

# Esophageal Microenvironment: From Precursor Microenvironment to Premetastatic Niche

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**Abstract:** Esophageal cancer (EC) is the sixth most deadly cancer, and its incidence is still increasing year by year. Although the researches on the molecular mechanisms of EC have been widely carried out and incremental progress has been made, its overall survival rate is still low. There is cumulative evidence showing that the esophageal microenvironment plays a vital role in the development of EC. In precancerous lesions of the esophagus, high-risk environmental factors can promote the development of precancerous lesions by inducing the production of inflammatory factors and the recruitment of immune cells. In the tumor microenvironment, tumor-promoting cells can inhibit anti-tumor immunity and promote tumor progression through a variety of pathways, such as bone marrow-derived suppressor cells (MDSCs), tumor-associated fibroblasts (CAFs), and regulatory T cells (Tregs). The formation of extracellular hypoxia and acidic microenvironment and the change of extracellular matrix stiffness are also important factors affecting tumor progression and metastasis. Simultaneously, primary tumor-derived cytokines and bone marrow-derived immune cells can also promote the formation of pre-metastasis niche of EC lymph nodes, which are beneficial to EC lymph node metastasis. Further research on the specific mechanism of these processes in the occurrence, development, and metastasis of each EC subtype will support us to grasp the overall pre-cancerous prevention, targeted treatment, and metastatic assessment of EC.

**Keywords:** esophageal cancer, tumor precursor microenvironment, tumor microenvironment, premetastatic niche

## Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-related deaths and one of the eight most common malignancies. According to different tissue subtypes, it can be mainly divided into esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). The incidence rate of ESCC is increasing globally, but it is rapidly rising in some European countries for EAC and has even exceeded incidence rate of ESCC in some regions,<sup>1,2</sup> which may be related to different lifestyles in different countries. Although the diagnosis and treatment of EC have been gradually improved, the five-year survival rate (19%) for EC is the same as that for lung cancer (19%) and is second only to liver cancer (18%) and pancreatic cancer (9%)<sup>3</sup> according to the latest statistical analysis. This is mainly due to the late diagnosis and metastatic tendency.<sup>4</sup> The current standard treatments of EC mainly include chemoradiotherapy, surgery, and endoscopic resection.<sup>5</sup> Although the Food and Drug Administration (FDA) has approved some targeted drugs such as programmed cell death protein 1 (PD-1) inhibitors, human epidermal

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growth factor receptor-2 (HER2), and anti-vascular endothelial growth factor (VEGF) monoclonal antibodies for the treatment of EC, the survival rate of most patients with advanced EC is still low,<sup>6</sup> which requires us to conduct a more systematic evaluation of the exact molecular pathogenesis of EC in order to develop novel biomarkers and therapeutic targets.

According to previous reports, the dynamic process of cancer immunity includes three steps: elimination, equilibrium, and escape.<sup>7</sup> These steps can be corresponding to the occurrence, development, and invasion of cancer.<sup>8</sup> Similarly, we assume that the EC dynamic microenvironment could be roughly divided into three stages: tumor precursor microenvironment, tumor microenvironment (TME), and pre-metastatic niche. TME is a vital space for the metabolism of tumor cells. Nowadays, numerous studies have shown that TME could mainly promote the growth, proliferation, migration, and metastasis of EC. The main components of TME are a variety of adaptive and innate immune cells, fibroblasts, adipocytes, endothelial cells, and extracellular matrix (ECM) components, which have been extensively studied in a variety of tumors.<sup>9-12</sup> TME is not a fixed tumor survival environment, but a dynamic environment that is constantly remodeled by tumors and tumor-related cells to adapt to the survival of tumor cells. Therefore, the tumor precursor microenvironment and pre-metastasis niche should also be taken seriously as the microenvironment before tumorigenesis and pre-metastasis, respectively. A comprehensive and systematic study of this dynamic process will not only enable us to better understand the occurrence and development of EC but also provide us with a variety of therapeutic targets and biomarkers, and guidance for future diagnostic and therapeutic directions. In this review, we summarized the main research advances in EC tumor precursor microenvironment, TME, and premetastatic niche.

## Tumor Precursor Microenvironment Barrett's Esophagus (BE) Microenvironment

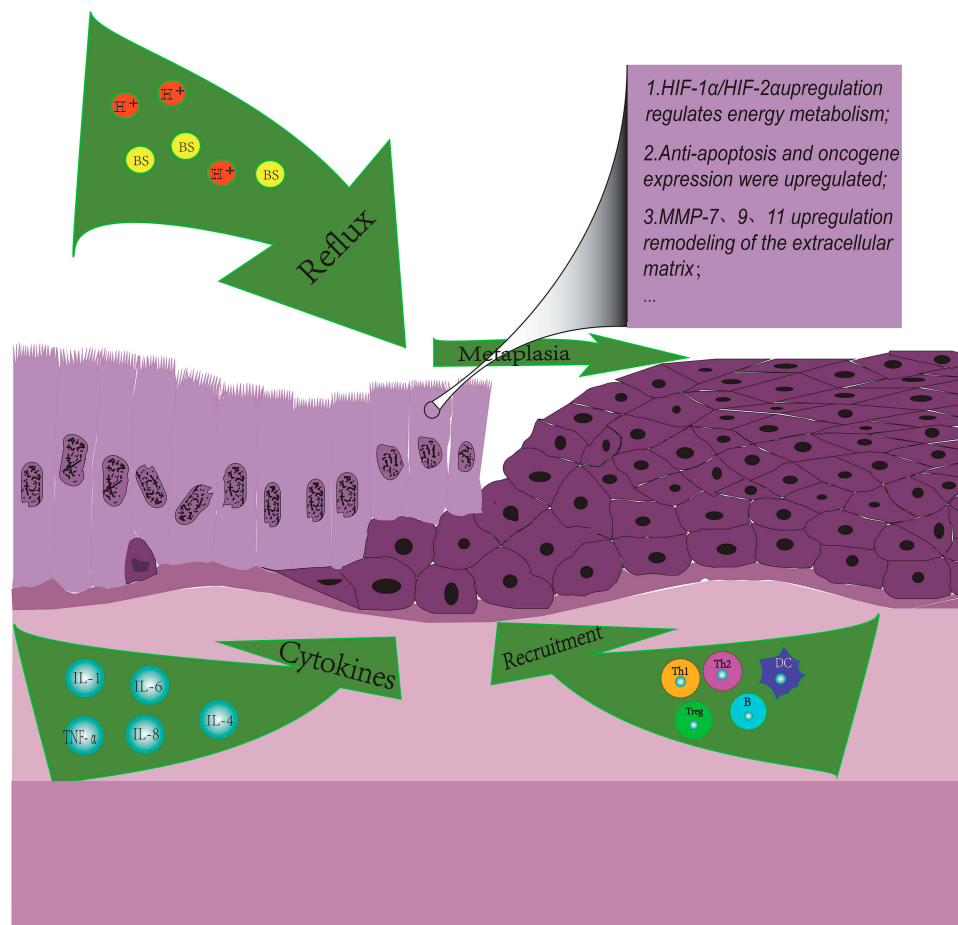
BE is a well-known precancerous lesion of EAC, and high-risk environmental factors are the promoters of this process. We also found that TME-like changes have partially occurred in the BE microenvironment (Figure 1) as follows: the production of various inflammatory factors, immunity cell infiltration, remodeling of ECM, etc.

The tumor hypoxia microenvironment may be formed in BE previously. Studies have shown that esophageal

ulcers and ischemia caused by reflux can result in hypoxia in injured esophageal tissues and increase the expression of hypoxia-induced factor 1 $\alpha$  (HIF-1 $\alpha$ ), which regulates the expression of VEGF and promotes angiogenesis and ulcer healing in turn.<sup>13</sup> However, we are not sure whether HIF-1 $\alpha$  is friend or foe in BE. For example, in other studies, it has been demonstrated that HIF-1 $\alpha$  could highly express in metaplastic esophageal epithelial cells, nevertheless, the expression of HIF-1 $\alpha$  did not increase further with the progress of BE epithelial dysplasia and was not associated with histopathological parameters or survival rate.<sup>14</sup> It is also worth mentioning that the level of glycolytic metabolism is more significantly increased in advanced BE cell lines than in early BE cell lines.<sup>15</sup> However, HIF-1 $\alpha$  is well known as the main regulator of tumor glycolysis metabolism. This process of BE cells continuously adapting to changes in the microenvironment by regulating energy metabolism pathways may be the origin of tumor aerobic glycolysis, but more evidence would be needed to verify this hypothesis.

The production of inflammatory factors has played an important role in the progress of BE. For example, gastroesophageal reflux can induce the up-regulation of inflammatory factors such as IL1B, IL4, and IL6. These inflammatory factors can activate cardia stem cells by inflammatory response and promote columnar epithelial metaplasia in the distal esophagus.<sup>16,17</sup> In addition, the inflammatory factor IL-6 can significantly increase the anti-apoptotic rate of Barrett epithelial cells in vitro through the IL-6/STAT3 pathway.<sup>18,19</sup> More than that, obesity, which is a high-risk factor for BE, can also up-regulate some inflammatory factors. Hypertrophic fat cells can lead to tissue hypoxia, which ultimately induces the up-expression of IL-6 and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ),<sup>20</sup> which can not only mediate the up-regulation of IL-8 expression, promote angiogenesis and migration of ECM by activating endothelial cells<sup>21,22</sup> but also increase the expression of  $\beta$ -catenin-mediated oncogene c-myc in BE epithelial cells.<sup>23</sup> It is noteworthy that TNF- $\alpha$  expression increases with the progression of metaplastic-proliferative-adenocarcinoma sequences.

Changes in the composition of immune cells are an important feature of BE microenvironment, and the formation of immunosuppressive BE microenvironment may be a major factor in the progress of BE. In a flow cytometric analysis, it was found that the proportion of immune cells in the BE microenvironment had decreased overall, and the immune environment had changed from a T-cell-based



**Figure 1** Immune landscape in Barrett's Esophagus. Influenced by environment factors, several immune cells and cytokines are involved in the progress of BE microenvironment. Cytokines IL-1B, IL-4, and IL-6 can activate cardiac stem cells to promote columnar metaplasia. Cytokine IL-6 can promote the anti-apoptosis of Barrett epithelial cells through the IL-6/STAT3 pathway. TNF- $\alpha$  can promote angiogenesis and the expression of oncogene c-myc by upregulating the expression of IL-8 and  $\beta$ -catenin, respectively. The proportion of overall immune cells and the ratio of Th1/Th2 in the BE microenvironment decreased significantly. MMP-7, MMP-9, and MMP-13 are up-regulated in BE epithelial cells and can promote cell migration and matrix remodeling.

microenvironment to a B-cell-based microenvironment.<sup>24</sup> Similar to EAC, the ratio of Th1/Th2 is significantly reduced in EC, and the Th2-based immune performance was shown in the BE microenvironment, which may promote tumorigenesis.<sup>25,26</sup> In addition, some studies have reported that the expression of forkhead box P3 (FOXP3) +, the major regulator of Tregs immunosuppressive function, was significantly up-regulated in BE tissues.<sup>27</sup> However, in the relatively early metaplasia stage of BE, acids and bile salts can stabilize the expression of HIF-2 $\alpha$  in esophageal epithelial cells, thereby upregulating pro-inflammatory cytokines and recruiting T lymphocytes and other lymphocytes to damage the esophagus through the Nuclear factor-kappaB (NF- $\kappa$ B) pathway.<sup>28,29</sup> In summary, the composition of immune cells in the BE microenvironment is relatively complex and constantly changing. Of course, more researches will be needed to elucidate the

development process and mechanism from early inflammatory infiltration injury to final immunosuppression.

The transformation of the ECM is also a major feature of changes in the BE microenvironment. Matrix metalloproteinases (MMPs) are enzymes involved in inflammation, ECM remodeling, and tumor metastasis. Previous studies have shown that MMP-7 in BE epithelial cells could stimulate stromal cell migration and invasion and remodel the microenvironment under the regulation of PI3-K kinases.<sup>30</sup> In addition, MMP9 and MMP13 are also up-regulated in BE.<sup>31</sup> It is noteworthy that MMP13 is more highly expressed in BE, while MMP-9 is more highly expressed in EAC. Once BE is converted to EAC, the expression ratio of MMP13/MMP9 in epithelial cells is significantly decreased. The study on the transition mechanism may be helpful in exploring novel therapeutic targets.

The BE microenvironment is also a complex inflammatory microenvironment. In the process of exploring its mechanism of inflammation microenvironment, the viewpoint is put forward of anti-inflammatory treatment of BE,<sup>22,27</sup> but the exact effect and specific mechanism need to be further clarified. The above discussions show that BE, as a precancerous lesion of EAC, is inextricably linked with EAC in the microenvironment. In-depth exploration and application of BE microenvironment in different stages will be a powerful method to prevent EAC.

### Esophageal Squamous Epithelium Dysplasia (ESD) Microenvironment

In a multi-region whole-exome sequencing, it was found that atypical proliferative epithelial cells have polyclonality, significant heterogeneity, and severe mutations similar to ESCC cells,<sup>32</sup> and a large amount of evidence indicates that ESD is a precursor lesion of ESCC.<sup>33</sup> Smoking, drinking, and eating very hot food or beverages are high-risk environmental factors for ESCC.<sup>34</sup> Although there are few studies on ESD microenvironment, we can still find some valuable information about that. For example, it was reported that glucose transporter 1 (GLUT-1), an important glycolytic pathway enzyme, is highly expressed not only in ESCC tumor cells but also in tumor precursor cells.<sup>35</sup> Similar to the above-mentioned BE, this may indicate that ESD cells also continuously adapt to changes in the microenvironment by transforming energy metabolism pathways. The occurrence of glycolysis and local inflammatory reactions will promote the oxygen consumption of the precursor microenvironment and the accumulation of acidic metabolites.<sup>36</sup> Under acidic conditions, mutations and heterogeneity of ESD cell may increase again, and further lead to the accumulation of carcinogenic mutations.<sup>37</sup> With the accumulation of carcinogenic mutations, the cell polyclonality also evolves, which increases the oxygen energy supply burden in the interstitial space, leads to a vicious cycle of hypoxia and acidic microenvironment, and eventually results in the occurrence of cancer and the formation of TME. The cumulative process of carcinogenic mutations is extremely long, which may also be the reason for the relatively long period of precancerous lesions.<sup>38</sup> However, more evidence will be needed to prove the evolution of this cycle. Finally, the occurrence of EC is the result of the interaction between the precancerous microenvironment and ESD. At present, there are many studies on the high-risk factors of ESCC, ESD itself and post-cancer, and we also should pay attention to the research on ESD

microenvironment. This will be a powerful weapon for our cancer prevention.

In short, the occurrence of cancer as a continuous and uninterrupted process, we should pay attention to all stages of its occurrence and development, and prevention as the “biblical” of humans against disease should receive more attention. At this time, the abundant signal molecules produced by the tumor precursor microenvironment and precursor cell interactions, as well as cell composition and function changes, have become our favorable biomarkers and targets for preventive treatment.

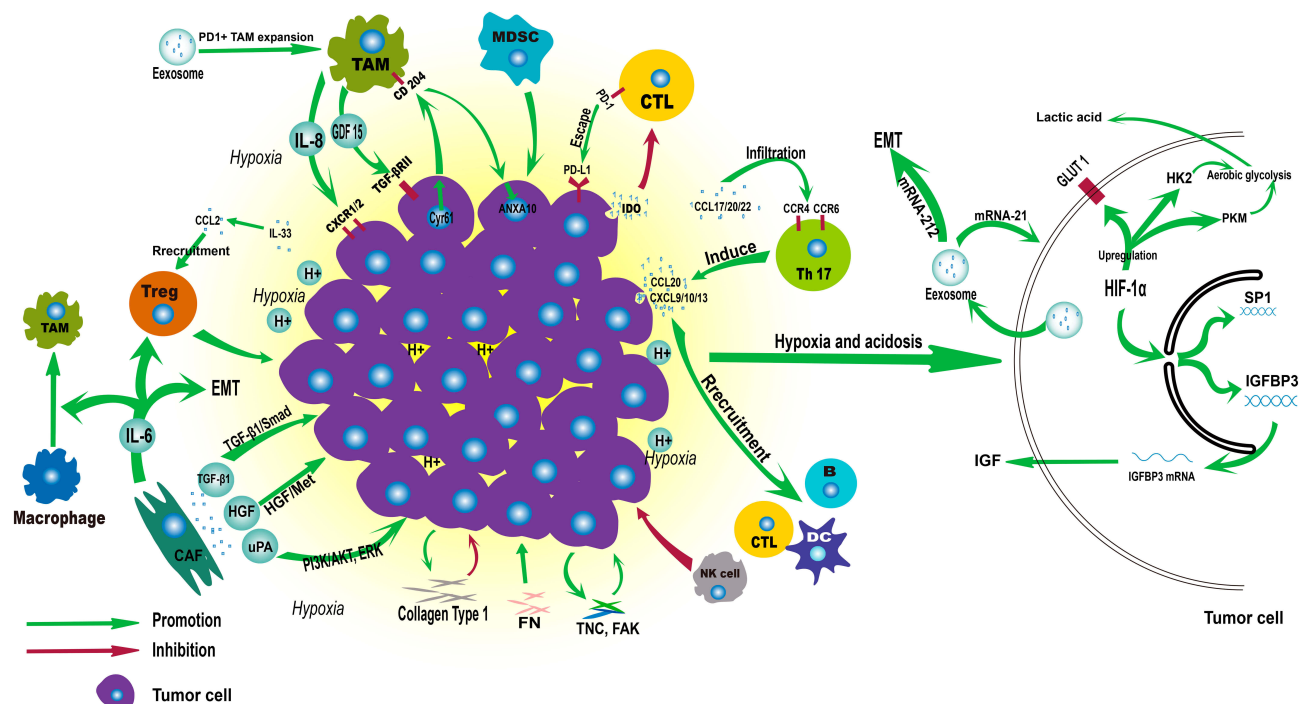
### Tumor Microenvironment

As the oncogenic mutation of tumor precursor cells continues to accumulate, the precancerous lesions further develop into cancer. Cancer tissues continue to deteriorate by interacting with TME and eventually lead to the occurrence of metastasis. In the following, we will review the research progression on the interaction between the main components of TME and EC tissue (Figure 2).

#### Hypoxia, Acidosis, and Nutrient Depletion are the Basic Characteristics of TME

Hypoxia and acidosis are common phenomenon in TME and can cause a series of metabolic changes in tumors. When we talk about the hypoxic microenvironment, the famous Warburg effect and HIF-1 should be mentioned. Under hypoxic conditions, prolyl-hydroxylase 2 (PHD-2) cannot hydroxylate HIF-1 $\alpha$ , which blocks the degradation of HIF-1 $\alpha$ .<sup>39,40</sup> The Warburg effect means that even in a microenvironment with sufficient oxygen, tumor cells obtain energy mainly through glycolysis rather than oxidative phosphorylation.<sup>41</sup> This metabolic transformation is caused by the HIF-1 transcriptional regulatory factors, which up-regulate GLUTs, hexokinase isoform 2 (HK2), pyruvate kinase isoform M (PKM) and other key factors of glycolytic metabolism, so as to transform the cancer metabolism into a mainly aerobic glycolytic metabolic pathway.<sup>40-42</sup> The theory is also applicable to EC. For instance, GLUT-1 highly expressed in ESCC tumor cells and pre-cancerous tissues of dysplasia,<sup>35</sup> in order to improve the efficiency of glucose transport in response to hypoxia microenvironment. PKM2, an important enzyme that converts phosphoenolpyruvate to pyruvate during glycolysis, can promote the aerobic glycolysis metabolism of ESCC under the regulation of the mTOR pathway.<sup>43</sup>

Another consequence brought about by glycolysis is the accumulation of its metabolite lactic acid in cells.<sup>44</sup> In



**Figure 2** Immune landscape of ESCC tumor microenvironment.

order to prevent intracellular pH imbalance, tumor cells trigger the proton transport mechanism to maintain the intracellular environment.<sup>45</sup> Carbonic anhydrase IX (CAIX) is a target gene of HIF-1 $\alpha$ , which mainly maintains the pH value of cells under hypoxic conditions. It is found that its expression increased in ESCC, and CAIX can also promote the migration and metastasis of ESCC cells while maintaining intracellular pH stability.<sup>46</sup> Elevated CAIX expression is predictive of greater aggressiveness and shorter survival of EC.<sup>47,48</sup> In addition, a recent EC study found that the vacuolar H<sup>+</sup>-adenosine triphosphatase (ATPase) subunit V0C (ATP6V0C), whose main function is to maintain a constant pH and induce acidification of organelles, can interact with PKM2 to upregulate the expression of glycolytic enzymes and increase extracellular acidification rate.<sup>49</sup> Finally, the accumulation of metabolites and the depletion of interstitial nutrients pose great challenges for immune cells to play an anti-tumor role in TME, but cancer cells themselves are not affected by microenvironment selection.<sup>40</sup>

Importantly, as a crucial intermediate molecule in the regulation of tumor metabolism, HIF-1 $\alpha$  also plays tumor-promoting functions in many different types of tumors, including EC, such as inducing and regulating angiogenesis,<sup>50,51</sup> promoting tumor growth, proliferation, recurrence and metastasis and remodeling ECM. First, the

regulation of HIF-1 $\alpha$  on angiogenesis in esophageal tissues has been reported in the BE microenvironment.<sup>13</sup> In EC, HIF-1 $\alpha$  protein expression is also significantly correlated with VEGF protein expression and related to poor prognosis of EC.<sup>52–54</sup> Secondly, as a key transcription factor in tumor cells, HIF-1 can specifically bind to various other promoters to regulate the progress of EC. For example, it can bind to the SP1 promoter and regulate its transcription program in order to enhance the ability of ESCC cells to migrate and invade and promote tumor recurrence and metastasis.<sup>55</sup> Other studies have mentioned that it also could bind to the hypoxic response element, which located at the upstream of the Insulin-like growth factor binding protein 3 (IGFBP3) transcription initiation site, and then induce continuous translation of IGFBP3 mRNA<sup>44</sup> and promote ESCC cell growth and proliferation through the insulin-like growth factor-independent pathway.<sup>56</sup> Finally, it can also directly bind to the promoter-specific hypoxic response element of ECM metalloproteinase inducer (EMMPRIN), and further promote EC cell migration and epithelial–mesenchymal transition (EMT).<sup>57</sup> In addition, another effect of HIF-1 $\alpha$  is to inhibit E-cadherin and increase the expression of MMP-2 to stimulate EC infiltration and metastasis,<sup>58</sup> and its mechanism may be related to the upstream regulation of Nuclear factor erythroid-2-related factor 2 (an important cytoprotective factor).<sup>59</sup> The

consistency of these results shows that HIF-1 $\alpha$  also has a regulatory effect on ECM components in EC TME. In summary, overexpression of HIF-1 can be produced for EC through a variety of regulatory mechanisms and is closely related to a variety of adverse prognostic factors such as tumor invasion depth, lymph node metastasis, and resistance to chemoradiation.<sup>60,61</sup> However, some studies have shown that HIF-1 $\alpha$  has different effects on the prognosis of ESCC and EAC, respectively.<sup>62</sup> This suggested that more detailed and precise researches were required on different cell and molecular subtypes.

In recent years, researches have particularly focused on extracellular vesicles (EVs). The EVs in tumor TME play an important role in the information transmission and material exchange of tumor cells and mediating the local or remote mutual regulation of cancer cells. Studies have shown that tumor cells can release more EVs in TME under hypoxic conditions.<sup>63</sup> According to the latest ESCC miRNA expression profiling analysis, the miRNAs in EVs secreted by ESCC cells would change significantly under hypoxic conditions. A total of 10,810 miRNAs were detected in EVs. Fifty miRNA was up-regulated and 34 miRNA was down-regulated significantly in hypoxic-treated cell lines, and these differentially expressed miRNAs are mainly involved in cancer-related and phospholipase D signaling pathways.<sup>64</sup> Base on the studies of kidney cancer and breast cancer cells, it was speculated that the enhanced phospholipase D activity was necessary for stable expression of HIF-1 and HIF-2 and aerobic glycolysis of cells.<sup>65–67</sup> Exosomes are special types of EVs with a diameter of 50–200nm. In previous studies, ESCC cell exosomes cultured under hypoxic conditions could promote the proliferation and invasion of endothelial cells in vivo and in vitro, and show significant angiogenic effects compared with non-hypoxic conditions.<sup>68</sup> ESCC-derived exosomes can also promote the expansion of PD-1 + TAM and the expression of CD206 to promote the progress of ESCC.<sup>69</sup> In addition to the study of the number and general function of exosomes, we are also committed to the study of the contents of exosomes such as miRNA, DNA, or protein molecules. For example, high expression of exosomal microRNA-21 in ESCC can significantly reduce the sensitivity of cisplatin to chemotherapy<sup>70</sup> and promote tumor cell migration and invasion.<sup>71,72</sup> Exosome microRNA-212 can promote the movement and invasion of ESCC cells by promoting EMT and degrading ECM.<sup>73</sup> Studies of exosomes in EC have been reported in detail in several reviews.<sup>74–76</sup> Although many studies have proved

that exosomes have great potential as markers for early diagnosis, treatment, or assessing prognosis in EC, there is no standard method for the isolation and purification of exosomes, and the functional mechanism of their inclusion bodies is not fully understood. In summary, anoxic micro-environment is the basis of important mechanisms of action in many tumors. Many targeted studies on HIF and its regulatory pathways, key enzymes of glycolysis, proton transport-related pathways, or exosomes have been actively carried out,<sup>77–79</sup> but there is still a huge gap in this field (Table 1).

## Myeloid Cell Line is an Important Regulatory Cell Population for TME

### MDSCs

**Table 2** MDSCs are immature bone marrow cells produced under persistent inflammatory conditions such as cancer, chronic disease, or persistent infection.<sup>80</sup> MDSC can be mainly divided into two subgroups: polymorphonuclear (PMN)-MDSC and monocyte (M)-MDSC.<sup>81</sup> The morphological and phenotypic characteristics of PMN-MDSC are similar to neutrophils, while M-MDSC is similar to monocytes.<sup>82,83</sup> Numerous studies have proven that MDSCs have a powerful inhibitory effect on tumor immunity, including inhibition of T cell activity<sup>84</sup> and NK cell function,<sup>85</sup> induction of Tregs activation,<sup>86</sup> promotion of fibroblasts to CAFs,<sup>87</sup> and tumor resistance. High infiltration of MDSCs is a poor prognostic marker for many types of tumors.<sup>81,88,89</sup>

On the one hand, the interaction of cytokines with MDSCs plays a key role in the progress of EC. These cytokines mainly include IL-4, IL-6, IL-8, IL-13, and so on. For example, tumor-associated gene MAEL is over-expressed in EC tissues and up-regulates the inflammatory factor IL-8 to promote the recruitment of PMN-MDSCs in tumor tissues for tumor-promoting, while the up-regulated PMN-MDSCs can promote MAEL expression through the TGF- $\beta$ /Smad pathway.<sup>90</sup> In addition, Th2-derived cytokines IL-4, IL-6, and IL-13 can significantly enhance the immunosuppressive function of MDSCs in EC by inducing the expression of arginase-1 (ARG1).<sup>81,89,91</sup> On the other hand, the heterogeneity of MDSCs is also paid special attention. A study found that MDSCs with high CD38 expression in EC had stronger activated T cell immunosuppressive and tumor-promoting functions, and the anti-CD38 monoclonal antibody Daratumumab could inhibit esophageal tumor cell growth in vitro and in vivo.<sup>92</sup> At present, Daratumumab has been clinically used to treat

**Table I** The Role of Hypoxia and Acidosis in the TME of Esophageal Carcinoma

Entry	Cancer Type	Finding	Effects on Tumors	Ref.
Hypoxia and acidosis	ESCC	Induction of high expression of GLUT-1 in response to hypoxia and insufficient energy supply	Acceleration	[35]
	ESCC	Regulation of the expression of the key aerobic glycolytic enzyme PKM2 through the mTOR pathway	Acceleration	[43]
	ESCC	The induction of high expression of CAIX maintains intracellular pH stability and promotes tumor cell invasion and migration	Acceleration	[46–48]
	EC	ATP6V0C up-regulates glycolytic enzyme expression and increases extracellular acidification rate through interaction with PKM2	Acceleration	[49]
	ESCC, EC	HIF-1 $\alpha$ and VEGF synergistically induce angiogenesis	Acceleration	[50–54]
	ESCC	HIF-1 $\alpha$ specifically binds to the SPI promoter and regulates its transcription program, thereby enhancing migration and invasion	Acceleration	[55]
	ESCC	HIF-1 $\alpha$ can bind to the hypoxic response element upstream of the IGFBP3 transcription initiation site, and then induce continuous translation of IGFBP3 mRNA and promote growth and proliferation through the insulin-like growth factor-independent pathway	Acceleration	[44,56]
	EC	HIF-1 directly binds to specific hypoxic response elements of the EMMPRIN promoter to promote EC cell migration and epithelial-mesenchymal transition	Acceleration	[57]
	EC	HIF-1 $\alpha$ can stimulate EC infiltration and metastasis by inhibiting E-cadherin and promoting MMP-2 expression	Acceleration	[58]
	ESCC	Up-regulation of exosomes in ESCC promote angiogenesis and PD1 + TAM expansion	Acceleration	[64,68,69]
	ESCC	MicroRNA-21 in exosomes induces chemotherapy resistance, promotes migration and invasion	Acceleration	[70–72]
	ESCC	MicroRNA-212 in exosomes promotes epithelial-mesenchymal transition and ECM degradation	Acceleration	[73]

multiple myeloma with acceptable safety and efficacy.<sup>93</sup> Therefore, the application of this drug in EC may be a promising treatment direction. The functional research of MDSCs in different subgroups may become a novel development direction.

#### Tumor-Associated Macrophages (TAMs)

Macrophages can differentiate into two cell types with completely different functions: tumor suppressor macrophages (M1) and tumor-promoting macrophages (M2). M1 macrophages play a role in tumor rejection as “classic” activated macrophages, while M2 macrophages can promote tumor progression. M2 macrophages can be activated and promoted by tumor cell Th-2 cytokines such as IL-4 and IL-13.<sup>94,95</sup> TAMs are M2-like macrophages, and some of them overexpress CD163, CD206, and CD204. The progression of EC can be promoted by TAMs through inducing

tumor-associated lymphangiogenesis,<sup>96</sup> tumor cell proliferation and invasion,<sup>97</sup> and EC immune escape.<sup>95</sup> It has been also demonstrated that TAMs infiltration in EC TME was a predictor of poor prognosis.<sup>98,99</sup>

The complexity of the interaction between TAMs and cancer cells also reveals its importance to cancer cells, and the expression of various cytokines is an important way for TAMs to participate in tumor regulation. For instance, several studies by Naoki et al have shown that in vitro cultured ESCC cell lines, Growth Differentiation Factor 15 (GDF15) derived from TAMs and tumor cells could indirectly activate TGF- $\beta$ R2 receptors in cancer cells, thereby inducing PI3K/Akt and MEK/Erk pathways and increase ESCC cell proliferation and migration.<sup>100,101</sup> In addition, TAM-like peripheral-blood monocyte (PBMo)-derived macrophages were found in co-culture to express IL-8 and phosphorylate the Akt and Erk1/2 pathways by binding to CXCR1,

**Table 2** The Role of Myeloid Cells in the TME of Esophageal Carcinoma

Entry	Cancer Type	Finding	Effects on Tumors	Ref.
MDSCs	EC	The tumor-associated gene MAEL over-expresses and up-regulates IL8 to recruit PMN-MDSCs, while the recruited PMN-MDSCs can up-regulate MAEL expression through the TGF- $\beta$ /Smad pathway	Acceleration	[90]
	EC	Th2 derived cytokines IL-4, IL-6 and IL-13 can significantly enhance the immunosuppressive function of MDSCs in EC by inducing the expression of ARG1	Acceleration	[81,89,91]
	EC	MDSCs overexpressing CD38 have stronger activated T cell immunosuppressive and tumor-promoting functions, and this effect can be inhibited by CD38 monoclonal antibody Daratumumab	Acceleration	[92]
TAMs	ESCC	GDF15 derived from TAMs activate TGF- $\beta$ RII receptor in cancer cells to induce the PI3K/ Akt and MEK/Erk pathways to increase proliferation and migration	Acceleration	[100,101]
	ESCC	Macrophage-derived IL-8 binds to CXCR1 and CXCR2 of ESCC cells and phosphorylates Akt and erk1/2 pathways to promote tumor migration and invasion	Acceleration	[102]
	ESCC	Cyr61 can be expressed in ESCC cells and TAMs, which promotes macrophage migration and CD204 expression in macrophages through the MEK/ERK pathway	Acceleration	[105]
	ESCC	CD204+TAM induced increased expression of ANXA10 in cancer cells and promoted ESCC cell proliferation through phosphorylated Akt and erk1/2 pathway	Acceleration	[106]
	EAC	Different TAMs subpopulations play diverse tumorigenic functions in EAC	Acceleration	[113]
DCs	EC	LAMP-3 can be expressed instantaneously when the DC is mature and can be regarded as a specific marker of mature DC, indicating a good prognosis	Inhibition	[115–117]
	EC	DC vaccine can improve the cytotoxicity of CTL and indirectly exert anti-tumor effect	Inhibition	[126–128]

CXCR2 expressed in ESCC cell lines and to promote tumor migration and invasion.<sup>102</sup> It is also noteworthy that CD204 + subtype TAM shows a significant tumor-promoting effect in EC. In previous report, CD204 + macrophages in ESCC tissues were closely related to microvessel density, clinical stage, vascular invasion, depth of tumor invasion, lymph node metastasis, and disease-free survival.<sup>103</sup> Simultaneously, Cyr61, an angiogenesis inducer of the CCN protein family,<sup>104</sup> can be expressed in ESCC cells and TAMs, which can not only promote macrophage migration through the MEK/ERK pathway but also promote CD204 expression in macrophages.<sup>105</sup> CD204 + TAM can also increase the expression of tumor suppressor protein annexin A10 (ANXA10) in cancer cells, and promote the proliferation of ESCC cells through phosphorylation of Akt and Erk1/2 pathways.<sup>106</sup> The high expression of ANXA10 in cancer cells has been found to promote cancer cell growth and proliferation in a variety of tumors including lung cancer,<sup>107</sup> ovarian cancer,<sup>108</sup> and prostate cancer.<sup>109</sup> Indeed, various secreted factors such as Cyr61,<sup>105</sup>

GDF15,<sup>100,101,110</sup> TGF- $\beta$ RII,<sup>100,111,112</sup> and ANXA10<sup>106</sup> have been reported to be associated with poor prognosis of ESCC. This series of studies revealed that TAMs could promote ESCC progression through strong secretory functions, and demonstrated the huge potential of TAMs-related pathways and molecules as novel therapeutic targets and biomarkers. Meanwhile, CD204 + subtypes of TAMs show more obvious tumor-promoting effects, which requires further studies on the specific mechanism of the interaction between TAMs of different functional subgroups and tumor cells. In addition, TAMs of different subtypes have been reported to have different tumor-promoting effects on EAC, but the specific mechanism remains to be studied in the future.<sup>113</sup>

#### Mature Dendritic Cells (DCs)

DCs can not only secrete cytokines to regulate immune function in TME but also induce adaptive immunity through its powerful antigen-presenting ability, exerting immune-stimulating effect further. DCs present in



esophageal tissue belong to Langerhans cells, which are bone marrow-derived dendritic cells.<sup>114</sup> When DCs capture antigens and mature, lysosomal-associated membrane glycoprotein 3 (LAMP-3) is significantly up-regulated. LAMP-3 can transiently express and participate in the loading and transport of MHC class II peptides to the cell surface.<sup>115,116</sup> Therefore, it can be regarded as a specific marker of mature dendritic cells. In an analysis of specimens from 80 postoperative patients with EC, it was found that CD8 + T cells clustered around LAMP-3 DC and formed LAMP-3 DC-CD8 + T cell clusters. In the same pathological stage, the 5-year survival rate of patients with high infiltration of LAMP-3 was higher than with low infiltration of LAMP-3.<sup>117</sup> However, the expression of LAMP-3 in non-DC cells has different clinical results, and many reports have shown that the expression of LAMP-3 in non-DC (including EC) is closely related to poor prognosis.<sup>118–121</sup> This may be related to the activation of its downstream signaling pathway.

Antigen presentation function is one of the major functions of DCs cells, and the application of vaccines based on DCs antigen presentation function in cancer has been widely studied. However, these studies are only performed primarily in patients with prostate cancer, melanoma, renal cell carcinoma, and glioblastoma nowadays.<sup>122</sup> For example, Sipuleucel-T is the first antigen-presenting cell-based therapeutic cancer vaccine approved by the FDA and has been used clinically for prostate cancer treatment.<sup>123</sup> The DCs vaccine in EC has also been partially reported.<sup>124,125</sup> It was shown that the strategy of using DCs loaded with autologous tumor RNA to improve the cytotoxic response was effective in the treatment of EC.<sup>126</sup> Another in vitro experiment confirmed that DC vaccine could activate SART1 peptide-specific CTLs,<sup>127</sup> nevertheless, exact clinical efficacy and survival benefits have not been observed in clinical trials. However, in a Phase I clinical trial of 40 EC patients, the 1-year (82.1% vs 50.0%,  $P=0.04$ ) and 2-year (67.8% vs 33.3%,  $P=0.04$ ) survival rates of DC vaccine combined with radiotherapy were significantly improved compared to patients not receiving the DC vaccine therapy.<sup>128</sup> This result confirms its reliability and therapeutic potential from a clinical perspective. These contradictory results may suggest that it was needed to develop individualized treatment standards for patients and prepare higher quality DC vaccines. The role of DC vaccine in the treatment of EC needs to be determined by further researches.

## Tumor-Infiltrating Lymphocytes (TILs) are Important Components of Anti-Tumor Immunity

TILs are the crucial components of tumor antagonism in TME, including T lymphocytes, B lymphocytes, and NK cells. Most of TILs are associated with good prognosis in a variety of solid tumors such as EC,<sup>129–132</sup> melanoma,<sup>133</sup> breast cancer,<sup>134</sup> and gastric cancer.<sup>135</sup> In the following, we will explain the main types of TILs in EC and their research progression (Table 3).

### T Lymphocytes

**CD8+T Lymphocytes.** CD8 + T lymphocytes are essential immune components which exert anti-tumor cell immunity. The primary cells differentiated and matured into cytotoxic T lymphocytes after interacting with DCs, CD4 + T cells, and NK cells, and mediated their cytotoxic response through the perforin-granzyme pathway and FASL pathway. CD8 + T cells have also been proven to be positive predictors of both ESCC<sup>136</sup> and EAC,<sup>137</sup> and T cell suppression is an important mechanism for tumor immune escape. Previous studies have shown that the expression of indoleamine 2,3-dioxygenase (IDO) in ESCC was associated with decreased the number of CD8 + TILs infiltration and poor prognosis.<sup>138,139</sup> This may be due to IDO-mediated tryptophan catabolism, thus suppressing T lymphocyte proliferation.<sup>140</sup> In addition, it has been reported that CAFs and microvascular endothelial cells were the major IDO expressing cells in the ESCC interstitial.<sup>141</sup> This finding indicates that IDO and its pathway may become new therapeutic targets and prognostic markers.

The exploration of immune checkpoints has always been the focus of our research. PD-1 is mainly expressed on the surface of activated T cells and can bind to programmed death ligand-1 (PD-L1) expressed in tumor cells to make tumor cells escape from the cytotoxicity of CTL.<sup>142</sup> In ESCC, multiple studies including a meta-analysis have shown that the increased expression of PD-L1 was associated with poor prognosis,<sup>143–146</sup> while a study of EAC seemed to suggest that upregulation of PD-L1 was conversely a favorable prognostic factor.<sup>147</sup> Certainly, more studies are required to confirm the exact effect of PD-L1 expression in EAC, and it is also essential to conduct more research on PD-1/PD-L1 inhibitors in ESCC and EAC. So far PD-1/PD-L1 inhibitors have been applied clinically in more than 10 tumors. In a recent Phase III clinical trial<sup>148</sup> and previous Phase II clinical

trials,<sup>149</sup> the results consistently showed that pembrolizumab (anti-PD-1 antibody) can improve OS in patients with advanced ESCC. Based on these results, the clinical application of pembrolizumab in patients with relapsed metastasis or locally advanced ESCC has been approved by the FDA. Simultaneously, several phase III clinical trials exploring the application of such drug in other indications of EC are also ongoing.<sup>150</sup> Other immunotherapy strategies based on CD8 + T cells in EC are also promising. For example, the cytotoxicity of T lymphocytes can be enhanced by an exogenous pathway, such as the aforementioned DCs vaccine. Or develop other immune checkpoint inhibitors, such as PD-L2 and CTLA-4.<sup>151</sup> This will be the direction of researches for EC treatment.

**Th17 Lymphocytes.** Th17 lymphocyte is a branch of CD4 + helper T cells, and its main effector molecule is IL-17. However, the role of Th17 lymphocytes in antitumor immunity remains controversial. A meta-analysis showed that IL-17A overexpression was significantly associated with poor

prognosis in liver cancer and non-small cell lung cancer, but IL-17A expression was associated with a significant improvement in overall survival (OS) in patients with ESCC.<sup>152</sup> Similarly, we found in chemotaxis analysis that IL-17A expressed by Th17 cells can induce ESCC cells to produce chemokines such as CCL20, CXCL-9, CXCL-10, and CXCL13, thereby chemotactic effector T cells, DCs, B cells, and NK cells migrate to ESCC tissue and then exert antitumor effect.<sup>153,154</sup> In addition, Chen et al showed that the expression of chemokines such as CCL17, CCL20, and CCL22 in EC TME is up-regulated and can bind to CCR4 and CCR6 expressed by Th17 lymphocytes to induce Th17 lymphocytes infiltration.<sup>155</sup> In other studies, cytokines and chemokines can also promote the differentiation and expansion of Th17 lymphocytes in ESCC TME, and the increase of Th17 lymphocytes is positively correlated with more lymph node metastasis and later clinical stages.<sup>156</sup> However, studies in another EAC have found that IL-17A could activate MMP-2 and MMP-9 through the ROS/NF- $\kappa$ B signaling

**Table 3** The Role of Lymphocytes in the Tumor Microenvironment of Esophageal Carcinoma

Entry	Cancer Type	Finding	Effects on Tumors	Ref.
CD8 +T cell	ESCC, EAC	The infiltration of CD8+ T cells in both ESCC and EAC predicted a favorable prognosis.	Inhibition	[136]
	ESCC	IDO may inhibit T lymphocyte proliferation by mediating tryptophan catabolism	Acceleration	[138–140]
	ESCC	PD-L1 expressed in tumor cells binds to PD-1 on the surface of T cells to escape CTL cytotoxic effects	Acceleration	[143–146]
	EAC	Upregulation of PD-L1 in EAC is a favorable prognostic factor	Inhibition	[147]
Th17 cell	ESCC	IL-17A induced tumor cells to produce chemokines such as CCL20, CXCL-9, CXCL-10, and CXCL13, thereby recruiting effector T cells, DCs, B cells, and NK cells	Inhibition	[153,154]
	ESCC	Th17 cell infiltration and amplification is promoted by chemokines such as CCL17, CCL20 and CCL22 and is associated with poor prognosis	Acceleration	[155,156]
	EAC	IL-17A activates MMP-2 and MMP-9 through the ROS/NF- $\kappa$ B signaling pathway	Acceleration	[157]
Tregs	/	FOXP3 may play an immunosuppressive role by directly inhibiting the IL-2 gene or by promoting the expression of CTLA-4 and CD25	Acceleration	[158]
	ESCC	IL-33 promotes the expression of CCL2 through the NF- $\kappa$ B pathway, thereby recruiting Treg cells to promote tumor progression	Acceleration	[165,166]
B cell	EAC	Breg cell interacts with Treg, MDSC, TAM and other immune cells to promote tumor growth	Acceleration	[168,169]
NK cell	EC	Co-inhibitory receptor Tim-3 is highly expressed in NK cells and is associated with NK cell dysfunction	Acceleration	[174]
	ESCC	The co-cultured NK cells had a strong cytotoxic effect on ESCC cells expressing the NK cell activated receptor	Inhibition	[175]

pathway. MMPs can catalyze the degradation of ECM and promote the migration and metastasis of cancer cells.<sup>157</sup> The above results indicate that the role of Th17 lymphocytes is still controversial. Therefore, we need further research to clarify the role of Th17 lymphocytes, especially the role of Th17 lymphocytes in different pathological types of EC.

**Tregs.** Tregs are a subset of CD4 + helper T cells, which are distinguished from other lymphocytes mainly by the expression of CD25 and Foxp3. Tregs can promote tumor progression by inhibiting the maturation of antigen-presenting cells (APC), reducing the secretion of cytokines and killing effect of CTL cells, and promoting angiogenesis.<sup>158–160</sup> Many studies have reported that Foxp3 expression was increased in EC and closely related to poor prognosis.<sup>161–164</sup> One of the specific mechanisms may be that FOXP3 directly inhibited the IL-2 gene and promoted the expression of CTLA-4 and CD25.<sup>158</sup> It is also reported that IL-33 is highly expressed in ESCC tumor tissues, and IL-33 can promote CCL2 expression through the NF- $\kappa$ B pathway, thereby recruiting Tregs to promote tumor progression.<sup>165,166</sup> The current therapeutic strategies targeting Tregs signaling pathways are quite promising. Researches on candidate target inhibitors such as CD25, CTLA-4, CCR4, and IDO-1 are underway,<sup>158</sup> but their complex interactions in EC need further exploration.

### B Lymphocytes

Most TILs-related studies currently focus on T lymphocytes, while relatively little attention has been paid to the tumor immune function of B lymphocytes. B lymphocytes are another major component of TILs. They mainly exert antitumor immunity by secreting specific antibodies, presenting antigens to T cells, or directly killing cancer cells. Studies have shown that B lymphocytes can exert the above-mentioned tumor immune effects through the CCL19, 21/CCR7 axis, and CXCL13/CXCR5 axis,<sup>167</sup> but their specific effects in different cancer types and different stages have yet to be explored. In EC, studies have shown that infiltration of B cells and T cells in tumor tissues indicated a better prognosis.<sup>131</sup> However, specific subgroup analysis showed that the regulatory B cell (Bregs) subgroup in peripheral blood can promote the growth and proliferation of EAC tumors.<sup>168</sup> Bregs are a subset of lymphocytes that can be activated in TME and interact with immune cells such as Tregs, MDSCs, and TAMs to promote tumor growth.<sup>169</sup> There is no doubt that the study of this subgroup is a promising future direction. The targeted therapy

designed for B cells is mainly concentrated in the field of B cell malignancies. Ibrutinib is a BTK (part of B-cell receptor signaling pathway) inhibitor approved for the treatment of B-cell malignancies.<sup>170</sup> In addition, this drug can also increase the antitumor effect by inhibiting IL-2-inducible T-cell kinase (an important enzyme in Th2 cells), thereby changing the ratio between Th1 and Th2 cells.<sup>171</sup> Importantly, Ibrutinib showed a significant increase in antitumor efficacy when combined with PD-1/PD-L1 inhibitors. However, the biological function of B lymphocytes in TME of EC is relatively insufficient. The related mechanism needs to be further explored, which will become a new direction for the treatment of EC.

### Natural Killer Cell (NK Cell)

NK cells are very important cells, which are involved in anti-tumor immunity and related to the good prognosis of EC.<sup>131,172</sup> Impaired NK cell activity is one of the dominating mechanisms for tumor immune escape. Studies have shown that in EC TME, the expression of co-suppressor receptor Tim-3<sup>173</sup> in NK cells was up-regulated, and NK cells overexpressing Tim-3 appeared to be dysfunctional and suggested poor prognosis.<sup>174</sup> The increase of Tim-3 is related to the increase of the inflammatory factor TNF- $\alpha$  induced by the NF- $\kappa$ B pathway,<sup>174</sup> but the related mechanism remains to be further studied. Tim-3 related pathway inhibitors based on NK cell immune activity may be a promising therapeutic direction for EC. In addition, it is shown in previous studies that NK cells expanded and co-cultured with special cells could achieve high proliferation and high cytotoxicity. These NK cells have a stronger cytotoxic effect on ESCC cells expressing NK cell activation receptor NKG2D,<sup>175</sup> which provides a basis for the clinical application of NK cells. Of course, more biological functions of NK cells need further research.

### Tumor Stromal Cells are Imperative Supporters for TME

Tumor mesenchymal cells mainly include three types of mesenchymal stem cells (MSCs):<sup>176</sup> normal mesenchymal stem cells, tumor-associated mesenchymal stem cells, and CAFs. The role of MSCs in tumors is still controversial, but their supportive role in tumor progression is dominant.<sup>177,178</sup> The results of two studies in EC also show that MSCs promote progression of tumor in vivo,<sup>179,180</sup> which is a promising therapeutic direction, but the research on its specific mechanism and related effects need to be further studied.

CAFs, as the major components of TME interstitial cells, play a vital role in the process of remodeling TME and promoting the progression of many types of tumors.<sup>181–183</sup> Similarly, CAFs can remodel TME through various mechanisms such as promoting angiogenesis, secreting cytokines, inducing EMT, recombining matrix components, and recruiting inflammatory cells to promote EC progression.<sup>184,185</sup> In the EC field, multiple studies have consistently shown that CAFs were predictors of poor prognosis.<sup>186–189</sup> Common effector molecules expressed by CAFs, including platelet-derived growth factor receptor (PDGFR $\alpha$ ), PDGFR $\beta$ , smooth muscle actin (SMA), fibroblast activating protein (FAP), fibroblast-specific protein-1 (FSP1), etc.<sup>190</sup> More specifically, SMA is associated with ESCC T stage progression, lymph node metastasis, and poor prognosis; FSP1 is associated with lower survival rates; PDGFR $\beta$  expression is associated with poorly differentiated tumors; FAP expression is associated with increased frequency of deaths.<sup>186</sup> In addition, IL-6 cytokines secreted by FAP + CAFs not only promote ESCC cell growth and migration, EMT, and the occurrence of drug resistance<sup>191,192</sup> but also recruit FoxP3 + T cells<sup>193</sup> and induce M2 polarization of TAMs<sup>194</sup> to promote tumor immunosuppression. The tumor-promoting effect of IL-6 has been detected in the BE microenvironment mentioned above,<sup>18,19</sup> and it is a vital cytokine in multiple progression processes of EC. In addition, other signaling pathways have also been studied. The urokinase plasminogen activator (uPA) secreted by CAFs promotes the progression of ESCC through the PI3K/AKT and ERK pathways.<sup>195</sup> The high expression of the transcription factor Twist1 in CAFs promotes the secretion of CXCL12 itself and the EMT process of EC cells through the ERK/AKT pathway.<sup>196,197</sup> Two other important signaling molecules are hepatocyte growth factor HGF and transforming growth factor  $\beta$  (TGF- $\beta$ ), both of which are closely related to tumor cell invasion and metastasis. It has been confirmed that CAFs could express HGF and TGF- $\beta$ 1, and they promoted the progression and metastasis of EC through the HGF/Met<sup>198</sup> and TGF $\beta$ 1/Smad pathways,<sup>199,200</sup> which were associated with poor prognosis of EC.<sup>201</sup> Moreover, the mechanism by which TGF $\beta$ 1 and HGF play a role is related to the angiogenic effect of EGFR.<sup>202,203</sup> Due to the extensive expression of HGF and TGF $\beta$  in tumor stroma, targeted therapy for corresponding pathways has been widely studied.<sup>204–206</sup> The treatment direction of EC has a guiding role. In addition, other therapeutic strategies against CAFs, such as infrared photoimmunotherapy targeted therapy strategies for FAP + CAFs, have also been proven to be effective.<sup>207</sup> Infrared

photoimmunotherapy is a combination of a specific monoclonal antibody and a photosensitizer and can produce great cytotoxicity on cells expressing specific antigens.<sup>208</sup> Of course, the current research is still inadequate. CAFs show a strong tumor-promoting effect. The researches on the target of CAFs expression molecules and their corresponding pathways will be our new study direction (Table 4).

### ECM is a Complex and Integral Part of TME

A tumor cell population is dynamically changing that continuously reshapes TME to adapt to its own progression. Matrix reprogramming is an important part of TME reconstruction,<sup>209</sup> and the role of CAFs is essential for ECM reconstruction.<sup>181,210</sup> As the original “soil” for the survival of tumor and other cells, a variety of ECM proteins contained in ECM, such as type I collagen, Fibronectin (FN),<sup>211</sup> and Tenascin-C (TNC), can be up-regulated in tumor.<sup>212</sup> Type I collagen is the most abundant protein of ECM and its role in tumors is still controversial,<sup>213</sup> but it is increasingly believed that it played a major role in promoting tumor progression.<sup>214</sup> In a recent ESCC study, cancer cells can produce cancer-derived type I collagen and inhibit tumor growth in contrast to previous studies which discovered that CAFs-derived collagen fibers could promote tumor growth,<sup>215</sup> and these two type I collagens appear to have different molecular weights.<sup>216</sup> This result gives us new insights and treatment directions for EC. Fibronectin (FN) is a high-molecular-weight glycoprotein component of the ECM. It has been reported that the high-matrix FN environment could promote the movement and migration of ESCC cells through a series of signaling pathways.<sup>217</sup> TNC is an ECM protein secreted by tumor cells and myofibroblasts. It mainly promotes the movement and invasion of cancer cells by inhibiting cell adhesion.<sup>218</sup> Studies have shown that the expression of TNC in ESCC was significantly correlated with the expression of C4.4A, which was a glycolipid-anchored membrane protein associated with poor prognosis in many human malignancies.<sup>218</sup> In addition, TNC expressed in ESCC can also improve cancer stem cell characteristics and promote EMT-like changes through the Akt/HIF-1 $\alpha$  axis.<sup>219</sup> It is a predictor of poor prognosis in ESCC and a possible target for treatment.<sup>190</sup> In addition, the high expression of the gene ADAM12-L in ESCC can promote the activation of focal adhesion kinase (FAK), and then promote cancer cell metastasis and migration through the FAK/JNK/c-Jun axis.<sup>220</sup> Lysyl oxidase (LOX) also plays an important role in ECM reconstruction. The LOX protein family includes LOX and LOX-like 1–4

**Table 4** The Role of CAFs and ECM in the Tumor Microenvironment of Esophageal Cancer

Entry	Cancer Type	Finding	Effects on Tumors	Ref.
CAFs	ESCC	FAP+ CAFs-derived IL-6 promoted EMT and drug resistance, recruited FoxP3+ T cells, and induced M2 polarization of TAMs	Acceleration	[191–194]
	ESCC	uPA secreted by CAFs promotes ESCC progression through the PI3K/AKT and ERK pathways	Acceleration	[195]
	EC	The high expression of Twist1 in CAFs promotes the secretion of CXCL12 and EMT through the ERK/AKT pathway	Acceleration	[196,197]
	ESCC	HGF expressed by CAFs promotes progression and metastasis through the HGF/Met pathway	Acceleration	[198]
	ESCC	TGF- $\beta$ 1 expressed by CAFs promotes tumor progression through the TGF- $\beta$ 1/Smad pathway	Acceleration	[199,200]
ECM	ESCC	Cancer cells can produce cancer-derived type I collagen with a molecular weight different from CAFs-derived collagen and inhibit tumor growth	Acceleration	[216]
	ESCC	High matrix FN environment promotes movement and migration of ESCC cells	Acceleration	[217]
	ESCC	TNC can also improve cancer stem cell properties and promote EMT-like changes through the Akt/HIF1 $\alpha$ axis	Acceleration	[219]
	ESCC	High expression of ESCC cell gene ADAM12-L can promote FAK activation and promote cancer cell metastasis and migration through FAK/JNK/c-Jun axis	Acceleration	[220]
	ESCC	Increased expression of LOX, LOXL4 and 5-LOX is associated with poor prognosis	Acceleration	[223–227]
	EAC	Elevated APE1 expression can induce ARF6 activity and up-regulate MMP-14, and up-regulation of MMP-14 can activate MMP-2 to mediate ECM degradation	Acceleration	[230,231]

(LOXL1–4), whose main function is to maintain the homeostasis of the ECM by catalyzing the oxidative deamination of lysine in matrix proteins.<sup>221,222</sup> Current studies in ESCC have shown that expression of LOX,<sup>223,224</sup> LOXL4,<sup>225</sup> and 5-LOX<sup>226,227</sup> was elevated and predicted shorter survival and poor prognosis for patients. Its specific mechanism is not clear. The unique mechanism of action of different LOX proteins is our future research direction.

MMPs play an important role in tumor ECM remodeling, which can promote tumor spread and metastasis mainly by regulating the degradation and remodeling of matrix protein networks. MMPs include matrix proteases, collagenases (MMP-1, -8, and -13), and gelatinases (MMP-2 and MMP-9) according to their structural and functional specificity.<sup>228,229</sup> Studies have found that the expression of apurinic/apyrimidinic endonuclease (APE1) was abnormally increased in EAC cell lines and induced ARF6 activity in a redox-dependent manner to up-regulate MMP-14 (also known as membrane-type matrix metalloproteinase MT1-MMP).<sup>230</sup> The increased expression of MMP-14 can activate MMP-2 and then mediate ECM degradation,<sup>231</sup> thereby promoting tumor migration. In addition, a number of other studies have shown that

MMP-2,<sup>232,233</sup> MMP-3,<sup>234</sup> MMP-7,<sup>235</sup> MMP-9,<sup>232,236</sup> MMP-12,<sup>237</sup> MT2-MMP,<sup>238</sup> and MMMP-16 (namely MT3-MMP)<sup>239</sup> all play negative regulatory roles in the process of EC through their different mechanisms. Among the nearly 30 different subtypes of MMPs, MMP-2 and MMP-9 are most closely related to EC. The MAPK pathway seems to be the core mechanism regulating MMP-2 and MMP-9 expression.<sup>209,240</sup> In short, ECM is a space for information exchange and growth between tumor cell subgroups in tumors. It contains a large number of signal pathways and involves hundreds of signal molecules. Our current research is still the tip of the iceberg (Table 4).

### The Interaction Between Immune Cells is an Indirect Promoter of Tumor Progression

In this complex network mechanism of tumor microenvironment, there is also important information exchange between each immune cell apart from the interaction between various immune cells and esophageal cancer cells, which indirectly promotes the progress of esophageal cancer (Figure 3). For example, a variety of cytokines (IL-4, IL-6, IL-13) derived from Th-2 can promote the recruitment

of MDSCs in the tumor microenvironment of EC<sup>81,89,91</sup> and the polarization of macrophages into M2 macrophages.<sup>94,95</sup> In addition, as an important component of TME, CAFs can also promote the development of ESCC by secreting cytokines such as IL-6 to recruit Tregs<sup>193</sup> and promote the polarization of M2 macrophages.<sup>194</sup> Moreover, MDSCs, especially MDSCs with high expression of CD38, can strongly inhibit the cytotoxic effect of activated T cells in EC.<sup>92</sup> The activity of NK cells is also inhibited by MDSCs through inhibiting NK cell perforin, instead of granular enzyme through direct contact-dependent pathway.<sup>85</sup> Other functions of MDSCs include inducing the activation of Tregs,<sup>86</sup> promoting the differentiation of CAFs,<sup>87</sup> etc. The activated Tregs can further inhibit the antigen-presenting cells and reduce the secretion of cytokines, thereby indirectly reducing the cytotoxic effect of CTL.<sup>158–160</sup> Additionally, the activation of the immune system mediated

by both the familiar specific and non-specific immune systems plays a crucial part in antitumor immunity. For example, as the main cellular component of antitumor immunity, CTL can be activated by DCs, CD4+T cells, B cells, or NK cells and other immune cells, so as to further exert its cytotoxic effect. The infiltration of B cells, NK cells or DCs has also been reported to predict a good prognosis in EC.<sup>117,131,167,172</sup> Therefore, it is not hard to draw the conclusion that there exists a balance and antagonism between anti-tumor immunity and pro-tumor immunity. With the progress of tumor, the balance of tumor immunity gradually shifted to the direction of anti-tumor immunity and finally became completely unbalanced. Therefore, the enhancement of anti-tumor immunity and the weakening of anti-tumor immunity will be a promising therapeutic development direction. However, for esophageal cancer, especially for different subtypes of EC (ESCC and EAC), the specific

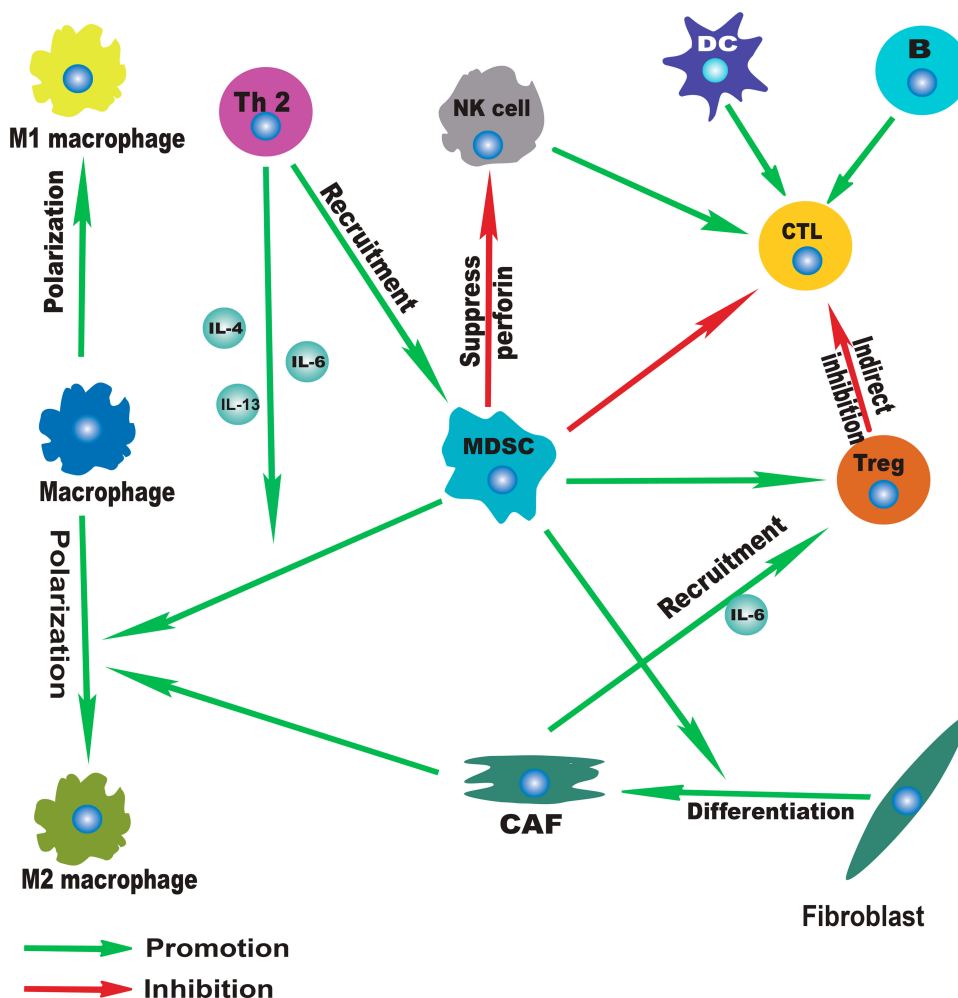


Figure 3 Immune landscape of immune cell interactions.

mechanism and degree of these interactions at different stages of the tumor remain to be explored.

## Premetastatic Niche

Metastasis is the leading cause of cancer death and the final stage of cancer development. Metastasis is a step in which circulating tumor cells colonize other tissues or organs and become diffuse tumor cells. However, only 0.01% of circulating tumor cells were reported to successfully colonize and develop into spreading tumor cells.<sup>241,242</sup> The main reason is that circulating tumor cells are severely affected by the local microenvironment. The well-known “seed and soil” hypothesis can help us understand this tumor metastasis process: tumor cells in situ (“seeds”) tend to settle on specific target organs (“soil”). The target organ microenvironment is formed before tumor cell metastasis and facilitates the colonization and metastasis of tumor cells, which is the pre-metastatic niche. The formation mechanism of the niche before metastasis mainly includes the following points: 1) Primary tumor secretion factors: tumor-derived secretion factors,<sup>243</sup> EVs,<sup>244</sup> etc. 2) Bone marrow-derived cell infiltration: MDSCs,<sup>245</sup> Tregs or Th cells,<sup>246</sup> TAMs,<sup>247</sup> CAFs,<sup>248</sup> etc. 3) Remodeling of the matrix microenvironment.<sup>249</sup> Although research on the ecology before metastasis has been gradually paid attention to, its relationship with EC is still insufficiently understood.

It is well known that lymph nodes are the most common metastatic organs of EC, and lymph node metastasis is the most important prognostic factor of EC.<sup>250</sup> Several studies have shown that this is closely related to the dense lymphatics and special lymphatic drainage directions in the anatomy of the esophagus.<sup>251,252</sup> However, EC lymph node metastasis is not simply a process of direct cancer cell migration. Otto et al<sup>253</sup> reported that the lymph node niche has changed significantly before EC lymph node metastasis. And another previous study found that ESCC could inhibit the cytotoxicity of lymphocytes in lymph nodes through factors other than metastasis.<sup>254</sup> Both of these results have hinted at the existence of the lymph node pre-metastasis niche in EC. Otto et al<sup>253</sup> also showed that the immune status of lymph nodes in patients with pN0 and pN1 was completely different. The lymph nodes in pN0 group showed obvious immune activation, while the non-metastatic lymph nodes in pN1 showed reduced immune response. A clear pattern of immune response activation was observed in the pN0 group. In contrast, lymph nodes in the pN1 group exhibited significant suppression patterns such as reduced immune response, decreased proliferation, and increased apoptosis. This finding is of great significance. It is assumed that in

the relatively early stage of EC, tumor antigen drainage to lymph nodes led to the development of anti-tumor immunity. However, with the immune regulation of tumor secretion factors and the recruitment of immunosuppressive cells, the immune status of the lymph nodes can be transformed from an anti-tumor response to a tumor-promoting mode until the first colonization of the tumor cells, the formation of micro-metastases. It has also been found in previous studies that melanoma cells injected directly into lymph nodes can induce CD8 + cytotoxic T cell responses and exert anti-tumor immunity.<sup>255</sup> Similarly, the formation of advanced micro-metastasis also implies the formation of an immunosuppressive microenvironment in lymph nodes.<sup>256,257</sup> In summary, the formation of the microenvironment before lymph node metastasis is the only precondition for lymph node metastasis. The study of its mechanism about action can not only provide us with new therapeutic targets to prevent cancer metastasis but also find biomarkers before lymph node metastasis. Guide the scope of lymph node dissection during surgery, which will be one of the development directions of our treatment.

## Conclusion

In this review, we divide the dynamic evolution of the microenvironment into three stages: tumor precursor microenvironment, TME, and premetastatic niche. The related research progression in EC is reviewed separately. Under high-risk environmental factors, such as gastroesophageal reflux, drinking, smoking and eating overheated food, metaplasia, and dysplasia can occur in normal esophageal epithelium. At the same time, environmental factors have also led to a partial remodeling of the epithelial microenvironment. Mild atypical epithelial hyperplasia can continuously accumulate malignant mutations and form the tumor precursor microenvironment under the induction of high-risk environmental factors. When it reaches a certain stage (with the removal of atypical hyperplasia in patients with high-risk environmental factors still irreversible), the tumor precursor cells and microenvironment can form a vicious cycle. For example, ESD can increase epithelial cell mutation and heterogeneity in an acidic microenvironment, and the increase in mutations promotes the formation of a highly proliferative state of cells and can produce more acidic metabolites, thereby forming a vicious cycle and eventually leading to the appearance of cancer. Therefore, the formation of malignant microenvironment changes dynamically with the change of precancerous cells in the microenvironment, which has also been confirmed in a small amount of

relevant literature on breast ductal carcinoma in situ microenvironment.<sup>258,259</sup> After tumorigenesis, tumors and tumor-related cells can remodel the distal metastatic environment by remote regulation such as secretion of cytokines or exosomes to prepare for metastasis while tumor cells continue to reshape TME. In EC metastasis, we mainly explored the microenvironment of lymph node metastasis and demonstrated that the pre-metastatic ecology of lymph nodes occurred before tumor cell colonization. All in all, understanding the entire dynamic evolution of the microenvironment is important for tumorigenesis and development. Of course, research on TME is helpful for targeted treatment of cancer. However, the study of the early tumor precursor microenvironment will become an important direction for cancer prevention, and the study of the pre-metastasis niche will become an important method of re-evaluating treatment strategies and preventing metastasis. Research at these stages is equally significant.

Some aspects need to be pointed out that may guide the future development of the EC field. 1). Regarding the EC precursor microenvironment, most of the current studies focus on the microenvironment of EAC precancerous lesion (BE), while little is known about the microenvironment of ESCC precancerous lesion. 2). We mainly discussed the associations between primary but not secondary EC microenvironment and tumor. The significance of secondary EC for this study lies in: whether there are some differences between the microenvironment of secondary EC and that of primary EC. More importantly, when other tumor cells colonize the esophageal tissue, whether the premetastatic niche of the esophagus has formed and plays a role in the process of secondary metastasis. 3). With respect to TME, most of the current studies focus on ESCC or all types of EC. However, it is essential to separate studies on ESCC and EAC, and more attention should be paid to the research on EAC. In general, TME is a relatively complex, wide-ranging, and promising field. However, there are still a lot of gaps in the field of EC. More research and exploration in this area is essential and critical for us to further elucidate the occurrence and development of EC.

## Disclosure

The authors have no conflicts of interest to disclose.

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