of naïve T cells towards Treg cells.[89] Upon TCR engagement, Smad3 interacts with the enhancer region of Foxp3 (CNS1).[90] Besides, Smad3 could upregulate Foxp3 transcription by forming enhanceosome complex with NFATc2 and CREB.[91] The level of $Foxp3^+$ Treg cells is remarkably reduced in Smad3 knockout mice.[92] Although Smad2 could not directly interact with CNS1, T cells lacking Smad2 showed reduced Foxp3 expression.[93] The effect of Smad2 on Foxp3 level was proposed to depend on the interaction between Smad2 and Smad3.[54] Apart from Smad pathway, TGF-β could indirectly promote Foxp3 expression by counteracting Foxp3 transcription inhibitory factor such as Gfi-1.[94]

DC

DC is the key component of cancer-immunity cycle which captures antigenic materials from cancer cells and activates cancer-specific lymphocytes. TGF-β suppresses the antigen presentation of DC by decreasing the expression of MHCII.[95] Hypersecretion of TGF-β in tumor microenvironment propels the transformation of DC towards immature myeloid cell phenotype which exhibits potent immune inhibitory effect.[96] This transformation is attributed to multiple reasons. Firstly, TGF-β could upregulate the level of inhibitor of differentiation 1 which inhibits the differentiation of DC and proliferation of $CD8^+$ T cells.[96] Moreover, TGF-β promotes the formation of regulatory phenotype DC by inducing IDO in plasmacytoid DC and chemokine (C-C motif) ligand 22 (CCL22) chemokine in myeloid DC.[97,98]

Macrophage

TAM is the important source of TGF-β in tumor microenvironment.[99] Besides, TAM activates TGF-β from its precursor via extracellular integrin αvβ8 and matrix metalloproteinase 14.[99] It has been reported that TGF-β could inhibit NF-κB signaling-mediated inflammation response by promoting the degeneration of MYD88.[100] Besides, TGF-β inhibits the inflammation phenotype macrophages by the interaction between inhibitory molecule Smad7 and TNF signaling pathway, which might contribute to immunosuppressive tumor microenvironment.[52]

NK

NKs could rapidly respond to a broad range of pathogen challenges, detect, and kill malignant cells. It is notable that NKs kill cancer cells independent on priming and activation processes. Besides, NKs enhance anti-cancer effect of adaptive immunity by secreting cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α).[101] TGF-β substantially suppresses the functions of NKs by blocking the expression of IFN-γ.[80] Moreover, TGF-β downregulates the levels of NKG2D and NKp30 on NKs, which mediates the recognition of stressed cells.[102]

CAF

The ratio of CAF is usually increased in the microenvironment of advanced cancers. CAFs generate multiple materials including fibroblast activation protein, α-smooth muscle actin, extracellular matrix proteins (e.g., type I collagen and fibronectin), and various cytokines.[103] Actually, CAF is the main producer of TGF-β for multiple cancer types. Previous study showed that increased TGF-β secreted by CAF and other cells suppressed anti-tumor immune response.[104]

Besides, CAF could be activated by TGF-β as well. The transcriptome analysis of patients undergoing ICI treatment showed that TGF-β-activated CAF gene program was highly correlated with ICI resistance.[22] However, anti-TGF-β treatment could effectively convert T cell exclusion and enhance the efficacy of ICIs.[22] The exact mechanisms by which TGF-β-activated CAFs lead to cancer immune escape are attributed to three reasons. Apart from the contribution of CAF to the upregulation of TGF-β, TGF-β propels the morphological alterations in CAFs which is helpful to the release of active TGF-β from latent complex.[52] Moreover, activated TGF-β signaling pathway hampers the infiltration of T cells by remodeling the matrix architecture in the tumor stroma.[52,105]

Dual Blockade Of TGF-β And Immune Checkpoint

In tumor microenvironment, cancer cells usually hijack multiple immune inhibitory pathways to escape immune surveillance. Dysregulated TGF-β signaling pathway impaired multiple processes in anti-cancer immune response including antigen presentation, T cell infiltration, and tumor-killing activity. Therefore, ICI monotherapy might fail to restore robust anti-cancer immune response (Figure 2). Feun et al reported the results of NCT02658019 and found the baseline plasma TGF-β levels were significantly correlated to the poor outcomes of pembrolizumab-treated advanced hepatocellular carcinoma patients.[106] Given the potentially synergistic effect of TGF-β pathway and immune checkpoint in inducing immune tolerance, a series of studies were conducted to explore the efficacy of combination strategy of TGF-β inhibitor and ICI for cancer treatment (Table 1).[107]

TGF-β Inhibitor Plus ICI

By establishing human microsatellite stable-like colorectal cancers in mice, Tauriello et al found that ICI monotherapy could not effectively eliminate cancer cells.[104] However, additional TGF-β blockade remarkably enhanced the anti-cancer effect of ICI.[104] The further exploration indicated that TGF-β could induce immune suppression by promoting the formation of T cell-exclusion phenotype tumors which were prone too resistant to ICI treatment.[104] Simultaneously, Mariathasan et al noticed the similar phenomenon in patients with metastatic urothelial cancer.[22] For metastatic urothelial cancer patients receiving atezolizumab treatment, the treatment response was

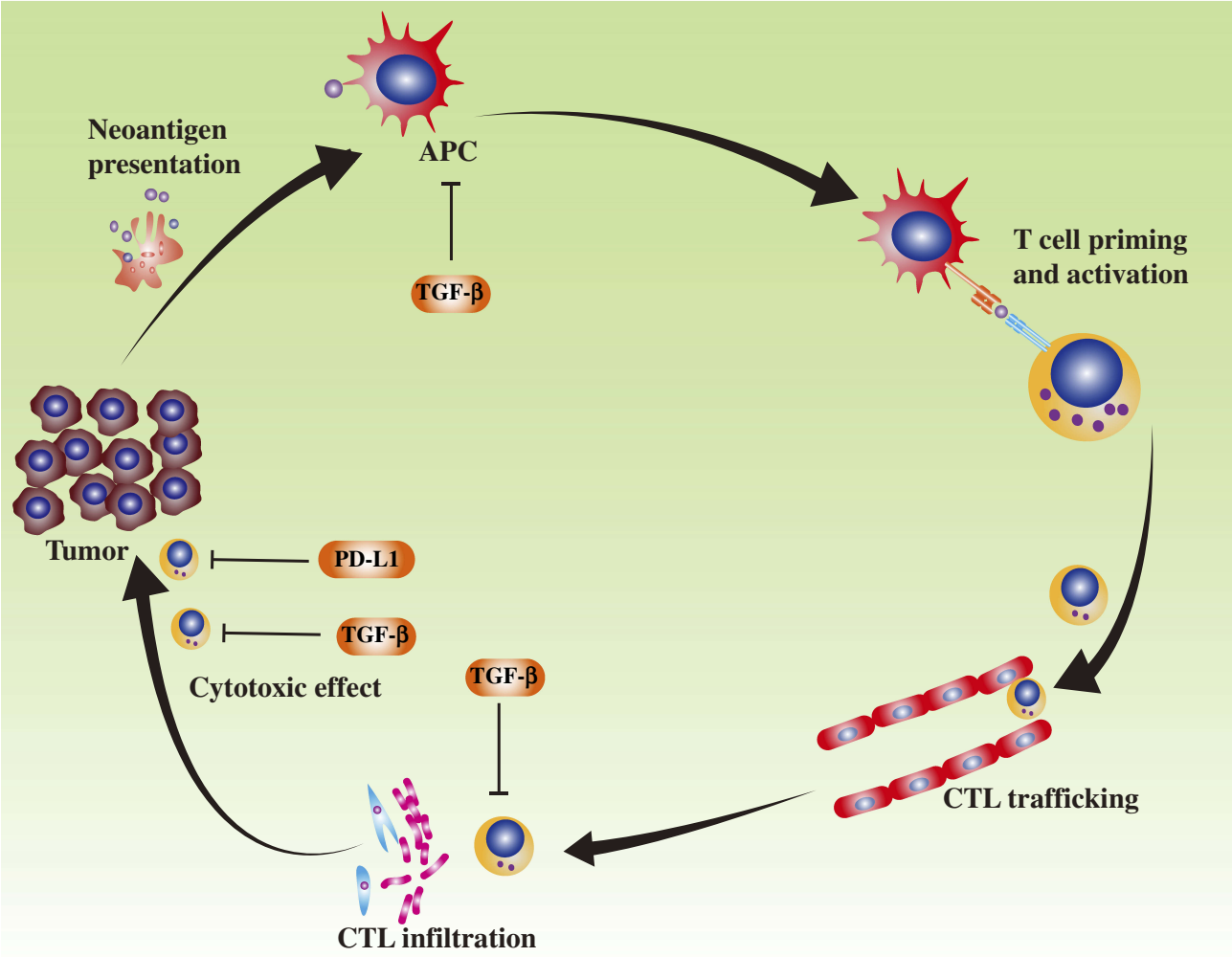

Figure 2 The synergistic effect of TGF-β pathway and immune checkpoint in inducing immune tolerance. Dysregulated TGF-β signaling pathway impaired multiple processes in anti-cancer immune response including antigen presentation, T cell infiltration, and tumor-killing activity. Hyperactive TGF-β signaling together with increased PD-1/PD-L1 signal axis undermine anti-cancer immune response.

Table 1 Clinical Trials Of Dual Blockade Of TGF-β And Immune Checkpoint

Study	Combination Strategy	Cancer Type	Phase	Status
NCT03821935	ABBV-151 and ABBV-181	Advanced solid tumors cancer	I	Recruiting
NCT03631706	M7824	NSCLC	II	Recruiting
NCT03840915	M7824	Carcinoma, NSCLC	I/II	Recruiting
NCT03427411	M7824	HPV associated malignancies	II	Recruiting
NCT03840902	M7824	NSCLC	II	Recruiting
NCT03833661	M7824	Biliary tract cancer	II	Recruiting
NCT03579472	M7824	Triple negative breast cancer	I	Recruiting
NCT03451773	M7824	Adenocarcinoma of the pancreas	I/II	Recruiting
NCT03436563	M7824	Colorectal cancer or advanced solid tumors with microsatellite instability	I/II	Recruiting
NCT02947165	NIS793 and PDR001	Advanced malignancies	I	Recruiting
NCT03192345	SAR439459 and Cemiplimab	Advanced solid tumors	I	Recruiting
NCT03724851	Vactosertib and Pembrolizumab	Colorectal or gastric cancer	I/II	Not yet recruiting
NCT03732274	Vactosertib and Durvalumab	NSCLC	I/II	Not yet recruiting
NCT02423343	Galunisertib and Nivolumab	Advanced refractory solid tumors and in recurrent or refractory nsclc, or hepatocellular carcinoma	I/II	Active, not recruiting
NCT02734160	Galunisertib and Durvalumab	Pancreatic cancer	I	Active, not recruiting

Notes: NSCLC, non-small cell lung cancer. All data in Table 1 are available in https://www.clinicaltrials.gov/.

highly related with the activity of TGF-β signaling pathway in fibroblasts, especially in patients with abundant $CD8^+$ T cells enriched in peritumoral stroma rather than tumor center (immune excluded phenotype).[22] Researchers recapitulated the immune-excluded phenotype tumors with EMT6 mouse mammary and MC38 mouse colon carcinoma model. Although neither following atezolizumab nor TGF-β blockade could reduce tumor burden, the combination therapy of atezolizumab and TGF-β blockade potently eradicated cancer cells in the two models.[22] Further investigation suggested that the synergistic effect of dual-blockade was attributed to enhanced T cell infiltration into tumor center and anti-tumor immune response.[22]

In addition to increased T cell infiltration, Terabe et al found that blocking TGF-β1 and TGF-β2 significantly elevated cancer vaccine-induced Th1-type immune response, upregulated IFN-γ production, and increased T-bet expression of tumor-infiltrating $CD8^+$ T cells.[108] Besides, TGF-β blockade could enhance the treatment effect of cancer vaccine and anti-PD-1 antibody in mice models.[108] Similarly, Chen et al found that TGF-β secreted by MDSCs was highly associated with PD-1/PD-L1 inhibitor resistance by inducing PD-1 upregulation on $CD8^+$ T cells in tumor bed.[109] TGF-β blockade significantly promoted the lytic function of tumor antigen-specific $CD8^+$ T cells in vivo and in vitro.[109]

Fusion Antibody Simultaneously Targeting TGF-β And Immune Checkpoint

In 2018, Lan et al designed a bifunctional fusion protein (M7824) which consisted of a PD-L1 monoclonal antibody and the extracellular domains of human TGFβR-II.[110,111] M7824 could simultaneously block cancer cell extrinsic and intrinsic immunosuppressive pathways.[110] Under the guidance of PD-L1 monoclonal antibody moiety, M7824 could preciously locate into tumor microenvironment and further neutralize the abundant TGF-β. In vitro study showed that partial M7824 would be internalized after incubation with 293 cells ectopically expressing PD-L1.[110] In vivo study M7824 exhibited potent tumor control effect superior to isotype control, trap control, as well as PD-L1 monoclonal antibody.[110] Besides, M7824 treatment effectively prolonged survival time in mice bearing EMT6 or MC38 carcinomas.[110] Subsequent investigation showed that M7824 elevated both quantity and immune ability of tumor-infiltrating lymphocytes such as $CD8^+$ T cells, NKs, DCs, and macrophages.[110] Later, Ravi et al reported another bifunctional fusion protein

targeting CTLA-4 and TGFβR-II.[112] This bifunctional fusion protein could inhibit the differentiation of Tregs and Th17 cells, and increase tumor-specific IFN-γ^+ effector and memory cells.[112] The anti-cancer effect of anti-CTLA4-TGFβR-II fusion protein was superior to CTLA-4 or PD-1 inhibitors.[112]

Conclusion

The biologic effects of TGF-β signaling pathway are highly context-dependent. In addition to the pleiotropic function as a tumor promoter or suppressor, TGF-β pathway could induce immune suppression and participate in immune homeostasis or immune evasion. For normal tissues especially for ones consistently exposure to antigenic materials, intact TGF-β pathway could decrease the risk of inflammation-related malignant transformation. However, for advanced tumor tissues, hyperactive TGF-β pathway undermines immune surveillance and promotes tumor immune escape. TGF-β pathway broadly inhibits multiple anti-cancer producers including T cell priming and activation, immunosuppressive lymphocyte differentiation, cytotoxic function of effectors. Thus, additional TGF-β blockade might effectively enhance the ICI therapy for TGF-β-enriched tumors. We believe that the dual blockade of TGF-β and immune checkpoint would be a promising strategy in clinical practice in the future.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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