The multidimensional regulatory network of the PD-1/PD-L1 axis in the esophageal squamous cell carcinoma microenvironment: Implications for novel combination therapies and precision immunotherapy (Review)

HAOYUE LAI[1], LU QI[1], ZHIXUAN LIN[2] and ZHONGWEN LI[1]

[1]Department of Head and Neck Oncology, Second Clinical College, The Second Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou 563000, P.R. China; [2]Zunyi Medical University, Guizhou 563000, P.R. China

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Abstract. Esophageal cancer is a highly prevalent malignancy worldwide. Although immunotherapy, particularly programmed cell death-1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors, has notably improved patient outcomes, the overall response rate remains limited. This limited efficacy is largely attributed to complex immunosuppressive networks within the tumor microenvironment (TME). The present review systematically dissects the multifaceted regulatory mechanisms of the PD-1/PD-L1 signaling axis in the TME of esophageal squamous cell carcinoma (ESCC), and its impact on immunotherapeutic efficacy. Emerging evidence indicates that multiple immunosuppressive mechanisms within the TME shape the response to immune checkpoint inhibitors: Regulatory T cells enhance immunosuppression via the TGF-β-PD-1/PD-L1 axis; IL-6/STAT3 signaling upregulates PD-L1 expression and mitochondrial remodeling and amino acid network regulation exacerbate T cell exhaustion. Meanwhile, tertiary lymphoid structure (TLS) maturation is positively associated with clinical prognosis by promoting tissue-resident memory T cell activation and enhancing antitumor immunity. By contrast, the predictive value of tumor mutational burden (TMB) is constrained by TME heterogeneity. Emerging strategies highlight the predictive potential of TLS maturity and TMB, although the predictive relevance of TMB in ESCC remains inconsistent. Combination approaches show promise in reversing T/natural killer cell exhaustion and remodeling immunosuppressive TMEs. Future research should combine multi-omics data with clinical information to develop personalized immunotherapy models for ESCC.

Correspondence to: Dr Zhongwen Li, Department of Head and Neck Oncology, The Second Affiliated Hospital of Zunyi Medical University, Xinlong Avenue and Xinpu Avenue Interchange, Honghuagang, Zunyi, Guizhou 563000, P.R. China
E-mail: ker885888@sina.com

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1. Introduction

Esophageal cancer (EC), one of the major malignancies of the digestive system, poses a pronounced global health burden. According to the most recent global cancer statistics, EC ranked as the 11th most commonly diagnosed cancer and the 7th leading cause of cancer-related mortalities worldwide in 2022 (1). Epidemiological data indicate that ~511,000 new cases and 445,000 mortalities occurred globally in that year, underscoring the substantial clinical and public health challenges associated with this disease (1). Historically, EC is classified into two main subtypes: Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC is primarily associated with lifestyle risk factors such as tobacco smoking and alcohol consumption, while EAC is more commonly associated with gastroesophageal reflux disease and obesity (2). ESCC accounts for >90% of EC cases worldwide (3). These subtypes exhibit distinct molecular profiles: ESCC is characterized by frequent increased expression of CCND1, SOX2 and/or TP63, whereas EAC more often involves amplification of ERBB2, VEGFA and members of the GATA gene family (GATA4/6) (4). Although the overall incidence of EC has shown a downward trend, the prognosis remains poor (5). This is mainly due to the asymptomatic nature of early-stage disease, the lack of specific diagnostic biomarkers and the limitations of current detection methods. As a result, the majority of patients are diagnosed at an advanced stage, with a 5-year overall survival (OS) rate of <20% (6). The advent of immune checkpoint inhibitors (ICIs) has ushered in a new era in EC therapy, particularly monoclonal antibodies targeting programmed cell death protein 1 (PD-1) and its ligand (PD-L1). These agents have demonstrated promising efficacy across multiple malignancies, including melanoma, non-small cell lung cancer, renal cell carcinoma, head and neck squamous cell carcinoma and several types of gastroesophageal cancer (7).

Clinical studies have shown that PD-1/PD-L1 inhibitors markedly improve OS in advanced EC, with particularly notable benefits in the ESCC subtype (8,9). Currently approved or investigational anti-PD-1/PD-L1 agents include nivolumab, pembrolizumab, JS001, SHR-1210, durvalumab and SHR-1316 (10). However, the response rate to PD-1/PD-L1 blockade remains limited. Emerging evidence indicates that immunosuppressive signaling within the tumor microenvironment (TME) carries out a key role in limiting efficacy, primarily by persistently impairing T cell function through aberrant activation of inhibitory pathways (11). In fact, immunosuppression represents the primary source of resistance to immunotherapy (12). To comprehensively decipher the molecular landscape of tumors, researchers have extensively integrated multi-dimensional data, including genomics, transcriptomics, proteomics and metabolomics, to deeply explore the intricate interactions between tumor and immune cells within the TME, thereby providing direction for personalized cancer immunotherapy. In this context, combination therapies are widely regarded as a key strategy to overcome resistance to immunotherapy. Among them, unleashing the therapeutic potential of ICIs in combination with targeted therapy, cytokines, metabolic immunotherapies, chemotherapy, radiotherapy and multifunctional nanoparticles has gained recognition (13). However, although a study has shown that anti-PD-L1 inhibitors combined with radiotherapy can elicit systemic antitumor immunity by remodeling the immune microenvironment in mouse models of ESCC, the role of the spleen and tumor-draining lymph nodes in radioimmunotherapy remains to be elucidated. Moreover, to the best of our knowledge, these studies have not thoroughly analyzed the relationship between PD-1 expression and CD8$^+$ T-cell function (14).

It is noteworthy that the complex composition of different types of cancer and TMEs collectively shapes the multidimensional mechanisms of immune regulation. Therefore, determining the optimal combinations between immunotherapies and TME-modulating agents remains a major challenge. The present review aims to systematically dissect the complex immunosuppressive network mechanisms in ESCC based on an in-depth analysis of its TME and further discuss the translational potential of combination immunotherapy in this setting.

2. PD-1 and PD-L1

The dual role of PD-L1 in EC tumorigenesis and immunotherapy. Driven by breakthroughs in immunological theory, T lymphocytes, as central effector cells, have been widely used in tumor immunotherapy (15). PD-1 was first discovered in 1992 and is functionally recognized as a key molecule regulating both adaptive and innate immune responses (16).

The PD-1 gene, PDCD1, is located on human chromosome 2q37 and encodes a 288 amino acid type I transmembrane glycoprotein belonging to the CD28 immunoglobulin superfamily (16,17). PD-1 is highly expressed on the surface of activated T cells and is also dynamically distributed across various antigen presenting cells (APCs), including B cells, macrophages, dendritic cells (DCs) and monocytes. Its primary biological function is to negatively regulate T cell effector activity, carrying out a key role in maintaining immune homeostasis, preventing autoimmunity and modulating anti-tumor immune responses (18,19). PD-1 exerts its signaling through interaction with two specific ligands, PD-L1 and PD-L2. PD-L1 is widely expressed on APCs, activated lymphocytes and a variety of tumor cells, whereas PD-L2 expression is more restricted and is primarily observed on activated DCs and macrophages (20). Notably, aberrant overexpression of PD-L1 in the TME represents a major mechanism of immune evasion. Its expression is directly regulated by abnormal activation of the nuclear factor erythroid 2-related factor 2 (NRF2) signaling pathway and is positively associated with tumor node-metastasis staging, lymphatic metastasis and poor prognosis (19). At the molecular level, PD-L1 binds to PD-1 on T cells, effectively suppressing the proliferation of PD-1+ immune cells, thereby dampening anti-tumor immunity and facilitating tumor progression (21,22).

In normal tissues, PD-L1 displays tissue-specific expression patterns, primarily localized on the surface of CD4$^+$Foxp3$^+$ regulatory T cells (Tregs). By inhibiting the phosphorylation cascade of the mTOR/AKT signaling pathway, PD-L1 promotes Treg development, maintains their homeostasis and supports their immunosuppressive functions (23). During the malignant transformation of esophageal tissues, PD-L1 expression undergoes considerable reprogramming. From precancerous lesions such as Barrett's esophagus and low-/high-grade intraepithelial neoplasia to invasive gastroesophageal junction adenocarcinoma, the PD-L1 positivity rate increases progressively (24). Among them, IFN-γ can directly induce PD-L1 expression in esophageal epithelial cells via activation of the JAK/STAT signaling axis, thereby establishing a molecular barrier to immune surveillance (25,26). Clinical data show that ~40% of patients with EC exhibit PD-L1 overexpression, and its expression level is positively associated with disease progression. In cohorts treated with ICIs, the objective response rate (ORR) in EC ranges from 9.9 to 33.3%, further confirming the clinical value of PD-L1 as a therapeutic target (27). Importantly, PD-L1 expression is negatively associated with both progression-free survival (PFS) and OS in patients with EC, supporting its potential as an independent prognostic biomarker (28). These findings define the dual role of PD-L1 in EC: It acts both as a key driver of immune evasion and as a predictive biomarker for immune therapy response. This dual function provides a molecular basis for the development of stratified treatment strategies.

PD-1-mediated immunosuppressive signaling network and immune evasion mechanisms in ESCC. Evidence has confirmed that the PD-1/PD-L1 axis mediates immunosuppressive effects by directly interfering with T cell receptor (TCR) signaling. In the TME, PD-1 binding on activated T cells to its PD-L1 ligand triggers phosphorylation of tyrosine

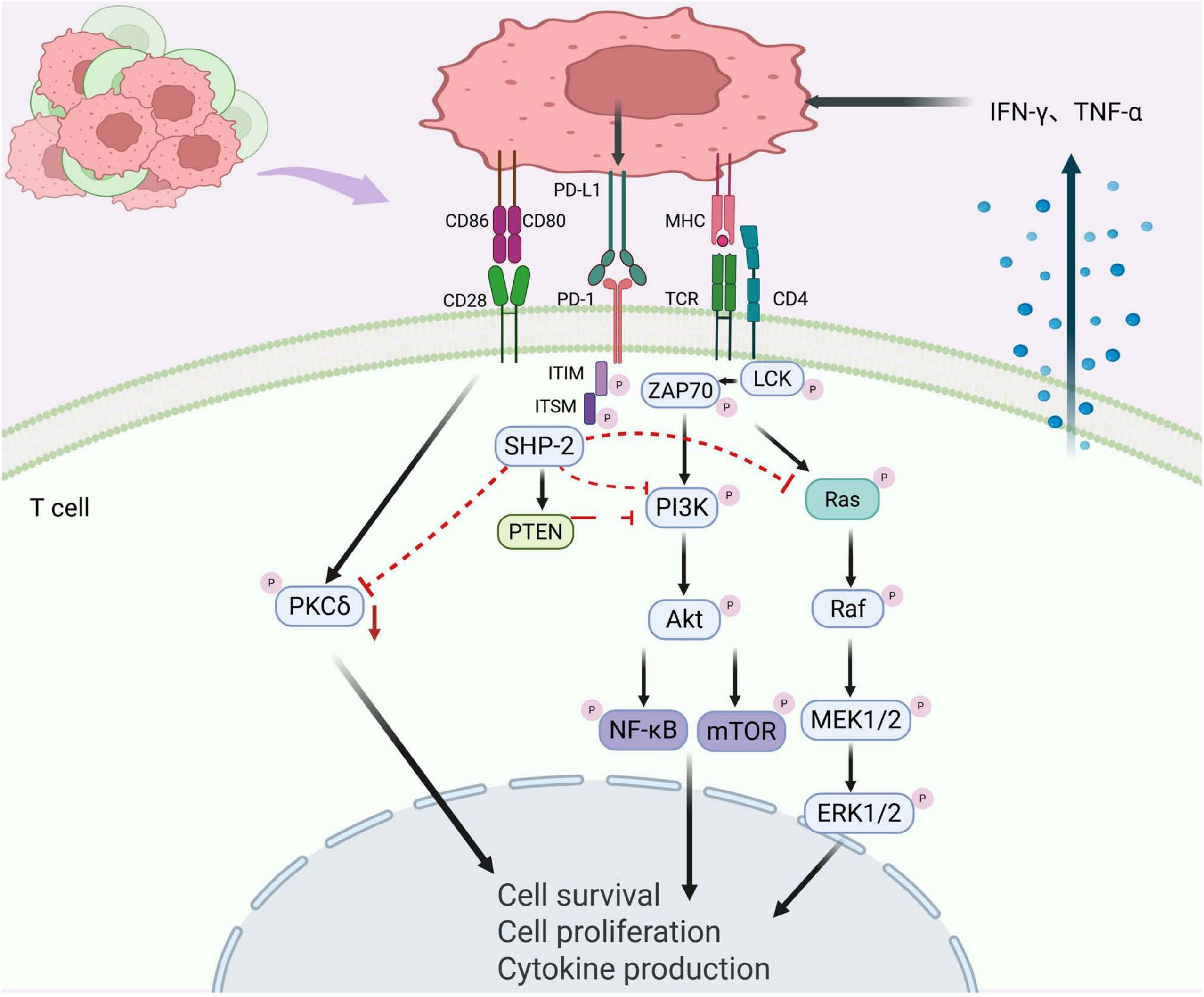

Figure 1. Mechanisms of PD-1/PD-L1-mediated immunosuppression in TMEs. Binding PD-1 on activated T cells to PD-L1 in the TME triggers immunoreceptor tyrosine-based switch motif phosphorylation and recruitment of SHP-1/2 phosphatases, leading to inhibition of PI3K and subsequent suppression of PI3K/Akt signaling and impairs proliferation and effector function. PD-1 also inhibits the RAS-ERK1/2 and PKCδ pathways and activates PTEN, further dampening TCR signaling. IFN-γ and TNF-α upregulate PD-L1 expression, establishing a positive feedback loop that enhances immune evasion. PD-L1, programmed death ligand 1; SHP-2, Src Homology 2 domain-containing phosphatase-2; ZAP70, zeta-chain-associated protein of 70 kDa; LCK, lymphocyte-cellular kinase; PKCδ, protein kinase C delta δ; MHC, major histocompatibility complex; TCR, T-cell receptor; Ras, rat sarcoma virus oncogene; Raf, rapidly accelerated fibrosarcoma kinase; TME, tumor microenvironment.

residues within the immunoreceptor tyrosine switch motif (ITSM). This conformational change recruits SH2 domain tyrosine phosphatases SHP-1 and SHP-2 to ITSM. These phosphatases inhibit the phosphorylation cascade of spleen tyrosine kinase and phosphoinositide 3-kinase (PI3K), ultimately inhibiting PI3K/Akt signaling. This results in downregulation of the anti-apoptotic protein Bcl-xL, initiation of apoptosis in T lymphocytes and suppression of T cell proliferation, survival and effector function, thus establishing a key molecular basis for tumor immune escape (21,29).

The immunosuppressive function of PD-1 is mediated through multiple regulatory pathways: i) Inhibition of the RAS-ERK1/2 signaling cascade blocks T cell cycle progression; ii) downregulation of protein kinase C δ activity reduces the secretion of cytokines such as IL-2, weakening the immune response; iii) activation of PTEN inhibits TCR-induced PI3K/AKT signaling (17). Additionally, inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) in the TME can markedly upregulate PD-L1 expression on immune and stromal cells, creating a positive feedback loop that reinforces immune suppression and enhances tumor immune evasion (30) (Fig. 1). Unlike cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which primarily regulates early T cell activation in lymph nodes, PD-1 primarily functions at later stages in peripheral tissues, where it inhibits effector T cell activity through multifaceted mechanisms. This spatial and temporal distinction suggests that the PD-1/PD-L1 axis carries out a central role in the effector phase of tumor immune escape (31).

T cell exhaustion serves as a key driver of immune evasion in ESCC. Although PD-1 blockade can partially reverse the dysfunctional state of exhausted T cells, this therapeutic effect is generally transient and limited within the complex immunosuppressive TME of ESCC (32). This persistent exhausted state is reinforced by multiple mechanisms within the TME. For instance, fibroblast-derived growth factor (FGF)-2 exacerbates T cell exhaustion by upregulating Sprouty RTK signaling antagonist 1 (33). Concurrently, non-coding RNAs carry out pivotal regulatory roles: The long non-coding RNA FOXP4-AS1 stabilizes PD-L1 via the deubiquitinase ubiquitin-specific protease (USP) 10, thereby suppressing $CD8^+$ T cell viability, promoting their exhaustion and consequently driving immune evasion (34). Meanwhile, the circular RNA circNF1 upregulates PD-L1 expression through dual mechanisms, activating the IL-6-induced JAK-STAT3 pathway and inhibiting USP7-mediated deubiquitination of annexin A1, thereby enhancing PD-L1-mediated immunosuppression (35). Beyond direct regulation of T cells, their interactions with myeloid cells are key. In patients with ESCC with stable disease, $PD\text{-}1^+$ $CD8^+$ T cells frequently interact with macrophages; targeting the iron-sulfur cluster assembly enzyme in macrophages can repolarize them, enhance $CD8^+$ T cell cytotoxicity and synergize with anti-PD-1 therapy (36). Furthermore, melanoma-associated antigen C3 amplifies IFN-γ signaling and upregulates PD-L1 by binding to IFN-γ receptor 1 and enhancing its interaction with STAT1, while also promoting epithelial-mesenchymal transition, ultimately leading to immune evasion (37).

Finally, the CD47-SIRPα axis, a key regulator of innate immune surveillance, promotes immune evasion by delivering a 'don't eat me' signal that inhibits macrophage phagocytosis of tumor cells. Elevated SIRPα expression is associated with poor prognosis in patients with ESCC, potentially due to impaired phagocytic function and subsequent suppression of antitumor immunity (38-40). The PD-1/PD-L1 axis represents a core mechanism of tumor immune evasion. Although PD-1 blockade can partially reverse T cell exhaustion, its efficacy is often constrained and prone to resistance within the multi-mechanistic, multidimensional immunosuppressive microenvironment of ESCC. Therefore, in-depth dissection of this regulatory network and the subsequent development of rational combination targeting strategies are key directions for overcoming immunotherapy resistance in ESCC.

Regulation of DNA damage and repair signaling by PD-L1 in ESCC. Beyond its well-known role in inhibiting anti-tumor immunity by binding PD-1 to immune cells, tumor-intrinsic PD-L1 signaling also shapes tumor immunogenicity. Surface PD-L1 may engage PD-1 on tumor cells themselves, promoting proliferation through enhanced phosphorylation of ribosomal protein S6. Cytosolic PD-L1 regulates mRNA stability of DNA damage response (DDR) genes and activates key pathways such as MAPK/ERK and STAT3 through protein interactions. Nuclear PD-L1 may modulate transcription of immunogenicity-related genes or influence chromosomal stability through mechanisms such as sister chromatid cohesion (29).

Deficiencies in DDR are commonly observed in ESCC, and DDR alterations may influence mutational processes and immune cell infiltration (41). Increased expression of DDR genes contributes to ESCC progression and suppresses the tumor immune microenvironment, while targeting DDR pathways has demonstrated potent efficacy in inducing immune activation and improving patient outcomes (42). Moreover, a study investigating the impact of DDR on the TME have confirmed that wild-type p53 overexpression suppresses DNA damage pathways and reduces PD-L1 expression in prostate cancer (43). Additionally, it has been suggested that oxidative stress may induce DNA damage signaling that upregulates PD-L1 expression. Both activation of DNA damage signaling and DDR deficiencies carry out important roles in promoting PD-L1 expression (44). Notably, DNA damage can stimulate PD-L1 mRNA expression, leading to increased PD-L1 levels on the cell surface. For example, DNA double-strand breaks (DSBs) activate the STAT1/3-IRF1 signaling axis, leading to PD-L1 upregulation (45). In addition, DSBs induced by chemotherapy and/or radiotherapy may activate DDR. While excessive DNA damage leads to tumor cell death, it also promotes the release of damage-associated molecular patterns, which in turn elicit anti-tumor immune responses. These observations highlight the strong potential of targeting DDR pathways in cancer immunotherapy. Combining DDR-targeted agents with ICIs represents a promising therapeutic strategy. For example, colorectal cancer with mismatch repair deficiency exhibit high sensitivity to anti-PD-1 checkpoint blockade therapy (46). A subset of patients with ESCC harbor DDR gene mutations. These patients with DDR gene alterations show considerable associations with immunological biomarkers, suggesting the potential feasibility of combining DDR-targeting agents with immunotherapy for DDR-deficient patients in future therapeutic strategies (47).

3. Current landscape of ICI therapy and TME in EC

TME acts as a key mediator of the dynamic interplay between tumor cells and host immune defense. Accumulating evidence suggests that resistance to ICIs stems primarily from the intricate crosstalk within the tumor-TME network (48). The following sections of the present review aim to systematically explore the multifaceted regulatory mechanisms of the PD-1/PD-L1 axis in ESCC TMEs, with particular attention to T and natural killer (NK) cell exhaustion, immunosuppression, angiogenesis, tertiary lymphoid structures (TLS) and tumor-driving mutations. This exploration holds promise for identifying potential strategies to improve therapeutic outcomes in EC.

Exhaustion of NK cells. The exhausted state in NK cells is characterized by the loss of cytotoxic capacity and cytokine production (49). In patients with ESCC, PD-1 expression is markedly elevated on the surface of NK cells, and this expression is negatively associated with patient prognosis (50). Subsets of $PD\text{-}1^+$NK cells exhibit stronger functional activity than $PD\text{-}1^-$counterparts, suggesting that this subpopulation may represent NK cells with the highest effector potential. This observation helps explain why, despite PD-1 being expressed on only a small proportion of NK cells, its interaction with PD-L1 on tumor cells can still effectively suppress NK cell responses (51). Similar to regulatory mechanisms in T cells, the PD-1/PD-L1 axis impairs NK cell activation thresholds, proliferation capacity and cytokine secretion, ultimately leading to an exhausted NK phenotype and weakened anti-tumor immune surveillance (52). Mechanistically, this inhibition is mediated primarily by blocking the PI3K/AKT phosphorylation cascade (50).

In addition, T cell immunoreceptor with Ig and ITIM domains (TIGIT) is often co-expressed with PD-1 on tumor-infiltrating T and NK cells, forming a synergistic inhibitory network. A study has confirmed that high TIGIT expression associates with an exhausted state in tumor-infiltrating NK cells (53). Experimental evidence indicates that TIGIT promotes lymphocyte exhaustion through two mechanisms: i) Inhibition of AKT pathway activation and ii) inhibition of forkhead box protein O1 phosphorylation (54). Notably, blocking TIGIT can effectively reverse exhaustion of NK cells within the TME and restore their effector function (55). In a study based on peripheral samples from surgically resected patients with ESCC, PD-1 expression revealed a notable positive association with TIGIT levels (53). Correspondingly, an ESCC clinical trial demonstrated that the combination of the TIGIT-targeting antibody vibostolimab with pembrolizumab exhibited encouraging clinical activity without requiring biomarker prescreening; particularly, PD-L1 high-expressing patients, defined by a combined positive score ≥10, achieved higher ORR (53). Both TIGIT and T cell immunoglobulin and mucin domain-3 (Tim-3) are associated with poor survival outcomes in ESCC (56). Similarly, Tim-3, which is upregulated on NK cells across various types of cancer, is a hallmark of lymphocyte exhaustion. *In vitro* inhibition of Tim-3 not only enhances NK cell cytotoxicity but also improves tumor cell killing efficiency (57). Research has confirmed that functionally impaired Tim-3^+ NK cells in EC associates with poor patient prognosis, and the formation of this cellular subset is mechanistically driven by TNF-α through NF-κB signaling pathway-mediated TIM-3 expression (58). Multiple TIM-3-targeting immunotherapeutic agents are currently undergoing phase 1/2 clinical trials, including Cobolimab, Sabatolimab, BGB-A425, BC3402, TQB2618, NB002, AZD7789, LB1410 and INCAGN02390 (59). PD-1^+NK cells in the TME frequently exhibit a PD-1^+/Tim-3^+ double-positive depleted phenotype, suggesting potential synergistic suppression (60).

Although substantial fundamental mechanistic research supports TIM-3 as a key target for improving NK cell function and enhancing cancer immunotherapy efficacy, with its individual blockade or combination with PD-1 inhibitors considered to hold considerable potential (59,61,62). The field still contains debatable complexities worthy of deeper investigation. This controversy is highlighted by an *in vitro* study by Astaneh *et al* (49), which reached contrasting conclusions. In early-stage chronic lymphocytic leukemia, researchers found that pre-treatment of NK cells with anti-PD-1 and anti-Tim-3 blocking antibodies failed to effectively restore or enhance NK cell functional characteristics, while also not promoting the production of key pro-inflammatory cytokines TNF-α and IFN-γ. In summary, these studies reveal the complex interactions among PD-1, TIM-3 and TIGIT in driving NK cell dysfunction in ESCC. These findings not only elucidate the multidimensional regulatory mechanisms of NK cell exhaustion but also provide a theoretical foundation for developing PD-1 inhibitor combination therapies with TIM-3 or TIGIT blockade, demonstrating notable translational potential. However, the efficacy of TIM-3/PD-1 co-targeting strategies should not be simplistically generalized, as their effectiveness highly depends on specific tumor types and disease stages. Future research should validate this strategy in ESCC and other cancer types across different disease stages, while further clarifying the intrinsic mechanisms of treatment resistance.

Multi-source TGF-β in the TME drives PD-1 expression and NK cell exhaustion. In the ESCC microenvironment, PD-1 expression on NK cells is dynamically regulated by various cytokines. Experimental data show that IL-12 and IL-15 markedly upregulate PD-1 expression in patient-derived NK cells in a time-dependent manner (50). Notably, TGF-β, a key inhibitory cytokine for NK cells, promotes NK cell exhaustion by upregulating the expression of the inhibitory receptor PD-1 on their surface (63,64). Mechanistically, abnormal accumulation of TGF-β in the TME results from the coordinated secretion by multiple cell types: In addition to tumor cell-autonomous secretion (65), immunosuppressive subpopulations such as cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and Tregs collectively form a cascading TGF-β secretion network, shaping a hostile microenvironment that promotes NK cell dysfunction (63,66). A mechanistic study by Thangaraj *et al* (67) demonstrated that in tumor models with high TGF-β expression, effective blockade of the TGF-β signaling pathway is a prerequisite for restoring NK cell anti-tumor activity. It is noteworthy that elevated TGF-β in the TME contributes to cancer immune evasion. The PD-1 and TGF-β pathways function independently yet interactively, collectively facilitating immune escape by tumor cells. Co-blockade of TGF-β and PD-L1 has been validated to enhance the efficacy of PD-L1 monoclonal antibodies and overcome therapeutic resistance (68). Furthermore, bispecific anti-PD-(L)1/TGF-β inhibitors can reinvigorate the effector functions of CD8+ T and NK cells, hinder Treg expansion and increase M1 macrophage density. Additionally, anti-PD-(L)1/TGF-β inhibitor therapy can be safely administered in combination with vaccination, radiotherapy and chemotherapy (69). Research by Lucarini *et al* (70) demonstrated that low-dose mitoxantrone combined with anti-TGF-β and PD-1 blockade therapy enhances anti-tumor immunity by remodeling the tumor immune landscape and overcoming the immunosuppressive microenvironment in aggressive neuroblastoma.

These findings validate the central role of the TGF-β-PD-1 regulatory axis in NK cell exhaustion and offer an experimental basis for targeting the TME to restore immune competence. They also offer theoretical guidance for exploring the therapeutic potential of dual TGF-β and PD-1/PD-L1 blockade in ESCC immunotherapy.

HIF-1α drives NK cell metabolic reprogramming and PD-L1 positive feedback. Impaired metabolic functioning of NK cells is a major factor contributing to their reduced activity, and the TME carries out a key role in this process. The extensive consumption of nutrients (such as glucose and glutamine) and the continuous release of TGF-β in the TME inhibit NK cell glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), leading to decreased functional activity (61,71). Additionally, a study has confirmed that tumor cells in the TME can secrete TGF-β, which further induces the upregulation of fructose-1,6-bisphosphatase expression in NK cells, thereby inhibiting glycolysis and ultimately impairing NK cell cytotoxicity and vitality (72). Beyond the reduced glucose concentration, other characteristic factors of the TME also suppress NK cell function, such as hypoxia and acidic pH (61). Research has shown that in hypoxic prostate tumors, hypoxia-inducible factor 1α (HIF-1α) mediates the overexpression of miR-224, which suppresses NCR1 signaling, markedly reducing NK cell cytotoxicity (73). Moreover, elevated expression levels of HIF-1α in patients with ESCC are associated with poor prognosis (74). Based on this association, HIF-1α could serve as a potential biomarker for predicting the prognosis of ESCC (75).

Additionally, PD-L1 is not only expressed on tumor cells in the TME but also on T cells and NK cells. HIF-1α, as a response element of the PD-L1 promoter, can promote and sustain PD-L1 expression, further enhancing its function through a positive feedback mechanism (76). Research by Ding *et al* (77) demonstrated that in gliomas, hypoxia-induced overexpression of HIF-1α promotes PD-L1 expression, confirming the positive association between the two. In their study, the combination of anti-PD-L1 antibody and HIF-1α inhibitors in a mouse glioma model considerably increased the infiltration of immune cells (CD4+ T, CD8+ T and CD11c+ DC) into the tumor while reducing PD-L1 expression, suggesting that this combination strategy could effectively reverse the immunosuppressive microenvironment in tumors. Furthermore, Shurin *et al* (78) suggested that HIF-1α inhibition has a synergistic anti-tumor effect with anti-PD-1 therapy. In a mouse melanoma model, inhibiting HIF-1α transcriptional activity led to increased infiltration of NK cells and CTLs mediated by CCL2 and CCL5, markedly enhancing the anti-tumor effect of PD-1 blockade antibodies.

T cell exhaustion. T cell exhaustion is a dynamic process characterized by a gradual transition from stem-like self-renewal to terminal exhaustion. Persistent expression of PD-1 inhibitory receptor on T cells is a hallmark of exhaustion (79). As previously mentioned, one of the key signaling pathways targeted by PD-1 is the TCR signaling pathway. In exhausted T cells, the function of the TCR signaling pathway is impaired (80).

IL-2/tryptophan hydroxylase 1 (TPH1)-aryl hydrocarbon receptor (AhR) axis drives T cell exhaustion mechanism and IL-2/PD-1 combination therapy as a novel strategy to reverse immune suppression. In ESCC TMEs, IL-2 levels are notably increased when compared with normal tissues (81). IL-2 promotes CD8+ T cell proliferation and its decline is one of the key markers of T cell exhaustion. However, despite the decline in IL-2 levels, IL-2 receptor β (IL-2Rβ) associated with PD-1 expression is upregulated in exhausted CD8+ T cells (79). In the early stages of tumor growth, IL-2 induces CD8+ T cell differentiation through autocrine signaling. In the mid to late stages of tumor growth, CD4+ T cells continuously secrete IL-2, which further acts on CD8+ T cells. The mechanism is that IL-2 continuously activates STAT5 in CD8+ T cells, inducing the expression of tryptophan hydroxylase 1 (TPH1), which catalyzes the conversion of tryptophan to 5-hydroxytryptophan (5-HTP). The 5-HTP then activates AHR nuclear translocation, leading to coordinated upregulation of inhibitory receptors such as PD-1 and TIM-3, while inhibiting cytokine and effector molecule production, ultimately leading to T cell exhaustion (82,83) (Fig. 2). A study by Hashimoto *et al* (84) showed that PD-1+TCF-1+ CD8+ T cells (precursors of exhausted CD8+ T cells) are not fate-locked to the exhausted path and their differentiation can be regulated by IL-2 signaling. The researchers suggested that PD-1+IL-2 combination therapy could markedly alter the differentiation program of PD-1+TCF-1+ stem-like CD8+ T cells.

To address the clinical toxicity associated with IL-2 therapy, antibody-cytokine fusion proteins such as PD1-IL2v have been developed. These conjugates enhance the cytotoxic and proliferative functions of stem-like precursor exhausted CD8+ T cells (85). Accordingly, the antibody-cytokine fusion protein eciskafusp alfa (PD1-IL2v) has been engineered, wherein the PD-1 antibody delivers an IL-2R agonist to PD-1-expressing T cells via a fused monomeric IL-2 variant (IL-2v), providing cis-binding that reportedly generates superior effector populations (86). However, integrating both mechanisms into a single molecule presents considerable challenges due to competing requirements for PD-1 engagement and IL-2 receptor signaling. Researchers including Mure *et al* (87) have developed a novel bispecific antibody-cytokine fusion protein, ANV600, which delivers IL-2Rβγ agonists specifically to PD-1+ cells while preserving the binding site for PD-1 checkpoint inhibitors. This approach provides a new strategy with enhanced antitumor activity and reduced toxicity. By targeting PD-1, ANV600 selectively expands tumor antigen-specific CD8+ T cells, particularly progenitor exhausted (Tpex) and cytotoxic exhausted subsets, while sparing Treg and NK cells. Furthermore, ANV600 produces additive effects when combined with either pembrolizumab or nivolumab.

Separately, Ye *et al* (88) designed a novel fusion protein, AWT020, similarly aimed at reducing systemic immune cell activation and thereby mitigating the severe toxicity typically associated with IL-2 therapy. The anti-PD-1 moiety of AWT020 serves a dual purpose: It precisely delivers an IL-2c payload to tumor-infiltrating T cells while simultaneously blocking PD-1 pathway-mediated immunosuppressive signals. Furthermore, research by Gadwa *et al* (89) demonstrated that combining radiotherapy with PD1-IL2v induces durable systemic tumor control and improves survival in an orthotopic head and neck squamous cell carcinoma model. In patients with pancreatic ductal adenocarcinoma, Piper *et al* (90) reported that PD1-IL2v notably improves local and metastatic survival rates and markedly increases tumor-infiltrating CD8+ T cell subsets.

In summary, T cell exhaustion represents a key immunosuppressive phenomenon in the TME, particularly prominent during the pathogenesis and progression of ESCC. PD1-IL2v molecules such as ANV600 and AWT020, which modulate T cell status by targeting PD-1, provide novel insights and strategic approaches for ameliorating T cell exhaustion in ESCC, enhancing anti-tumor immune responses and improving survival outcomes.

Mitochondrial metabolic reprogramming and PD-1/PD-L1-amino acid network regulate T cell exhaustion. Exhausted T cells exhibit notable heterogeneity in phenotype and function, mainly classified into two major subsets: Tpex cells and terminally exhausted T cells. Tpex cells have a stem-like exhaustion phenotype characterized by markers such as PD-1,

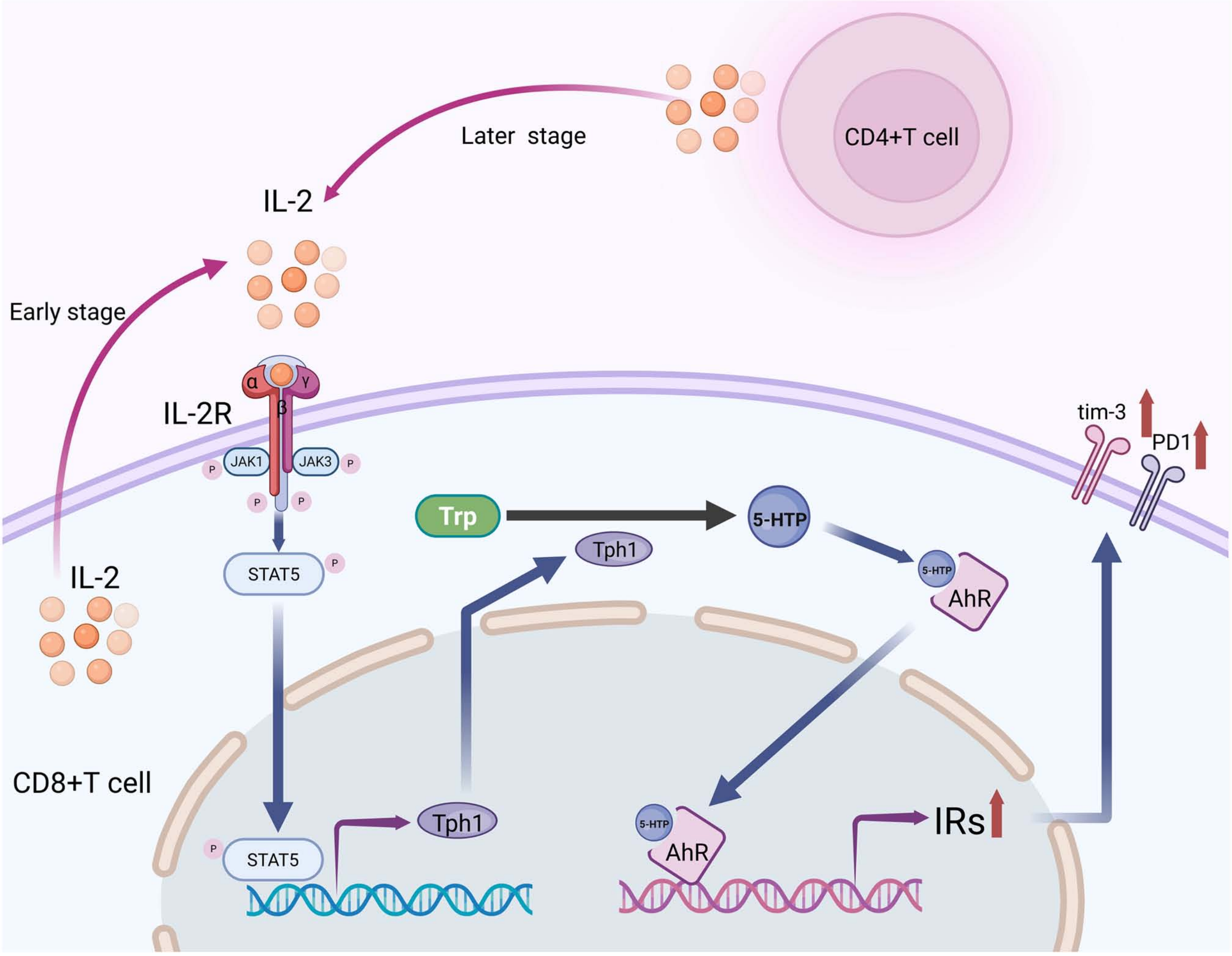

Figure 2. IL-2-driven CD8$^+$ T cell depletion in the ESCC TME. IL-2 levels are elevated in ESCC TMEs compared with normal tissues. In early tumor stages, IL-2 promotes CD8$^+$ T cell differentiation through autocrine signaling. During tumor progression, sustained IL-2 secretion by CD4$^+$ T cells chronically activates STAT5 in CD8$^+$ T cells, inducing TPH1 expression and converting tryptophan to 5-HTP. 5-HTP triggers AhR nuclear translocation, upregulating inhibitory receptors such as PD-1 and tim-3 while suppressing cytokine and effector molecule production, ultimately driving T cell exhaustion. Trp, tryptophan; Tph1, tryptophan hydroxylase 1; AhR, aryl hydrocarbon receptor; 5-HTP, 5-hydroxytryptophan; IRs, immunoreceptor signaling; PD1, programmed death 1; TME, tumor microenvironment; tim-3, T cell immunoglobulin and mucin-domain containing-3.

CD127, chemokine receptor CXCR5 and high levels of TCF-1 (encoded by Tcf7). Tpex cells rely primarily on mitochondrial fatty acid oxidation and OXPHOS for metabolism. However, prolonged antigen stimulation leads to changes in mitochondrial structure, which in turn impairs both glycolysis and OXPHOS function (91). Furthermore, a reduction in mitophagy or impaired OXPHOS can lead to the accumulation of reactive oxygen species in mitochondria and increased accumulation of damaged mitochondria, which causes the loss of mitochondrial membrane potential and depolarization, ultimately leading to mitochondrial dysfunction, increased hypoxic stress, induced epigenetic reprogramming and irreversible terminal T cell exhaustion (92,93). The PD-1/PD-L1 axis carries out a particularly key role in T cell exhaustion, particularly in regulating mitochondrial function. The PD-1 signaling pathway inhibits phosphorylation of dynamin-related protein 1 by modulating extracellular protein kinases 1/2 and mTOR pathways, blocking mTOR-mediated mitochondrial fission, thereby reducing mitochondrial numbers and weakening their function. This process ultimately suppresses T cell activation, proliferation and infiltration (94). Current research indicates that immune-related pathways and mitochondrial function are associated with immunotherapy sensitivity in ESCC (95). To overcome the immunosuppressive TME, characterized by hypoxia and lipid raft formation, which considerably suppress T cell infiltration and function, Chen *et al* (96) developed a stable nano-formulation named Abstatin. This formulation combines partially denatured albumin with fluvastatin, effectively mitigating mitochondrial respiratory suppression and disrupting lipid raft stability in non-small cell lung cancer cellular and animal models. Notably, Abstatin notably enhanced the efficacy of anti-PD-1 therapy with minimal toxicity, demonstrating promising clinical safety prospects. This approach, which integrates cellular metabolic regulation with immune sensitization, broadens the potential applications of immunotherapy and offers novel insights for ESCC treatment. Similarly, in manganese-based STING-activating cancer immunotherapy, efficacy is limited by T cell exhaustion, with mitochondrial dysfunction being a key contributing factor. A study shows that spermidine can enhance mitochondrial function in T cells, thereby providing a viable strategy to reverse T cell exhaustion in manganese-based immunotherapy (97).

Additionally, supplementation with nicotinamide riboside has been shown to improve T cell mitochondrial function, consequently enhancing their responsiveness to PD-1 inhibitors (98). Recent evidence further emphasizes mitochondria as key hubs for enhancing anti-tumor immune responses in 'immune desert' tumors (99). For instance, targeted induction of mitochondrial DNA release can activate the cyclic GMP-AMP synthase-stimulator of interferon genes (STING) pathway, thereby sensitizing tumors to ICI therapy (100). This opens new perspectives for enhancing tumor immunogenicity in ESCC.

In addition, various amino acids regulate the PD-1/PD-L1 signaling axis through different mechanisms. For example, glutamine is abundant in EC cells and other tumor cells, glutamine deficiency lowers intracellular glutathione levels in tumor cells, thereby upregulating PD-L1 expression to suppress T cells. Additionally, glutamine depletion irreversibly inhibits T cell proliferation and cytokine secretion, creating a dual inhibitory effect (101,102). In tryptophan metabolism, IDO+ DCs activate Tregs, upregulate PD-L1 on DCs and enhance immune suppression. Multiple amino acid deficiencies inhibit mTOR/Akt signaling, activate the FOXO-PD-1 pathway in Tregs and form a PD-1-PTEN-PI3K/Akt negative feedback loop, further reinforcing the immune suppression state (101). Serum metabolomic analyses indicate that elevated serum L-tryptophan levels are associated with reduced risk of ESCC (103). Dysregulated tryptophan metabolism conversely promotes progression and metastasis (103). While metabolism carries out a pivotal role in tumorigenesis and cancer progression, the regulatory mechanisms of tryptophan metabolism within the EC microenvironment remain largely unknown. Finally, from the emerging perspective of metabolic intervention and immune sensitization, serine metabolism reveals another mechanism. Serine occupies a central position in maintaining metabolic homeostasis of cancer cells. Research by Saha *et al* (104) confirmed that restricting both endogenous and exogenous serine supply in colorectal cancer cells induces mitochondrial dysfunction, which unexpectedly enhances tumor sensitivity to PD-1 ICIs. This discovery not only reveals a novel immune mechanism underlying the anti-tumor effects of serine deprivation but also demonstrates the pronounced potential of targeting amino acid metabolism to reverse immunotherapy resistance. It outlines a blueprint for transitioning ESCC treatment from 'immune monotherapy' to 'multidimensional immune-metabolic combination therapy'. Future clinical breakthroughs in ESCC will likely emerge from combination therapies designed against its specific metabolic vulnerabilities. By precisely intervening in the 'metabolic battle' within the TME, the full anti-tumor potential of the immune system can be unveiled and improve patient survival outcomes.

Immune suppression

Tregs cooperate with TGF-β-PD-1/PD-L1 axis to drive immune suppression. In the TME, Treg cells, MDSCs, TAMs-M2 type, neutrophils and CAFs cooperate to promote immune suppression through cytokine networks (105). In ESCC, Treg cells are central to the TME, maintaining immune homeostasis while exerting immune suppressive effects (106). Tregs suppress immune cell function through the secretion of immunosuppressive cytokines, such as IL-10, IL-35 and TGF-β, and through direct cell-to-cell contact (107). The PD-1/PD-L1 signaling axis carries out a key role in regulating Treg differentiation and function, specifically by reducing STAT activation in Th1 cells through SHP-1/2 phosphatases, promoting Th1 cell to Treg conversion. In addition, Tregs may affect the efficacy of PD-1/PD-L1 blockade therapy (108). Additionally, the expression of PD-1 can suppress the immunosuppressive capacity of Tregs and promote the production of IFN-γ. Conversely, PD-L1, promotes the conversion of naïve CD4+ T cells to Tregs by downregulating Akt, mTOR and ERK2, and upregulating PTEN (108).

Among these secreted proteins, TGF-β can impair the cytotoxicity of effector T cells or NK cells, while upregulating immune checkpoints on regulatory immune cells (such as TAMs and MDSCs) and Tregs (109). TGF-β not only stimulates Treg differentiation and expansion but also converts normal T cells into Tregs (107,110). Notably, blocking MDSC-derived TGF-β can enhance the efficacy of PD-L1/PD-1 inhibition therapy and induce an immune response from MAGE-A3+ specific CD8+ T cells in ESCC. Therefore, combining T cell-based therapies with dual blockade of the PD-1/PD-L1 and TGF-β signaling pathways may be a promising cancer treatment strategy (111).

IL-6/STAT3-PD-L1 axis drives immune suppression. As another important immunosuppressive secretory factor in TME, IL-6 is overexpressed in both ESCC and EAC, and its levels associate positively with disease progression. IL-6 carries out a key role in ESCC by upregulating PD-L1 expression (112). IL-6 inhibits DC function in antigen presentation by activating STAT3, thereby inhibiting tumor immune response (113). In addition, other cytokines carry out a key role in T cell exhaustion. For example, IL-6 recruits MDSCs in the TME, which inhibit T cell activity via IFN-γ and promote partial CD8+ T cell exhaustion. IL-6 released by MDSCs also interacts with IL-6/STAT1 and is associated with multidrug resistance (114). In addition, activated fibroblasts secrete IL-6, which further promotes ESCC cell proliferation and enhances cisplatin resistance (115). The mechanism involves IL-6 secreted by CAFs upregulating the expression of CXCR7 via the STAT3/NF-κB pathway, thereby promoting chemotherapy resistance (116). More importantly, IL-6 expression was positively associated with PD-L1 expression levels. Metformin downregulates PD-L1 expression by inhibiting the IL-6/JAK2/STAT3 signaling pathway in ESCC, thereby enhancing antitumor immune responses (117). Furthermore, radiation therapy considerably upregulates PD-L1 expression levels via the IL-6/STAT3 signaling pathway, suggesting that combining anti-PD-1/PD-L1 immunotherapy with radiation therapy may improve the treatment outcomes (118). Based on clinical and experimental evidence, drugs targeting IL-6 signaling provide a reasonable theoretical basis for combination therapy of patients with patients with ICIs (119). As a tumor-derived and systemic immune checkpoint, IL-6 can evade the killing effects of tumor-reactive CD8+ T cells activated by ICI therapy through tumor hijacking mechanisms. Therefore, combining IL-6 signaling pathway inhibitors with ICIs is well supported by clinical evidence (120).

In ESCC, IL-6 carries out a particularly prominent role: It not only serves as a pivotal factor shaping the immunosuppressive microenvironment but also possesses clear clinical

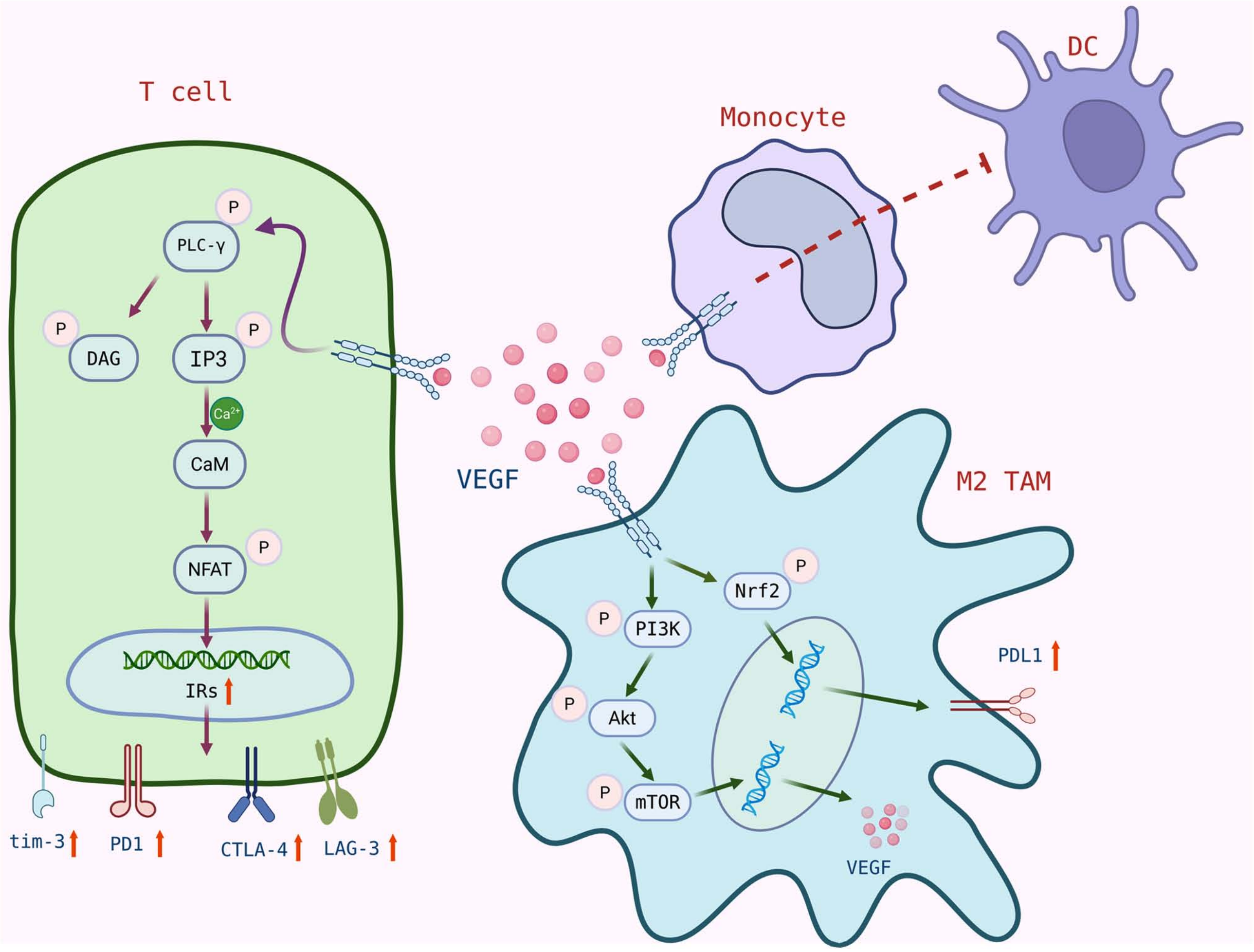

Figure 3. VEGF/VEGFR-2-mediated immunosuppression and immune evasion in ESCC. VEGF promotes CD8$^+$ T cell exhaustion by upregulating PD-1, CTLA-4, TIM-3 and LAG-3 via the VEGFR-2-PLC-γ-calmodulin-NFAT pathway. In M2-type TAMs, VEGFR-2 activation enhances VEGF secretion through PI3K/Akt/mTOR and induces PD-L1 expression through Nrf2 activation. VEGF/VEGFR2 binding also inhibits monocyte-to-DC differentiation and reduces DC maturation and antigen presentation, driving immune escape. PLC-γ, phospholipase C-γ; P, phosphate group; DAG, diacylglycerol; Cam, calmodulin; NFAT, nuclear factor of activated T-cells; Irs, immunoreceptor signaling; Tim-3, T cell immunoglobulin and mucin-domain containing-3; PD1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte associated protein-4; LAG-3, lymphocyte-activation gene 3; VEGF, vascular endothelial growth factor; DC, dendritic cell; TAM, tumor-associated macrophage; PDL1, programmed cell death ligand 1.

predictive value. Specifically, IL-6 overexpression may serve as a potential biomarker for predicting response to PD-1 inhibitors (121); concurrently, elevated serum IL-6 levels markedly associate with increased risk of immune-related adverse events and poor overall patient prognosis (122). These findings collectively establish the key position of IL-6 in ESCC immunotherapy and provide further rationale for the clinical application of combination blockade strategies in this patient population.

Angiogenesis. Angiogenesis is key for the progression of ESCC (123). For patients with advanced who have failed standard first-line therapy, subsequent treatment options remain limited, making targeted anti-angiogenesis drugs an area of extensive interest. Vascular endothelial growth factor (VEGF) is one of the key mediators of angiogenesis, with overexpression of VEGF found in 24-74% of patients with ESCC (124). Combined use of ICIs and therapies targeting VEGF/VEGFR is considered a synergistic treatment strategy that may have a positive regulatory effect on the TME (125).

Innate immune cell-driven multi-molecular proangiogenesis network. In the TME, various innate immune cells participate in angiogenesis regulation through multiple molecular mechanisms. Macrophages, N2-type neutrophils, MDSCs and masT cells not only directly promote the formation of new blood vessels by secreting VEGF, but also mediate the release of VEGF-A through the secretion of MMP-9. Additionally, MDSCs produce FGF-2 and Bv8 molecules, which, together with FGF-2, IL-8, TGF-β and TNF-α secreted by masT cells, form a complex pro-angiogenesis signaling regulatory network that carries out a key role in the TME (126). In turn, VEGF can inhibit the maturation, differentiation and antigen presenting abilities of professional APCs, such as DCs, NK cells and T cells, while enhancing the immunosuppressive functions of Treg cells, TAMs and MDSCs (48). Notably, VEGF inhibition has been shown to increase the number of tumor-infiltrating lymphocytes (127). In addition, other mediators of angiogenesis, such as platelet-derived growth factor and FGF signaling pathways, also carry out roles in regulating angiogenesis, tumor growth and metastasis. Therefore, when

VEGF signaling is blocked, compensatory mechanisms may come into play, suggesting that anti-angiogenesis therapy needs to block not only VEGF/VEGFR signaling pathways but also other key pathways involved in angiogenesis and tumor growth (128).

Mechanistic analysis of VEGF/VEGFR2 axis and translational potential of PD-1/VEGF combined blockade in ESCC immunotherapy. Most importantly, VEGF expression shows notable association with EC progression and prognosis, and contributes to improved survival outcomes in advanced ESCC and EAC (129). VEGF increases the expression of PD-1 and other immune checkpoints (such as CTLA-4, tim-3 and Lag-3) on CD8+ T cells via the VEGFR-2-PLCγ-calmodulin phosphatase-activated T cell nuclear factor NFAT pathway, thereby promoting CD8+ T cell exhaustion and facilitating tumor immune evasion (130,131). Additionally, high expression of VEGFR-2 is considered a poor prognostic factor in patients with ESCC, with activation of the Akt/mTOR signaling pathway carrying out a key role in the tumorigenesis, progression and prognosis of ESCC cells (132). Upon activation of VEGFR-2 on M2-type TAMs, VEGF secretion is induced via the Akt/mTOR/PI3K pathway and PD-L1 expression is upregulated by activation of Nrf2 (133). The binding of VEGF/VEGFR2 also inhibits the differentiation of monocytes into DCs and reduces DC maturation and antigen-presenting function through inhibition of the NF-κB-mediated pathway, driving immune evasion and promoting PD-L1 expression on DCs (134). Studies have shown that blocking PD-L1 can reactivate DC function and enhance anticancer T cell immune responses (135) (Fig. 3). These findings demonstrate that anti-angiogenic agents can effectively enhance tumor immunogenicity, thereby improving responses to immunotherapy. Zhang *et al* (PMID: 31704735) reported that VEGF-A induces the expression of the transcription factor TOX in T cells, driving specific exhaustion transcription programs within T cells. Through combined *in vitro*, *ex vivo* and *in vivo* mouse experiments, they demonstrated that a combination of PD-1 and VEGF-A blockade could restore the anti-tumor function of T cells (136).

Substantial clinical investigations have been conducted in this area. For instance, Hou *et al* in a retrospective propensity-matched cohort study of 135 patients with metastatic clear cell renal cell carcinoma, found that anti-PD1 therapy following anti-angiogenic treatment considerably improved OS and PFS (137). In a separate study, Lochrin *et al* (138) retrospectively analyzed the clinical characteristics, treatment and outcomes of patients with mucosal melanoma treated with Axtinib (a selective VEGF inhibitor) with or without anti-PD-1 therapy at a single US referral center between 2018 and 2021. They found that 78% of patients received Axtinib combined with PD-1 inhibitors, with a median treatment duration of 3.2 months. The overall response rate was 13%, disease control rate was 26%, median PFS was 3.2 months and median OS was 8.2 months. Overall, this regimen was well-tolerated. Additionally, a phase II study demonstrated that neoadjuvant toripalimab combined with Axtinib shows promise in reducing inferior vena cava tumor thrombus staging and decreasing the need for extensive surgery in patients with clear cell renal cell carcinoma and inferior vena cava tumor thrombus, while also establishing the safety profile of this combination therapy (139). Furthermore, Atkins *et al* (140) conducted a non-randomized phase 1b clinical trial in treatment-naive patients with advanced renal cell carcinoma, showing that the combination of Axtinib (a selective VEGF inhibitor) and pembrolizumab (an anti-PD-1 therapy) was safe, well-tolerated and exhibited some anti-tumor activity. However, trials of this scale are insufficient to demonstrate substantial clinical benefit, and further validation in larger trials may be required. Notably, a previous case analysis reported a patient with ESCC who experienced disease recurrence after chemoradiotherapy and achieved a durable complete remission confirmed by PET/CT evaluation following treatment with the PD-1 inhibitor camrelizumab combined with the anti-angiogenic agent apatinib, with manageable treatment-related toxicity (141). However, a study in other cancer types such as metastatic renal cell carcinoma, such as the combination of lenvatinib with pembrolizumab compared with sunitinib, did not show notable improvement in OS (137). These results suggest that the clinical efficacy of combining immunotherapy with anti-angiogenic strategies may be heterogeneous across different tumor types, drug combinations and patient populations. Additionally, both anti-angiogenic drugs and ICIs have their own side effect profiles, and adverse events become more complex with combination therapy (142). The synergistic mechanisms between ICIs and anti-angiogenic agents have not been fully elucidated, and identifying potential beneficiary populations for ICI combined with anti-angiogenic therapy in ESCC and other types of cancer remains a key clinical challenge.

TLS. TLS have a positive prognostic value in the majority of solid tumors, carrying out a important role in creating a microenvironment with a unique cytokine profile (143). TLS is characterized by its temporary formation at tumor sites, rather than in secondary lymphoid organs, and these ectopic lymphoid tissues consist mainly of follicular DCs, B cell zones, T cell zones and high endothelial venules (144). TLS formation begins with the release of CXCL13 and IL-7 by stromal cells (143). Deguchi *et al* (144) showed that high TLS presence was associated with improved prognosis in patients with early-stage ESCC, but no notable difference was observed in advanced cases at stage III or higher. This may be due to the increased presence of Treg cells and M2 macrophages, which exert immunosuppressive functions in the vicinity of TLS or locally within the tumor. In addition, studies have found that in ESCC immunotherapy, the maturity of TLS is markedly associated with improved prognosis, with mature TLS (MTLS) being an independent prognostic factor for ESCC (145,146). MTLS contribute to improved treatment outcomes in patents with ESCC who undergo various neoadjuvant therapies, and T cells reactivated by PD-1 inhibitors within TLS may promote TLS maturation through interactions and activation with B cells. As TLS matures, both humoral and adaptive anti-tumor immune responses are effectively activated. MTLS accelerates the spread of T cells from TLS to tumor sites, enhancing tumor cell elimination (146).

In gastric cancer, the presence of tissue-resident memory T (Trm) cells or TLS is associated with a favorable prognosis (147). Hu *et al* (148) demonstrated that activated B cells enhanced the secretion of CXCL13 and granzyme B by CD103+CD8+ Trm cells. Furthermore, the presence of

TLS and CXCL13+CD103+CD8+ Trm cells was associated with a tumor necrosis factor receptor 2-dependent effective response to PD-1 inhibitor treatment and CD103+CD8+Trm cells in the high TLS group showed markedly increased PD-1 expression levels. Additionally, Zhang *et al* (149) identified the presence of HLA-A+ TLS through multiplex immunohistochemistry and showed that as TLS matured, the resident cells within TLS gradually expressed HLA-A. Spatially distinct tumor-infiltrating lymphocyte (TIL)-T cells and single-cell RNA-sequencing data from 60 ESCC tumor tissues showed through digital spatial analysis that as TLS matured, CXCL13-expressing exhausted TIL-T cells within TLS were reactivated and features of antigen-presenting machinery were upregulated. Further experiments by Zhang *et al* (149) confirmed the presence of HLA-A+ TLS in ESCC tumor tissues and HLA-A+ TLS, along with their major cell components, TIL-Ts and TIL-Bs, were associated with clinical benefits from ICI treatment in ESCC. These observations suggest that integrating tumor mutational burden (TMB) with PD-L1 expression, TLS maturity and other multidimensional parameters may improve ESCC patient stratification and therapeutic decision-making. This implies that combining ICIs with agents targeting these inhibitory cells could potentially alleviate microenvironmental suppression and restore or enhance the anti-tumor function of TLS, thereby offering promising prospects for improving treatment outcomes in patients with advanced ESCC.

TMB. TMB, as a biomarker for predicting responses to anti-PD-1 therapy, represents the total number of mutations in all coding regions of the tumor genome (150). Multiple studies have shown that TMB is relatively high in locally advanced or metastatic ESCC and that TMB is markedly associated with ORR and OS (151,152). Immunologically 'hot' tumors typically exhibit (TMB-H) (TMB-H), which implies a higher load of neoantigens and upregulation of PD-L1 expression, thereby promoting increased TILs (153). For example, in cancer types such as melanoma and colorectal cancer, CD8+ TIL-T cell levels are positively associated with neoantigen load and tumors with TMB-H are more responsive to immune checkpoint blockade (ICB) (154). TMB-H can optimize the spatial distribution of T cells by promoting APCs to present neoantigens, thereby activating T cells and enhancing their infiltration into the TME. As such, TMB was initially proposed as a predictive biomarker for ICB responses (155,156).

However, subsequent research has shown that TMB-H is not universally applicable to all solid tumors, including some cases of ESCC, and its predictive value is limited. This inconsistency can be attributed to notable differences in immune cell infiltration density and immune activity between low-TMB and high-TMB subtypes in these types of cancer (155,157). Concurrent chemoradiotherapy (CCRT) can reduce TMB and induce non-specific inflammation in ESCC cells. When ICIs are administered before or in combination with CCRT, they may initiate and reactivate immune cells, triggering a more robust immune response (158).

Although TMB, as an indicator of genomic instability (152) holds potential value in predicting ICB responses. Its effectiveness is influenced by a combination of tumor type, microenvironmental characteristics (such as immune infiltration and PD-L1 expression levels), genetic mutations and clinical factors (such as stage of disease and alcohol consumption history). In the future, integrating multi-omics data to guide personalized immunotherapy decisions will be a key direction (159,160).

4. Discussion

PD-1/PD-L1 ICIs have brought transformative advances in the treatment of EC, yet their limited efficacy highlights the need for a deeper understanding of complex regulatory networks within the TME. The central role of the PD-1/PD-L1 axis in mediating immune evasion in the EC has been elucidated, conversely, PD-L1 impairs T cell function by promoting exhaustion and apoptosis through TCR signaling; on the other hand, the synergistic reinforcement of multiple mechanisms within the TME leads to persistent immune evasion in ESCC, collectively resulting in limited response rates and frequent resistance to PD-1/PD-L1 monotherapy. The TME in ESCC exhibits high heterogeneity and multidimensional immunosuppressive characteristics, comprising various immune cells (such as Tregs, MDSCs and TAMs) and their secreted factors (such as TGF-β and IL-6), non-coding RNAs (such as FOXP4-AS1 and circNF1), metabolic reprogramming (such as amino acid metabolism and mitochondrial dysfunction), innate immune checkpoints (such as CD47-SIRPα), angiogenic factors (such as VEGF) and the maturation status of TLS. These multifaceted immunosuppressive components interact and function synergistically, forming an intricate immunosuppressive network that ultimately undermines the therapeutic efficacy of PD-1/PD-L1 single-agent targeting. Given the heterogeneity and complexity of the TME, single ICB is unlikely to achieve curative outcomes. Future research should focus on systematically characterizing the dynamic regulatory networks within the TME. Rapid translation of these fundamental discoveries into clinical benefits is key, necessitating the promotion of more prospective clinical trials exploring novel combination therapies and utilizing advanced models such as patient-derived organoids for preclinical efficacy evaluation. This includes exploring intelligent combinations of PD-1/PD-L1 inhibitors with other targeted agents, such as co-targeting additional immune checkpoints in the TME (such as TIGIT and TIM-3), antagonizing key immunosuppressive factors (such as TGF-β and IL-6), synergizing with anti-angiogenic drugs and leveraging DDR pathway-targeting agents to enhance tumor immunogenicity. Concurrently, developing targeted drug delivery systems for specific TME components, employing IL-2 signaling to regulate the differentiation of stem-like exhausted T cells and creating novel PD1-IL2v fusion proteins (such as nanoformulations), along with in-depth investigation of resistance mechanisms in combination therapies and integration of multi-omics data with clinical variables to build personalized immunotherapy decision models, hold promise for gradually dismantling the immune defense network of ESCC and offering patients more durable and effective treatment options.

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HL contributed to conceptualization, project administration, writing-original draft, writing-review and editing. LQ contributed to investigation, supervision, writing-review and editing. ZLin contributed to investigation, writing-review and editing. ZLi contributed to conceptualization, funding acquisition, supervision, writing-review and editing. Data authentication not applicable.

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Competing interests

The authors declare that they have no competing interests.

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