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Review

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Deciphering the tumor microenvironment in esophageal cancer: cellular heterogeneity and functional crosstalk

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Abstract: Esophageal cancer (EC) is a highly prevalent malignant tumor worldwide, with a particularly high incidence in regions such as Asia. The poor prognosis of EC is attributed mainly to late detection, high invasiveness, and limited treatment efficacy. Growing evidence has revealed a close association between the adverse outcomes of EC and the tumor microenvironment (TME). This review comprehensively summarizes advances made from 2020 to 2025 in understanding the TME of EC based on single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics. First, we illustrate the heterogeneity of major cellular components within the TME, with a focus on subtype classification, dynamic phenotypic changes, and the clinical significance of T cells, B cells, tumor-associated macrophages (TAMs), dendritic cells (DCs), and cancer-associated fibroblasts (CAFs). Then, we highlight the functional crosstalk mechanisms underlying anti-tumor immunity and the dual regulatory role of tertiary lymphoid structures (TLS) in the TME. Finally, we address current challenges in EC TME research, including inconsistencies in cell subtype classification, significant inter-patient heterogeneity, and incomplete understanding of cellular crosstalk signaling networks. We aim to provide a foundation for further mechanistic exploration of EC TME and to support the development of novel therapeutic strategies.

Key words: Esophageal cancer; Tumor microenvironment; Single-cell RNA sequencing; Cellular heterogeneity; Cellular interaction

1 Introduction

Esophageal cancer (EC) is one of the most lethal malignant tumors and the seventh leading cause of cancer-related deaths globally (Fitzmaurice et al., 2018). Esophageal squamous cell carcinoma (ESCC) accounts for about 90% of EC, and is usually diagnosed at an advanced stage (Rustgi and El-Serag, 2014). In Asia, particularly in China, the incidence of ESCC is relatively high; in contrast, among Western populations, esophageal adenocarcinoma (EAC) accounts for a higher proportion of EC cases (Zhang et al., 2012; Zhu et al., 2024). Although surgery, chemotherapy, radiotherapy, and immunotherapy have been applied solely or in combination, the outcome for patients with EC remains poor (Baba et al., 2018; Fang et al., 2022). A deep understanding of how EC arises and acquires drug resistance is crucial to developing more effective drugs and

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therapeutic strategies (Fang et al., 2022).

Cancer has been defined as an evolutionary and ecological process, driven by continuous and dynamic interactions between cancer cells and the tumor microenvironment (TME) (Merlo et al., 2006; Schiffmann et al., 2021; Wang et al., 2022). The TME encompasses all non-cancerous host cells and acellular components within the tumor (Jin and Jin, 2020; Bejarano et al., 2021; Xiao and Yu, 2021), as summarized in Fig. 1. Immune cells, generally divided into myeloid cells and lymphocytes, continually reshape the TME, thereby governing the performance of cancer cells. Myeloid cells include tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), and tumor-associated neutrophils (TANs), while lymphoid populations include helper ($CD4^+$) T cells, cytotoxic ($CD8^+$) T cells, regulatory T cells (Tregs), B cells, and natural killer (NK) cells (Zhang et al., 2021; Guo et al., 2022). In addition to immune cells, the stromal cells are another cell population within the TME, including cancer-associated fibroblasts (CAFs), vascular endothelial cells, smooth muscle cells and others (Dinh et al., 2021). The acellular components of the TME include the extracellular matrix (ECM) and soluble products, such as chemokines (including cytokines), growth factors and extracellular vesicles (Najafi et al., 2019). The TME influences tumor progression through multiple mechanisms, rendering it one of the focal points in recent cancer research. Single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics now allow us to depict the TME in unprecedented detail and to dissect how TME-cancer cell interactions drive tumorigenesis and therapeutic resistance. Given that non-tumor cells within the TME are genetically more stable, they are more appropriate for the exploration of therapeutic targets to improve clinical outcomes for cancer patients (Que et al., 2022; Shi et al., 2025; Xie et al., 2025).

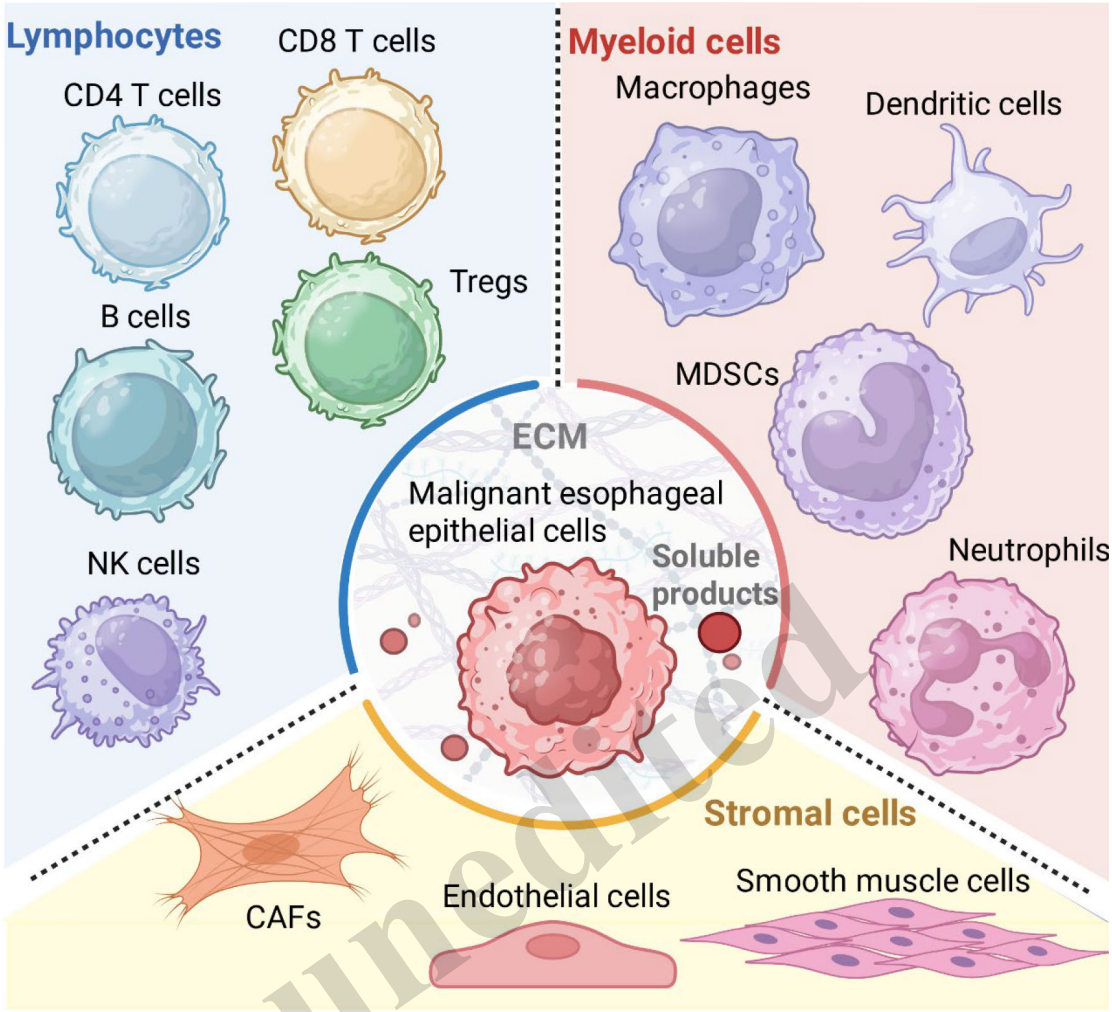

Fig. 1 Composition of the tumor microenvironment (TME) in esophageal cancer

Tregs: regulatory T cells; NK cells: natural killer cells; MDSCs: myeloid-derived suppressor cells; CAFs: cancer-associated fibroblasts; ECM: extracellular matrix. The figure was created with BioRender (https://BioRender.com).

However, like tumor cells, the TME is highly heterogeneous, both between patients and within the same tumor, posing a fundamental obstacle to TME-targeted therapeutic strategies (Zhang et al., 2022). Within the TME, each cell type differs in spatial layout, relative abundance, and molecular subsets, further compounding the challenge of TME-targeted therapy. For example, the differences in the quantity and function of lymphocytes infiltrating the tumor determine the efficacy of immune checkpoint inhibitors (Plesca et al., 2020). Unlike bulk RNA-seq, which returns an averaged transcriptional profile across thousands of cells, scRNA-seq resolves gene-expression heterogeneity at single-cell resolution, accurately identifies cell subsets and their dynamic functional states, and overcomes the loss of cell-specific characteristics caused by the averaging effect of traditional bulk RNA-seq (Huang et al., 2023). Spatial transcriptomics enables the analysis of cellular gene expression profiles while preserving the original spatial positional information of cells within tissues, thereby overcoming the limitation of "losing spatial context" inherent in single-cell transcriptomics (Rao et al., 2021). Consequently, scRNA-seq and spatial transcriptomics have been widely applied in the field of tumor research to refine TME cell subtypes, dissect drug-resistance mechanisms, and discover predictive biomarkers of therapeutic efficacy and patient prognosis (Ho et al., 2018; Wu et al., 2023).

There has been no systematic summary of the TME in EC. This review systematically summarizes

research advances in EC with a focus on the TME. We describe the cellular constituents of the EC TME, and clarify how several well-studied, functionally pivotal cell types (such as T cells, B cells, macrophages, DCs and CAFs) in anti-tumor immunity either drive or restrain tumor progression, as well as describing their interactions with malignant cells and other cellular components.

2 Core Cellular Components of the TME in EC

2.1 T cells

T cells dominate the EC microenvironment, dictating tumor initiation, progression, and therapeutic response. T cells are divided mainly into $CD8^+$ cells and $CD4^+$ cells. $CD8^+$ cells are referred to mainly as cytotoxic $CD8^+$ lymphocytes (CTLs), while $CD4^+$ cells are further divided into helper $CD4^+$ cells (Th1, Th2, Th17, Tfh, etc.) and $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs). In addition, each type can be further subdivided into numerous subsets. Different T cell subpopulations may play opposing roles in the TME, either exerting anti-tumor effects or promoting tumor progression. $CD8^+$ T cells exert core anti-tumor immune effects by specifically recognizing tumor antigens and lysing tumor cells. The helper $CD4^+$ T cells activate immune responses by activating dendritic cells, sustaining $CD8^+$ T cell expansion and effector function, and acquiring direct cytotoxic activity. In contrast, Tregs mediate tumor immune evasion by suppressing the activity of effector immune cells (Antony et al., 2005; Ahrends and Borst, 2018).

With tumor progression, $CD8^+$ T cells gradually differentiate into an exhausted state (Tex), which is characterized by the sustained expression of multiple immune checkpoint molecules. Tex cells promote tumor progression through their immunosuppressive effects. Two states of Tex cells have been identified: a progenitor exhausted state (expressing *CST7*, *GZMK*, etc.) observed in early-stage intramucosal ESCC (Liu et al., 2023a), and a terminally exhausted state (expressing immune checkpoint molecules like PD-1 and TIM-3) in advanced-stage ESCC. Despite discrepancies among studies regarding the checkpoint molecules highly expressed on Tex, all consistently include TIGIT and CTLA4, pointing to a core molecular signature of $CD8^+$ Tex cells amid the heterogeneity of the TME (Chen et al., 2021; Dinh et al., 2021). Notably, at the gene expression level, ESCC-derived $CD8^+$ T cells exhibit high expression of both checkpoint molecules (exhaustion markers) and immune effector genes (chemokines and cytotoxicity-related genes). This duality has been widely reported recently, indicating that the effector functions of $CD8^+$ T cells are impaired by co-inhibitory factors (Chen et al., 2021; Dinh et al., 2021). The functional fates of $CD8^+$ T cell subsets, whether they become anti-tumor effector T cells or pro-tumor Tex, are determined by specific environmental signals. Various cells in the TME, including tumor cells and macrophages, can influence $CD8^+$ T cell function, as discussed below. In addition, progenitor-like exhausted $SPRY1^+CD8^+$ T cells, recently identified in ESCC, activate B cell functions through pathways such as the IFNG-IFNGR axis to promote antigen presentation. Additionally, these Tex cells secrete cytokines or chemokines like IFNG and TNF to induce the proinflammatory polarization of macrophages, thereby exerting antitumor immune effects (Liu et al., 2023b). Functionally, these Tex cells resemble conventional effector T cells. In conclusion, $CD8^+$ T cells exhibit high heterogeneity and functional plasticity within the TME.

The response of $CD8^+$ T cells to anti-cancer therapies differs slightly between ESCC and EAC. In studies of EAC from Western cohorts, the efficacy of anticancer treatment is associated with the alleviation of the immunosuppressive TME. After neoadjuvant chemotherapy, the cytotoxicity, exhaustion, and expression level of immune checkpoints all decrease (Croft et al., 2022). Additionally, the increased abundance of $CXCL13^+CD8^+$ Tex is associated with poor response to neoadjuvant chemotherapy (Croft et al., 2022). In contrast, some findings in ESCC are contradictory. Neoadjuvant chemoradiotherapy (neoCRT) induces exhaustion in the $CD8^+$ T cell population and a reduction in $CXCL13^+CD8^+$ T cells within ESCC tumors (Wen et al., 2022; Yin et al., 2024). The variation in treatment responses between these two cancers may arise from subtle disparities in treatment protocols or fundamental biological differences between the malignancies,

underscoring the need for further in-depth investigation. Furthermore, a study investigating gender differences in both ESCC and EAC revealed a higher infiltration of both $CD8^+$ Tex and $CD8^+$ effector memory T cells in male patients than in females (Yan et al., 2024). This finding underlies the improved response to immunotherapy in male patients.

$CD4^+$ T cells differentiate into several subsets, such as Th1, Th2, and Th17 cells, which perform distinct immune functions. Th1 cells mainly mediate cellular immune responses and play a key role in tumor surveillance (Braumüller et al., 2013). Th2 and Th17 cells are involved mainly in immunity against extracellular pathogens (Bettelli et al., 2007). In addition, $CD4^+$ T cell subsets expressing immune checkpoint molecules, together with regulatory Tregs, exert immunosuppressive effects, thereby contributing to the formation of an immunosuppressive TME. For instance, the $CD4^+HAVCR2^+$ T cell subset in ESCC expresses the checkpoint molecules PDCD1 and TIGIT (Shi et al., 2022a), promoting an immunosuppressive TME by inhibiting the activation and function of effector T cells. The immunosuppressive and regulatory phenotype of $CD4^+$ T cells worsens as ESCC progresses, a trend consistent with that of $CD8^+$ T cells (Yao et al., 2020; Zhang et al., 2021; Chang et al., 2025). Furthermore, with the progression of ESCC, the $CD4^+$ Th subsets undergo a proportional shift, characterized by a progressive decline in Th1 cells and a concomitant increase in Th2 and Th17 cells. This shift indicates that the immune focus of $CD4^+$ T cells transitions from targeting tumor cells toward responding to extracellular pathogens (Yao et al., 2020). Interestingly, the infiltration of $CD4^+$ T cells is higher in female EC patients than in male patients (P=0.05) , and this trend is consistent across all stages and different histological types (Yan et al., 2024), though further validation is still required.

A reduction in the proportion of Tregs after anticancer treatment is generally associated with better therapeutic efficacy. This conclusion holds true for both ESCC and EAC. For example, in ESCC, the reduction in the Treg-to-Th cell ratio after neoCRT indicates a preferential differentiation into Th cells rather than Treg cells, which contributes to a less immunosuppressive TME (Wen et al., 2022). Additionally, the proportion of total T cells increases after neoadjuvant chemoimmunotherapy (NACI), and a decreased proportion of Tregs is associated with a better pathological response (Ji et al., 2024). According to studies from Western cohorts, EAC tumors show a high density of Treg cell infiltration prior to neoadjuvant chemotherapy, which shows a notable reduction following chemotherapy (Croft et al., 2022). These results collectively confirm that anti-cancer therapies can ameliorate an immunosuppressive TME.

In addition to the formation of an immunosuppressive TME, T cells exert anti-tumor effects or promote tumor progression through direct interactions with ESCC tumor cells. In terms of immune responses, most are mediated by interactions involving MIF-associated ligand-receptor pairs. Immunosuppression, by contrast, is achieved mainly through immune checkpoint molecule interactions between tumor cells and T cells, particularly $CD8^+$ T cells. For example, interactions between T cells and tumor cells involving the ligands MIF and COPA and their receptor CD74 have been identified (Chen et al., 2021). Malignant epithelial cells also engage in strong interactions with T cells via the ACKR3-MIF axis, thereby promoting immune infiltration (Guo et al., 2025). Additionally, tumor cells interact with T cells via EGFR-TGFB1, CD2-CD58, and MIF-TNFRSF10D, exerting immunostimulatory effects (Chen et al., 2021). In terms of immunosuppression, the widely identified immune checkpoint pairs, such as TIGIT-NECTIN2 (Chen et al., 2021; Guo et al., 2025), TIGIT-PVR (Ji et al., 2024), and LGALS9-HAVCR2 (Chen et al., 2021; Ji et al., 2024), are consistent with the previously described mechanisms of immunosuppressive microenvironment formation. Moreover, the Notch, *KRAS*-related, and Wnt/β-catenin pathways are activated in $CD8^+$ T cells (Chen et al., 2021; Shi et al., 2022a; Yin et al., 2024), and these pathways promote cancer progression through multiple mechanisms (Xiu et al., 2021; Liu et al., 2022; Ma et al., 2025). Collectively, T cells combat or promote tumors through multiple pathways.

Not only are ESCC cells influenced by T cells, but they can also actively educate T cells, thereby establishing an immunosuppressive TME. Mechanistically, O-GlcNAc transferase derived from exosomes of ALDH-positive EC stem cells can be internalized by adjacent $CD8^+$ T cells, leading to increased PD-1 expression in $CD8^+$ T cells, which contributes to cancer cell immunity evasion (Yuan et al., 2021). Furthermore, tumor cells overexpress TRPM8 (transient receptor potential melastatin 8). Both TRPM8 overexpression and

TRPM8 agonists can reduce the cytotoxic effect of $CD8^+$ T cells on EC cells (Lan et al., 2019). Tumor cells can also recruit Tregs via the eomesodermin-CCL20-CCR6 pathway, thereby forming an immunosuppressive TME and promoting tumor proliferation (Lian et al., 2021). In conclusion, tumor cells influence T cells through mechanisms such as inducing the conversion of $CD8^+$ T cells into Texs and recruiting Tregs (Fig. 2).

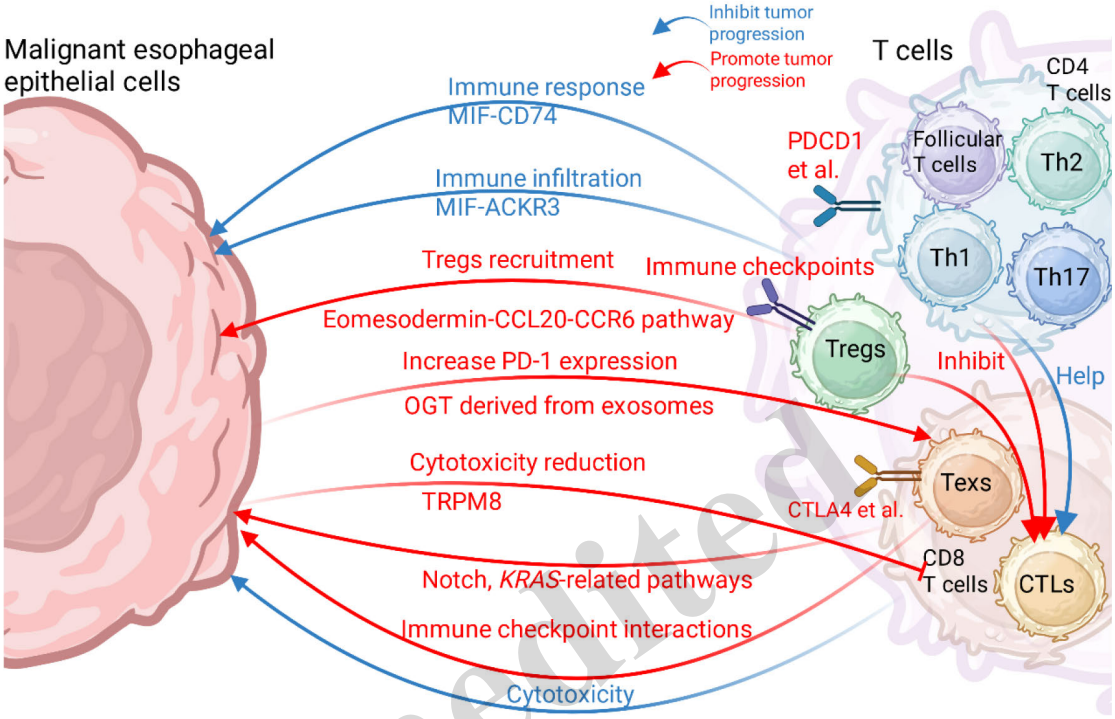

Fig. 2 Interactions between T cells and tumor cells within the esophageal cancer TME

Th: helper T cells; CTLs: cytotoxic $CD8^+$ lymphocytes; Tex: exhausted T cells. Eomes: Eomesodermin. OGT: O-GlcNAc transferase. Red and blue lines denote interactions that promote and inhibit tumor progression, respectively. The line orientation denotes the direction of action. Arrows and inhibitory marks denote promotive and inhibitory actions, respectively. The effect of each interaction is shown above the line, with the involved molecules or pathways listed below. The figure was created with BioRender (https://BioRender.com).

Tertiary lymphoid structures (TLS) stimulate adaptive anti-tumor immunity in ESCC (Zhang et al., 2021; Teillaud et al., 2024). The presence of TLS is associated with favorable outcomes in tumor therapy (Huang et al., 2024). As T cells constitute a major component of TLS, recent studies have begun to characterize their spatial distribution, subset proportions, and transcriptional profiles within TLS in ESCC. Most T cells are located at a distance of more than 150 μm from TLS (Nakamura et al., 2023a; Huang et al., 2024). TLS^+ tumors exhibit a higher overall T cell content compared to normal tissues, with notably increased proportions of infiltrating $CD8^+$ T cells and follicular helper T (Tfh) cells (Nakamura et al., 2023a; Huang et al., 2024). At the molecular level, $CD8^+$ T cells in TLS^+ tumors express significantly higher levels of cytotoxic markers (*GZMK* and *PRF1*) than TLS^- tumors, indicating that their anti-tumor immunity is activated (Nakamura et al., 2023a). In contrast, Tfh cells in TLS^+ tumors highly express *CXCL13*, *SEMA4D*, and other germinal center (GC) reaction-related genes (Nakamura et al., 2023a). Elevated *CXCL13* expression in T cells is associated with poor prognosis in ESCC (Liu et al., 2023a), and *SEMA4D* is also frequently highly expressed in various solid tumor types (Evans et al., 2015). In conclusion, TLS display a dual and complex role in ESCC. They not only demonstrate therapeutic potential by enriching specific anti-tumor T cell subsets and enhancing cytotoxic immune responses,

but also promote tumor progression through the elevated expression of immunosuppressive and other molecules associated with poor prognosis.

2.2 B cells

B cells participate in tumor immunity mainly through antibody production, antigen presentation, and secretion of cytokines, thereby regulating antitumor immune responses and tumor progression (Laumont and Nelson, 2023). As a cornerstone of adaptive immunity, B cells show significant functional heterogeneity within the TME. They contribute to anti-tumor immune responses through various mechanisms, including antibody secretion, antigen presentation, and coordinated interactions with T cells (Gilbert et al., 2011; Hladíková et al., 2019). Conversely, B cells can be co-opted by the tumor to facilitate progression through immunosuppression and angiogenesis promotion (Baba et al., 2020). In this section, we summarize recent advances in understanding the spatiotemporal distribution of B cells within tumors, their mechanisms of anti-tumor immunity, and their association with TLS (Fig. 3).

ESCC is characterized by sparse B cell infiltration compared with other types of cancer (Zheng et al., 2020). This scarcity may underpin the attenuated interactions between malignant cells and B cells, which are significantly less frequent than those between malignant cells and other immune cells (Chen et al., 2021). The number of infiltrating B cells in early stage intramucosal ESCC is higher than that in advanced ESCC (Liu et al., 2023a). Compared to the adjacent tissues, the level of infiltrating B cells is higher in early-stage ESCC, but lower in late-stage ESCC (Liu et al., 2023a; Guo et al., 2025). Collectively, as ESCC progresses, B cell infiltration within the TME decreases, with a concomitant shift in localization from the tumor tissue to the surrounding stromal regions. The spatial distribution of distinct B cell subsets within the TME has also been investigated. For instance, three subsets of B cells (C1-C3) have been identified as being enriched in metastatic samples of ESCC (Guo et al., 2025). Additionally, more plasma B cells are present in ESCC tissues than in adjacent non-tumor tissues, whereas follicular B cells are found mainly in non-malignant esophageal samples (Chen et al., 2021).

In terms of the response of B cells to anti-cancer therapy, ESCC and EAC show opposite results. After neoadjuvant chemotherapy (NACT), the proportion of antibody-secreting cells (ASCs) in ESCC tumors increases, while the expression level of *IGHD*, a key marker of naive B cells, decreases (Nakamura et al., 2023b). However, studies from Western cohorts have shown that, prior to treatment, the proportion of switched memory B cells ($S100A10^+$) is decreased, while the population of plasmablasts secreting IgA and IgG is expanded in EAC. This trend is reversed following NACT, with a concurrent increase in the total B cell numbers (Croft et al., 2022). In summary, NACT promotes the differentiation of B cells into ASCs in ESCC, but inhibits this process in EAC. These seemingly contradictory effects suggest that the existence of unexplored, context-dependent mechanisms underlying B cell response to treatment.

In recent years, the anti-tumor effects of B cells have been extensively documented. As antibody secretion represents a major mechanism of B cell-mediated immunity, plasma B cells and ASCs have become a central focus of research in this field. In ESCC tumors, antibody-secreting genes in ASCs are significantly enriched (Nakamura et al., 2023b). Correspondingly, the expression levels of *MZB1*, *FKBP11*, and *IGHG3* are elevated in plasma B cells, all of which are associated with antibody secretion (Chen et al., 2021). Moreover, the upregulation of differentially expressed genes (*XBP1*, *IGHG1*) in ASCs is positively correlated with prolonged survival (Nakamura et al., 2023b). Additionally, in metastatic lymph nodes, a group with higher expression of *IGKC* (one of the genes highly expressed in ASCs) exhibited higher overall survival (OS) and recurrence-free survival (RFS) than a low-expression group (Nakamura et al., 2023b). Collectively, these findings confirm the anti-tumor effect of ASCs in the TME of ESCC. Interestingly, gene set variation analysis (GSVA) indicates that plasma B cells play an important role in cell proliferation and differentiation (Chen et al., 2021), which differs from the traditional understanding of plasma cells and thus requires further research.

In ESCC, B cells also exert immune functions through interactions with other immune cells, such as T cells. For example, IL23 derived from NEIL1-expressing follicular B cells can activate $CD8^+$ $SPRY1^+$ Tex cells by

targeting genes (e.g., *IRF1*, *PRDM1*, *REL*, *BRD2*, and *NFKBIZ*) associated with T cell activation, expansion, and effector functions (Liu et al., 2023b). NEIL1-expressing follicular B cells can also promote the M1 polarization and antitumor phenotype of macrophages via the IL-23-TNF axis, and further mediate the recruitment and activation of $CD8^+$ T cells through the CXCL9/CXCR3 axis in downstream macrophages (Liu et al., 2023b). Tfh cells, on the other hand, are recruited to B cell follicles by expressing *CXCR5*, thereby promoting the differentiation and maturation of B cells (Zhang et al., 2021). More B cell subsets have been characterized and their functions are being gradually explored. A mature B cell (MBC) subset, $ITGAX^+$ MBC, is characterized by high expression of *ITGAX*, *CLECL1*, *HLA-DPB1* and key genes involved in CD40 signaling, such as *ICAM1*, *CD80*, *CD86*, and *CFLAR*, which are associated with cell adhesion and antigen presentation to T cells (Ryan et al., 2002; Harjunpää et al., 2019; Nakamura et al., 2023b). The $ITGAX^+$ MBC subset also shows high expression of CCL17 and CCL22. These chemokines are secreted following CD40 signaling and recruit Tfh cells to the GCs via binding to CCR4 on their surface (Liu et al., 2021; Nakamura et al., 2023b). Notably, this subset also exhibits elevated expression of *MYC* pathway genes and *PD-1* signaling components, suggesting a potential dual role in tumor immunity (Nakamura et al., 2023b). Furthermore, the naive B cell subset, characterized by *NF-κB* expression, expresses genes that activate the *NF-κB* pathway, a signaling axis critical for B cell maturation and survival (Siebenlist et al., 2005; Nakamura et al., 2023b). This subset also exhibits elevated expression of *SLAMF1*, a surface molecule that sustains interactions with Tfh cells to facilitate GC formation (Corcoran et al., 2014). The discovery of new subsets reveals the extensive interactions between B cells and T cells, which will provide new insights into understanding the mechanisms of anti-tumor immunity.

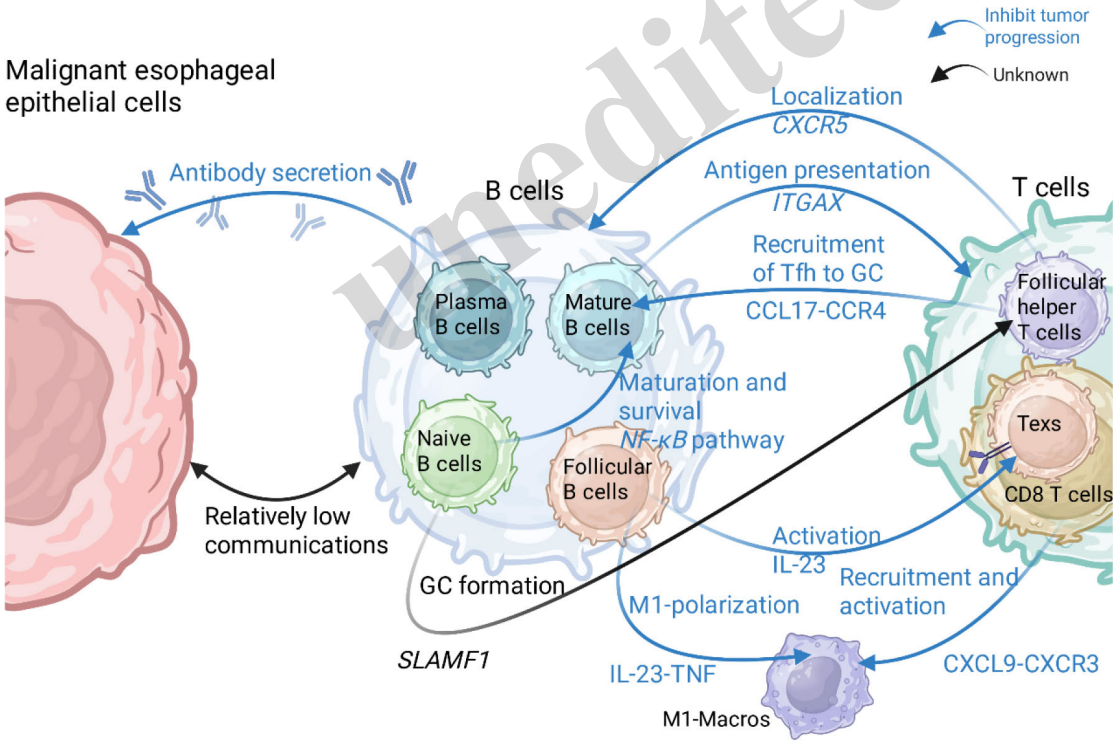

Fig. 3 B cell-centered cellular interactions within the TME of esophageal cancer

GC: germinal center. Tfh: follicular helper T cells. Blue lines indicate inhibitory interactions, whereas black lines represent interactions with undetermined effects on tumor progression. The line orientation denotes the direction of action. The effect of each interaction is shown above the line, with the involved molecules or pathways listed below. The figure was created with BioRender (https://BioRender.com).

B cells, together with T cells, represent a major cellular component of TLS (Nakamura et al., 2023b). The diversity of B cell subsets within TLS implies their multifaceted roles in anti-tumor immunity. A subtype of B cells, germinal center B cells (GCBs), are located mainly in the GCs of secondary lymphoid organs and the TLS of ESCC (Victora and Nussenzweig, 2022; Nakamura et al., 2023a). Additionally, ESCC tumors with TLS exhibit an increased plasmocyte content compared to those without TLS (Shi et al., 2022a). Mechanistically, in ESCC tumors with TLS, the density of IGKC-positive cells is significantly increased, which indicates that TLS promote the maturation of B cells into ASCs, as IGKC is a marker of ASCs, thereby facilitating the secretion of anti-tumor antibodies (Nakamura et al., 2023a). Nevertheless, the mechanisms through which TLS promote anti-tumor immunity in EC are not fully understood, underscoring the need for deeper mechanistic investigation to elucidate their immune regulatory functions.

2.3 Tumor-associated macrophages (TAMs)

Macrophages play dual roles in anti-tumor immunity. On the one hand, they can exert anti-tumor effects through mechanisms such as phagocytosing tumor cells and presenting antigens. On the other hand, they may be induced by the TME to acquire a pro-tumor phenotype that promotes tumor growth and metastasis. Multiple methodological approaches have consistently shown that TAMs and their precursor cells constitute the predominant population of myeloid infiltrate within the TME across most human solid malignancies (Gentles et al., 2015; Chevrier et al., 2017; Cassetta et al., 2019). Moreover, the TAM compartment is highly dynamic and heterogeneous both within and between tumors (Chevrier et al., 2017; Cuccarese et al., 2017; Chen et al., 2021). The heterogeneity is evident in phenotypic, metabolic, and functional profiles of TAMs, which exhibit a continuum of polarization states ranging from pro-inflammatory (M1-like) to anti-inflammatory (M2-like) (Mills et al., 2000; Cassetta and Pollard, 2018). M2-like TAMs are associated with poor outcomes by fostering tumorigenesis, angiogenesis, and immunosuppression, thereby driving disease progression (Mantovani and Allavena, 2015; Geng et al., 2024). In contrast, M1-like TAMs exert tumoricidal functions and enhance the effectiveness of anticancer therapies, including immunotherapy and chemotherapy (Arnold et al., 2014; Mantovani and Allavena, 2015). While the classical binary M1/M2 model provides a useful conceptual framework for understanding TAM biology in tumors (Vitale et al., 2019), the *in vivo* reality is far more complex. Both M1-like and M2-like TAMs coexist in the TME of ESCC before and after neoadjuvant chemoradiotherapy (Wen et al., 2022). In ESCC, however, macrophages undergo a phenotypic transition from M1 to M2, and then to an intermediate phenotype (Chen et al., 2021). To fully elucidate the functional impact of macrophages on tumors, the development of more refined and specific molecular markers is essential for precise characterization of TAM phenotypic and functional states.

Recent studies have widely identified and characterized SPP1-expressing TAMs ($SPP1^+$ TAMs) as a pro-tumorigenic subset in ESCC. These $SPP1^+$TAMs exhibit classical TAM-like signals (Nakamura et al., 2023a), and express matrix metalloproteinases (MMPs), which are closely associated with cancer progression (Egeblad and Werb, 2002; Kudo et al., 2012). This macrophage subset promotes tumor progression and confers resistance to neoadjuvant chemoimmunotherapy (NACI) (Shi et al., 2022a; Geng et al., 2024). Other mechanisms by which TAMs promote tumor progression are also being gradually elucidated. For example, TAMs secrete CCL18, which promotes the proliferation of ESCC through the *JAK2*/*STAT3* pathway (Sui et al., 2023). Macrophages and epithelial cells also promote cancer progression through MIF-CD74 interactions (Li et al., 2024). In addition to the stimulatory interactions between malignant cells and M2/anti-inflammatory macrophages, inhibitory interactions have been detected recently, including those involving LGALS9-SORL1, LGALS9-SLC1A5, and LGALS9-PTPRK (Chen et al., 2021). These findings suggest that the role of TAMs in TME may be more complex than previously recognized. Moreover, as ESCC progresses, the expression of *DTX3L* in epithelial cells and of *BST2* in stromal cells gradually increases. This upregulation is associated with enhanced M2 macrophage polarization, as well as increased tumor proliferation and migration (Li et al., 2024).

In addition to the interactions with cancer cells, macrophages interact with T cells to shape the TME of ESCC (Dinh et al., 2021; Oyoshi H, 2023). TAMs mediate immune suppression through interactions with Tregs

and CD8$^+$ Tex cells, secrete inflammatory factors to activate immune cells, and engage in a closed-loop activation via crosstalk with CD4$^+$ T cells. The cell numbers of CD163$^-$CD68$^+$ macrophages and FOXP3$^+$CD4$^+$ Tregs are positively correlated both before and after neoadjuvant chemoradiotherapy (Wen et al., 2022). These findings reveal a close functional interaction between macrophages and Tregs. Specifically, TAMs and Tregs interact through the ligand-receptor pairs of TNF-TNFSF1B, CCL4-CCR8, and IL1β-IL1R2 (Zheng et al., 2020). These three ligand-receptor pairs play crucial roles in suppressing local inflammation, recruiting Tregs, and inhibiting the activation of effector T cells, respectively (Mercer et al., 2010; Liu et al., 2015; Zheng et al., 2020). Furthermore, Tregs may polarize macrophages toward an M2 phenotype (characterized by CD163 and PD-L1 expression) and suppress TNFα production by macrophages through the HLA-LILRB1 interaction, thereby facilitating tumor progression (Zheng et al., 2020). C1QC$^+$ TAMs strongly interact with CD8$^+$CXCL13$^+$ Tex cells, in addition to Treg cells (Yin et al., 2024). Consistent with findings in other epithelial carcinomas, such as uveal melanoma and basal cell carcinoma, the immunosuppressive interactions between C1QC$^+$ TAMs and CD8$^+$CXCL13$^+$ Tex cells in EC are also mediated by the MIF-CD74 axis (Yin et al., 2024). Beyond mediating immunosuppression via genes such as *TGFB1* and *COX2* (Zhang et al., 2021), macrophages present immunostimulatory functions. M1-like C1QC$^+$ TAMs express high levels of inflammatory cytokines, including CXCL1, CXCL2, CXCL3, and CXCL8, which promote the recruitment and activation of immune cells (Geng et al., 2024). C1QC$^+$ TAMs are prevalent in ESCC samples following neoadjuvant chemoimmunotherapy (Geng et al., 2024). Notably, C1QC$^+$ TAMs are negatively correlated with chemotherapy survival rates (Yin et al., 2024), suggesting the complex role of macrophages modulating anti-tumor immunity. Additionally, a positive feedback loop between macrophages and CD4$^+$ T cells, mediated by the *Il1b-Il1r1* ligand-receptor pair has been identified in a mouse ESCC model and shown to promote anti-tumor immune responses (Yao et al., 2020). The interactions among macrophages, cancer cells, and T cells are summarized in Fig. 4.

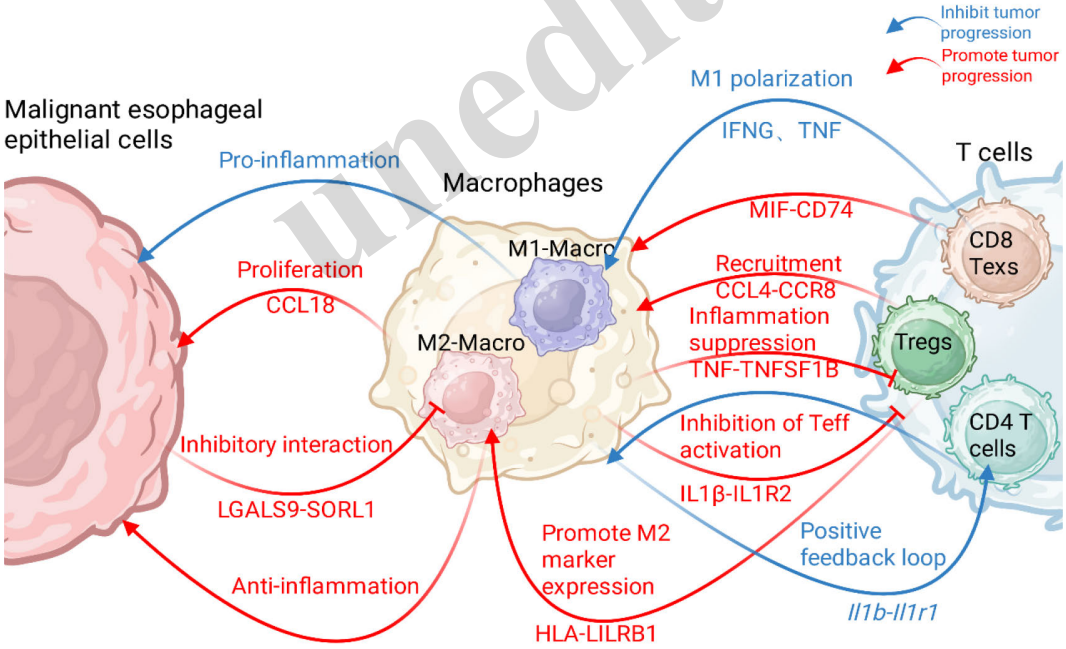

Fig. 4 Macrophage-centered cellular interactions in anti-tumor immunity within the TME of esophageal cancer

Red and blue lines denote interactions that promote and inhibit tumor progression, respectively. The line orientation denotes the direction of action. Arrows and inhibitory marks denote promotive and inhibitory actions, respectively. The effect of each interaction is shown above the line, with the involved molecules or pathways listed below. The figure was created with BioRender (https://BioRender.com).

Finally, a new TAM subset, TREM2$^+$ TAM, was characterized in ESCC samples by the upregulated expression of *TREM2*, *C1QC*, *C1QB*, *C1QA*, *SPP1*, and *APOE* (Li et al., 2023b). The abundance of this subset in tumor tissues is higher than that in normal tissues. An immunosuppressive environment is observed in contexts with elevated TREM2$^+$ TAMs (characterized by upregulation of the Tex marker CTLA4), enrichment of pathways associated with therapy resistance and apoptosis, and an increase in immunosuppressive cell populations (Hugo et al., 2016; Bagaev et al., 2021; Li et al., 2023b). Analytical results from melanoma cases have also confirmed the immunosuppressive effect of TREM2$^+$ TAMs (Li et al., 2023b). Furthermore, TREM2$^+$ TAMs are associated with poor responses to immune checkpoint blockade, highlighting their potential as biomarkers for predicting the efficacy of immunotherapy (Li et al., 2023b). Moreover, TREM2$^+$ macrophages secrete type I interferons, which contribute significantly to lymph node metastasis in ESCC (Guo et al., 2025). Interestingly, these TAMs also highly express soluble factors such as SPP1, APOE, C1QA, C1QB, and C1QC, indicating their involvement in exocrine or paracrine signaling processes that help establish a pro-invasive and pro-metastatic TME (Li et al., 2023b). In addition, studies have consistently shown that SPP1, APOE, C1QA, C1QB, and C1QC are significantly elevated in the plasma of cancer patients (Xing et al., 2017; Zhang et al., 2019b). Consequently, such genes represent promising targets for non-invasive liquid biopsy in ESCC detection, serving as a valuable complement to traditional biopsy (Ignatiadis et al., 2021). In summary, TREM2$^+$ TAMs promote ESCC progression through immunosuppressive mechanisms and also hold potential as predictive biomarkers for immunotherapy response and as targets for non-invasive diagnostic strategies.

2.4 Dendritic cells (DCs)

DCs take up, process, and present antigens to T cells, thereby initiating and regulating adaptive immune responses. They serve as a key bridge connecting innate and adaptive immunity (Everts and Pearce, 2014; Sesti-Costa et al., 2020). Three major subtypes of DCs are commonly identified in the TME: conventional DCs (cDCs), tolerogenic DCs (tDCs), and plasmacytoid DCs (pDCs) (Zhang et al., 2021). cDCs are responsible for the uptake of antigens from tumor cells and the presentation of these antigens to T cells (Wang et al., 2023a). The cDC population can be further classified into two well-defined subtypes: cDC1 and cDC2. Since tDCs are a specialized subtype of DCs that suppress immune responses and promote immunological tolerance in the TME, their presence inhibits the anti-tumor immune response and promotes tumor progression (Takenaka and Quintana, 2017). pDCs are involved in antiviral immunity and regulate immune responses (Venet et al., 2023). However, due to the high heterogeneity of DCs within the TME (Dinh et al., 2021), these three major subtypes are not consistently identified across all studies (Zhang et al., 2021; Wen et al., 2022; Shi et al., 2024). Based on their evolutionary trajectory, the three DC subtypes occupy distinct differentiation stages: cDCs represent an early differentiation stage, while tDCs reflect a late stage of differentiation. In contrast, pDCs span both early and late differentiation stages with a more active cell cycle (Shi et al., 2024). The evolutionary trajectory of the three subtypes indicates that with tumor progression, DCs are gradually transformed into an immunosuppressive phenotype (tDCs).

Following anticancer therapy, changes in DC subtypes are indicative of enhanced anti-tumor immunity. In ESCC, neoadjuvant chemoradiotherapy (neoCRT) promotes the maturation of cDC1s, which present mainly endogenous antigens like tumor antigens, and reduces the abundance of cDC2s, which present mainly exogenous antigens (Wen et al., 2022). Moreover, in EAC from Western cohorts, NACT counteracts the pre-treatment trend of pDC expansion and cDC reduction (Croft et al., 2022). The above findings demonstrate that neoadjuvant anticancer therapies can augment both the abundance and functional capacity of specific anti-tumor cDC subtypes. However, pDCs decrease in frequency following treatment, and the underlying mechanisms for this reduction require further investigation.

In anti-tumor immunity against ESCC, cDCs are responsible for antigen presentation and are involved in immune regulation (Zheng et al., 2020; Zhang et al., 2021; Shi et al., 2024). A subset of DCs expressing *LAMP3* has been extensively studied recently; some studies classify it as cDCs, while others do not (Zheng et al., 2020;

Wen et al., 2022). This subset highly expresses the DC activation markers *LAMP3* and *CCR7* (Shi et al., 2022a), and exhibits a higher activation and migratory capacity than other DC subsets in ESCC and hepatocellular carcinoma (Zhang et al., 2019a; Zheng et al., 2020). Activated DCs are generally beneficial for anti-cancer processes and are associated with a favorable prognosis in ESCC (Shi et al., 2022a). However, $LAMP3^+$ DCs express various immunoregulatory molecules, including IDO1, EBI3, CD274 (PD-L1), and IL10, imparting a tolerogenic phenotype that can suppress anti-cancer immune responses (Maier et al., 2020; Zheng et al., 2020). $LAMP3^+$ cDCs can also interact with moderately activated/exhausted $CD8^+$ T cells via immune checkpoint pairs such as TIGIT-NECTIN2, contributing to an immunosuppressive TME. The immunosuppression is improved following NACT (Wen et al., 2022). Furthermore, $LAMP3^+$ DCs highly express *RELB*, whose downstream targets are associated with cell migration and DC differentiation (Zheng et al., 2020). RELB also regulates the development of cDCs through a hematopoietic extrinsic mechanism (Briseño et al., 2017). In conclusion, $LAMP3^+$ DCs exhibit dual functional characteristics: both immunostimulatory (anti-cancer) and tolerogenic (pro-cancer), and play a role in regulating DC development.

tDCs exert immunosuppressive effects by suppressing $CD8^+$ and $CD4^+$ T cells in the TME of ESCC. The abundance of tDCs is elevated in tumor tissues compared to normal tissues (Zhang et al., 2021; Shi et al., 2024), and increased tDC infiltration is positively correlated with poor prognosis in ESCC (Shi et al., 2024). Functionally, tDCs exist in a semi-mature state: they retain the ability to present antigens but lack cytokine secretion capacity (Zhang et al., 2021). Additionally, tDCs highly express CCR7, a key chemokine receptor involved in both immune activation and tolerance (Förster et al., 2008; Shields et al., 2010; Wculek et al., 2020). Nonetheless, the role of tDCs in immunity appears to be predominantly immunosuppressive. For instance, tDCs highly express multiple immune checkpoint genes, including *PD-L1* (Zhang et al., 2021). tDCs also inhibit the proliferation of $CD8^+$ T cells and reduce their production of the cytokines IL-12 and IFN-γ (Zhang et al., 2021). Moreover, DCs treated with IFN-γ and LPS upregulate *PD-L1* and *IDO*, acquiring a tDC-like phenotype. When co-cultured with $CD4^+CD45RA^+$ naive T cells, their ability to induce T cells to express *FOXP3* is enhanced (Zheng et al., 2020). In other words, tDCs can induce the differentiation of naive T cells into Tregs, thereby promoting an immunosuppressive TME. Collectively, tDCs interact with both $CD8^+$ T cells and $CD4^+$ T cells, promoting their differentiation into exhausted or regulatory phenotypes, thereby suppressing anti-tumor immunity.

In contrast, research on pDCs is relatively limited. pDCs exhibit a dual role in tumor immunity: on the one hand, they are involved in the expansion of Tregs; on the other hand, they can exist in an activated state that exerts immune effects. pDCs specifically express the ICOS ligand, which is involved in the expansion of Tregs (Zhang et al., 2021). Additionally, the differentially expressed genes (DEGs) of pDCs are enriched in pathways related to “ribonucleoprotein complexes”, which may be associated with their plasmacytoid characteristics (Shi et al., 2024). The abundance of pDCs in ESCC samples is higher than in normal samples, yet their presence is associated with a favorable prognosis (Shi et al., 2024). This suggests that pDCs within the TME of ESCC may adopt an activated state capable of exerting anti-tumor immune effects. Similarly, an increased abundance of pDCs was also observed in EAC samples from Western cohorts, a phenomenon that was reversed after chemotherapy (Croft et al., 2022). The currently understood molecular mechanisms of pDCs, including their immune regulatory pathways and cellular characteristics, remain only partially elucidated and are insufficient to fully explain their functional complexity. The precise mechanisms underlying pDC function in anti-tumor immunity and their complex interactions with other immune components require further exploration. The interactions among DCs, tumor cells, and T cells are shown in Fig. 5.

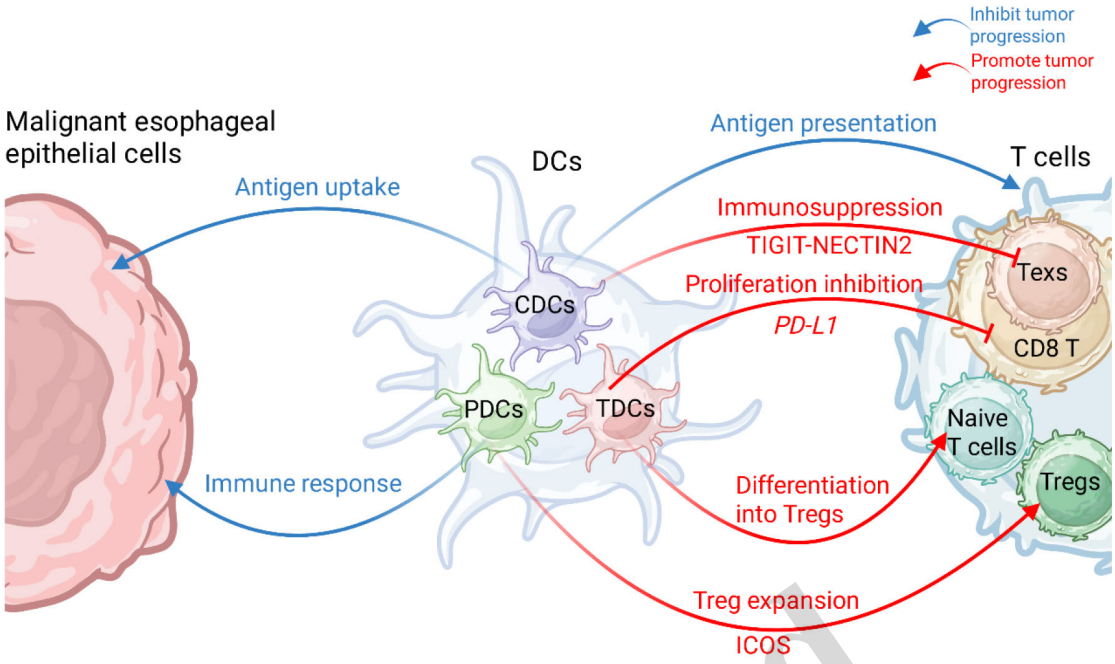

Fig. 5 The dendritic cell (DC)-centered cellular interactions within the TME of esophageal cancer
Red and blue lines denote interactions that promote and inhibit tumor progression, respectively. The line orientation denotes the direction of action. Arrows and inhibitory marks denote promotive and inhibitory actions, respectively. The effect of each interaction is shown above the line, with the involved molecules or pathways listed below. The figure was created with BioRender (https://BioRender.com).

Regarding the relationship between DCs and TLS in ESCC, infiltrating DCs are found at higher densities in TLS-positive tumors and are localized mainly within the GCs of TLS (Nakamura et al., 2023a). Furthermore, compared with TLS-negative tumors, TLS-positive tumors exhibit enhanced transcriptional signatures related to DC activation and migration and antigen presentation (Nakamura et al., 2023a). Moreover, follicular dendritic cells (fDCs), which play a critical role in antibody affinity maturation and long-term immune memory, are arranged in a reticular pattern within TLS. In ESCC samples with mature TLS (classified as $MTLS^{+}$, representing the most advanced stage of TLS maturation), the abundance of fDCs is higher than in $MTLS^{-}$ tumors (Huang et al., 2024). In surgery-alone (SA) and neoadjuvant chemotherapy (NCT) groups, the proportion of fDCs showed a positive correlation with both OS and disease-free survival (DFS) (Huang et al., 2024). In summary, TLS exert anti-tumor effects by recruiting and activating DCs, and by supporting fDCs to promote adaptive immunity. However, further studies are required to elucidate the underlying molecular mechanisms.

2.5 Cancer associated fibroblasts (CAFs)

Fibroblasts are the most prevalent cell type in human connective tissues, responsible mainly for synthesizing ECM components, such as collagen and elastic fibers. They play a crucial role in maintaining tissue structure and facilitating tissue repair and regeneration (Tomasek et al., 2002; Parsonage et al., 2005; Kalluri and Zeisberg, 2006; Watt and Fujiwara, 2011). Within the TME, fibroblasts can be activated by tumor cells and transformed into CAFs, which in turn promote tumor growth, angiogenesis, invasion and metastasis, as well as ECM remodeling (Mao et al., 2021).

In ESCC, four CAF subsets (CAF1, CAF2, CAF3, and CAF4) have been identified and broadly categorized into two functional groups. The first group, comprising CAF1 and CAF2, expresses pro-inflammatory chemokines (e.g., CXCL1 and CXCL6) and other cytokines that promote immune cell recruitment and induce an activated inflammatory state. The second group, consisting of CAF3 and CAF4, is characterized by high expression of myofibroblast marker genes, including *TAGLN*, *ACTA2*, and *ACTG2* (Zhang et al., 2021). Cancer-associated myofibroblasts (myCAFs) exert tumor-promoting effects in ESCC. MyCAFs are more abundant in ESCC tissues than in normal tissues (Chen et al., 2021), and their prevalence is further increased in advanced ESCC relative to early-stage ESCC (Zhang et al., 2021). High expression levels of two myofibroblast-specific genes, *MRVI1* and *MYLK*, are associated with poor prognosis in ESCC patients (Shi et al., 2022a). Additionally, $MMP3^{+}IL24^{+}$ myofibroblasts promote the metastasis of ESCC by degrading ECM and facilitating angiogenesis (Guo et al., 2025). As cancer progresses, CAFs exhibit a transition from the first to the second group (Zhang et al., 2021). The TGF-β pathway is prominently activated in CAF1 and CAF2 subsets, driving the transition of fibroblasts to myofibroblasts (Webber et al., 2015; Zhang et al., 2021). PDGFB secreted by endothelial cells also recruits pericytes through the PDGFB-PDGFRB pathway and induces their transformation into myofibroblasts (Zhang et al., 2021). In conclusion, during ESCC progression, CAFs appear to be gradually domesticated and transformed into myCAFs that support tumor growth and invasion.

More importantly, CAFs directly interact with ESCC cells. For example, CAFs communicate specifically with $KRT15^{+}$ tumor cell subpopulations (Li et al., 2023a). Notably, GSVA revealed that myofibroblasts exhibit upregulation of the apical junction related pathways (Chen et al., 2021; Zhang et al., 2021). The apical junction, essential for maintaining epithelial polarity, barrier function, and intercellular communications between epithelial and endothelial cells, has been implicated in tumor invasion and metastasis (Takahashi et al., 2022). MDK (midkine) was upregulated in esophageal epithelial cells during the malignancy change and interacted with NCL (nucleolin) in CAFs to promote their proliferation (Liu et al., 2023a). These findings further support the existence of functional crosstalk between CAFs and tumor cells in ESCC. To further elucidate the critical interactions between CAFs and epithelial cells, the concept of the CAF-Epi niche has been proposed, defined as a unique cellular compartment composed of CAFs and invasive epithelial cells (Chang et al., 2025). The CAF-Epi niche promotes the synthesis of CXCL1/CXCL8 through the *JAG1-NOTCH1* signaling pathway, thereby recruiting and activating CAFs. Additionally, a dense fibrotic ECM is formed around the CAF-Epi niche, as a barrier to impede immune cell infiltration and suppress their anti-tumor function (Chang et al., 2025). Through the least absolute shrinkage and selection operator (LASSO) Cox regression, a "CAF-Epi-immune" score based on 26 genes was established, which shows a strong correlation with prognosis in ESCC. Furthermore, the CAF-Epi niche has potential prognostic significance in pan-squamous cell carcinoma (Chang et al., 2025). Collectively, CAFs promote ESCC progression through multiple mechanisms, including direct paracrine signaling to cancer cells and the remodeling of a dense and fibrotic ECM, which not only fosters immunosuppression but also creates a physical barrier that impedes drug delivery.

CAFs also participate in multiple biological processes by secreting exosomes, thereby promoting the proliferation, invasion, and migration of ESCC cells. Two key differentially expressed exosomal proteins (TNFRSF10B and ILF3) were used to construct an effective prognostic model based on myCAF-derived exosomal proteins (Wang et al., 2023b). LINC01410, secreted by CAF-derived exosomes, can also promote metastasis and epithelial-mesenchymal transition (EMT) by sponging miR-122-5p and increasing the level of PKM2 in ESCC cells (Shi et al., 2022b). In addition, the exosome-derived miR-3656 induces the downregulation of ACAP2, which further promotes the activation of the PI3K/AKT and β-catenin signaling pathways, ultimately facilitating the occurrence and progression of ESCC (Jin et al., 2021).

Various prognostic models have been established based on CAFs (Ren et al., 2023; Wang et al., 2023b; Huang et al., 2025). For example, in a model established using myCAFs, the favorable prognosis group showed an increased infiltration of immune cells, including DCs, NK cells, and $CD4^{+}$/$CD8^{+}$ T cells, most of which are associated with anti-tumor immunity (Pittet et al., 2023; Zhao and Wucherpfennig, 2024). These findings suggest potential functional crosstalk between CAFs and immune cells within the TME. The number of Treg

cells in ESCC was positively correlated with CAF subsets 2, 3 and 4, suggesting a potential interaction between CAFs and Tregs (Zhang et al., 2021). In a mouse model of ESCC, fibroblasts promote immune cell recruitment by upregulating complement components and chemokine expression (Yao et al., 2020). Specifically, fibroblast-derived PI16 induces the differentiation of naïve $CD4^+$ T cells into Tregs through a DOCK2-dependent mechanism, thereby promoting the formation of an immunosuppressive TME (Suo et al., 2025). $CST1^+$ myofibroblasts regulate Tregs via the TGF-β pathway, and their abundance is correlated with shorter OS and DFS (Dinh et al., 2021). Fibroblasts also receive immune signals from $CD8^+$ Texs and Tregs through GZMA and its receptor F2R, which may be involved in the regulation of immunosuppression in the TME (Liu et al., 2023a). Furthermore, during the progression of ESCC, the CAF-Epi niche supports the differentiation of $SPP1^+$ macrophages, which are considered a pro-tumorigenic subset (Chang et al., 2025). In summary, specific CAF subsets in the TME of ESCC have been found to interact with immune cells such as $CD8^+$ Tex, Tregs, and $SPP1^+$ macrophages. Potential interactions with other immune cells, including NK cells and DCs, remain to be elucidated.

A novel subset of CAFs, termed antigen-presenting fibroblasts (AP-Fibros), has been widely identified across multiple malignancies, including ESCC, pancreatic cancer, and gastric cancer, as well as in normal tissues. This subset is uniquely characterized by the exclusive expression of *MHC II* genes compared with other fibroblast populations (Elyada et al., 2019; Dominguez et al., 2020; Dinh et al., 2021; Song et al., 2025). AP-Fibros preferentially localizes in the vicinity of TLS (Dinh et al., 2021; Song et al., 2025). Although they express MHC-II, the levels of co-stimulatory genes in AP-Fibros are lower than those in canonical antigen-presenting cells, suggesting a distinct mode of immune regulation (Dinh et al., 2021). Mechanistically, AP-Fibros promote T cell activation, enhance their cytotoxicity and proliferative capacity, and induce macrophage polarization toward a pro-inflammatory phenotype. In turn, these polarized macrophages further support the differentiation and maintenance of AP-Fibros, establishing a positive feedback loop to amplify the anti-tumor immune response (Song et al., 2025). In conclusion, AP-Fibros interact with both T cells and macrophages to coordinately promote anti-tumor responses, though the full scope of their mechanisms requires further elucidation. Figure 6 describes the interactions among CAFs, tumor cells, and T cells.

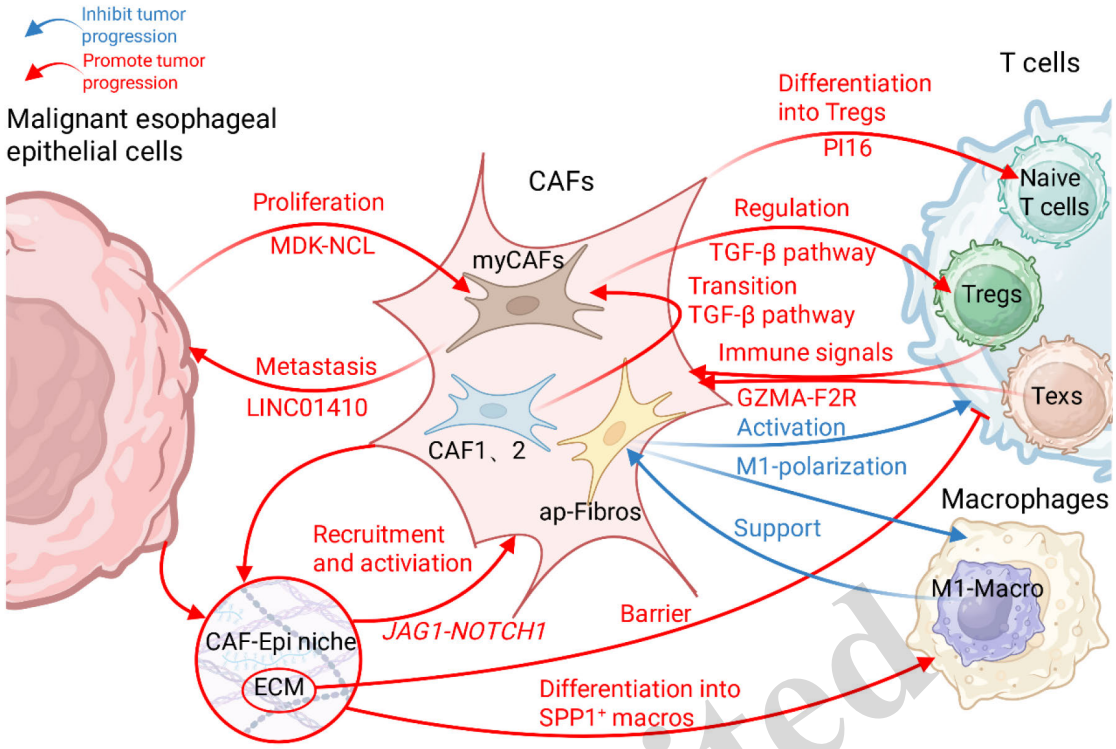

Fig. 6 Cancer-associated fibroblast (CAF)-centered cellular interactions within the TME of esophageal cancer

MyCAFs: myofibroblastic cancer-associated fibroblasts. AP-Fibros: antigen-presenting fibroblasts. Red and blue lines denote interactions that promote and inhibit tumor progression, respectively. The line orientation denotes the direction of action. Arrows and inhibitory marks denote promotive and inhibitory actions, respectively. The effect of each interaction is shown above the line, with the involved molecules or pathways listed below. The figure was created with BioRender (https://BioRender.com).

2.6 Dynamic Regulation of Core Cellular Composition in the Esophageal Cancer TME Under Therapeutic Interventions

From the characteristic analysis of core cell lineages (including T cells, B cells, TAMs, DCs, and CAFs) in the EC TME discussed above, it can be seen that clinical interventions such as neoadjuvant chemotherapy, chemoradiotherapy, and immunotherapy do not only target tumor cells themselves, but also significantly reshape the cellular composition and functional status of the TME, and this reshaping effect exhibits distinct subtype-specific differences between ESCC and EAC. To summarize the dynamic patterns of change in these key cell lineages across different treatment phases, the core findings are compiled in Table 1.

Table 1 Dynamics of key cell types in the tumor microenvironment of ESCC/EAC in response to different treatments

Cell Type	Pre-Treatment Status	Post-Neoadjuvant Chemotherapy (NACT)	Post-Neoadjuvant Chemoradiotherapy (neoCRT)	Post-Neoadjuvant Immunotherapy (NACI)
ESCC-T cells	High $CD8^+$ Tex & Tregs; decreased Th1, increased Th2/Th17	Not available	Increased $CD8^+$ Tex; decreased $CXCL13^+CD8^+$ T cells; reduced Treg/Th ratio	Increased total T cells; decreased Tregs

ESCC-B cells	Low abundance, migrated to stroma; plasma cells dominant in ESCC tissues	Decreased IGHD (naive B cell marker); B cell differentiation into ASCs	Not available	Not available
ESCC-TAMs	Coexistence of M1/M2-like phenotypes; M1→M2 transition; $CD163^{-}CD68^{+}$ macrophages correlated with Tregs	Not available	Coexistence of M1/M2-like phenotypes; correlation with Tregs maintained	High proportion of M1-like $C1QC^{+}$ TAMs
ESCC-DCs	High cDCs/pDCs; immunosuppressive interaction between $LAMP3^{+}$ cDCs and $CD8^{+}$ T cells	Reduced immunosuppressive interaction between $LAMP3^{+}$ cDCs and $CD8^{+}$ T cells	Promoted cDC1 maturation; decreased cDC2	Not available
ESCC-CAFs	High myCAFs; subsets 2/3/4 correlated with Tregs; presence of AP-Fibros	Not available	Not available	Not available
EAC-T cells	High Tregs	Decreased $CD8^{+}$ T cell cytotoxicity, exhaustion & checkpoint expression	Decreased Tregs proportion	Not available
EAC-B cells	Decreased $S100A10^{+}$ switched memory B cells; increased IgA/IgG-secreting plasmablasts	Inhibited B cell differentiation into ASCs; increased total B cell count	Not available	Not available
EAC-DCs	pDC expansion, cDC reduction	Reversal of pre-treatment pDC expansion & cDC reduction	Not available	Not available

Abbreviations: ESCC: esophageal squamous cell carcinoma, EAC: esophageal adenocarcinoma, Tex: exhausted T cells, Tregs: regulatory T cells, ASCs: antibody-secreting cells, TAMs: tumor-associated macrophages, DCs: dendritic cells, CAFs: cancer-associated fibroblasts.

Table 1 above clarifies the heterogeneous responses of TME cells to different treatments between ESCC and EAC and provides a direct reference for exploring subtype-specific therapeutic targets. However, note that the data are derived mainly from bulk RNA-seq and scRNA-seq studies, and the spatial distribution characteristics of these cells during treatment still require further validation through spatial transcriptomics evidence.

3 Discussion

Esophageal cancer is a globally prevalent gastrointestinal malignancy, with a notably high incidence in certain regions, such as China. Due to the lack of specific measures for early diagnosis and effective treatment, EC has a poor prognosis and a high mortality rate (Nakamura et al., 2023a). Since the TME regulates tumor cell proliferation, invasion, metastasis, and response to therapy through dynamic crosstalk with tumor cells, it is a pivotal determinant of tumor initiation and progression, clinical prognosis, and the formulation of therapeutic

strategies. However, due to the strong heterogeneity of the TME, it is challenging to dissect tumor immune regulatory mechanisms, develop precise medical strategies, and evaluate therapeutic responses (Dinh et al., 2021; Zhang et al., 2021).

Single-cell RNA sequencing overcomes the limitations of bulk RNA sequencing by preserving cell-specific gene expression profiles, thereby enabling a more detailed characterization of the TME in EC. Spatial transcriptomics technology, on the other hand, enables the analysis of cellular gene expression profiles while preserving the original spatial positional information of cells within tissues. Although scRNA-seq and spatial transcriptomics have revealed extensive cellular heterogeneity within the TME and enabled the identification of novel cell subtypes and subsets, classification systems remain inconsistent across studies. Due to high TME heterogeneity and variations in analytical algorithms, definitions and functional annotation of putatively identical cell subtypes often differ considerably. For instance, among macrophage subtypes, transitional states showing hybrid features of both M1- and M2-like phenotypes are frequently observed. Currently, the lack of precise classification criteria for these intermediate cells makes it challenging to systematically elucidate their functional roles and integrate findings regarding their impact on tumor progression (Wen et al., 2022). Note that cell subtypes may not simply exist as discrete “either/or” states; rather, they might actually form continuous lineage differentiation trajectories. Thus, in the future, it may be necessary to aggregate a large volume of single-cell sequencing EC data to construct a more standardized and comprehensive cell subtype annotation system.

Inter-patient heterogeneity also represents a significant challenge. Due to differences in genetic backgrounds, etiological factors of onset, clinical stages, and other aspects among EC patients, the TME exhibits highly individualized differences. For instance, studies have found that the heterogeneity level of the ESCC ecosystem is associated with patients’ gender, age, and alcohol consumption status (Zhang et al., 2021; Yan et al., 2024). Most studies rely on single-center cohorts with limited sample sizes, which often fail to capture the full spectrum of clinical phenotypes. To derive generalizable insights, multi-center collaborations are essential to integrate larger and more diverse datasets. Such efforts will enable systematic analysis of the relationships among TME cellular composition, molecular characteristics, and clinical outcomes among different patients, ultimately laying the groundwork for precision medicine strategies.

Additionally, regarding the downstream signaling pathways of cellular crosstalk in the TME of EC, only some key nodes have been identified, and how these pathways integrate to form a regulatory network remains unclear. For example, the molecular mechanisms underlying signal transmission between immune cells and tumor cells, whether relying simply on a few pathway cascades or functioning through a complex network of synergistic interactions, remains ambiguous. From a holistic perspective, the construction of the TME involves highly complex and integrated cellular interactions that remain insufficiently understood. Within the TME, diverse cell types, including tumor cells, immune cells, stromal cells, and other components, do not act in isolation but engage through paracrine signaling, direct contact, and other mechanisms. However, how these interactions synergistically drive the formation of a microenvironment that either supports or suppresses tumor progression remains incompletely understood. Constructing a comprehensive and dynamic model of these cellular crosstalks will be essential for guiding future mechanistic and therapeutic studies.

The construction of predictive models based on key genes has become a common approach in this field. However, the foundational criteria for building these models are highly inconsistent across studies (Ren et al., 2023; Shi et al., 2024; Chang et al., 2025). Their performance also varies considerably when applied to evaluate prognosis and treatment response in EC. To improve accuracy and clinical relevance, there is a growing requirement to integrate multi-omics data, including genomics, transcriptomics, proteomics, and other layers of molecular information, into model development. Such integrated models should ultimately capture the complex interplay among TME cellular composition, molecular characteristics, and clinical outcomes to better inform patient stratification and therapeutic strategy.

Across studies, technical heterogeneity can lead to inconsistent results. Specifically, dissociation bias may distort the in situ cellular composition. Variations in tissue preservation methods, for example comparing

formalin-fixed paraffin-embedded (FFPE) samples with fresh tissues, can affect transcriptomic fidelity. Additional factors that can introduce substantial bias include differences in detection platforms (e.g., 10x Genomics and Smart-seq for single-cell RNA sequencing; Visium and CosMx/MERFISH/GeoMx in spatial transcriptomics), which differ in resolution and gene-capture efficiency; inconsistent batch-correction and doublet-detection approaches; and the widespread absence of matched spatial validation. Addressing these sources of technical variability is therefore essential for improving the comparability and robustness of transcriptomic studies in EC.

While single-cell sequencing has advanced our understanding of the TME in EC, further breakthroughs remain to be made in several key areas, including refining cell subtype classification, deciphering cellular heterogeneity, elucidating functional pathways, designing integrative models of microenvironmental crosstalk, developing clinically applicable predictive tools, and addressing technical variability across studies. Advances in these domains will accelerate the translation of basic findings into clinical practice and provide a more robust foundation for precise diagnosis and treatment of EC.

In summary, in this review we have synthesized key research findings on the TME of EC over the past five years, leveraging emerging technologies, such as scRNA-seq. By tracing the major cellular components within the TME, we have outlined both promotive and inhibitory roles of various cell types in anti-tumor immunity and the interactions between cell subtypes, providing a reference for exploring further the regulatory mechanisms of the TME in EC and developing novel therapeutic strategies.

Data availability statement

This is a review article that does not present original research data. All relevant data and findings summarized herein are derived from previously published studies cited in the reference list. The original datasets can be accessed through the corresponding Digital Object Identifiers (DOIs) or public repositories provided by the authors of the cited works. For further inquiries regarding data sources, please contact the corresponding author.

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Author contributions

Zongru ZHOU: investigation, visualization, writing – original draft, writing – review and editing. **Jiaxin LIU**: investigation, writing – review and editing. **Jie JIA**: visualization, writing – review and editing. **Ziyi ZHANG**: writing – review and editing. **Zhuoyu CHENG**: writing – review and editing. **Xueyi JIANG**: writing – review and editing. **Chunyan LI**: conceptualization, supervision, writing – review and editing, funding acquisition. All authors have read and approved the final manuscript.

Compliance with ethics guideline

Zongru ZHOU, Jiaxin LIU, Jie JIA, Ziyi ZHANG, Zhuoyu CHENG, Xueyi JIANG and Chunyan LI declare that they have no conflict of interests.

This review does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the manuscript preparation, the authors used the online AI tools Doubao (https://www.doubao.com) and DeepSeek (https://chat.deepseek.com) exclusively to optimize language expression and enhance readability, with no involvement in the generation of original data or core content. The authors have thoroughly reviewed, edited, and verified all content to ensure its accuracy, academic rigor, and consistency with the research context. The authors take full responsibility for the final manuscript.

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