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RESEARCH

Distinct immune signatures for predicting the immunotherapy efficacy of esophageal squamous cell carcinoma or adenocarcinoma

Peng Wu[1] · Guohui Qin[1] · Jinyan Liu[1] · Qitai Zhao[1] · Xueke Zhao[7] · Xin Song[7] · Lidong Wang[7] · Shengli Yang[1,6] · Yi Zhang[1,2,3,4,5]

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Abstract

Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are distinct histological subtypes of esophageal cancer. The tumor microenvironment of each subtype significantly influences the efficacy of immunotherapy. However, the characteristics of the tumor microenvironments of both subtypes, as well as their specific impacts on immunotherapy outcomes, still require further elucidation. Through the integration of gene expression profiles from ESCC and EAC obtained from The Cancer Genome Atlas database, alongside tumor tissues derived from Chinese patients, we identified TNFSF10, CXCL10, IL17RB, and CSF2 as pivotal immune molecules with significant prognostic implications. Elevated expression levels of TNFSF10 correlated with adverse outcomes in individuals diagnosed with ESCC. In contrast to patients from other geographical regions, CXCL10, IL17RB, and CSF2 exhibited distinct prognostic implications in Chinese patients with esophageal cancer. The Cox risk scores derived from the molecules TNFSF10 and CXCL10 for ESCC and IL17RB and CSF2 for EAC were used to assess their predictive capacity for immunotherapy efficacy. The results indicate that patients with lower Cox risk scores demonstrated an enhanced response to immunotherapeutic interventions. This study revealed significant disparities in the expression and functionality of immune-related molecules between ESCC and EAC and highlighted the potential of Cox risk scores derived from immune-related molecules to predict the efficacy of immunotherapy in patients. The findings underscore the clinical relevance of these biomarkers and emphasize the necessity for developing ethnic-specific biomarkers to guide personalized immunotherapy strategies between ESCC and EAC.

Keywords Esophageal squamous cell carcinoma · Esophageal adenocarcinoma · Tumor microenvironment · Chinese patient characteristics · Immunotherapy

Introduction

Globally, esophageal cancer ranks seventh among the deadliest cancers, with approximately 20% of patients surviving for at least five years after diagnosis [1]. Unlike in Western

Peng Wu and Guohui Qin have contributed equally to the present study.

✉ Shengli Yang
slyang@caeshc.com.cn

✉ Yi Zhang
yizhang@zzu.edu.cn

1 Biotherapy Center and Cancer Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China

2 State Key Laboratory of Esophageal Cancer Prevention and Treatment, Zhengzhou 450052, Henan, China

3 School of Life Sciences, Zhengzhou University, Zhengzhou 450052, Henan, China

4 Tianjian Laboratory of Advanced Biomedical Sciences, Academy of Medical Sciences, Zhengzhou University, Zhengzhou 450052, Henan, China

5 School of Public Health, Zhengzhou University, Zhengzhou 450052, Henan, China

6 Center for Translational Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China

7 State Key Laboratory of Esophageal Cancer Prevention & Treatment and Henan Key Laboratory for Esophageal Cancer Research of The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, Henan, China

countries, where esophageal adenocarcinoma (EAC) constitutes over 80% of esophageal cancer cases, esophageal squamous cell carcinoma (ESCC) constitutes the vast majority, exceeding 97%, whereas EAC constitutes only 1.5% in China [2, 3]. In addition, there are wide discrepancies in the epidemiological characteristics, risk factors, and gene mutation profiles between China and Western countries [4, 5].

As tumorigenesis is closely correlated with mutations, mutated genes continue to be investigated as ideal targets for targeted therapies. The findings from whole exome sequencing have revealed a scarcity of common driver mutations in esophageal cancer, which poses a challenge for the development of targeting therapies [6]. Furthermore, the advent of immunotherapy, which enhances or activates the patient's immune system to combat cancer cells, has instilled new hope in patients diagnosed with esophageal cancer. Consequently, a comprehensive investigation into the immune characteristics associated with the onset and progression of esophageal cancer is instrumental in enhancing the development of effective treatment strategies for this malignancy.

Studies on esophageal cancer mainly focus on understanding the genetic traits and signaling pathways of malignant cells in both ESCC and EAC, particularly considering regional variations in disease prevalence [7]. However, there has been limited attention on the variations in immune factors. This study aimed to clarify the tumor microenvironment characteristics of these two subtypes by analyzing sequencing data from The Cancer Genome Atlas (TCGA) and tumor samples from Chinese cohorts. Key immune molecules, including TNFSF10, CXCL10, IL17RB, and CSF2, were identified as having significant prognostic implications. Elevated expression levels of TNFSF10 correlated with unfavorable outcomes in patients with ESCC. Notably, CXCL10, IL17RB, and CSF2 displayed distinct prognostic profiles among Chinese patients with esophageal cancer compared to those from other regions. Cox regression models were used to calculate risk scores. This model incorporated TNFSF10 and CXCL10 for ESCC immune scores, along with IL17RB and CSF2 for EAC immune scores. The results showed that patients with lower scores experienced better clinical outcomes after receiving immunotherapy. This study highlights the significant disparities in immune-related molecular expression and functionality between ESCC and EAC, while emphasizing the clinical relevance of these biomarkers in predicting immunotherapy efficacy, thus guiding the development of ethnicity-specific biomarkers for personalized immunotherapy strategies.

Materials and methods

Clinical samples

The samples were obtained and histopathologically confirmed as ESCC or EAC by the State Key Laboratory of Esophageal Cancer Prevention & Treatment at Zhengzhou University (Zhengzhou, Henan, China). None of the enrolled patients had received chemotherapy, radiotherapy, or any other treatment before surgery. After post-fixation in a 4% paraformaldehyde fixative, the tissue samples were embedded in paraffin, and subsequently, sections of a 5-um thickness were used for tissue microarray preparation.

Common platforms and R packages for data analysis

The disparities in gene expression between tumor tissues (ESCC or EAC) and adjacent non-tumor tissues (normal tissues) were evaluated using the limma package (version 3.60.3) in R (version 3.56.2). To control the false discovery rate and thereby ensure accuracy in the differential gene expression analysis, the Benjamini–Hochberg method was applied to adjust the P-values. Enrichment analysis for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms, as well as Venn analysis, were performed using the Xiantao platform (https://www.xiantaozi.com/). The OmicsBean platform (http://www.omicsbean.cn) was used to generate a protein–protein interaction (PPI) network, considering gene/protein expression levels, KEGG pathway enrichment, and biological process enrichment. The mutated genes were visualized in R using the maftools package (version 2.18.0).

Tumor tissue microarray and immunohistochemical staining

Tumor microarrays were subjected to immunohistochemical analysis after fixation in 4% paraformaldehyde and embedding in paraffin. Sections were dewaxed with xylene and graded alcohol concentrations, followed by antigen retrieval by heating in 0.01 mol/L citrate buffer. After cooling, endogenous peroxidase activity was quenched with 3% H_2O_2. The tissue sections were incubated with primary antibodies against TNFSF10 (Catalog No. ab2056; Abcam), CXCL14 (catalog no. ab137541; Abcam), CXCL8 (catalog no. ab106350; Abcam), IL17RB (Catalog No. AF1207; R&D Systems), CXCL10 (Catalog No. ab306587; Abcam, Cambridge, UK), HIF1α (catalog no. ab51608; Abcam), CSF2 (catalog no. ab316862; Abcam), and NOS2 (catalog no. ab283655; Abcam) (diluted at 1:500 in phosphate-buffered saline) overnight at 4℃. The following day, after incubation

at 25℃, the samples were incubated with a secondary antibody conjugated to horseradish peroxidase for 30 min. Color development was achieved using DAB (ZSGB-BIO, #ZLI-9017), and the nuclei were counterstained with hematoxylin (Solarbio; #G4491). After dehydration in a graded series of alcohol solutions and clearance with xylene, the slides were examined using the Pannoramic DESK, P-MIDI, and P250 systems. The evaluation was subsequently scored using the Pannoramic Scanner software.

Survival analysis

The association between the prognostic relevance of the identified molecules and survival outcomes in patients with esophageal cancer was assessed using Kaplan–Meier survival analysis. Survival rates were compared using the log-rank test. The groups were categorized based on the optimal cut-off value. The most favorable cut-off value for survival data was calculated with the survminer R package (version 0.4.9).

Immune infiltration score and correlation analysis

Gene expression profiling serves as a foundation for estimating immune cell populations within the tumor microenvironment. This was achieved by leveraging the Immunedeconv R package (version 2.0.0) along with single-sample gene set enrichment analysis (ssGSEA), xCell, and CIBERSORT methods. Spearman's correlation was used to assess the relationships between various groups, whereas hierarchical clustering with Euclidean distance was employed for data aggregation. The P-values obtained were subsequently adjusted using the Benjamini–Hochberg procedure to mitigate false discovery rates.

Construction of prediction model

Preliminary screening identified molecules (TNFSF10, CXCL14, CXCL10, and HIF1α in ESCC; CXCL8, IL17RB, CSF2, and NOS2 in EAC) with significant survival correlations. Sequencing data from five datasets (GSE91061, GSE100797, GSE93157, PRJEB23709, and PRJNA482620), which have been standardized and filtered to exclude low-expression genes, were retrieved from the Tumor Immunotherapy Gene Expression Resource (TIGER) database (http://tiger.canceromics.org/). Following the verification of the structural integrity and correctness of the data through the str () function, the survival package (version 3.7.0) was utilized for survival analysis, whereas the dplyr package (version 1.1.4) facilitated data processing. The Cox proportional hazards model was fitted using the coxph function, incorporating various signature genes as covariates to assess their potential impact on survival time. The regression coefficients for these genes were estimated using a Cox proportional hazards model, with gene expression serving as the predictor and survival duration as the outcome. By utilizing these coefficients along with the expression levels of the signature genes, individual patient Cox risk scores were computed.

Statistical analysis

Once the normality and homogeneity of variance were evaluated, statistical significance was ascertained by employing the Mann–Whitney U test, with $P < 0.05$ serving as the threshold for significance unless otherwise stipulated. Computations for these analyses were performed using the Xiantao platform. Data presentations in graphical form are depicted as the mean ± standard error of the mean (SEM). The significance levels are as follows: ns: no significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

Results

Immune infiltration and functional enrichment of differentially expressed genes in esophageal cancer tissues

In conjunction with the distribution characteristics of various histological subtypes of esophageal cancer, gene mutation analysis conducted on ESCC and EAC patients within TCGA revealed that in addition to *TP53* and *TTN*, the mutation rates for other genes did not exceed 30% (Fig. S1a, b). Comparative analysis of differentially expressed mRNA profiles between ESCC and EAC tissues versus normal esophageal mucosa revealed varying gene counts (Fig. 1a, b), with KEGG pathway enrichment highlighting a notable enrichment of overexpressed genes involved in cytokine-cytokine receptor interactions (Fig. 1c, d). Further, GO enrichment analysis revealed that most of the evaluated genes were enriched in cytokine activity in both ESCC and EAC (Fig. S2a, b). Detailed enrichment information for the differentially expressed and downregulated genes is shown in Fig. S2c, d. Based on these results, it can be concluded that cytokines or cytokine receptors may represent promising targets for investigating the prognosis of esophageal cancer.

To comprehensively evaluate the tumor microenvironment in esophageal cancer, immune infiltration analysis was performed. The findings revealed that, compared to normal tissues, the tumor microenvironments of ESCC and EAC displayed a significant enrichment of activated dendritic cells (aDCs), regulatory T cells (Tregs), T helper 1 (Th1) cells, and Th2 cells, whereas the infiltration levels of $CD8^+$ T cells, mast cells, and natural killer

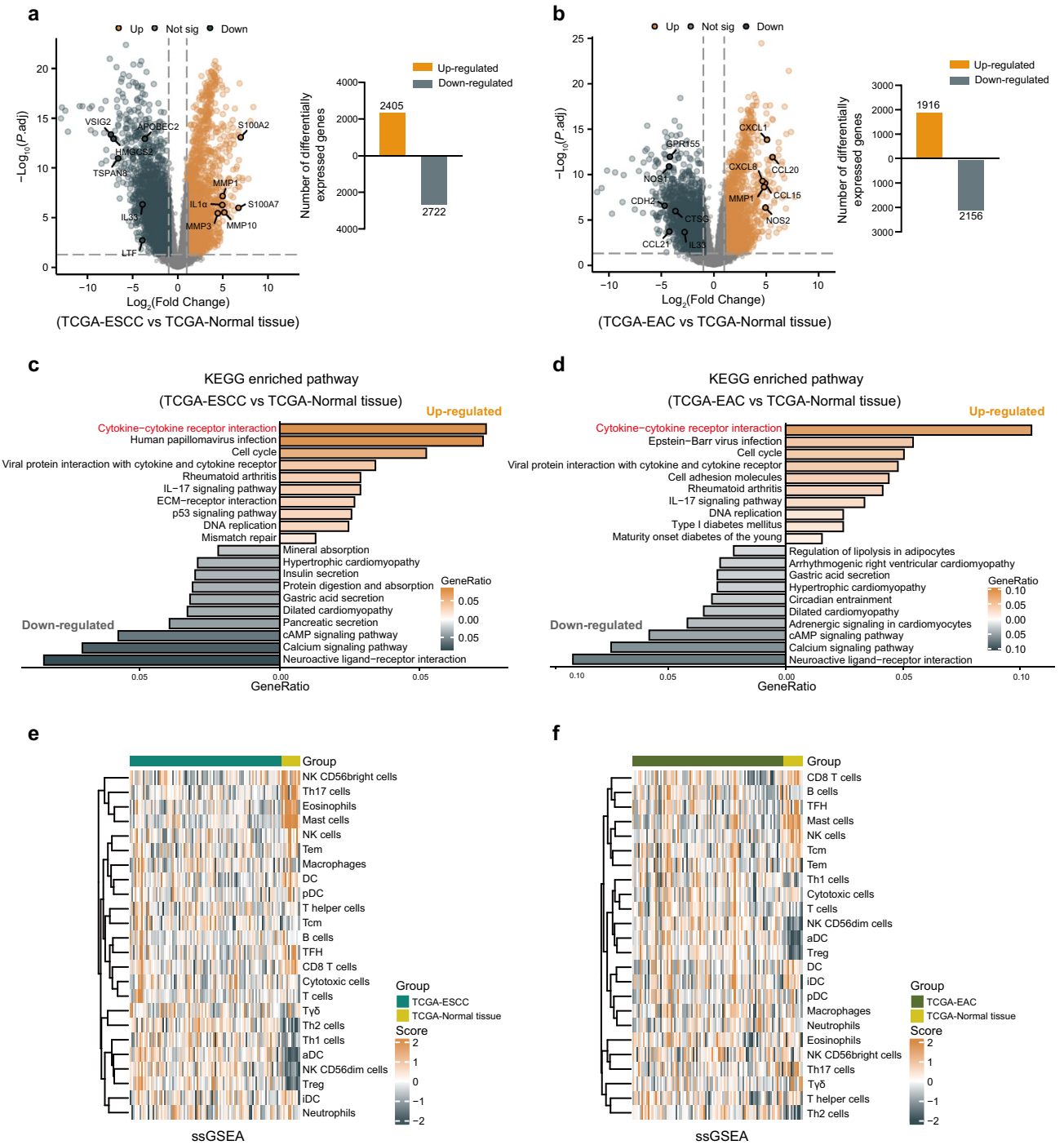


Fig. 1 Pathway enrichment of differential genes in esophageal carcinoma tissues and the characteristics of immune infiltration. **a, b** Left: Volcano plot shows significant gene expression differences between ESCC/EAC vs. normal tissues in TCGA. Right: Histograms count upregulated and downregulated genes in ESCC and EAC (|log2 Fold change|≥ 1, P < 0.05). **c, d** Top 10 KEGG pathways enriched by differentially expressed genes in ESCC/EAC from TCGA. **e, f** Heatmaps show immune-infiltrating scores of various immune cells in ESCC, EAC, and normal tissues from TCGA, calculated by ssGSEA. KEGG: Kyoto Encyclopedia of Genes and Genomes; ssGSEA: single-sample Gene Set Enrichment Analysis

cells (NKs) were markedly diminished (Fig. 1e, f). The observed levels of immune cell infiltration were further substantiated by immune infiltration scores derived from CIBERSORT (Fig. S2e, f), and xCells (Fig. S2g, h) methodologies. Importantly, while sharing certain commonalities, the tumor microenvironments of ESCC and EAC

exhibit distinctive immune infiltration characteristics. Notably, central memory T (Tcm) cells were significantly enriched in ESCC tissues but demonstrated an opposite infiltration trend in EAC tissues (Fig. 1e, f). Moreover, the infiltration of eosinophils and effector memory T (Tem) cells was significantly diminished in ESCC tissues compared to their normal counterparts (Fig. 1e), whereas the recruitment of immature dendritic cells (iDC) was markedly reduced in EAC tissues (Fig. 1f). Furthermore, a notable enhancement in macrophage recruitment was observed in the ESCC samples (Fig. S2g). The above analysis of esophageal cancer elucidated gene mutation signatures and immune infiltration patterns in both ESCC and EAC, highlighting cytokines and their receptors as promising prognostic targets. The unique immune landscape associated with these malignancies indicates that personalized immunotherapeutic approaches may yield significant therapeutic benefits.

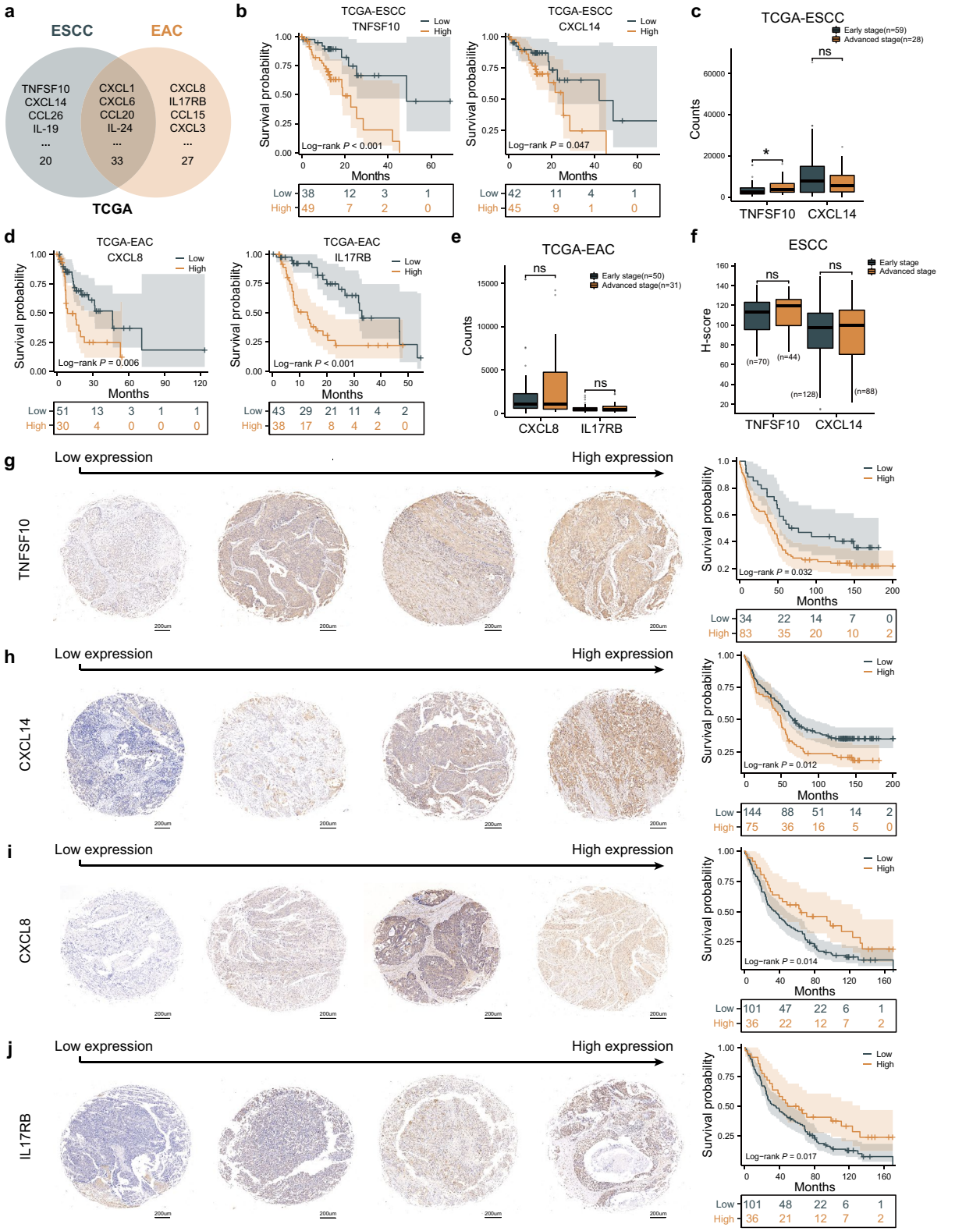
Identifying distinct cytokines associated with clinical outcomes in ESCC and EAC

Venn analysis of TCGA data revealed distinct profiles of upregulated cytokines and cytokine receptors in ESCC and EAC tissues (Fig. 2a). Kaplan–Meier survival analysis demonstrated a correlation between higher expression of TNFSF10 or CXCL14 and poorer prognosis in patients with ESCC (Fig. 2b), yet, elevated IL17RB or CXCL8 levels were significantly negatively associated with survival in patients with EAC within TCGA (Fig. 2d). Clinicopathological analysis showed that only TNFSF10 was upregulated in advanced-stage ESCC samples (Fig. 2c, e). To further investigate the role of these molecules, we conducted immunohistochemical analyses of tissue samples from Chinese patients with esophageal cancer and found that the expression levels of TNFSF10, CXCL14, CXCL8, and IL17RB varied among individuals (Fig. 2g–j). While the expression levels of TNFSF10 and CXCL14 were not significantly different between patients with early and advanced-stage ESCC in China (Fig. 2f), survival analysis conducted after stratifying patients based on the H-scores of TNFSF10 or CXCL14 indicated that their increased expression contributed to the poor prognosis (Fig. 2g, h). In contrast, increased CXCL8 and IL17RB levels in Chinese patients with EAC were associated with improved overall survival (Fig. 2i, j). The prognostic effects of CXCL8 and IL17RB exhibited regional variation. Therefore, it has been suggested that cytokines and cytokine receptors play distinct roles in the tumor microenvironment of different esophageal cancer types and across diverse ethnic populations.

Tumor microenvironment-related genes present diverse expression patterns in ESCC and EAC

In a previous analysis of differentially expressed genes and tumor microenvironment characteristics related to esophageal cancer, diminished levels of immune infiltration by $CD8^+$ T cells and other cytotoxic cells were noted at tumor tissue sites. Conversely, Tregs, macrophages, and other cells were significantly recruited to these tumor regions (Fig. 1 e, f). Consequently, based on the molecules that modulate the tumor microenvironment, specifically those associated with effector T cell aggregation and activation, immune checkpoints, and key factors that promote an immunosuppressive environment, 69 genes were selected to elucidate the differences in the immune status of ESCC and EAC after evaluating their expression levels compared with adjacent normal tissues (Fig. S3a, b). These genes encode various types of molecules, such as chemokines, cytokines, transcription factors, immune checkpoints, and surface markers. In comparison to normal esophageal mucosa, the quantities of differentially expressed genes associated with the regulation of the tumor microenvironment in ESCC and EAC were different (Figs. 3a, 4a). PPI network analysis indicated that molecules such as CD38, FOXP3, and CD14 were significantly overexpressed in ESCC, suggesting increased infiltration of immunosuppressive cells within the tumor microenvironment (Fig. 3b). In contrast, most upregulated molecules in EAC tissues were associated with rheumatoid arthritis and TNF signaling pathways, indicating a potential link between EAC development and inflammatory processes (Fig. 4b). The correlation between EAC pathogenesis and gastroesophageal reflux disease or Barrett's esophagus further supports this observation [8]. These results imply that the mechanisms driving the formation of the immunosuppressive tumor microenvironment differ between ESCC and EAC.

The association between the upregulated differentially expressed genes and patient outcomes was further investigated. Elevated CXCL10 levels were associated with decreased overall survival in patients with ESCC in TCGA database (Fig. 3c). In Chinese patients with ESCC, high expression of CXCL10 prevented tumor progression, in contrast to the results in other races (Fig. 3d). In alignment with the results from patients of nonChinese background with ESCC, elevated HIF1α expression in Chinese patients was linked to an unfavorable outcome (Fig. 3e, f). Elevated CSF2 expression was significantly associated with poor prognosis in patients with EAC from other countries (Fig. 4c). However, EAC samples from Chinese patients showed that high CSF2 expression prolonged



overall survival (Fig. 4d). Analysis of patients with EAC in China and other countries revealed that those in the high NOS2 expression group exhibited prolonged survival (Fig. 4e, f). These findings indicate that important immune molecules may have different effects on esophageal cancer in the Chinese and other ethnic groups.

◂**Fig. 2** Detecting unique cytokines linked to clinical outcomes in esophageal squamous cell carcinoma and adenocarcinoma separately. **a** Venn diagram shows genes in the cytokine-cytokine receptor pathway for ESCC and EAC from TCGA, annotated with gene counts in distinct and overlapping categories. **b** Overall survival of patients with TCGA-ESCC and different expression levels of TNFSF10 and CXCL14. **c** TNFSF10 and CXCL14 expression comparison between early and advanced ESCC tissues (TCGA). **d** Survival probabilities of patients with EAC classified by CXCL8 and IL17RB expression levels (TCGA). **e** CXCL8 and IL17RB expression during EAC progression from early to advanced stages. **f** TNFSF10 and CXCL14 expression in early and advanced ESCC tissues of Chinese patients. **g, h** The left panel presents immunohistochemical images illustrating TNFSF10 or CXCL14 staining in tissue microarrays obtained from patients diagnosed with ESCC, arranged sequentially from left to right based on ascending expression levels quantified by the H-score. On the far left, the lowest expression level is shown, followed by a sample in the lowest 33.33 percentile, then a moderate expression sample in the next 33.33 percentile, and finally, the highest expression level on the far right. The right panel displays survival curves stratified by the expression of TNFSF10 and CXCL14, categorized into low and high expression groups based on their H-scores. **i, j** The left panel displays immunohistochemical staining for CXCL8 or IL17RB in tissue microarrays from patients with EAC, with samples ordered from left to right by increasing H-scores. The sequence starts with the lowest expression level, followed by a sample in the first 33.33 percentile, then a sample with moderate expression in the next percentile, and ends with the highest expression level on the far right. P values were calculated using the Mann–Whitney (log-rank) test. Early stage: Stage 0-II; Advanced stage: Stage III-IV; ns: no significance; $*P < 0.05$, Mann–Whitney test

Combinations of immune molecules could predict the response to immunotherapy in patients with cancer

Molecular signatures within the esophageal cancer microenvironment significantly affect the efficacy of immunotherapy. Correlation analyses among patients with ESCC and EAC revealed varying degrees of association between these molecules and immune checkpoints (Fig. S4a). Cox risk scores were used to assess the potential of immune-related molecules as predictive biomarkers for response to immunotherapy in solid tumors. Specifically, ESCC immune scores were defined by considering Cox risk scores obtained from various combinations of TNFSF10, CXCL14, CXCL10, and HIF1α. Similarly, EAC immune scores were determined by considering the Cox risk scores obtained from different combinations of CXCL8, IL17RB, CSF2, and NOS2. By integrating five immunotherapy-related datasets, it was found that, compared to other gene combinations, the ESCC immune scores derived from TNFSF10/CXCL10 or TNFSF10/CXCL10/HIF1α, and the EAC immune scores derived from IL17RB/CSF2 or IL17RB/CSF2/NOS2, were effective in distinguishing the response group (patients who benefited from immunotherapy) from the non-response group (patients who did not benefit from immunotherapy) (Fig. 5a, b) [9–13]. Because of insufficient expression levels for detection, HIF1α and NOS2 were excluded, leading to the selection of Cox risk scores for TNFSF10 and CXCL10 as ESCC immune scores, and those for IL17RB and CSF2 as EAC immune scores. This ensured broader applicability of the scores to diverse immunotherapy-treated solid tumor patient cohorts.

Using receiver operating characteristic (ROC) curves, four datasets capable of distinctly differentiating responders from non-responders based on ESCC or EAC immune scores were selected for subsequent analyses. These findings indicate that patients with lower ESCC or EAC immune scores were more likely to benefit from immunotherapy (Fig. 5c, d), which was verified by subsequent survival analyses (Fig. 5e, f). The ESCC immune score exhibited a better predictive ability in distinguishing different immunotherapy responses of patients with melanoma at 1 or 3 years (Fig. S4b). Notably, in patients with non-squamous non-small cell lung cancer (NSQ-NSCLC) undergoing immunotherapy, there was no significant difference in overall survival between the high and low EAC immune score groups (Fig. 5f). However, the EAC immune score demonstrated high prognostic accuracy for patient outcomes within 1 year of treatment (Fig. S4c).

To elucidate the predictive mechanism of ESCC and EAC immune scores for immunotherapy efficacy, an immune infiltration analysis was conducted. Among patients with melanoma, the response group exhibited higher levels of plasma cells, macrophages (M1), activated NKs cells, $CD8^+$ T cells, and follicular helper T (Tfh) cells, whereas mast cells and macrophages (M0/M2) showed lower infiltration than the non-response group (Fig. S4d, e, g). In the NSQ-NSCLC cohort, no significant difference in immune cell infiltration was observed between the responders and non-responders (Fig. S4f). Correlation analysis revealed negative associations between ESCC immune scores and activated NKs cells, macrophages (M1), $CD8^+$ T cells, Tfh cells, and plasma cells (Fig. S5a, b), but a positive correlation with M2 macrophages (Fig. S5a). The EAC immune score showed a moderate correlation with M1 macrophages in the GSE93157 dataset but negative correlations with activated NKs, macrophages (M1), $CD8^+$ T cells, Tfh cells, and memory $CD4^+$ T cells in other datasets (Fig. S5c, d, e). In conclusion, the EAC and ESCC immune scores indicated varying degrees of decrease in the infiltration of anti-tumor immune cells across the datasets, with a high ESCC immune score indicating an increased infiltration of myeloid immunosuppressive cells.

Discussion

Immunotherapy has emerged as an effective approach for treating certain cancers; however, the impact of the tumor microenvironment on immunotherapy and its regulatory mechanisms warrant further investigation [14]. Therefore,

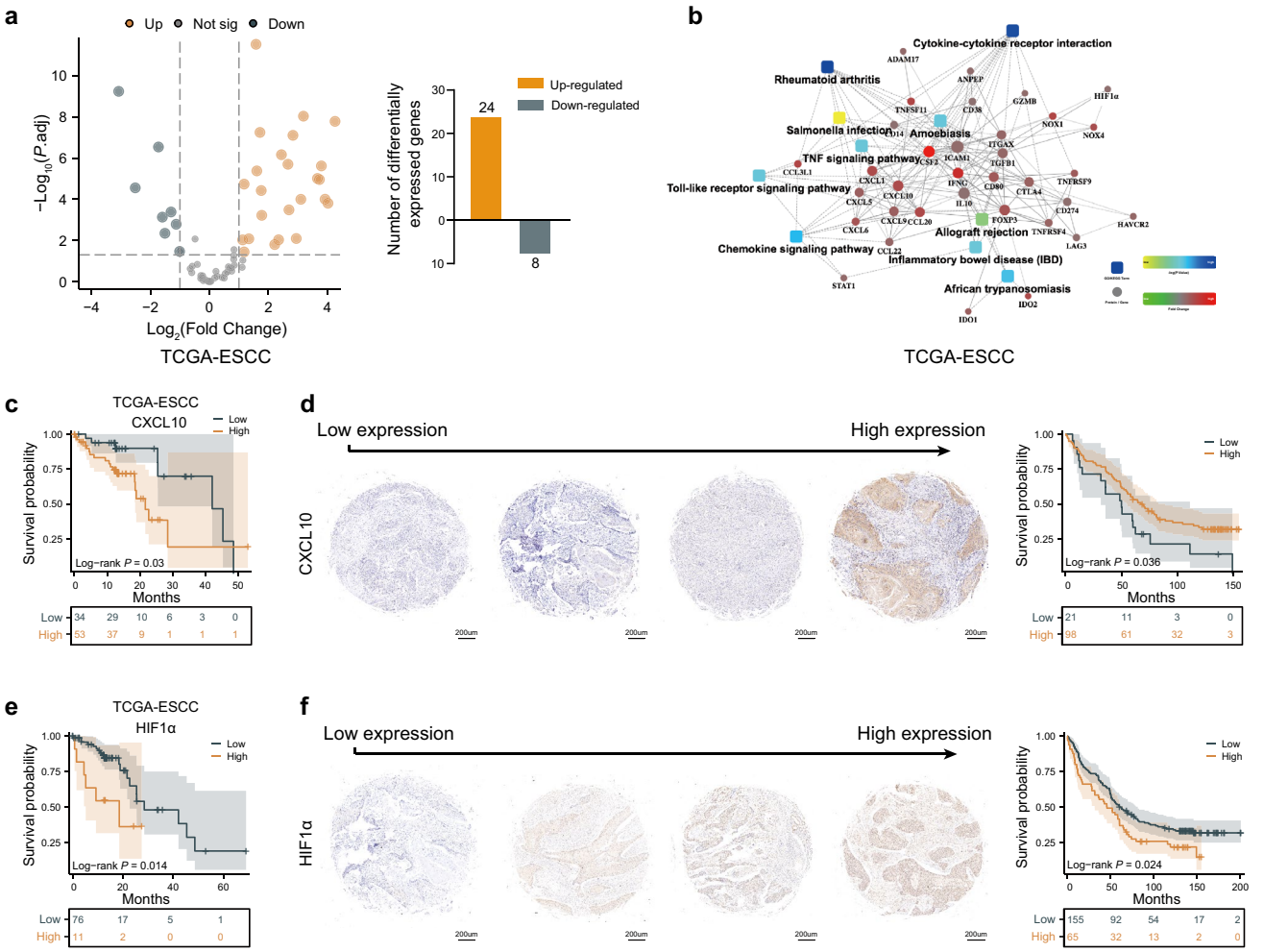


Fig. 3 Expression patterns of tumor microenvironment-related genes in ESCC. **a** Left: Volcano plot of TCGA-ESCC vs. normal tissues, focusing on tumor microenvironment-related differentially expressed genes. Right: Histogram showing upregulated and downregulated genes (|log2 Fold change|≥ 1, $P < 0.05$). **b** PPI network of functional enrichment for upregulated tumor microenvironment-related genes in TCGA-ESCC. **c** Overall survival of patients with TCGA-ESCC classified by CXCL10 expression levels. **d** Left: Immunohistochemical images of CXCL10 staining in ESCC tissue microarrays, arranged by H-score from lowest to highest expression. The far left shows the lowest expression level of CXCL10, followed by an individual in the lowest 33.33 percentile, then one in the next 33.33 percentile with moderate expression, and finally the highest expression level on the far right. Right: Overall survival of Chinese patients with ESCC by the expression of CXCL10 (low vs. high), classified based on H-score. **e** Overall survival of patients with TCGA-ESCC classified by HIF1α expression levels. **f** Left: Immunohistochemical images of HIF1α staining in ESCC tissue microarrays, arranged by H-score from lowest to highest expression. The far left shows the lowest expression level of HIF1α, followed by an individual in the lowest 33.33 percentile, then one in the next 33.33 percentile with moderate expression, and finally the highest expression level on the far right. Right: Overall survival of Chinese patients with ESCC by the expression of HIF1α (low vs. high), classified based on H-score. P-values calculated using the log-rank test. PPI: protein–protein interaction

an exhaustive exploration of immune traits within the esophageal cancer microenvironment is necessary to provide a valuable benchmark for clinical therapeutic decisions. The KEGG pathway enrichment results indicated that the cytokine–factor receptor interaction pathway played a crucial role in both histological subtypes. Clinical trials targeting cytokines have provided valuable data that may help reshape therapeutic approaches in oncology and other diseases. For instance, CXCL14 has been studied for its role in modulating immune responses in clinical settings. Its inhibition shows promise in enhancing the effectiveness of therapies for chronic myeloid leukemia (CML), suggesting that CXCL14 could be a valuable target for improving treatment outcomes [15]. CXCL8 is correlated with enhanced aggressiveness of tumor cells. And it is currently being tested in trials that focus on blocking its signaling pathways to reduce breast cancer metastasis [16]. Moreover, the expression of IL17RB in various cancers correlates with treatment resistance, indicating its potential as a target for new therapeutic interventions

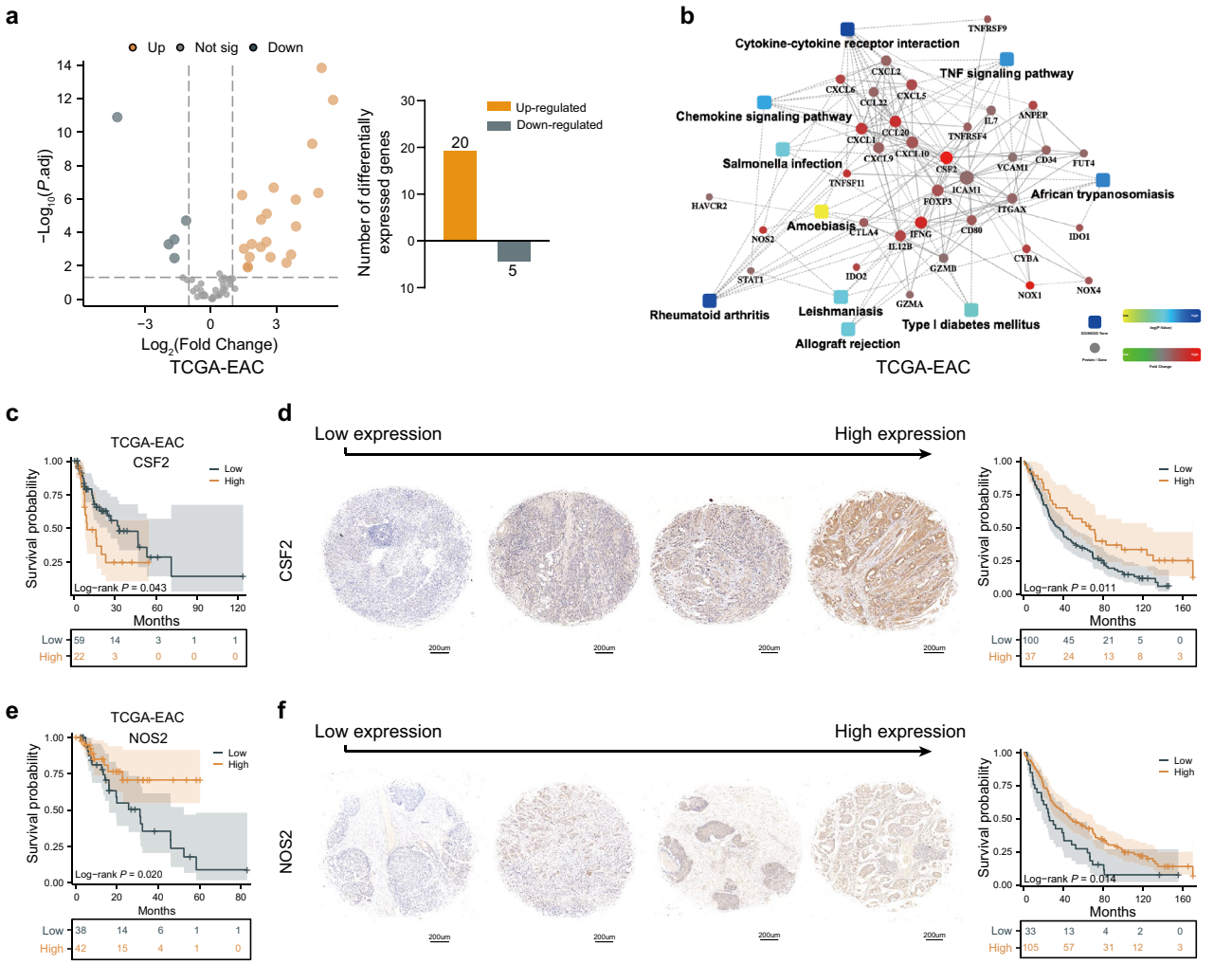


Fig. 4 Expression patterns of tumor microenvironment-related genes in EAC. **a** Left: Volcano plot of differentially expressed genes related to the tumor microenvironment in EAC vs. normal tissues from TCGA. Right: Histogram showing upregulation and downregulation of genes in EAC, significant at |log2 Fold change| ≥ 1 and $P \leq 0.05$. **b** PPI network illustrating functional enrichment of upregulated tumor microenvironment-related genes in TCGA-EAC cohorts. **c** Overall survival probabilities for patients with EAC in TCGA, stratified by high and low CSF2 expression levels. **d** Left: Immunohistochemical images depicting CSF2 staining in EAC tissue microarrays, organized by H-score from minimal to maximal expression. The far left illustrates the lowest expression level of CSF2, succeeded by an individual in the lowest 33.33 percentile, then one in the subsequent 33.33 percentile exhibiting moderate expression, and ultimately the highest expression level on the far right. Right: Overall survival of Chinese patients with EAC categorized by the expression of CSF2 (low vs. high), classified according to H-score. **e** Disease-specific survival probabilities for patients with EAC in TCGA, stratified by high and low NOS2 expression levels. **f** Left: Immunohistochemical images depicting NOS2 staining in EAC tissue microarrays, organized by H-score from minimal to maximal expression. The far left illustrates the lowest expression level of NOS2, succeeded by an individual in the lowest 33.33 percentile, then one in the subsequent 33.33 percentile exhibiting moderate expression, and ultimately the highest expression level on the far right. Right: Overall survival of Chinese patients with EAC categorized by the expression of NOS2 (low vs. high), classified according to H-score. P-values calculated using the log-rank test. PPI: protein–protein interaction

[17]. Ongoing clinical trials focusing on these cytokines are expected to yield substantial insights into their roles as therapeutic targets and ultimately improve patient outcomes through personalized treatment strategies.

Subsequent survival analysis of cytokines and immune-related genes demonstrated a significant disparity in the prognostic relevance of CXCL8, IL17RB, CXCL10, and CSF2 between Chinese and TCGA cohorts. Disparities among patient cohorts represent a critical factor contributing to inconsistent findings. Chinese patients diagnosed with esophageal cancer may exhibit significant differences in genetic [4, 5], lifestyle, and environmental factors compared to those within the TCGA database [18, 19]. These disparities can influence disease progression by altering the expression levels and functions of these molecules across different populations. Variations in biological functions

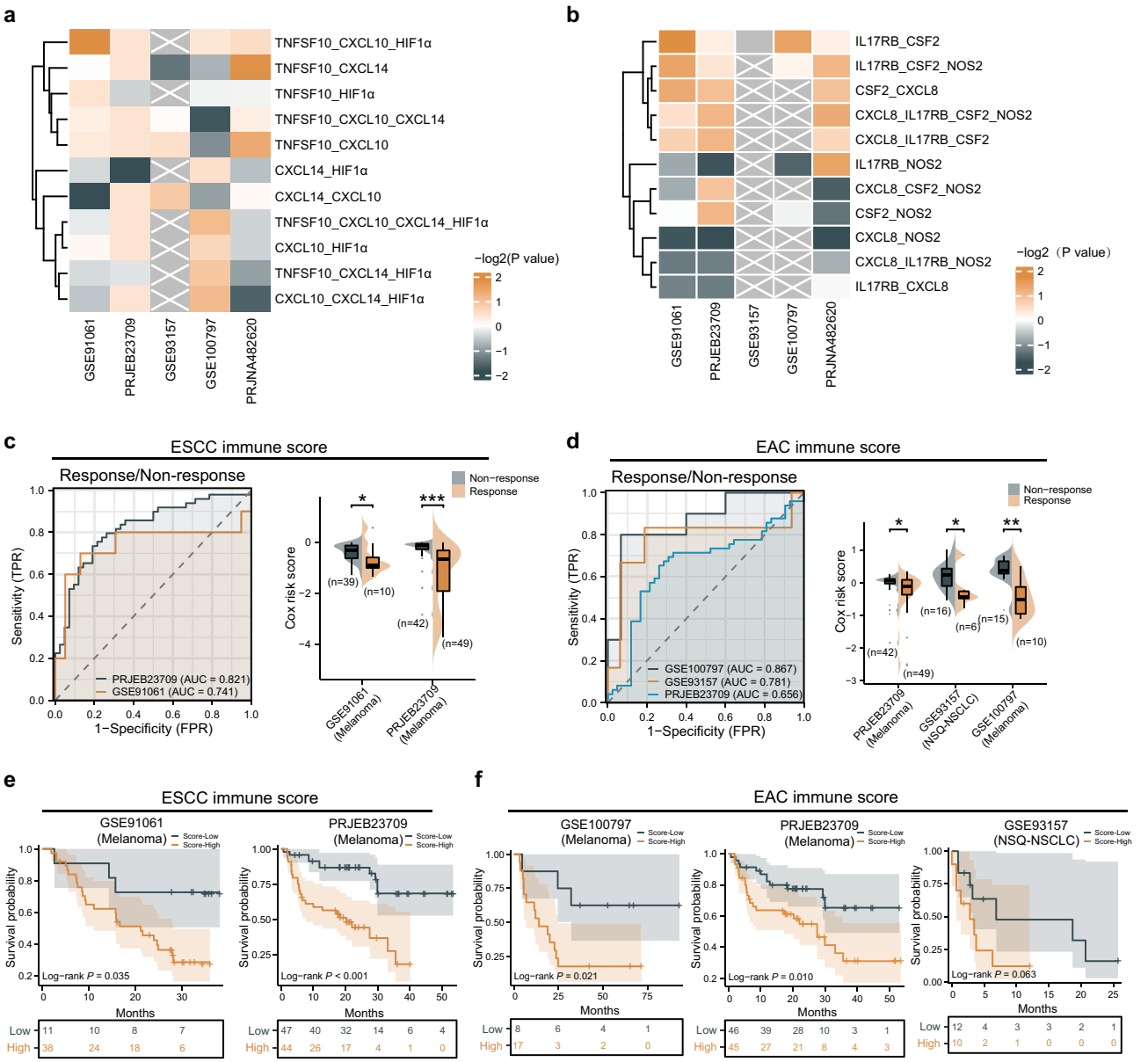


Fig. 5 Specific immune molecule combinations predict immunotherapy efficacy in patients with cancer. **a** Heatmap of P-values for Cox risk scores (ESCC immune scores) comparing gene combinations between immunotherapy response and non-response groups. **b** Heatmap of P-values for Cox risk scores (EAC immune scores) comparing gene combinations between immunotherapy response and non-response groups. **c** Left: ROC curves distinguishing immunotherapy response vs. non-response groups using ESCC immune scores. Right: Beanplots comparing ESCC immune scores between response and non-response groups in GSE91061 and PRJEB23709. **d** Left: ROC curves differentiating immunotherapy response vs. non-response groups using EAC immune scores. Right: Beanplots comparing EAC immune scores between response and non-response groups in GSE93157, GSE100797, and PRJEB23709. **e** Overall survival probabilities for high vs. low ESCC immune scores in GSE91061 and PRJEB23709. **f** Overall survival probabilities for high vs. low EAC immune scores in GSE93157, GSE100797, and PRJEB23709. Crosses indicate missing ESCC immune scores owing to gene absence, precluding P-value calculation. P-values were calculated using the Mann–Whitney (log-rank) test. ROC: receiver operating characteristic; Response: immunotherapy response group; Non-response: immunotherapy non-response group; $*P<0.05$, $**P<0.01$, $***P<0.005$

may also explain this discrepancy. CXCL8 is a multifunctional chemokine that plays a pivotal role in mediating the chemotaxis of neutrophils and monocytes [20]. IL17RB belongs to the IL-17 receptor family and plays a crucial role in inflammatory responses and immune regulation [21]. The functions of these two molecules within the tumor microenvironment are likely to differ based on tumor type, stage, and individual variability. For instance, elevated expression

levels of IL17RB facilitate the self-renewal of tumor stem cells, while simultaneously enhancing the immune functionality of Th2 cells [22, 23]. The upregulation of CXCL8 in tumors fosters immune evasion through disruption of glucolipid metabolism in $CD8^+$ T cells [24]. Furthermore, CXCL8 facilitated the migration of NKs to the tumor microenvironment, thereby boosting the immune response against tumors [25]. The variation in prognostic significance may be linked to the role of CXCL10 in recruiting different T-cell subsets [26, 27], alongside the role of CSF2 in both facilitating the generation of myeloid-derived suppressor cells (MDSCs) and promoting the maturation of antigen-presenting cells [28, 29]. Consequently, CXCL8, IL17RB, CXCL10, and CSF2 may have distinct biological functions in different populations.

In addition, the expression and activity of CXCL8 and IL17RB may be modulated by various cytokines, signaling pathways, and microenvironmental factors that can differ among patients and tumors [30–33]. Further investigation is necessary to explore the molecular mechanisms through which these factors affect CXCL8 and IL-17RB. The differential prognostic significance of CXCL8, IL17RB, CXCL10, and CSF2 across various esophageal cancer cohorts underscores the need for further exploration of the underlying biological mechanisms in future research. In addition, the generalizability of these findings requires further validation across diverse populations and larger patient cohorts in subsequent studies.

The prognostic significance of TNFSF10 and CXCL14 has been consistently observed across cohorts of patients with esophageal cancer in China and other regions. The induction of epithelial-mesenchymal transition in tumor cells by TNFSF10 and CXCL14 may contribute significantly to the unfavorable prognosis observed in these patients [34, 35]. Notably, although NOS2 serves as a crucial immunosuppressive molecule, its increased expression was significantly correlated with improved patient prognosis in both the Chinese and TCGA-EAC cohorts. This correlation may be attributed to the direct effects of nitric oxide on tumor cells [36, 37]. The pivotal molecules involved in the regulation of tumor microenvironment formation in ESCC and EAC offer a theoretical foundation for the development of targeted therapies for esophageal cancer.

The variable responsiveness of solid tumors to immunotherapeutic agents is ascribed to the immunosuppressive tumor microenvironment that shields neoplastic cells [38–40]. Patients exhibiting diminished ESCC or EAC immune scores are more likely to derive therapeutic benefits from immunotherapy, potentially because elevated ESCC or EAC immune scores may indirectly reflect enhanced infiltration of anti-tumor immune cells or suppression of their functionality. The molecules employed in the formulation of ESCC or EAC immune scores, such as CSF2 and IL17RB, have the potential to enhance the maturation and proliferation of MDSCs, thereby diminishing the immune response against tumor progression [41, 42]. Furthermore, CXCL10 possesses the capability to recruit not only MDSCs but also Tregs [27, 43]. Additionally, TNFSF10 directly mediates the dysfunction of $CD8^+$ T cells [44]. However, the specific mechanisms of action of these molecules require further investigation.

Although this study identified several potential prognostic markers for immunotherapy, it is not without limitations. The complex characteristics of the tumor microenvironment may lead to studies inadequately encompass all relevant factors, especially the heterogeneity inherent within tumors. Moreover, the limited availability of publicly accessible mRNA sequencing data from Chinese immunotherapy cohorts currently constrains the predictive capacity of ESCC and EAC immune scores to non-Chinese ethnic populations. For Chinese patients, it may be necessary to develop a specialized scoring system based on distinctive immune molecules to accurately predict the outcomes of immunotherapy. Future research should incorporate a diverse sample of patients from various ethnic backgrounds and geographic regions to enhance the generalizability of our findings, and it should also validate bioinformatic analysis results using laboratory techniques. Simultaneously, a thorough investigation of the interactions among different cells and molecules in the tumor microenvironment is essential for future research. Developing personalized immunotherapy strategies that account for each patient's unique immune microenvironment is also critical. Through these initiatives, we can achieve a more comprehensive understanding of the tumor microenvironment and offer patients more effective treatment options.

In conclusion, this comprehensive study revealed distinct regulatory molecules that play a crucial role in the tumor microenvironment of ESCC and EAC, thereby laying a solid foundation for the development of rational and targeted strategies aimed at effectively treating esophageal cancer. Furthermore, ESCC or EAC immune scores derived from the analysis of immune-related genes exhibit significant potential for clinical application in predicting the efficacy of immunotherapy in patients, thereby offering promising avenues for more personalized and effective treatment options in the future.

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Author contributions Y.Z. and S.Y. planned and guided this study. P.W. and G.Q. designed experiments. L.W., X.Z., and X.S. collected the samples and performed the experiments. P.W. and G.Q. wrote the manuscript. Y.Z., S.Y., L.W., J.L., and Q.Z. supervised the study and revised the manuscript. P.W. and G.Q. analyzed the data. All authors contributed to the writing and editing of the manuscript. All authors read and approved the final manuscript.

Data availability Data on mRNA sequencing from specimens of ESCC, esophageal adenocarcinoma (EAC), and healthy esophageal mucosa were sourced from the TCGA repository and are accessible via their official data portal (https://portal.gdc.cancer.gov/projects/TCGA-ESCA). Genetic mutation profiles of 86 ESCC and 68 EAC cases were obtained from the cBioPortal platform, a resource dedicated to Cancer Genomics (cBioPortal for Cancer Genomics). The clinical characteristics of patients with esophageal cancer were recorded on the cBioPortal website (http://www.cbioportal.org/study?id=esca_tcga#clinical). RNA-Seq datasets relevant to immunotherapy, namely GSE91061, GSE100797, GSE93157, PRJEB23709, and PRJNA482620, were retrieved from the TIGER database (http://tiger.canceromics.org/). Further information should be directed to and will be answered by the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (2019-KY-256).

Consent for publication The authors agree to the publication of all the data involved in this article.

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