

Bioinformatics-based analysis of the relationship between core genes and immune infiltration and tumor metastasis in esophageal cancer

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Abstract: We analysed four gene microarray datasets by GEO2R and obtained differential genes expressed in oesophageal cancer. To further elaborate the functions of DGEs, this study performed gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs. We constructed protein interaction networks of DGEs through the String database and screened core genes. We used the GEPIA online database with the Kaplan-Meier plotter database to verify the expression of Hub genes in expressed normal versus tumour tissues and the effect of Hub genes on overall and disease-free survival in oesophageal cancer. To further understand the relationship between Hub gene and tumour metastasis, we analysed the difference in Hub gene expression in patients without metastatic oesophageal cancer versus those with metastatic oesophageal cancer with the help of the HCMDDB database. The relationship between Hub genes and tumour immune infiltration was analysed by the TIMER database. We obtained a total of 149 DEGs, of which 49 were up-regulated genes and 100 were down-regulated genes. These DGEs were importantly enriched in IL-17 signalling pathway, ECM-receptor interactions, p53 signalling pathway, estrogen signalling pathway, complement and coagulation cascade response. We screened 10 Hub genes, MMP9, CXCL8, COL1A1, TIMP1, POSTN, MMP3, MMP1, COL3A1, SERPINE1, LUM, among 149 DGEs. hub genes were all up-regulated in expression in esophageal cancer tissues, in addition, MMP9, TIMP1, CXCL8, POSTN and The expression of COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 and TIMP1 was positively correlated with the infiltration of immune cells in the tumor microenvironment. In conclusion, our study identified 10 signature genes for oesophageal cancer. These genes are associated with the development, metastasis, prognosis and immune infiltration of oesophageal cancer and may be markers of development, metastasis and prognosis as well as targets for immunotherapy.

Key words: bioinformatics; esophageal cancer; data mining; GEO; immune infiltration.

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Abbreviations: GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; OS, overall survival; TME, tumor microenvironment; TILs, Tumor immune-infiltrating cells; DEGs, Differential genes; BP, biological processes; CC, cellular components; MF, molecular functions; MMPs, Matrix metalloproteinases; VEGF, vascular endothelial growth factor; TIDCs, Tumor-infiltrating dendritic cells; TILB, tumor-infiltrating B cells.

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Data Availability: The database and drawing software used in the article are free and open source.

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Introduction

Esophageal cancer is an aggressive gastrointestinal malignant tumor, occurring in esophageal epithelial tissue, according to its different pathological characteristics can be divided into esophageal phosphocell carcinoma and esophageal adenocarcinoma. The global incidence of esophageal cancer is reported as about 65 / 100,000, with a mortality rate of about 38 / 100,000, and about 500,000 new cases per year. In 2018, esophageal cancer ranked 7th and 6th in global malignancies, respectively [1]. The incidence of esophageal cancer patients in China accounts for about 46.6% of esophageal cancer patients worldwide, making one of the highest incidence and mortality rates in the world [2-3]. For patients with early oesophageal cancer, endoscopic treatment is the current effective treatment plan. Surgery and neoadjuvant chemoradiotherapy are suitable for patients with the muscle layer or not [4]. Unfortunately, early esophageal cancer lacks obvious clinical symptoms, many patients with esophageal cancer mostly develop metastases when diagnosed, and 5-year even surgically resectable overall survival (overall survival, OS) is only 16%~34% [5-6]. Relapse and metastasis are one of the high mortality rates in patients with esophageal cancer. Therefore, appropriate biomarkers are needed for the risk assessment of early diagnosis, treatment, recurrence and metastasis of oesophageal cancer.

The tumor microenvironment (TME) is composed of tumors, blood vessels, inflammatory factors, immune cells, and other extracellular matrix components [7]. Tumor immune-infiltrating cells (TILs) were first identified in 1863 and were initially considered as the source cells of inflammation-associated tumors [8]. Many current studies have shown that immune cells in the tumor microenvironment have anti-tumor or pro-tumour activity, correlated with tumor prognosis[9-10]. In this study, we obtained four datasets (GSE1420, GSE26886, GSE23400, GSE92396) from the GEO database to obtain differential genes in the dataset by GEO2R to identify genes for development and prognosis with esophageal cancer. The mechanism of DEGs was further elaborated by GO and KEGG enrichment analysis. This study used the PPI network to identify core genes in DEGs. The article performed validation and prognostic analysis of core genes using the GEPIA online database with Kaplan-Meier plotte. Finally, this study performed immune infiltration analysis of core genes through the TIMER database. This study aimed to find marker genes related in the development of esophageal cancer and to explore the relationship of these genes to immune infiltration and tumor metastasis.

2. Materials and Methods

2.1 Data Source

The study material was obtained from 4 datasets in the GEO database (GSE1420, GSE26886, GSE23400, GSE92396). The GSE1420 contains 8 normal esophageal tissue samples, 8 Barrett esophageal tissue samples and 8 esophageal cancer samples; GSE26886 contains 20 Barrett esophageal tissue samples and 19 normal esophageal tissue samples; GSE23400 contains 53 esophageal cancer tissue samples and 53 normal esophageal tissue samples, and 9 esophageal cancer tissue samples and 12 normal esophageal tissue samples in GSE92396. Only gene expression differences between normal tissues were compared to tumor tissues.

2.2 Differential genes (DEGs) analysis

We analyzed gene expression differences between tumor tissue versus normal tissues across the four datasets using the GEO2R online analysis tool. Correction P (adjust P-value) <0.05 and $|\log FC| > 1$ were used as screening criteria for differential genes, $\log FC > 1$ as upregulated and $\log FC < 1$ as downregulated. A common DEGs. was obtained using the intersection of DEGs from the four datasets The study shows the differential expression of the four dataset genes by volcano maps.

2.3 GO versus KEGG enrichment analysis for DEGs

GO enrichment and KEGG enrichment analysis of common DEGs by Metascape database. P value less than 0.01 and minimum enrichment of 3 were used as the screening criteria.

2.4 Protein interaction network of DEGs (PPI) analysis with screening of core genes (Hub)

The study imported DEGs into the String database to obtain the PPI network relationships of DEGs. Core genes of DEGs were obtained using the CytoHubba software package in Cytoscape3.7.1.

2.5 Hub gene validation and survival analysis

GEPIA online database was used to validate the expression between esophageal and normal tissues. The effect of Hub gene on overall and disease-free survival of esophageal cancer.

2.6 Immune infiltration and immune survival analysis of the Hub gene

The relationship between Hub gene and immune cells (B cells, CD4 + T cells, CD8 + T cells were analyzed by TIMER database, neutrophils, macrophages and dendritic cells) and the impact of immune infiltration on esophageal patients survival cancer.

2.7 Relationship between the Hub gene and the tumor metastasis

The HCMDB database included 29 tumors, 45 tumor subtypes, and 38 metastatic sites in the GEO database and the TCGA database, with a total of 124 data sets and 7,081 articles. The HCMDB database was used to analyze the relationship between Hub gene expression and tumor metastasis.

3. Results

2.1 Differential gene analysis

Studies used GEO2R to analyze the four dataset (GSE1420, GSE26886, GSE23400, GSE92396) differential genes in the GEO database and screen the differential genes with adjust P-value<0.05 and $|\log FC| > 1$. There were 1,734 differential genes in the GSE1420 dataset, Among these, 1,027 were upregulated genes, There were 707 downregulated genes; There were 7,791 differential genes in the GSE26886 dataset, Of these, 3932 upregulated genes, There were 3,859 downregulated genes; There were 727 differential genes in the GSE23400 dataset, Of these, 371 were the upregulated genes, There were 356 downregulated genes; There were 1,577 differential genes in the GSE92396 dataset, Of these, 781 were the

upregulated genes, There were 796 downregulated genes; There were 149 common differential genes in the 4 datasets, Of these, 49 were upregulated genes, There were 100 downregulated genes (Figure 1).

2.2 GO versus KEGG enrichment analysis

Study performed GO and KEGG enrichment analysis of 149 DEGs by the Metascape database. GO enrichment analysis showed that DEGs was mainly enriched in cell-matrix adhesion in biological processes (BP), positive regulation of cell movement, negative regulation of hydrolase activity, inflammatory response, epidermal development (Figure 2.a); cellular components involved in DEGs (CC) have extracellular matrix, keratinization envelope, collagen trimer complex, cell tip, intercellular connection, actin cytoskeleton (Figure 2.c); molecular functions (MF) with structural molecular activity, cell adhesion molecule binding, calcium ion binding, protease binding, serine-type peptidase activity and so on (Figure 2.b). KEGG pathway enrichment analysis showed that DEGs was mainly enriched in IL-17 signaling, ECM-receptor interaction, p53 signaling, estrogen signaling, complement, and coagulation cascade (Figure 2.d).

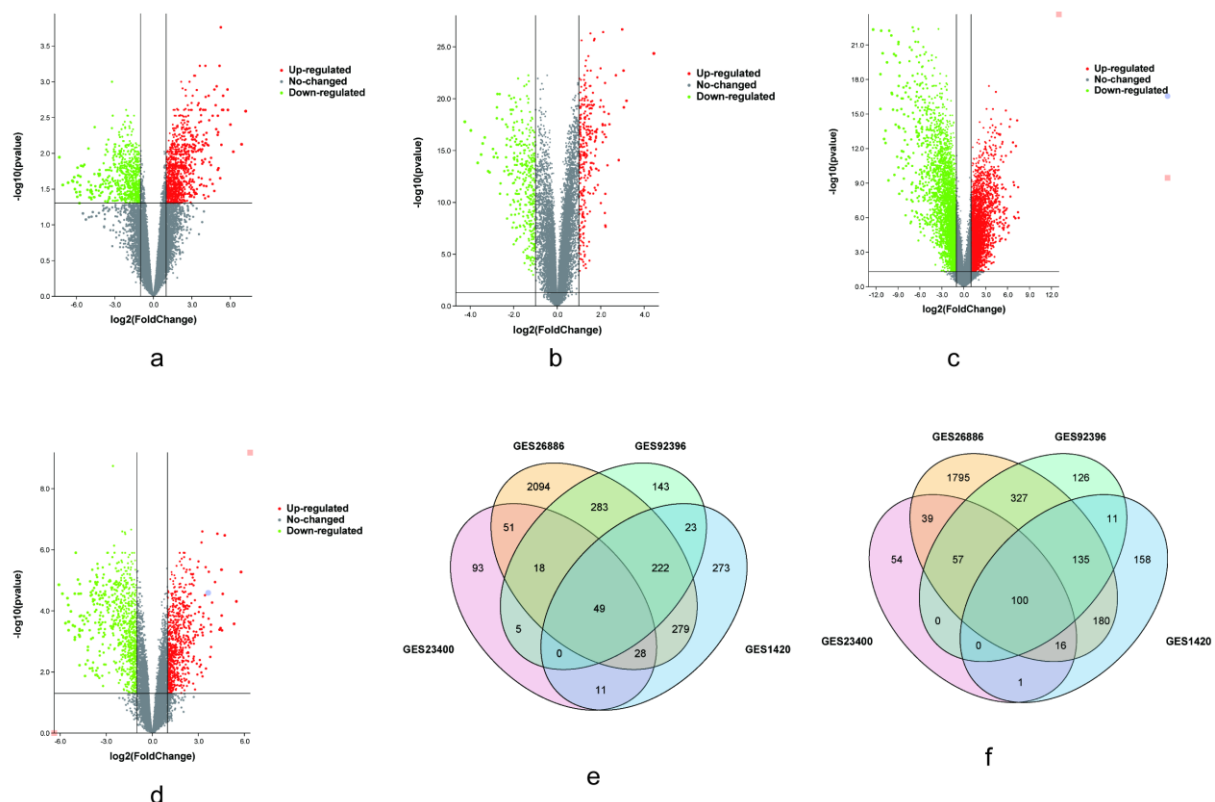


Figure 1: Heatmap of differential gene expression (a.GSE2140;b.GSE23400;c.GSE26886;d.92396); intersection genes of differential genes (e. upregulated genes; f. downregulated genes).

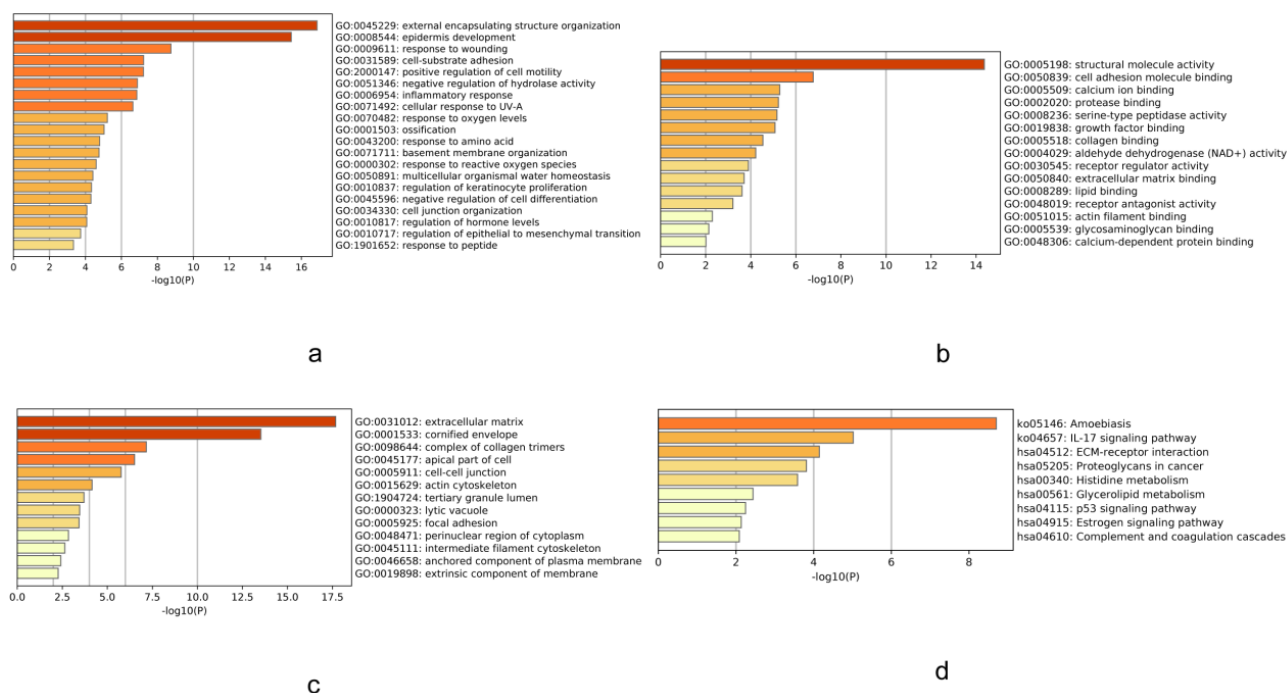


Figure 2: GO and KEGG enrichment analysis. (a.GO biological processes; b.GO molecular function; c.GO cellular components; KEGG signaling pathway).

2.3 Screening of PPI networks with Hub genes

To understand the relationship of protein interactions between Hub genes, the relationship between DEGs was analyzed by the String database to obtain the PPI network map (Figure 3). The PPI network was analyzed by the CytoHubba package in Cytoscape3.7.1, with the top 10 Degree genes as the core genes in DEGs (Figure 4). The core genes are: MMP9, CXCL8, COL1A1, TIMP1, POSTN, MMP3, MMP1, COL3A1, SERPINE1, LUM. These genes are highly expressed in esophageal cancer and are closely related to the occurrence and development of esophageal cancer.

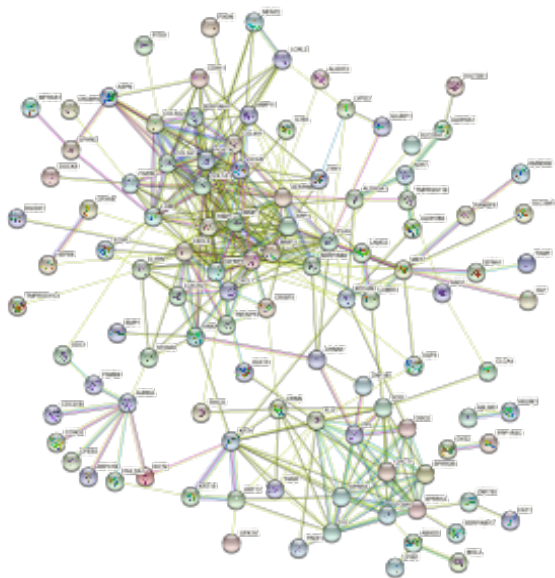


Figure 3: PPI network analysis.

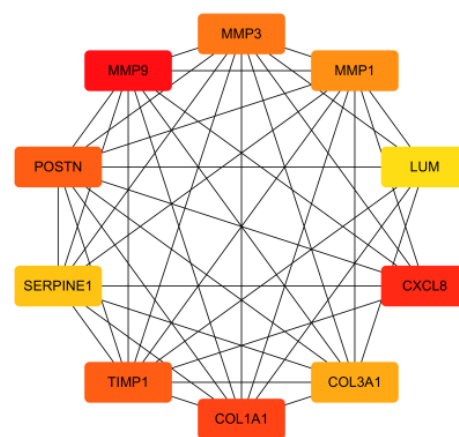


Figure 4: Core genes

2.4 Expression validation and survival analysis of the Hub genes

To further validate Hub gene expression, Hub genes were verified by the GEPIA online database. The GEPIA database shows a high expression of the Hub gene in tumor tissues (Figure 5). The expression of COL1A1, COL3A1, MMP9, TIMP1, LUM and POSTN in different stages of esophageal cancer (Figure 6), indicating that these genes are closely related to metastasis. To further understand the effect of the Hub gene on the survival of tumor patients, the effect of the Hub gene on esophageal cancer was analyzed by Kaplan-Meier plotter. The results showed that CXCL8, POSTN and LUM expression were inversely proportional to overall survival in esophageal cancer patients (Figure 7); MMP9, TIMP1, CXCL8

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and LUM expression were inversely proportional to disease-free survival in patients with esophageal cancer (Figure 8). MMP9, TIMP1, CXCL8, POSTN is

associated with LUM and prognosis and recurrence in patients with esophageal cancer.

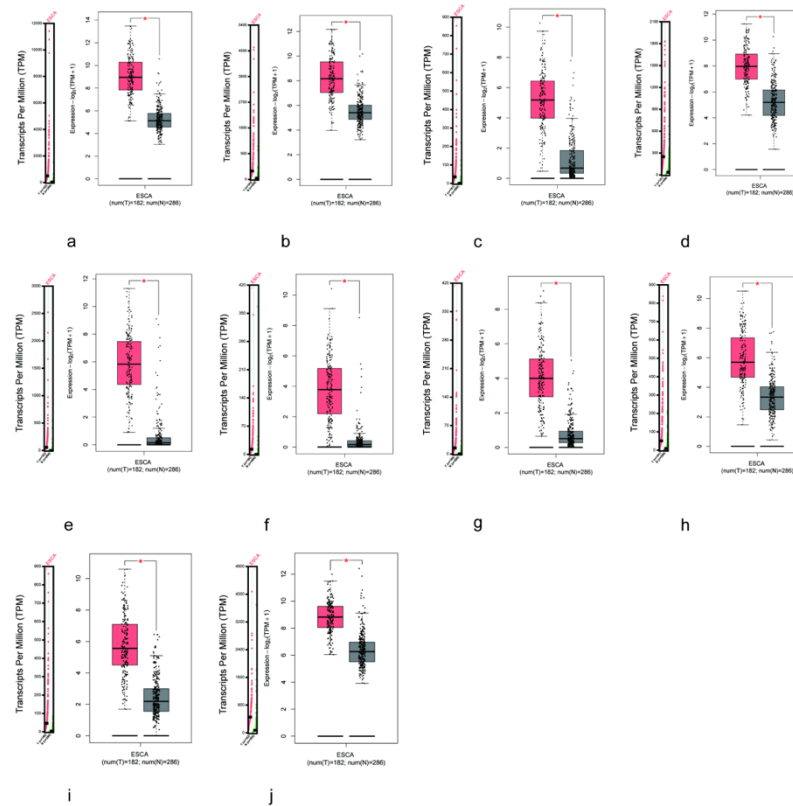


Figure 5: Hub gene expression validation (a.COL1A1; b.COL3A1; c.CXCL8; d.LUM; e.MMP1; f.MMP3; g.MMP9; h.POSTN; i.SERPINE1; j.TIMP1)

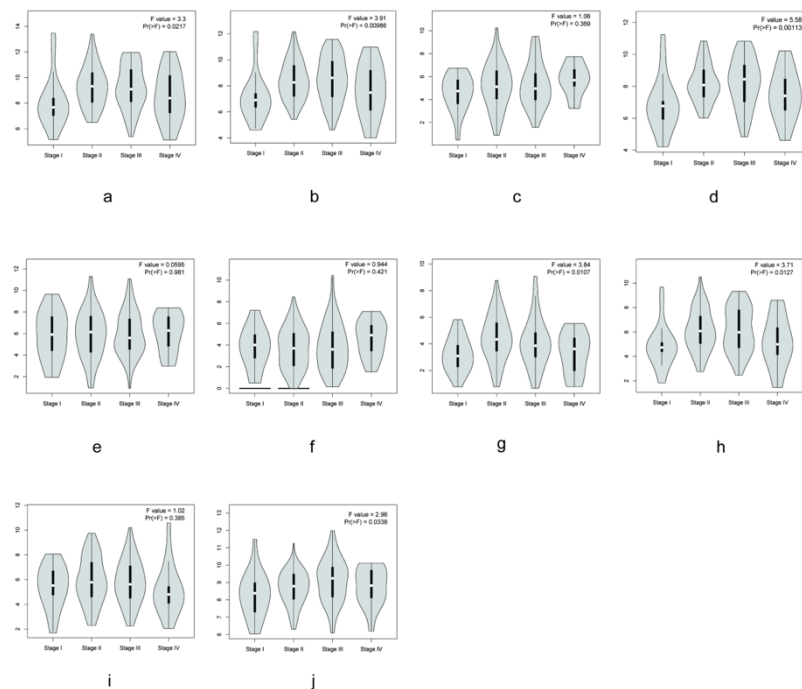


Figure 6: The expression of Hub genes during different tumor periods (a.COL1A1; b.COL3A1; c.CXCL8; d.LUM; e.MMP1; f.MMP3; g.MMP9; h.POSTN; i.SERPINE1; j.TIMP1)

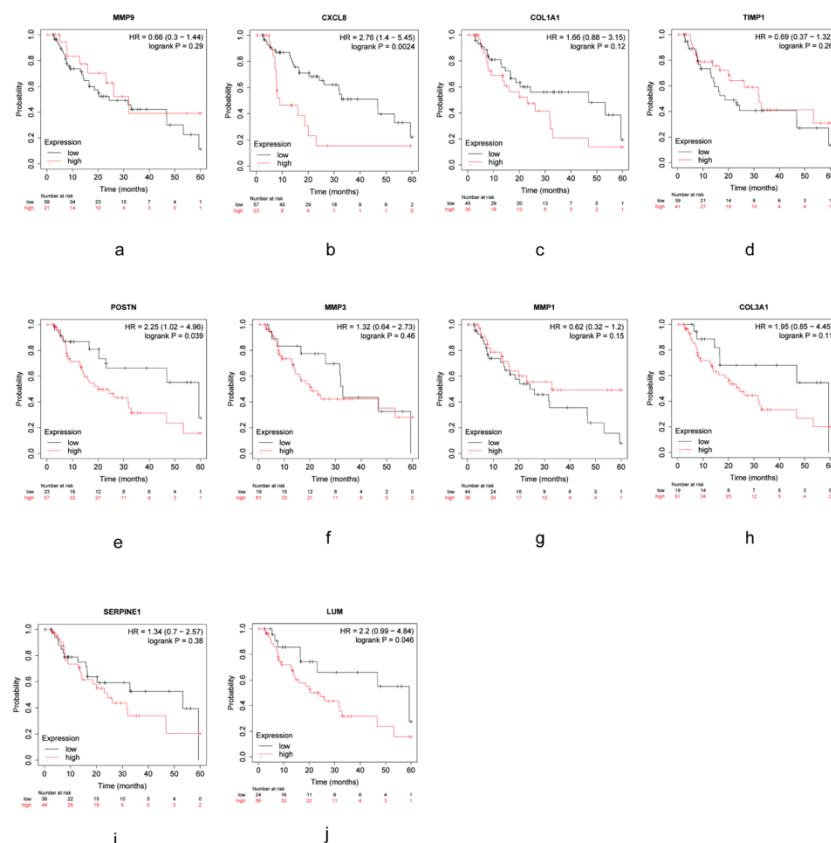


Figure 7: Total survival rate (a.MMP9; b.CXCL8; c.COL1A1; d.TIMP1; e.POSTN; f.MMP3; g.MMP1; h.COL3A1; i.SERPINE1; j.LUM)

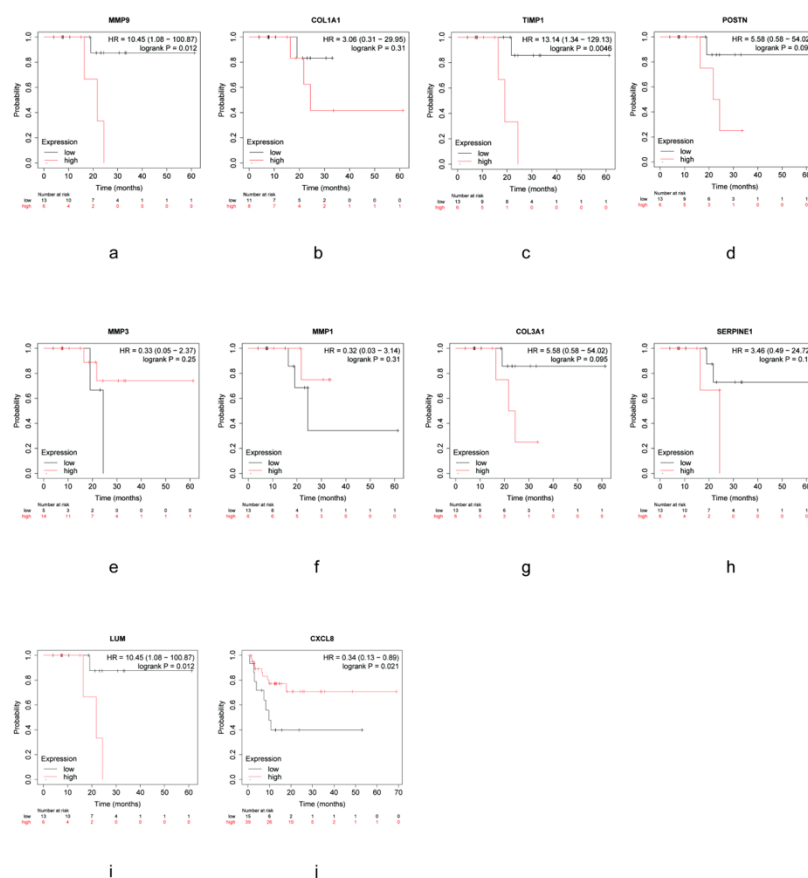


Figure 8: Disease-free survival rate (a.MMP9; b.COL1A1; c.TIMP1; d.POSTN; e.MMP3; f.MMP1; g.COL3A1; h.SERPINE1; i.LUM; j.CXCL8)

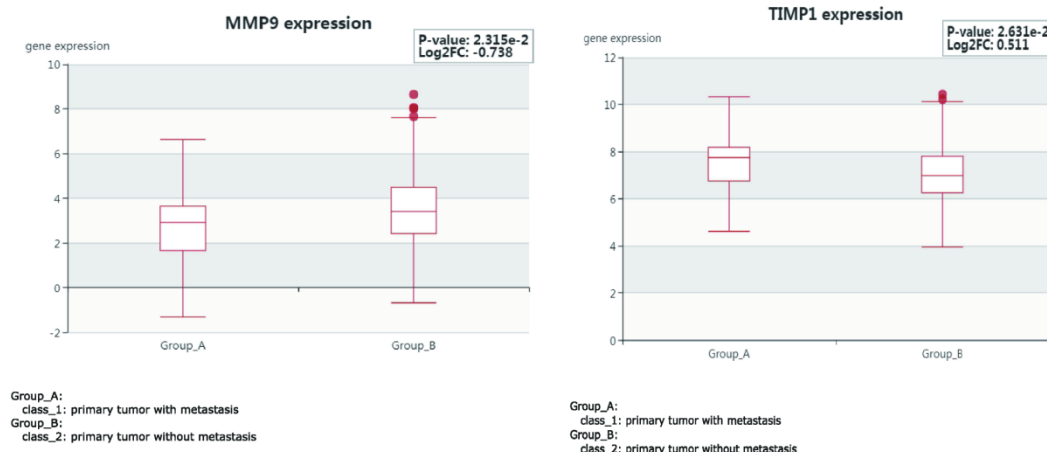


Figure 9: Expression of MMP9 and TIMP1 in both metastatic and nonmetastatic esophageal carcinoma

2.5 The Hub gene was associated with immune cell infiltration

The relationship of Hub gene expression and immune cell infiltration was analyzed by the TIMER database. The results showed that COL1A1 expression was positively associated with macrophage and dendritic cell infiltration, and both COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 and TIMP1 expression were negatively associated with tumor purity. COL3A1 expression was positively correlated with the infiltration of macrophages; LUM expression was compared with CD4⁺T cells were positively correlated with macrophage infiltration; MMP1 expression was associated with B cells compared with CD8⁺T cell infiltration was positively correlated; MMP9 expression levels versus CD4⁺T cells, macrophages, and neutrophils were positively associated with dendritic cell infiltration; POSTN expression was positively correlated with macrophage infiltration; SERPINE1 expression was with B cells, CD8⁺T cells, macrophages, and neutrophils were associated with DCs infiltration; TIMP1 expression was compared with CD4⁺T cells were positively correlated with macrophage infiltration. Expression of Hub genes can lead to infiltration of immune cells. The expression of COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 and TIMP1 can reduce tumor purity, indicating that the higher the expression of COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 and TIMP1, the higher the immune infiltration, the greater the chance of tumor mutation and metastasis [11](Figure 10).

2.6 The relationship between the Hub gene and the metastasis of oesophageal cancer

To further understand the relationship between the Hub gene and esophageal cancer metastasis, the expression of the Hub gene in patients with the primary and primary tumor with metastasis was analyzed by the

HCMDB database. The results showed that CXCL8, COL1A1, POSTN, MMP3, MMP1, COL3A1, SERPINE1 and LUM were not differently expressed in the primary tumor without metastasis in the Hub gene ($P > 0.05$). MMP9 expression was lower in patients with esophageal cancer with metastasis than in patients with tumor-free metastasis ($P < 0.05$, Log2FC:-0.738). TIMP1 expression was higher in patients with esophageal cancer with metastasis than in patients with tumor-free metastasis ($P < 0.05$, Log2FC:0.511). MMP9 is closely related to TIMP1 and esophageal cancer tumor metastasis, and can serve as a drug target to prevent tumor metastasis in esophageal cancer (Figure 9).

4. Discussion

Esophageal cancer is one of the deadliest malignancies in the world, and its incidence has risen sharply in recent years [12]. Early diagnosis of oesophageal cancer is difficult due to the lack of reliable cancer markers and specific clinical symptoms. At the same time, the metastasis rate and recurrence of esophageal cancer cause many patients to miss radical treatment, which is one of the reasons for the high mortality rate of esophageal cancer. In this study, differential analysis of four datasets by GEO2R yielded 149 DEGs, including 49 upregulated genes and 100 downregulated genes. We further explored the role of DEGs by GO with KEGG enrichment analysis. DEGs mainly has cellular components such as the extracellular matrix, keratinized envelope, collagen trimeric complexes, cell apical, intercellular junctions, and the actin cytoskeleton. The main pathways enriched in DEGs are IL-17 signaling, ECM-receptor interaction, p53 signaling, etc. Six members of the IL-17 family each exert different biological activity after binding to IL-17 receptors [13]. A lot of evidence suggests that IL-17 signaling is closely related to tumorigenesis and metastases [14-16]. IL-17 activates

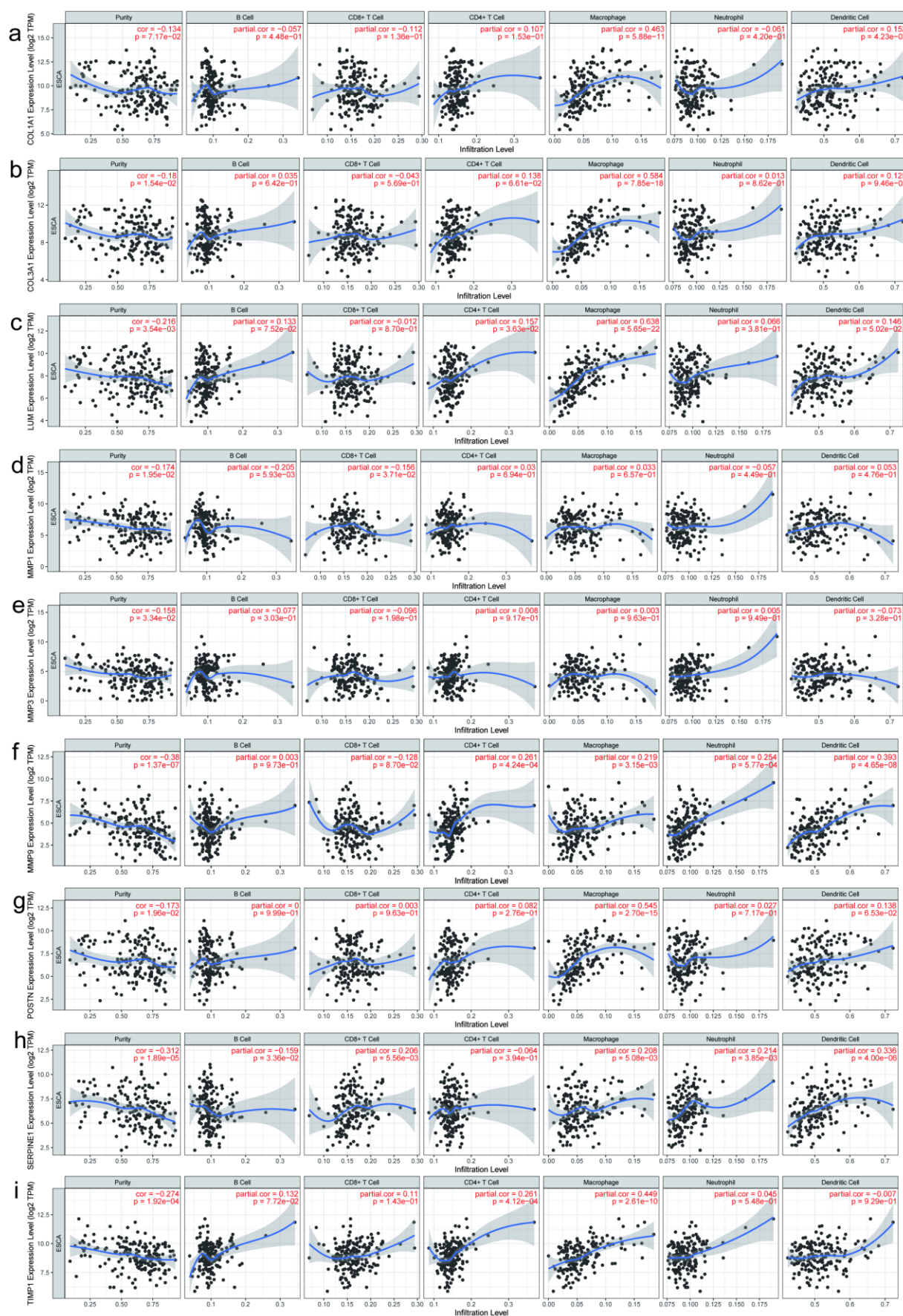


Figure 10: relationship between Hub gene expression and immune infiltration.

myeloid-derived cells through cytokines and chemokines, promoting angiogenesis and inhibiting tumor immunity in the body [17]. IL-17 can activate NF- κ B with P53 kinase and promote the expression of MMP2 with MMP9 [18]. Xiaoxia Li [19] found the IL-17-MMP7-ETM axis in prostate cancer was found by et al. IL-17 can activate MMP7 to promote ETM, is conducive to tumor metastasis [19]. P53 is an important tumor suppressor gene in the organism. The P53 signaling pathway is involved in biological processes such as cell cycle arrest, apoptosis, and repair of DNA damage [20]. The P53 gene is easily mutated, while the mutated P53 loses the function of DNA repair, which is one of the premises for tumorigenesis [21]. Mutations or loss of function of P53 were found in many tumors [22, 23]. At the same time, the mutant P53 is involved in the tumor proliferation and the formation of drug resistance [24, 25]. Many current studies have found that the restoration of mutant P53 to wild-type P53, suppresses tumor development and development by targeting P53 gene therapy [21]. 10 Hub genes (MMP9, CXCL8, COL1A1, TIMP1, POSTN, MMP3, MMP1, COL3A1, SERPINE1, LUM) were identified by the PPI network. These genes are highly expressed in esophageal cancer, are associated with tumor prognosis and metastasis, and can be used as marker genes for the diagnosis and prognosis of esophageal cancer.

Matrix metalloproteinases (MMPs) are a zinc-dependent family of proteases consisting of 24 members with the breakdown of the extracellular matrix. The expression of MMPs is closely related to tumor invasion and migration, cell apoptosis, angiogenesis, etc [26]. MMP1 expression can promote the degradation of the extracellular matrix, creating conditions for tumor metastasis [27]. In a clinical meta-analysis, MMP1 expression was closely related to multiple tumorigenesis, including lung cancer and colorectal cancer [27]. Both MMP3 and MMP9 have the role of resolving the extracellular matrix and thus promoting tumor metastasis. MMP3 expression can also activate MMP1 with MMP9, to further disrupt the intercellular matrix [28]. It has been experimentally shown that overexpression of MMP3 can promote the development of breast cancer in mice [29]. Okusha found that the proliferation of tumor cells knocking down MMP3 was inhibited compared with invasion and migration in vitro [30]. Knockdown of MMP3 also expands the area of tumor necrosis and inhibited tumor cell proliferation [31]. MMP1, MMP3 was found in different expression and MMP9 at different periods of esophageal cancer through the GEPIA online database, indicating that MMP1, MMP3 and MMP9 expression are one of the factors prone to esophageal cancer and metastasis. This is in agreement with the present experimental results: Liu Min [32], Li Hong [33], Cheng Yue [34] It is separately proved that the

expression of MMP1, MMP3 and MMP9 favors the value-added value, migration and invasion of esophageal cancer.

Metalloprotease inhibitor 1 (TIMP1) is a member of the metalloprotease inhibitor (TIMPs) family that encodes matrix metalloprotease inhibitors to inhibit MMPs function and prevent degradation of the extracellular matrix [35]. Meanwhile, TIMP1 also has nonmetalloproteinase-dependent functions, such as cell proliferation, apoptosis, etc [36]. TIMP1 expression can inhibit tumor metastasis by inhibiting MMPs [35, 37]. However, overexpression of TIMP1 can increase the transcription factors of epithelial-stromal transformation to promote epithelial-stromal transformation, upregulate MMPs expression, and contribute to tumor metastasis [38]. This shows that the relationship between TIMP1 and MMPs is complex and not completely positive and negative related. Many studies have found that TIMP1 is upregulated in tumor tissues and that high expression of TIMP1 is associated with poor tumor prognosis [39-42]. This may be associated with TIMP1 regulation of cell proliferation and apoptosis. TIMP1 can activate the PI3K/Ark pathway to promote cell proliferation [43]. Hayakawa found that TIMP1 can act as a growth factor to promote the proliferation of multiple tumor cells [44]. Guede L found that lymphoma cells with high TIMP1 expression were resistant to the induction of both endogenous and exogenous apoptosis, whereas TIMP1-negative lymphoma cells were not resistant to apoptosis induction [45].

CXCL8 is the gene encoding for interleukin 8 (IL-8). IL-8 is one of the mediators of the inflammatory response in the organism. In addition to being secreted by inflammatory cells such as macrophages, fibroblasts, and neutrophils, many tumor cells can also secrete IL-8 and be associated with tumor migration, invasion, and angiogenesis [46]. Many studies have found that CXCL8 expression is upregulated in tumors [47,48]. Wu et al found increased expression in bladder urothelial carcinoma and was positively associated with vascular endothelial growth factor (VEGF) and MMP9 expression, et al [49]. It shows that CXCL8 expression is closely related to tumor invasion and metastasis. Evidence has been shown that exogenous increased CXCL8 can promote the migration and invasion of hepatocellular carcinoma [50]. Thomas M et al found that CXCL8 induces angiogenesis and is a pro-angiogenic agent in tumor-fibroblasts [51]. CXCL8 can also promote MDSC infiltration together with the activation of regulatory T cells [52]. In this study, database validation revealed increased CXCL8 expression in esophageal cancer tissues compared with normal esophageal tissues. At the same time, high expression of CXCL8 was associated with OS in esophageal cancer and a poor prognosis of DFS. Consistent with our results, Hosono M found that

CXCL8 expression is upregulated in esophageal cancer and used to promote the metastasis and invasion of tumor cells by phosphorylation of Akt and Erk1/2 [53].

COL1A1 is the coding gene coding for type I collagen. Many studies have shown that COL1A1 expression correlates with tumour development [54, 55]. COL1A1 expression is increased in gastric cancer tissues and is associated with tumor invasion [56]. Zheyang Zhang found that COL1A1 expression was upregulated in colorectal cancer, while high COL1A1 expression could not only promote colorectal cancer invasion and metastasis, but also be associated with the poor prognosis of tumors [57]. COL1A1 can also regulate cell apoptosis through the Caspase-3, with PI3K/Akt pathway [58]. COL3A1 can encode type III collagen. Type III collagen and type I collagen are the components of the connective tissue. COL3A1 expression is upregulated in epithelial ovarian cancer and is a marker of a poor prognosis in tumors [59]. COL3A1 can regulate the extracellular matrix to promote tumor development [60].

SERPINE1 can encode a serine protease inhibitor that inhibits fibrin degradation. The present study has found that SERPINE1 expression is correlated with tumor proliferation, metastasis, and tumor cell resistance [61,62]. Expression of SERPINE1 promotes the migration of oral cancer with epithelial-interstitial transformation [63]. In NPC, downregulation of SERPINE1 expression can inhibit the metastasis of tumor cells and promote apoptosis [64]. SERPINE1 expression promotes the rate of HNSCC lymph node metastasis and the area of distant tumor dissemination [65]. It was also found that SERPINE1 could inhibit apoptosis in tumor cells by Fas/Fas-1 signaling with inhibitory Caspase-3 activation [66,67]. Qian et al found that SERPINE1 expression was associated with resistance to breast cancer and that SERPINE1 knockdown inhibited breast cancer resistance to purple oil and apoptosis of tumor cells [68]. High expressed SERPINE1 is also associated with poor prognosis in oral cancer [69]. Klimzak-Bitner AA et al found that SERPINE1 expression was upregulated in oesophageal cancer, which was also associated with the prognosis of oesophageal cancer [70].

POSTN can encode secreted extracellular matrix proteins and have a role in inducing cell adhesion and diffusion [71]. Many current studies have found increased POSTN expression in many tumors, and that the high expression of POSTN plays an important role in tumor invasion, metastasis and angiogenesis [72-74]. POSTN can promote the development of breast cancer and angiogenesis [75]. Sasaki H et al found increased POSTN expression in patients with breast cancer with bone metastasis, indicating that high expression of POSTN has a role in promoting tumor metastasis [76]. Hyeon Jeong et al found that POSTN expression was upregulated in colorectal cancer, especially in invasive

colorectal cancer [77]. The role of POSTN in tumor invasion may be associated with its regulation of E-cadherin and N-cadherin. POSTN can downregulate E-cadherin expression by Snail [78]. Soon Young et al found that knockdown of POSTN reduced glioma invasion with N-cadherin expression, thereby inhibiting tumor metastasis [79].

LUM can encode a leucine-rich small-molecule proteoglycan (Lumican). Lumican is one of the components of the extracellular matrix and is associated with cell proliferation, adhesion, metastasis and differentiation [80]. LUM is expressed in many tumors, and at the same time, LUM functions more complex and can act as promoting or inhibitory effects for different types of tumor [81]. It has been shown that LUM plays an inhibitory role in melanoma [82]. LUM can inhibit the expression and activity of MMP9, MMP14, and thereby inhibit melanoma metastasis [83]. LUM expression was upregulated in lung cancer, and it was correlated with pleural infiltration in lung adenocarcinoma [84]. In pancreatic cancer, Symeonidis et al [85]. It was suggested that LUM could inhibit the proliferation and expansion of pancreatic cancer, but we found that LUM expression promoted the growth of pancreatic cancer by Yamamoto et al [86]. In gastric cancer, LUM can promote tumor metastasis through FAK signaling [87]. High expression of LUM was associated with low survival in colorectal cancer and high metastasis, Wang et al [88]. The mechanism of role of LUM in tumorigenesis and development is temporarily unclear. This study found that LUM expression was correlated with the poor prognosis of esophageal cancer, and at the same time varied in esophageal cancer tissues during different periods. This suggests that LUM expression may be associated with metastatic recurrence of esophageal cancer, which can be one of the therapeutic targets of esophageal cancer.

To understand the relationship between core gene expression and immune infiltration in the tumor microenvironment, we performed immune infiltration analysis of core genes through the TIMER database. We found that COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 expression and TIMP1 was inversely associated with tumor purity, and that the expression of COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 and TIMP1 could promote the infiltration of immune cells in the tumor microenvironment. It has been found that TILs is closely related to tumor development and has a role in inhibiting or promoting tumor development [89]. CD8⁺T cells and NK cells can kill tumor cells and inhibit tumor cell proliferation [89]. CD8⁺Patients with high tumor infiltration of T cells and NK cells had a better prognosis [90]. Tumor-associated macrophages in TME can be classified into M1 and M2. Type M1 macrophages were found to inhibit tumor cells, and

type M2 macrophages can promote tumor development [91]. Tumor-infiltrating dendritic cells (TIDCs) are antigen-presenting cells that, by presenting the tumor antigen, enable T cells to recognize and kill the tumor [89]. Several studies have found that infiltration of TIDCs is associated with low metastasis rates of various tumors with prolonged survival [92]. It has also been found that the overall survival is better in colorectal cancer patients with a small number of TIDCs infiltrates [93]. The role of tumor-infiltrating B cells (TILB) in tumors is temporarily elusive. There is evidence that TILB correlated with lung cancer and good prognosis [94]. It has also been found to promote TILB promote tumor growth [95]. Tumor-associated neutrophils can promote the expression of matrix metalloproteinase (MMP) to promote tumor metastasis [96].

In conclusion, this study analyzed four datasets and obtained 149 DEGs, associated with the development of esophageal cancer, including 10 Hub genes. These Hub genes are all highly expressed in esophageal cancer and can serve as diagnostic markers in the early stage of esophageal cancer. In the Hub gene MMP9, TIMP1, CXCL8, POSTN expression of LUM is associated with poor prognosis in patients with esophageal cancer, and is a potential standard for poor prognosis of esophageal cancer. We also found that COL3A1, LUM, MMP1, MMP3, MMP9, POSTN, SERPINE1 expression with TIMP1 correlates with the infiltration of tumor immune cells. These genes may be key targets for esophageal cancer immunotherapy and may play a role in immune infiltration therapy.

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