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Possible biomarkers for predicting lymph node metastasis of esophageal squamous cell carcinoma: a review

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Abstract

Esophageal cancer is the eighth most common form of cancer worldwide, and esophageal squamous cell carcinoma (ESCC) is a major type of esophageal cancer that arises from epithelial cells of the esophagus. Local lymph node metastasis (LNM) is a typical sign of failure for ESCC clinical treatments, and a link has been established between LNM and the aberrant expression of specific biomarkers. In this review, we summarize what is known about nine factors significantly associated with LNM in ESCC patients: phosphatase and tensin homolog (PTEN), mucin 1, vascular endothelial growth factor-C, tumor necrosis factor alpha-induced protein 8 (TNFAIP8), Raf-1 kinase inhibitory protein, stathmin (STMN1), metastasis-associated protein 1, caveolin-1, and interferon-induced transmembrane protein 3. The function of these nine proteins involves four major mechanisms: tumor cell proliferation, tumor cell migration and invasion, epithelium–mesenchymal transition, and chemosensitivity. The roles of PTEN, STMN1, and TNFAIP8 involve at least two of these mechanisms, and we suggest that they are possible biomarkers for predicting LNM in ESCC. However, further retrospective research into PTEN, STMN1, and TNFAIP8 is needed to test their possibilities as indicators.

Keywords

Lymph node metastasis, esophageal squamous cell carcinoma, biomarkers, proliferation, migration, invasion, epithelial–mesenchymal transition, chemosensitivity

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Esophageal cancer is the eighth most common form of cancer, and 456,000 new cases and 400,000 deaths were reported in 2012 worldwide.[1] It occurs significantly more often in the developing world than in developed countries, with over half of newly diagnosed cases occurring in China.[1,2] Esophageal squamous cell carcinoma (ESCC) is the major pathological type of esophageal cancer which arises from esophageal epithelial cells. Indeed, over 90% of esophageal cancer patients in China are reported to have ESCC.[3] Although the incidence of esophageal cancer has decreased in recent years in China, the absolute quantity of patients remains high because of the large population size.

Radical surgery is regarded as the most preferable therapeutic choice for ESCC patients to achieve long-term survival. However, the majority of these ESCC patients still die from local–regional recurrence (LR) and/or distant metastasis regardless of the radical resection and extended lymph node dissection carried out. As such, 5-year survival rates for esophageal cancer are only 13% to 18%.[1,3,4] LR, especially regional lymph node metastasis (LNM), is the main sign of failure for ESCC patients after surgery.[3–6] Recently, studies have reported associations between microRNAs and long non-coding RNAs with ESCC metastasis,[7–9] suggesting the existence of direct connections between LNM and the aberrant expression of specific biomarkers. Thus, it is possible that high-risk LNM patients could be identified by detecting specific biomarkers after surgery, which would allow the precise administration of postoperative adjuvant treatments to improve the survival rate.

In this review, we identify the most significant biomarkers associated with predicting ESCC prognosis that have been reported in the PubMed and MEDLINE databases during the past 10 years. We also summarize the details of the biomarker signaling transduction pathways.

Large-scale ESCC retrospective studies

We searched for relevant retrospective research papers using the keywords "biomarkers", "lymph node metastasis", and "esophageal squamous cell carcinoma". We restricted our search to manuscripts published in the English language, with no limitations on the country of study. We identified six about LNM in over 70 ESCC patients. Seven potential biomarkers were considered to predict LNM in ESCC. The histological grade, number of research subjects, LNM markers, molecular analysis methods, and LNM rates are summarized in Table 1.

Sun et al. evaluated the LNM status of 82 patients with pN0 ESCC who underwent Ivor Lewis esophagectomy with two-field lymph node dissection. In the first 3 years after surgery, 37 patients had LNM based on mucin 1 (*MUC1*) mRNA expression (45.1%). The LNM rate of MUC1-positive patients was 73.9%, compared with 33.9% for MUC1-negative patients.[10] Sun et al. further demonstrated that MUC1 and vascular endothelial growth factor-C (VEGF-C) correlated with LNM in these 82 patients.[11] This suggested that overexpression of MUC1 and VEGF-C correlated with the LNM of pN0 ESCC patients. Shi et al.[12] also discovered that *MUC1* was upregulated by C-C motif chemokine ligand (CCL)21–C-C chemokine receptor (CCR)7 in 153 ESCC patients with LNM. The LNM rate for patients positive for both CCR7 and MUC1 was 59%, while it was 11.5% for patients negative for both factors. This indicated that MUC1 and CCR7 together predict disease prognosis more efficiently than a single factor.

Table 1. Large-scale retrospective studies of lymph node recurrence in ESCC patients

Reference	Grade	Cases	Markers	Analysis methods	Analysis tissue types	LNM rates in first 3 years after surgery
Sun[14]	pN0	74	PTEN	IHC	tumor tissue	PTEN-positive 36.1% PTEN-negative 60.5%
Sun[17]	pN0	82	MUC1	RT-PCR	lymph nodes	MUC1-positive 73.9% MUC1-negative 33.9%
Shi[9]	I-III	153	MUC1 CCR7	IHC	tumor tissue	CCR7/MUC1 LNM rates +/+ 59.0% +/− 10.3% −/− 11.5% −/+ 19.2%
Sun[8]	pN0	82	VEGF-C	RT-PCR	lymph nodes	VEGF-C positive 59.5% VEGF-C negative 30.0%
Sun[10]	pN0	122	TNFAIP8	IHC	tumor tissue	TNFAIP8 positive 43.8% TNFAIP8 negative 20.4%
Kim[11]	pN0	138	RKIP	IHC	tumor tissue	RKIP positive 56.5% RKIP negative 75.4%
Akhtar[13]	pN0	174	STMN1	IHC	tumor tissue	STMN1 high 52.0% STMN1 low 33.8%

RT-PCR: reverse transcription PCR; IHC: immunohistochemistry

Sun et al.[13] retrospectively studied 122 pN0 ESCC patients in 2016, and showed that tumor necrosis factor alpha-induced protein 8 (TNFAIP8) correlated with LNM in 73 patients. Additionally, Kim et al.[14] examined tissue specimens from 138 patients with thoracic ESCC, and found a significant inverse association of Raf-1 kinase inhibitory protein (RKIP) expression with LNM (P=0.002). Stathmin (STMN1) overexpression also predicted the LNM in pN0 ESCC patients,[15,16] with higher TNFAIP8 and STMN1 expression shown to be associated with a higher LNM rate 3 years after surgery. Moreover, phosphatase and tensin homolog (PTEN) was inversely associated with LNM,[17] with a higher expression of RKIP and PTEN correlating with a lower LNM rate in pN0 ESCC patients.

PTEN

PTEN is a member of the protein tyrosine phosphatase family, which acts as a tumor suppressor gene through the action of its phosphatase products.[18] It is involved in regulation of the cell cycle, thus preventing cells from growing and dividing too rapidly.[19] ESCC tumor tissue was shown to have lower PTEN expression compared with adjacent tissues,[17,20] with Li et al.[20] also reporting that lower PTEN expression levels were associated with later tumor (T), node (N), and tumor–node–metastasis (TNM) grades. However, Sun demonstrated that PTEN expression was not associated with ESCC grade.[17] Furthermore, the LNM of ESCC patients without PTEN expression was 60.5%, compared with 36.1% for those with PTEN expression.[17] Lu et al.[21] also concluded that PTEN-positive ESCC patients had higher 3-year overall survival rates and disease-free survival rates compared with PTEN-negative ESCC patients. This indicated that PTEN could be used as a predictor for LNM in pN0 ESCC.

PTEN is regulated by microRNA (miR) in ESCC. miR-21, -130b, -141-3p, -18a, and

RhoE can all reduce PTEN protein expression levels to activate the phosphoinositide-3-kinase (PI3K)/AKT pathway, and subsequently accelerate tumor cell growth, invasion, and migration.[21–25] miR-21 and 130b function by directly depressing PTEN protein expression, while miR-18a decreases PTEN expression to increase AKT S6K1 phosphorylation and cyclin D1 expression, resulting in tumor cell proliferation. Leucine-rich repeats and immunoglobulin-like domains protein 1 reduces PTEN expression by activating the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway in ESCC cell lines, which can be inhibited by the U0126 inhibitor.[26]

PTEN is also associated with epithelial–mesenchymal transition (EMT). Transforming growth factor-β1 (TGF-β1) activated the PTEN/PI3K pathway to induce EMT in ESCC cells,[27] while sine oculis homeobox homolog 1 maintained tumor basal cells via the TGF-β pathway and was associated with LNM of ESCCs in two sets of 42 and 85 ESCC patients.[28] Moreover, fractionated ionizing radiation mediated EMT in KYSE-150/RR cells through PTEN-dependent pathways accompanied by increased cell migration and invasion.[29]

Additionally, PTEN is linked with chemosensitivity. The chemoresistant ESCC cell line EC9706R can be reverted to chemosensitive towards fluorouracil and oxaliplatin by inhibiting miR-141-3p expression.[20] This reversion is caused by increased PTEN expression levels. Similarly, the inhibition of cyclin B1 increased ESCC cell sensitivity towards cisplatin and paclitaxel through the PTEN/AKT pathway.[30]

MUC1

MUC1 is a glycoprotein with extensive O-linked glycosylation of its extracellular domain.[31] Mucins line the apical surface of epithelial cells in the lungs, stomach, intestines, eyes, and several other organs. They protect the body from infection by preventing pathogens from reaching the cell surface.[32] MUC1 is not typically expressed in lymph nodes, so its expression in lymph nodes of ESCC patients is suggestive of local lymph node recurrence. Sun and Shi both demonstrated that ESCC patients with MUC1-positive lymph nodes after surgery had significantly higher LNM rates than MUC1-negative ESCC patients.[10–12,33–35] MUC1 is also positively correlated with T, N, and TNM grades.

CCR7 has two ligands: CCL19/ELC and CCL21.[36,37] CCR7 is expressed in various lymphoid tissues and activates B and T lymphocytes. CCR7 stimulates dendritic cell maturation, and is involved in the homing of T cells to the lymph nodes.[37–39] In ESCC cell lines, CCL21 binds to CCR7 and activates ERK1/2-Sp1, then Sp1 binds to the *MUC1* promoter to up-regulate gene expression. This promotes migration and invasion ability in tumor cells.[12] MUC1 also up-regulates matrix metalloproteinase 13 (MMP13) to induce metastasis of ESCC tumor cells.[33] miR-1291 was shown to negatively regulate *MUC1* expression by binding to its 3′-untranslated region.[40]

VEGF-C

VEGF-C is a member of the platelet-derived growth factor/ VEGF family. Its main function is lymphangiogenesis, in which it acts on lymphatic endothelial cells to promote tumor cell survival, growth, and migration.[41,42] Higher VEGF-C expression is associated with a higher T, N, and TNM grade. Moreover, the concentration of VEGF-C in the peripheral blood of ESCC patients was found to be significantly higher than in healthy volunteers, while the higher the VEGF-C concentration, the more likely LNM was to be present.[43,44] Additionally, Song et al.[45] demonstrated that ESCC patients positive for both CCR7 and

VEGF-C expression had significantly higher rates of LNM than those negative for both CCR7 and VEGF-C.

Lymphangiogenesis and chemotaxis migration are key for LNM. Liu et al.[46] demonstrated that VEGF-C short hairpin (sh) RNA decreased VEGFR-2 and VEGFR-3 phosphorylation, resulting in tumor growth inhibition in nude mice transplanted with ESCC cells. Transducin β-like 1 X-linked receptor 1 (TBL1XR1), A kinase-interacting protein 1 (AKIP1), hypoxia-inducible factor-1α, nuclear factor (NF)-κB, and sirtuin 1 were found to positively regulate VEGF-C expression, while NOTCH 1 negatively regulated it.[47–50] TBL1XR1 binds to the *VEGF-C* promoter and increases its expression, subsequently activating AKT and ERK and inducing lymphangiogenesis.[47] AKIP1 also binds to the *VEGF-C* promoter, and functions together with SP1, AP2, and NF-κB to increase *VEGF-C* expression.[48] Octamer-binding transcription factor 4 was reported to enhance the *VEGF-C* promoter activity, activate VEGFR-3, induce EMT, and finally promote esophageal cancer metastasis.[51] Moreover, Hong et al.[52] reported that VEGF-C down-regulated Dicer protein and miR326 expression, up-regulated cortactin expression, and stimulated ESCC cell invasion and migration.

Metastasis-associated protein 1 (MTA1)

MTA1 is associated with tumor progression and angiogenesis, and its protein expression was associated with the ESCC progression grade.[53–55] Thus, higher MTA1 expression correlated with later grades of ESCC and a higher possibility of LNM. High MTA1 expression was also linked to a high density of microvessels and poorly differentiated squamous carcinoma.[56] In the absence of MTA1, the 5-year overall survival rate was 69.9%, compared with 50.7% in its presence, suggesting that MTA1 could be a diagnostic and prognostic marker in esophageal cancer.

TNFAIP8

TNFAIP8 is a suppressor of TNF-α-mediated apoptosis, and its expression is induced by NF-κB activation.[57] In pN0 ESCC patients with high TNFAIP8 protein expression, LNM developed at a rate of 43.8% within 3 years of surgery, compared with a rate of 20.4% in those with low TNFAIP8 expression.[10] Following the use of small interfering (si)RNA for TNFAIP8, apoptosis was increased, cancer cell invasion and migration ability were decreased, and MMP1 and MMP9 expression was significantly decreased.[13] TNFAIP8 is also related to TNM grade, LNM, lymphatic invasion, and venous invasion,[58] suggesting it could be an effective future therapeutic target for ESCC.

RKIP

RKIP is a member of the phosphatidylethanolamine-binding protein family that disrupts regulation of the MEK1/2, ERK1/2, and NF-κB signaling pathways through its interaction with Raf-1 kinase.[58] RKIP was also shown to inhibit G protein-coupled receptor kinases (GRKs) when phosphorylated by protein kinase C.[59] RKIP regulates multiple cellular processes, and its down-regulation is associated with distinct human cancers including ESCC, in which RKIP protein expression is significantly lower than in adjacent tissues.[60,61] Promoter methylation in the 5′ CpG island of *RKIP* gene significantly induces gene silencing and ESCC development.[62] Moreover, low RKIP expression was significantly correlated with an increased risk of recurrence compared with high RKIP expression ($P < 0.001$),[58] and was also linked with shorter disease-free

survival and overall survival times in ESCC patients.[14,63]

RKIP was reported to down-regulate *GRK-2*, *LIN28*, and *MMP-14* mRNA expression levels in inhibiting the migration and invasion of TE-1 ESCC cells,[64] while decreased RKIP expression down-regulated E-cadherin and up-regulated NF-κB and p53, eventually stimulating cancer call invasion and migration.[61] RKIP expression is therefore an independent factor that affects overall survival in ESCC, and could be used as a predictor for disease outcome.

STMN1

STMN1, also known as metablastin and oncoprotein 18, is important for regulation of the cytoskeleton, which is required for cytoplasmic organization, cell division, and cell motility.[65] More specifically, STMN1 is crucial in regulating the cell cycle.[66] Out of 174 pN0 ESCC patients resected with Ivor Lewis esophagectomy, 57.47% had STMN1 overexpression accompanied by a significantly higher rate of LNM than patients with average STMN1 expression.[16] Jiang et al. demonstrated that STMN1 expression was significantly higher in tumor tissues compared with healthy tissues, while STMN1 overexpression was related to tumor length and depth of invasion, TNM grade, LNM, and prognosis.[15,67,68]

STMN1 overexpression was also reported to activate the PI3K pathway, with the PI3K inhibitor LY294002 found to reduce STMN1 protein expression, consistent with the down-regulation of p-Akt (S473).[67] *STMN1* shRNA administration silenced *STMN1* expression in ESCC EC9706 cells, arrested cells in the G2/M phase of the cell cycle, down-regulated Bcl-2 and survivin protein expression, activated caspase-3, and eventually induced apoptosis.[69]

STMN1 phosphorylation can be regulated by chemotherapeutic drugs. For example, the treatment of EC0156 cells with paclitaxel led to the stable phosphorylation of STMN1 and impaired cell migration.[70] Additionally, the level of STMN1 expression determines the sensitivity to chemotherapy. Thus, STMN1 silencing by the shRNA transfection of Eca109 and TE-1 cells increased sensitivity 191.4- and 179.3-fold to paclitaxel, and 21.3- and 28.4-fold to vincristine, respectively.[71] Zhu et al. reported that *STMN1* siRNA-transfected Eca109 cells had increased sensitivity towards paclitaxel and vincristine through the G2/M phase block.[72] Moreover, knockdown of *STMN1* enhanced ESCC cell line sensitivity to docetaxel and radiation.[68] STMN1 could therefore be a therapeutic target for ESCC treatment and a biomarker for predicting prognosis.

Caveolin-1

Caveolin-1 is a scaffold protein and the main component of the caveolae plasma membrane found in most cell types. Its integrin subunits link to the tyrosine kinase FYN to couple with the Ras–ERK pathway and promote cell cycle progression.[73] It is also a negative regulator of the Ras–p42/44 MAPK cascade.[74] Caveolin-1 expression in ESCC tumors was shown to be significantly higher than in adjacent healthy tissue,[75–77] while positive caveolin-1 staining was correlated with pathological stages pT, pN, pM, and pTNM.[76–78] ESCC patients with caveolin-1-positive staining also showed significantly shorter overall survival times than caveolin-1-negative patients ($P = 0.0105$,[75] $P = 0.0215$[76]).

High levels of caveolin-1 mRNA were detected in 15 esophageal cancer cell lines (TE 1–15), but not in the healthy esophageal epithelial cell line Het-1A.[78] Moreover, ESCC cell lines TE1 and TE13 were reported to have higher caveolin-1 expression than lines EC109 and Eca109. TE1 and

TE13 also had significantly stronger motility and migratory and invasion abilities than EC109 and Eca109 cells. Suppressing the Rho/ROCK pathway inhibited caveolin-1 and phosphorylated caveolin-1 expression, and decreased cancer cell migration and invasion. This suggested that Rho/ROCK pathway activation promoted ESCC metastasis by regulating caveolin-1.[77] Caveolin-1 could therefore be used as a prognosis marker to predict disease outcomes.

IFITM3

Interferon-induced transmembrane protein 3 (IFITM3) is an important member of the interferon-inducible transmembrane protein family that influences cell proliferation, migration, and invasion. It functions by modulating the Wnt/β-catenin signaling pathway and is implicated in controlling the cell cycle at the G0/G1 checkpoint.[79] Jia et al. found that IFITM3 was overexpressed in 56.7% of pN0 ESCC patient tumor tissues, and the 3-year survival rate of patients with high IFITM3 expression was 50.8%, compared with 26.7% in those with low expression.[79] More recently, 5-year survival rates for stage IIA ESCC patients with high IFITM3 expression were reported to be 78.7%, versus 64.9% in those with low IFITM3 expression.[80] Thus, IFITM3 has less of an influence on 5-year survival rates in stage IIA ESCC patients than in pN0 patients, suggesting it could only be a predictor for LNM in pN0 ESCC. Knockdown of IFITM3 expression significantly suppressed gastric cancer cell migration, invasion, and proliferation *in vitro*, arrested cells at the G0/G1 phase, and reduced numbers in S phase of the cell cycle.[81] Moreover, silencing of IFITM3 reversed the EMT process and reduced MMP-2 and MMP-9 expression. Taken together, IFITM3 could be a predictor for LNM in pN0 ESCC patients, as well as a potential therapeutic target for ESCC.

Discussion

In this review, we summarize information about nine biomarkers closely associated with LNM of ESCC. PTEN, MUC1, VEGF-C, TNFAIP8, RKIP, and STMN1 have been investigated in several large-scale retrospective studies, but less is known about MTA1, caveolin-1, and IFITM3.

For ESCC cells to migrate and/or invade local lymph nodes, four major biological mechanisms are involved: consistent tumor cell proliferation, enhanced tumor cell migration and invasion, EMT, and decreased chemosensitivity. Several key biomarkers have been linked with consistent tumor cell proliferation (Figure 1), with increased expression of IFITM3, MUC1, TNFAIP8, and caveolin-1, and decreased RKIP expression shown to stimulate ESCC cell migration and invasion. Moreover, increased VEGF-C expression stimulates lymphangiogenesis, which enables the invasion and migration of tumor cells. Three gene combinations, *MUC1* and *CCR7*, *MUC1* and *VEGF-C*, and *CCR7* and *VEGF-C*, predict the LNM of ESCC patients more accurately than any single gene, although alternative gene combinations with greater prediction accuracies should be investigated. ESCC patients positive for both of these two-factor gene combinations require more attention, such as shorter intervals between hospital visits, and more accurate chemical therapy drug doses.

EMT and chemosensitivity are other major mechanisms that influence the LNM of ESCC patients (Figure 2). ESCC cells with enhanced proliferative and EMT abilities and decreased chemosensitivity show more efficient migration and invasion.

PTEN is known to influence three of the four major mechanisms promoting LNM in

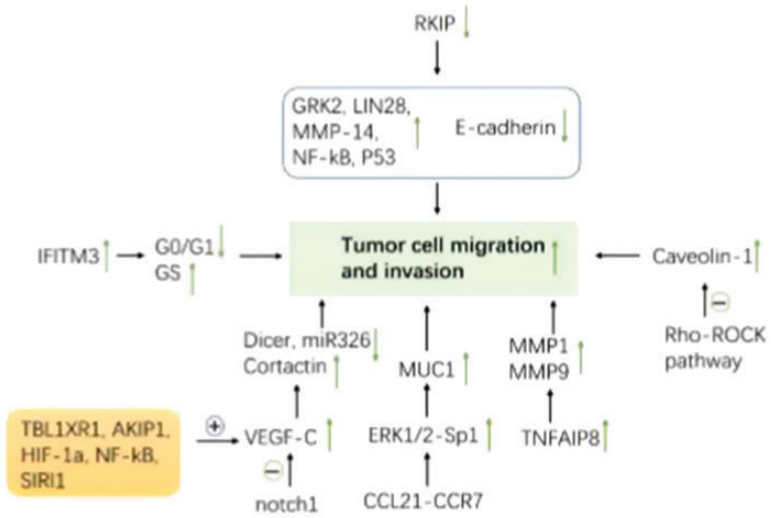

Figure 1. ESCC tumor cell migration and invasion abilities are regulated by six biomarkers. Caveolin-1, IFITM3, VEGF-C, MUC1, and TNFAIP8 stimulate tumor cell migration and invasion. RKIP is inversely associated with tumor cell migration and invasion abilities

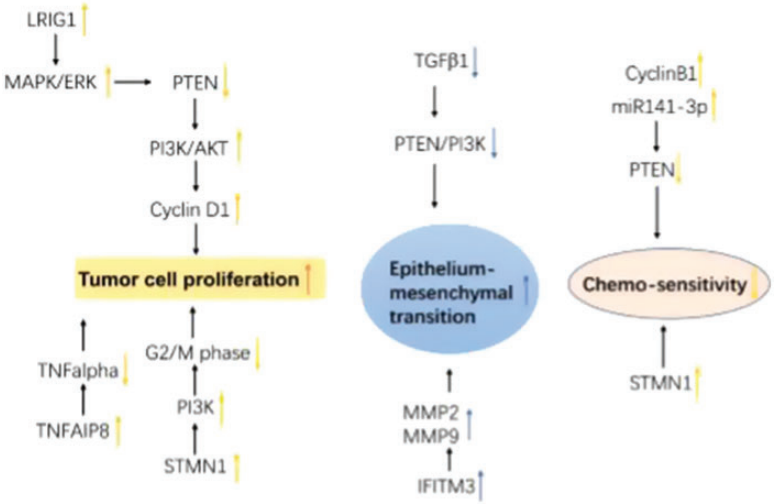

Figure 2. Three major mechanisms responsible for lymph node metastasis of ESCC. PTEN, STMN1, and TNFAIP8 influence tumor cell proliferation. PTEN and IFITM3 alter epithelium–mesenchymal transition. STMN1 and PTEN change tumor cell chemosensitivity

ESCC, while STMN1 and TNFAIP8 each alter two of them. This suggests that following changes to PTEN expression, the status of tumor cells is stimulated more widely than other factors, such that a chemotherapeutic drug targeting PTEN may reverse the LNM tendency. However, further study of PTEN, STMN1, and TNFAIP8

in large-scale retrospective investigations is required, and network analysis or GeneMANIA-based analysis are needed to confirm these genes as predictive biomarkers. Additionally, because the majority of patients in these studies were pN0 grade, the nine biomarkers should only be considered predictors for pN0 patients. Thus, additional studies on I–IV grade ESCC patients would be beneficial to extend the usefulness of the biomarker predictions.

In summary, we present the current knowledge of four major mechanisms and nine biomarkers that influence LNM in ESCC patients. PTEN, STMN1, and TNFAIP8 have wider influences on the prognosis of disease than the other six biomarkers, so may be more suited to predicting LNM following ESCC treatments.

Declaration of conflicting interest

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