Original Article

A bioinformatics analysis for diagnostic roles of the *E2F* family in esophageal cancer

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Background: Esophageal cancer (EC) is the eighth most commonly occurring cancer worldwide and the sixth leading cause of cancer-related deaths. The therapeutic effect of EC patients is not ideal, and new biomarkers are needed to guide diagnosis and prognosis of EC patients. *E2F* family transcription factors are among the most important links in the cell cycle regulatory network. *E2Fs* dysregulation not only promotes the early stages of tumor development but also the progression of benign tumors to malignant tumors. *E2F* is expected to be a new biomarker. The prognostic significance of the *E2F* family in EC requires further research.

Methods: We analyzed The Cancer Genome Atlas (TCGA), Gene Expression Profiling Interactive Analysis (GEPIA), and GeneMANIA databases to obtain RNA-sequencing data and clinical data. The clinical data included age, gender, race, stage, type, status, etc. The prognosis outcome included overall survival (OS) and progression-free interval (PFI). Subsequently, we conducted further research on gene expressions, enrichment analysis, interaction network, and prognostic values by R software, containing ggplot2, ComplexHeatmap, DESeq2, pROC R package, based on *t*-test, Wilcoxon rank sum test, Spearman rank correlation analysis, log-rank test and COX model.

Results: We found that mRNA transcription levels of *E2F1*, *E2F3-8* were more highly expressed in esophageal carcinoma (ESCA) tissues than in normal tissues. *E2F8* expression was correlated with tumor stage [Pr(>F)=0.00856]. *E2F*-related genes played a role in development and differentiation, and were prevalent in the endoplasmic reticulum lumen, Golgi lumen, and lipoprotein particle, catalyzing translation activities and lipid metabolism. Each gene was found to be related to each other to some degree. The GeneMANIA network analysis revealed links between E2Fs and other genes. We compared the correlations between 24 kinds of tumor-infiltrating immune cells and *E2Fs*. *E2F1* (AUC =0.945, CI: 0.890–1.000) and *E2F7* (AUC =0.958, CI: 0.920–0.996) exhibited higher predictive power accuracy. However, only *E2F7* was closely related to OS [HR =1.91 (1.16–3.16), P=0.011].

Conclusions: We discover that *E2F7* is a prognostic biomarker. *E2F* family may take part in the development of EC through lipid metabolism pathways, which is helpful to predict the prognosis of EC patients and guide accurate diagnosis and treatment.

Keywords: The Cancer Genome Atlas (TCGA); esophageal cancer (EC); *E2F*; prognosis

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Introduction

E2F family transcription factors are among the most important links in the cell cycle regulatory network, which regulate proliferation, cell differentiation, and apoptosis, and participate in a variety of physiological and pathological processes. The *E2F* family consists of eight members: *E2F1*, *E2F2*, *E2F3*, *E2F4*, *E2F5*, *E2F6*, *E2F7*, and *E2F8*. Previous studies have confirmed that the *E2F* family is related to multiple malignant tumors, including breast, colorectal, gastric, liver, and ovarian cancers, etc. These comprehensive studies have also revealed potential biomarkers and conducted prognostic evaluations. High expressions of *E2F1*, *E2F3*, and *E2F4* have been shown to be significantly associated with poor overall survival (OS) in gastric cancer; however, increased expressions of *E2F2*, *E2F5*, *E2F6*, and *E2F7* have been associated with favorable OS outcomes (1). Also, *E2F2*, *E2F5*, and *E2F8* might serve as potential prognostic biomarkers in ovarian cancer (2). Moreover, *E2F5*, *E2F3*, and *E2F6* have a poor effect on the OS and disease-free survival (DFS) in hepatocellular carcinoma (HCC) patients and can serve as a prognostic indicator for these patients (3). We have previously found that the *E2F* family plays an important role in the digestive tracts of many individuals. At present, there are numerous reports concerning *E2F*s in digestive tracts; however, those in esophageal cancer are lacking.

Esophageal cancer is the eighth most commonly occurring cancer worldwide and the sixth leading cause of cancer-related deaths (4). Esophageal squamous cell carcinoma and esophageal adenocarcinoma are the two main histological types of esophageal cancer. Esophageal cancer is characterized by its insidious onset, poor clinical prognosis, and high mortality rates. Surgery, chemotherapy, and radiotherapy are the mainstay of treatment. With the development of targeted therapy and immunotherapy, the treatment of esophageal cancer has shifted toward a precision medicine age. According to the genetic and molecular typing, the trend toward combination therapy using appropriate drugs has been confirmed. Indeed, the same is true for the screening of esophageal cancer. It is essential to identify new gene targets in esophageal cancer to discover potential therapeutic targets as well as prognostic and predictive biomarkers. Targeted therapy research in esophageal cancer mainly focuses on *EGFR*, *HER2*, *MET*, *VEGF*, *VEGFR*, etc., and immunotherapy has only recently become a first-line therapy (5). Due to tumor heterogeneity, there are currently still limited biomarkers that predict prognosis. Common tumor markers for esophageal cancer are CYFRA21-1, P53, Caspase-3, COX-2, E-cadherin, SCC-Ag, VEGF and CA199, which are not specific for ESCA and cannot be therapeutic targets. Therefore, there is a pressing need for predictive biomarkers, which can guide individualized therapy and improve prognosis.

*E2F*s have been shown to play an important role in the cell cycle. Among these, *E2F1*, which is the most studied, has demonstrated an increased expression that is associated with effective chemotherapy in esophageal squamous cell cancer. Up-regulated *E2F1* can induce increased expression of microRNA (*miR*)-*26b*, *miR-203*, and *miR-622*, thereby inhibiting the G1/S phase transition (6-8). Furthermore, previous studies have shown that *E2F2* is a target gene of *miR-98* and that *E2F2* and *miR-98* may be used as biomarkers for esophageal cancer (9,10). By using siRNAs (small interfering RNA) to respectively knock down *E2F1*, *E2F2*, and *E2F3*, Zhao *et al.* demonstrated that only *E2F3* inhibition could down-regulate the mRNA (messenger RNA) expression of *RACGAP1*, which predicts a better prognosis (11). Also, up-regulated *E2F4* promotes autophagy and increases the chemoresistance of esophageal squamous cancer cells (12). Moreover, *E2F5* is associated with a poor prognosis of esophageal squamous cancer (13). No clear correlation has been identified between *E2F6* and esophageal cancer yet. Silencing *E2F7* reduces the proliferation, migration, and invasion of esophageal cancer cells and induces apoptosis (14). Up-regulated E2F8 regulates important cell functions, including cell cycle progression and ESCC (esophageal squamous cell carcinoma) proliferation (15). In summary, *E2F* family play a key role in cell cycle progression and apoptosis, and when dysregulated can lead to cancer. Therefore, E2F family are closely related to tumor development and progression. The study about E2F family as biomarkers and drug targets is particularly important.

The role of the *E2F* family has only been partially established in the existing literature. We hypothesize that at least one *E2F* gene can predict the prognosis of esophageal cancer. In this study, we applied bioinformatics techniques to the integrative analysis of the *E2F* family to discover the potential prognostic and therapeutic targets against ESCA. Bioinformatics techniques can make large scale high-throughput screens combined with a larger number of clinical samples, which significantly improve the efficiency and reveal potential molecular mechanisms. It is highly desirable for clinical decision-making and individualized medical treatment.

Methods

Differential expression analysis

The differential expression analysis data were obtained from TCGA, GTEx (Genotype-Tissue Expression Project), and GEPIA databases. In the TCGA database, there were 848 cases of ESCA admitted (RNA-sequencing data of HTSeq-FPKM formats), including squamous cell neoplasms, adenomas, and adenocarcinomas. Differential expression analysis was performed using the ggplot2 R package (https://www.r-project.org/). A heat map was constructed using the ComplexHeatmap R package. GEPIA contains the RNA sequencing data of 9,736 tumors and 8,587 normal tissues from e Genotype Tissue Expression (16). The global and differential stage expressions were analyzed using the GEPIA database. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Molecular interactive networking

Enrichment analysis was conducted using the cluster Profiler R package and ID (IDentity) conversion was performed using the org.Hs.eg.db R package. Gene Ontology (GO) enrichment analysis was conducted to explore the functions of the targeted gene sets, including their molecular function (MF), biological process (BP), and cellular component (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed to explore one of the most commonly used metabolic pathway analyses. Visualization was displayed using the ggplot2 R package; the DESeq2 R package was used to screen molecules.

Functional clustering

The GeneMANIA database (http://genemania.org) was used to generate hypotheses about gene function, analyze gene lists, and prioritize genes for functional analysis. Additionally, it was also applied for gene function prediction; after searching for a specific gene, GeneMANIA identifies genes that are likely to share a function with it based on how the gene interacts with it.

A correlation heat map was constructed to demonstrate the connections between *E2F* family molecules using the ggplot2 R package. We drew an immune infiltration scatter plot using the GSVA R package by applying the ssGSEA (single-sample Gene Set Enrichment Analysis) algorithm.

Clinical significance

The pROC R package was used to perform data analysis, and the ggplot2 R package was used for data visualization. We estimated the area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, and specificity to assess the diagnostic value of the *E2F* family genes for ESCA. The genes of AUC >0.7 was considered to be diagnosis-related genes for ESCA patients. Statistical analysis of the survival materials was performed using the Kaplan-Meier analysis and was achieved using the survival R package and survminer R package (P<0.05).The prognosis outcome included OS and PFI. We extracted the data including age, gender, race, pathological stage, histological type, smoking history, alcohol history, and *E2F1-8*, to perform univariate and multivariate analysis (P<0.05).

Results

Analysis of gene expression differences

We first analyzed the expression of *E2F*s in different cancers and normal tissues using TCGA and GTEx databases. As is shown in *Figure 1*, significant differences were detected in *E2F*s in numerous cancers. We used the GEPIA database to compare the mRNA transcription levels of *E2F*s between ESCA tissues and normal esophageal tissues (*Figure 2*). The results suggested that *E2F1*, *E2F3*, *E2F4*, *E2F5*, *E2F6*, *E2F*7, and *E2F8* were more highly expressed in ESCA tissues than in normal tissues. Low expression was only observed in *E2F2*.

A more comprehensive comparison further highlighted the expression differences in ESCA (*Figure 3*). We also used the GEPIA database to analyze gene expressions that are correlated with the tumor stage. The result showed that only the expression *E2F8* varied significantly [Pr(>F)=0.00856], while that of the other *E2F* family members did not (*Figure 4*). We also analyzed the gene sets of *E2F1–8* using 848 gene probes, respectively, to export the heat map image (*Figure 5*); higher than average expression levels were marked in red, while lower than average levels were marked in blue.

GO/KEGG enrichment analysis and gene set enrichment analysis (GSEA)

We used the GO and KEGG databases for enrichment analysis of the *E2F* family. As shown in *Figure 5*, the color

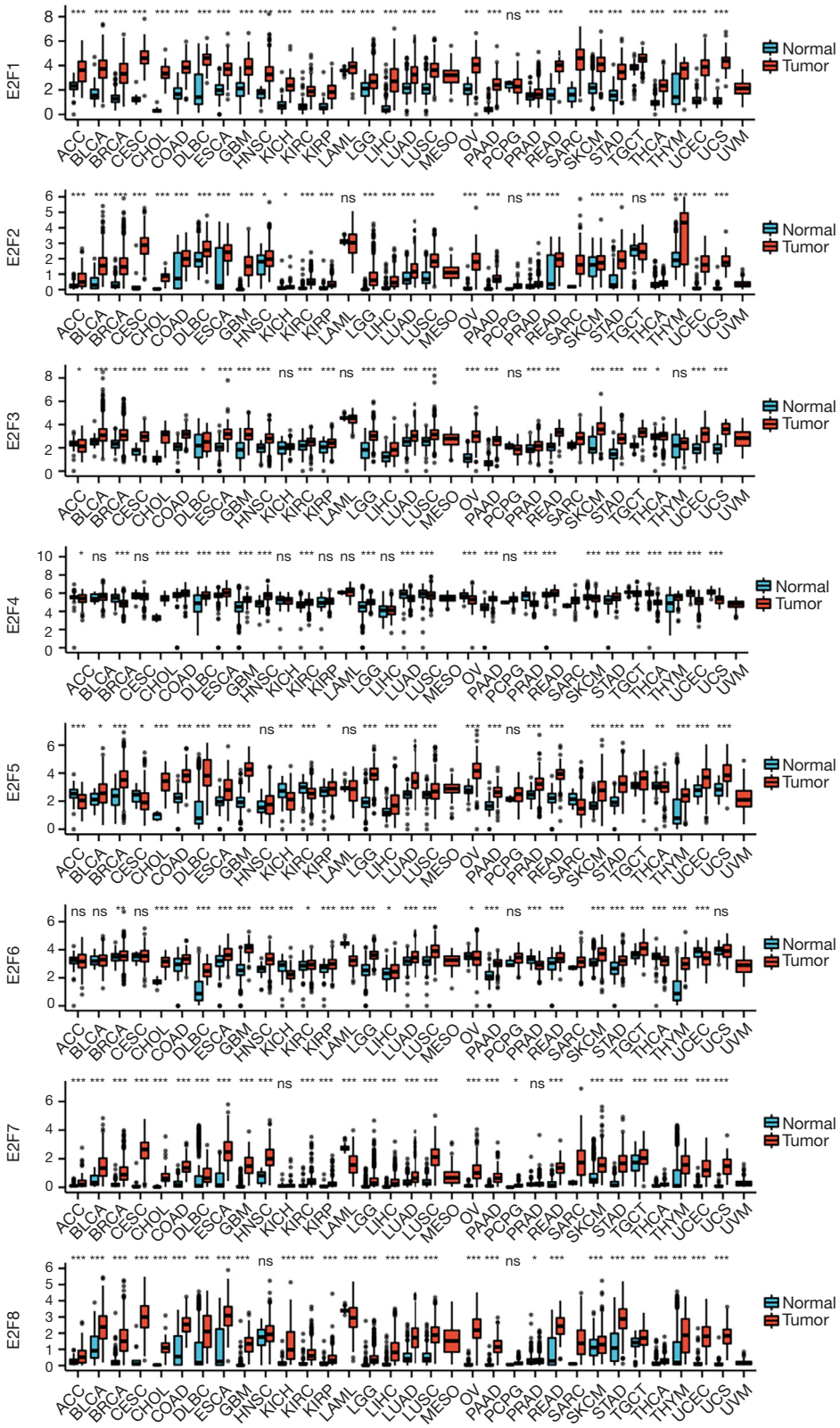

Figure 1 E2Fs expression differences in different kinds of cancers. ns, P>0.05; *P<0.05; **P<0.01; ***P<0.001.

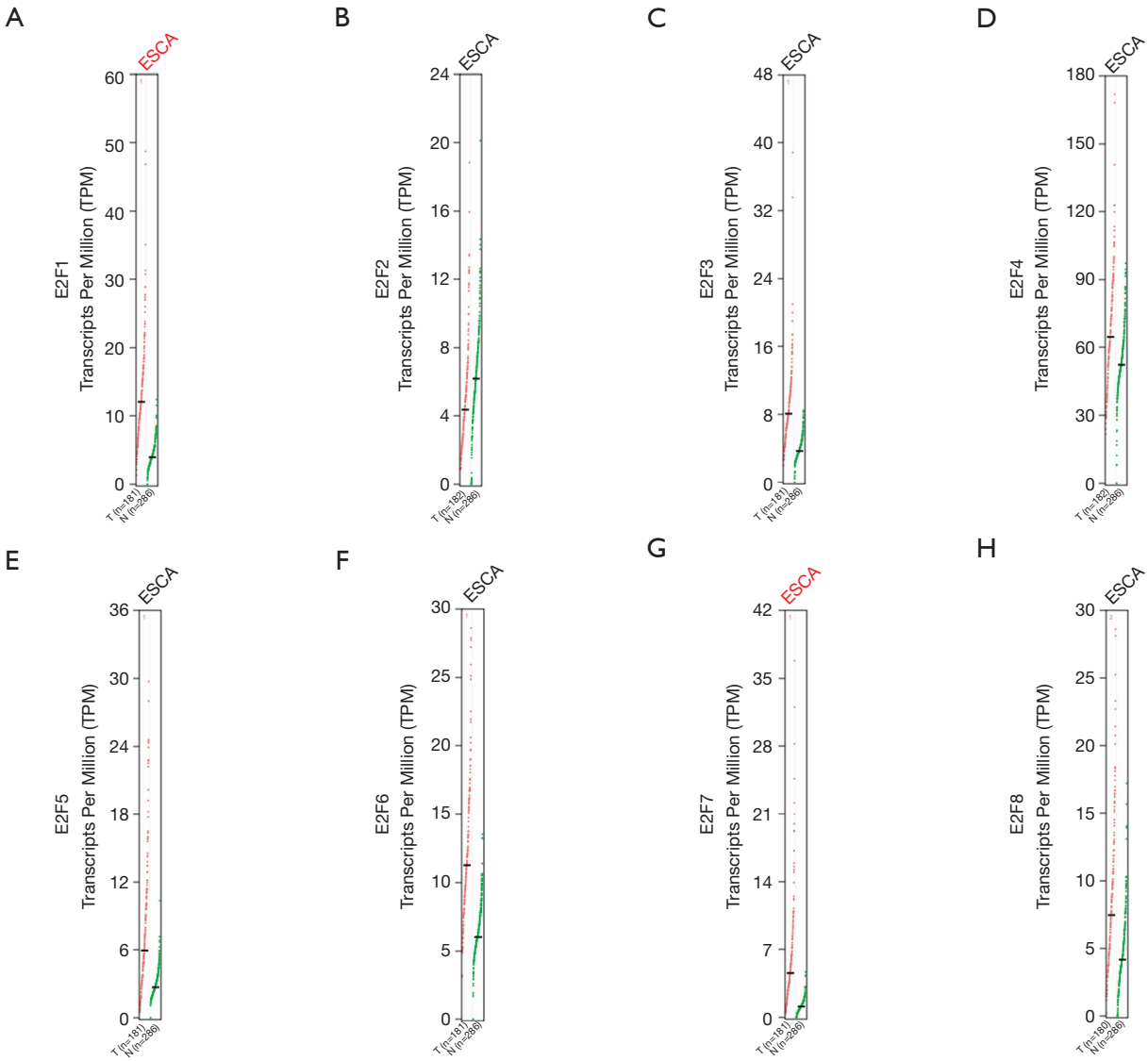

Figure 2 The expression of E2Fs in esophageal cancer (scatter diagram).

of bubbles is relevant to significance; the size represents the number of enriched gene sets. It was predicted that *E2F*-related genes play a role in development and differentiation (*Figure 6A*). Furthermore, *Figure 6B* showed that *E2F*-related genes exist in several kinds of particles, such as the endoplasmic reticulum lumen, Golgi lumen, and lipoprotein particles. Translation activator activity, lipoprotein particle receptor binding, translation regulator activity, etc. may be catalyzed by these *E2F*-related genes (*Figure 6C*). *Figure 6D* was based on the KEGG database; we screened out seven pathways that were most closely related to *E2F* gene function, among which lipid metabolic signaling pathways accounted for the majority, especially the *PPAR* (peroxisome proliferators-activated receptor) signaling pathway.

According to the NES (Normalized Enrichment Score) values in descending order (*Figure* 7), significant enrichment was observed in cell cycle checkpoint (NES =2.791, P. adj =0.016) (*Figure* 7*A*), DNA replication (NES =2.640, P. adj =0.016) (*Figure* 7*B*), mitotic metaphase and anaphase (NES =2.642, P. adj =0.016) (*Figure* 7*C*), and DNA repair (NES =2.506, P. adj =0.013) (*Figure* 7*D*). These gene sets were all enriched at the peak position and exhibited an up-regulated trend.

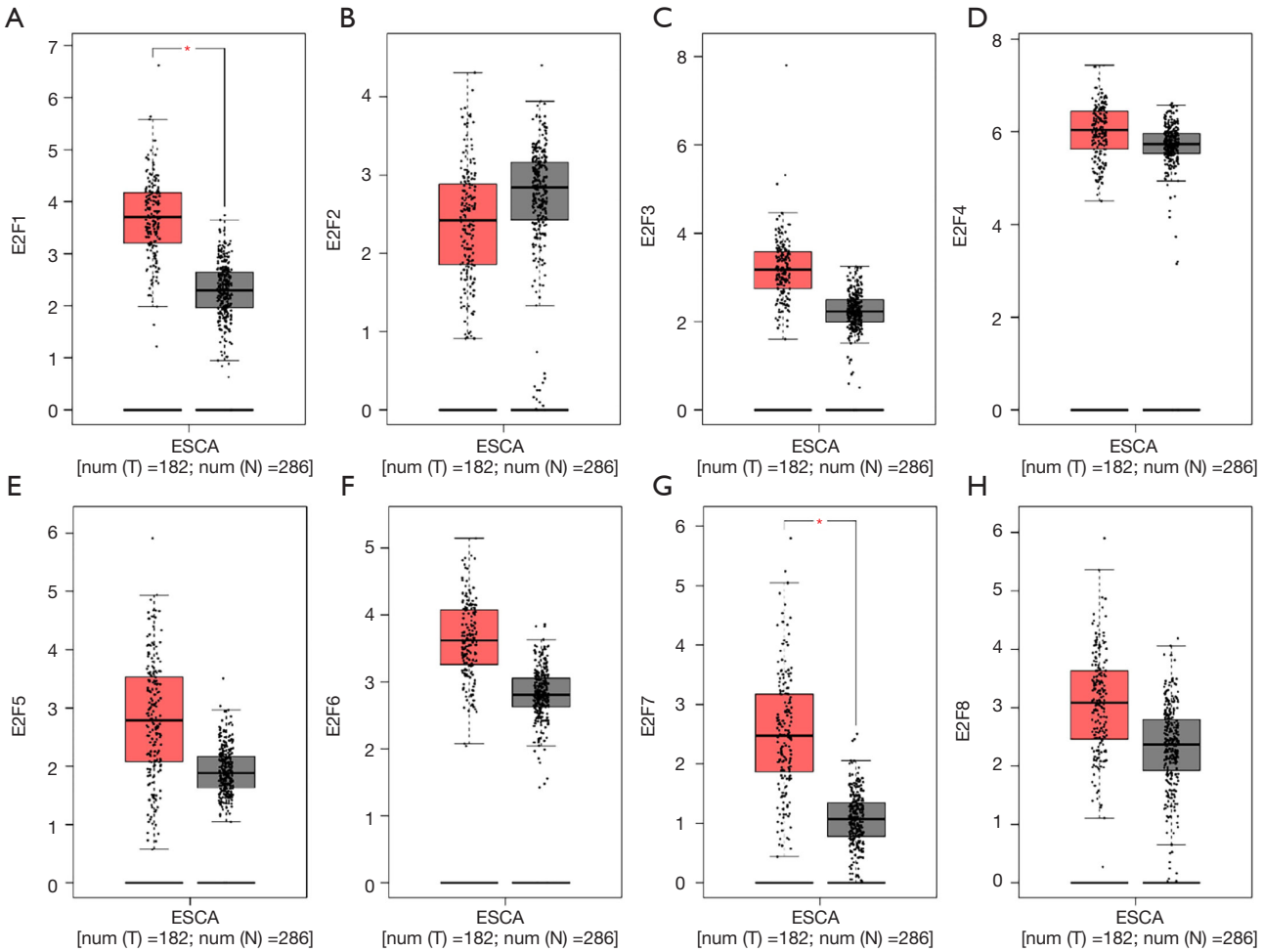

Figure 3 The expression of E2Fs in esophageal cancer (box plot). *P<0.05. ESCA, esophageal carcinoma.

Gene correlation analysis and immune infiltration

As shown in *Figure 8*, red denoted a positive correlation and blue represented a negative correlation. The "*" filled-in color indicated a significant correlation between genes (P<0.05), and no fill symbolized P>0.05. The results demonstrated the following positive correlations: *E2F1* with *E2F2*, *E2F3*, *E2F4*, *E2F5*, *E2F7*, and *E2F8*; *E2F2* with *E2F1*, *E2F3*, *E2F7*, and *E2F8*; *E2F3* with the other seven genes; *E2F4* with *E2F1*, *E2F3*, *E2F5*, and *E2F6*; *E2F5* with *E2F1*, *E2F3*, and *E2F4*; *E2F6* with *E2F3* and *E2F4*; *E2F7* with *E2F1*, *E2F2*, *E2F3*, and *E2F8*; and *E2F8* with *E2F1*, *E2F2*, *E2F3*, and *E2F7*. Weak negative correlations were observed between *E2F6* and *E2F2* and between *E2F6* and *E2F8*.

We use the GeneMANIA database to establish the gene interaction networks. *Figure 9* indicated that the network involved 502 total links between 8 *E2F*s and other 20 genes. The links included shared protein domains (77.98%), physical interactions (6.35%), predicted interactions (5.27%), genetic interactions (0.02%), pathway interactions (2.13%), co-localization (6.42%), and co-expression (1.83). We identified six main genes (*E2F1*, *E2F4*, *E2F7*, *E2F8*, *TFDP1*, *TFDP2*) that are involved in functional relationships: signal transduction by the p53 class mediator (blue), signal transduction involved in the cell cycle checkpoint (pink), positive regulation of the cell cycle process (green), DNA damage response, signal transduction by the *p53* class mediator (yellow), and cell cycle arrest (purple).

We compared the correlations between 24 kinds of tumor-infiltrating immune cells and the *E2F* family (*Figure 10*)

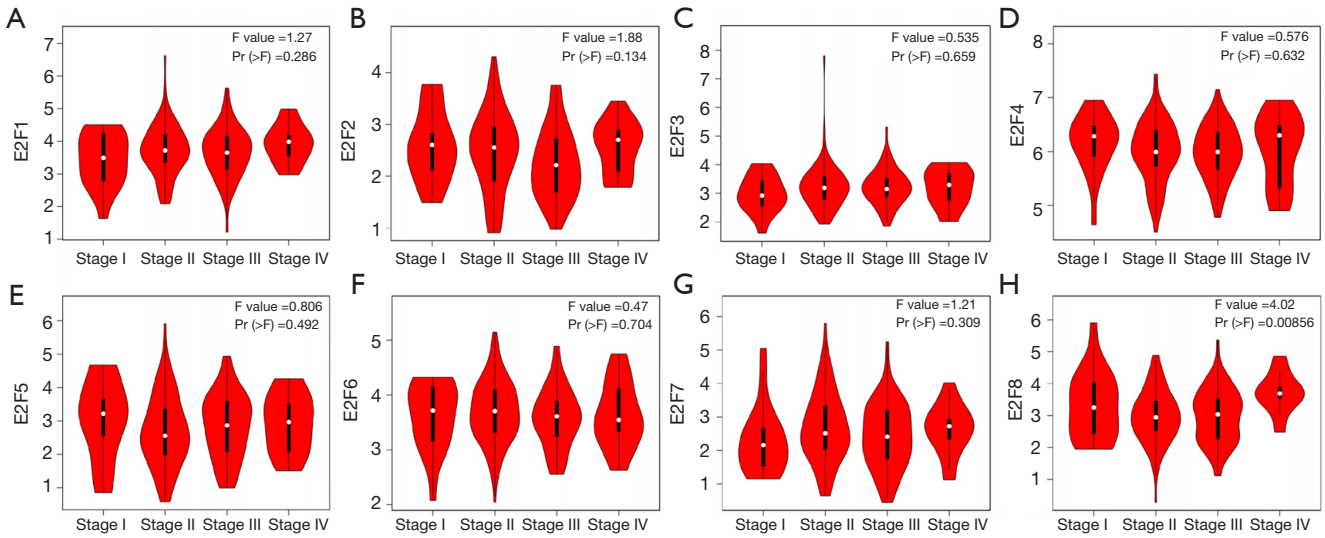

Figure 4 Relationship between E2Fs expression and tumor stage in esophageal cancer.

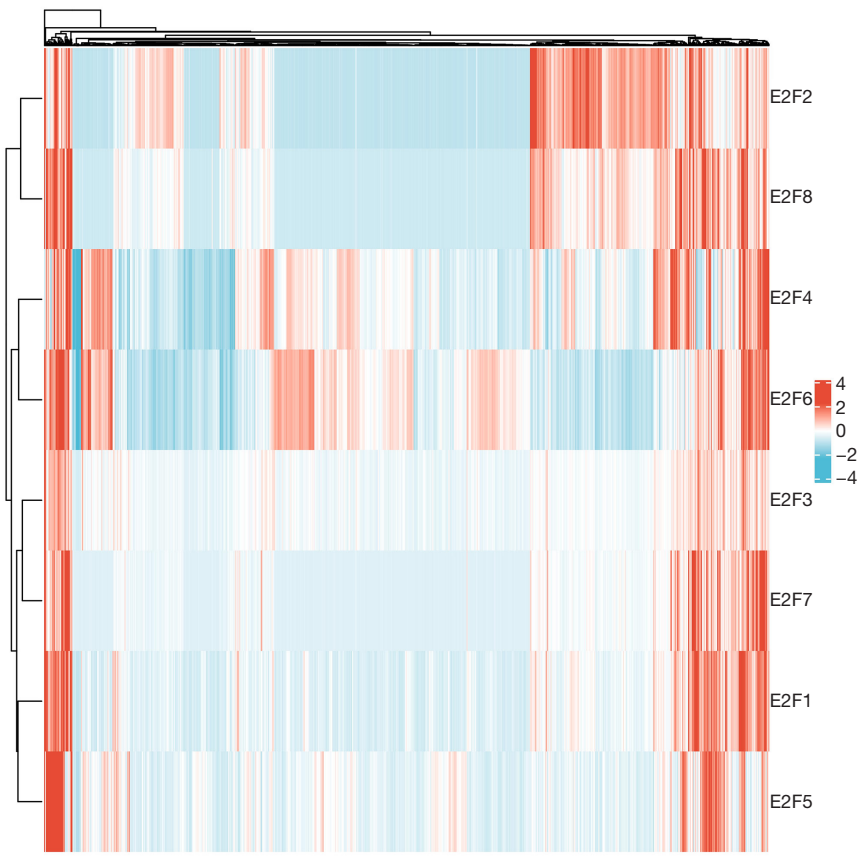

Figure 5 Heat map image. Higher expression levels than average are marked in red, while lower than average expression levels are marked in blue.

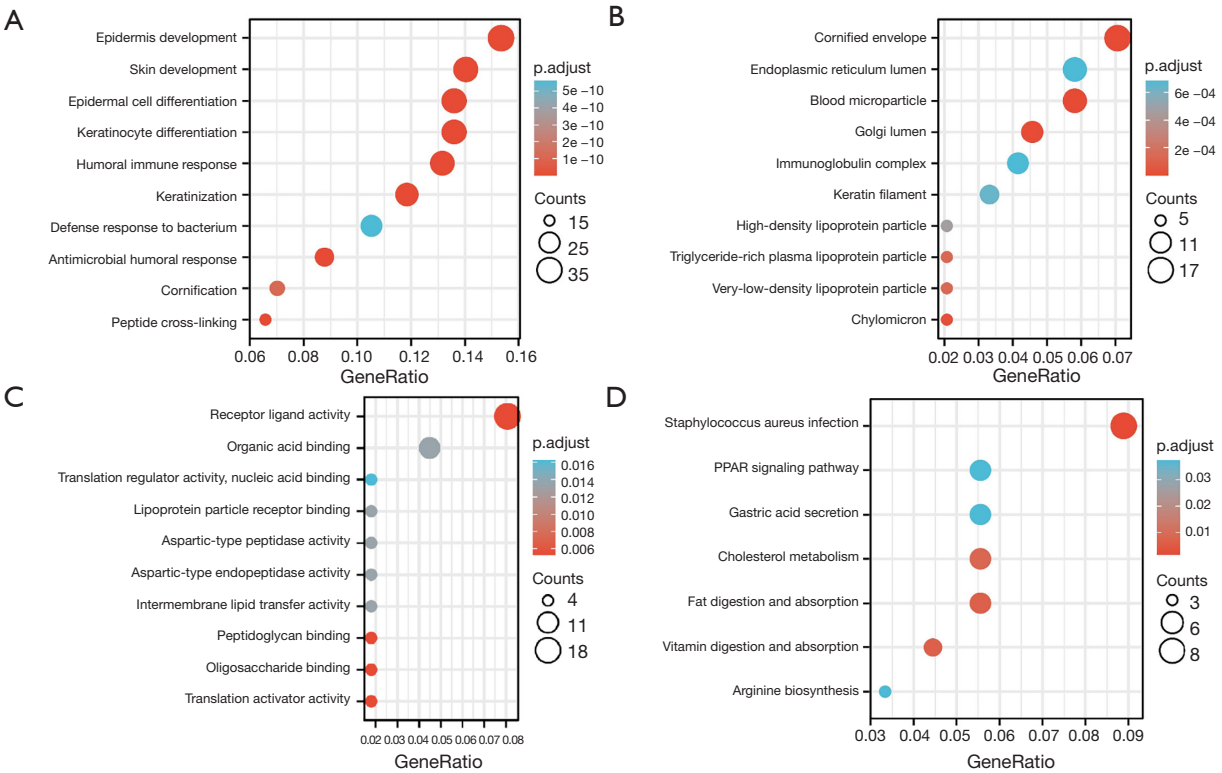

Figure 6 GO/KEGG performs enrichment analysis of the E2F family, including (A) BP, (B) CC, (C) MF, and (D) KEGG. BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

and found that *E2F1* is related to CD8 T cells, cytotoxic cells, mast cells, Th2 cells, etc.; *E2F2* is related to CD8 T cells, iDC, macrophages, mast cells, neutrophils, Th2 cells, etc.; *E2F3* is related to mast cells, Th2 cells, etc.; *E2F4* is related to mast cells, pDC, Th2 cells, etc.; *E2F5* is related to eosinophils, iDC, NK CD56bright cells, NK CD56dim cells, T helper cells, Tcm, Th17 cells, etc.; *E2F6* is related to cytotoxic cells, Th2 cells, etc.; *E2F7* is related to eosinophils, mast cells, pDC, Th17 cells, Th2 cells, etc.; *E2F8* is related to NK CD56dim cells, T helper cells, Th17 cells, Th2 cells, etc. (all P<0.001). Moreover, some other tumor-infiltrating immune cells were also meaningful.

ROC diagnostic curve, survival analysis and cox analysis

ROC curves were used to assess the diagnostic accuracy for esophageal cancer based on the *E2F*s. AUC values range from 0.5 to 1; the closer the AUC value is to 1, the better the diagnostic effect. However, AUCs ranging from 0.5 to 0.7 represented lower accuracy, while AUCs ranging from 0.7 to 0.9 denoted moderate accuracy. The accuracy of AUC greater than 0.9 is highest. Using the pROC and ggplot2 R packages, it was observed that in the predicted outcomes of the tumor and normal groups, *E2F1* (AUC =0.945, CI: 0.890–1.000) and *E2F7* (AUC =0.958, CI: 0.920–0.996) displayed higher predictive power accuracy. Also, *E2F2*, *E2F3*, *E2F4*, *E2F5*, *E2F6*, and *E2F8* exhibited moderate accuracy (*Figure 11*).

The prognostic analyses are shown in *Figure 12* (for OS) and *Figure 13* (for PFI). We found that only *E2F7* was closely related to OS [HR =1.91 (1.16–3.16), P=0.011], while the other seven genes were not. High *E2F7* expression was associated with worse OS; conversely, low expression was related to better OS. None of these genes exhibited significant differences in PFI, but it was observed that the P value was the smallest and showed a trend towards statistical significance.

Based on 162 clinical cases from the TCGA, the univariate and multivariate analysis about ESCA are performed in *Table 1*. The cox analysis reveal that Black or African American [HR =4.286 (1.249–14.717), P=0.021], stage III [HR =4.730 (1.365–16.391), P=0.014], stage IV

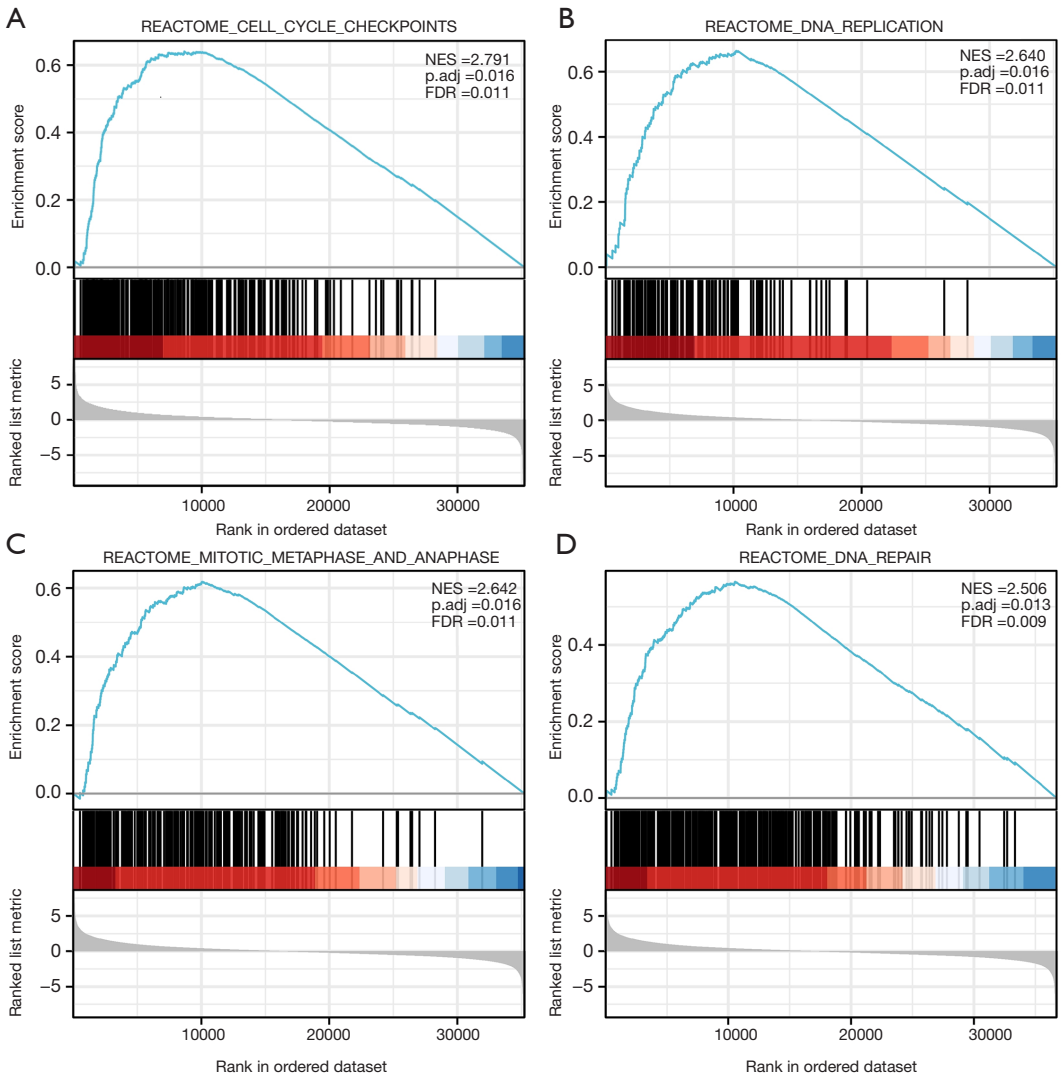

Figure 7 Visualization of GSEA. (A) Cell cycle checkpoint; (B) DNA replication; (C) mitotic metaphase and anaphase; (D) DNA repair. GSEA, gene set enrichment analysis.

[HR =13.716 (3.479–54.079), P<0.001], high expression of *E2F7* [HR =1.912 (1.157–3.161), P=0.011] are risk factors.

Discussion

Most *E2F* family genes have been studied in esophageal cancer. The mechanisms and pathways of cell cycle regulation have also been reported in previous studies. This paper is the first comprehensive analysis of the expression and prognosis of the entire *E2F* family in esophageal cancer and aims to provide a further basis for the accurate prognostic prediction of esophageal cancer patients and explore possible target pathways, thereby providing some new ideas concerning diagnosis and treatment.

A maximum of *E2F1* has been studied in previous studies. It has been confirmed that *E2F1* exerts a suppressive effect in esophageal adenocarcinomas (17) and a cancer-promoting effect in esophageal squamous cell cancer (18). In recent years, *E2F1*-related pathways have been studied extensively. Zhang *et al.* reported that *E2F1* binding to *miR-26b* could increase the expression of *miR-26b*, thereby inhibiting the proliferation of ESCC (6). A 2020 result from TCGA showed that *E2F1* is a potential regulator with good diagnostic and prognostic values in ESCA (19). Song *et al.*

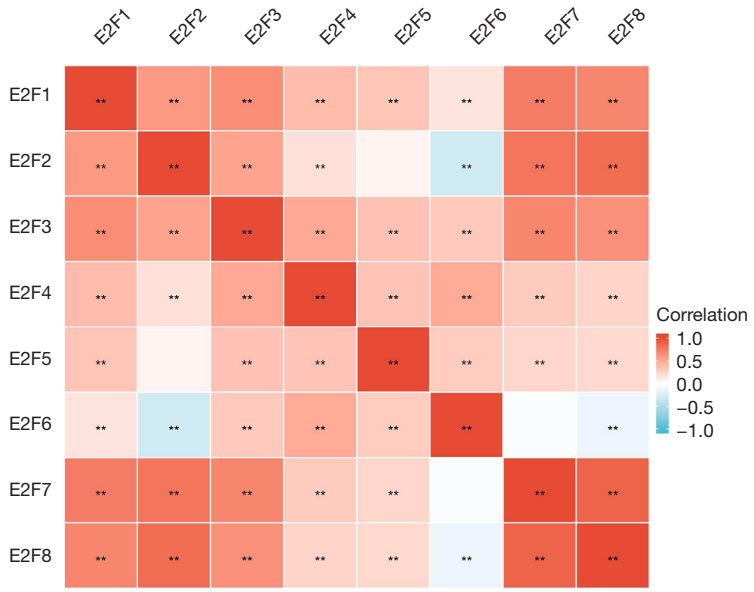

Figure 8 Correlations between E2Fs. **P<0.01.

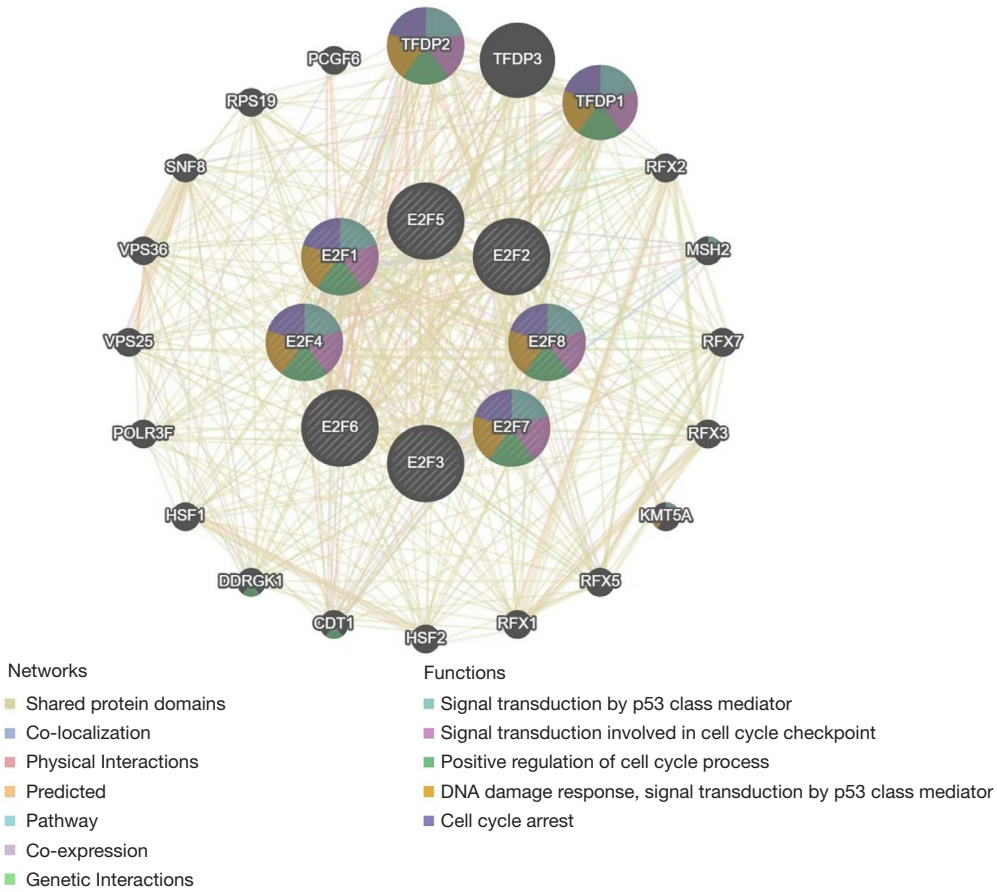

Figure 9 E2F interaction network.

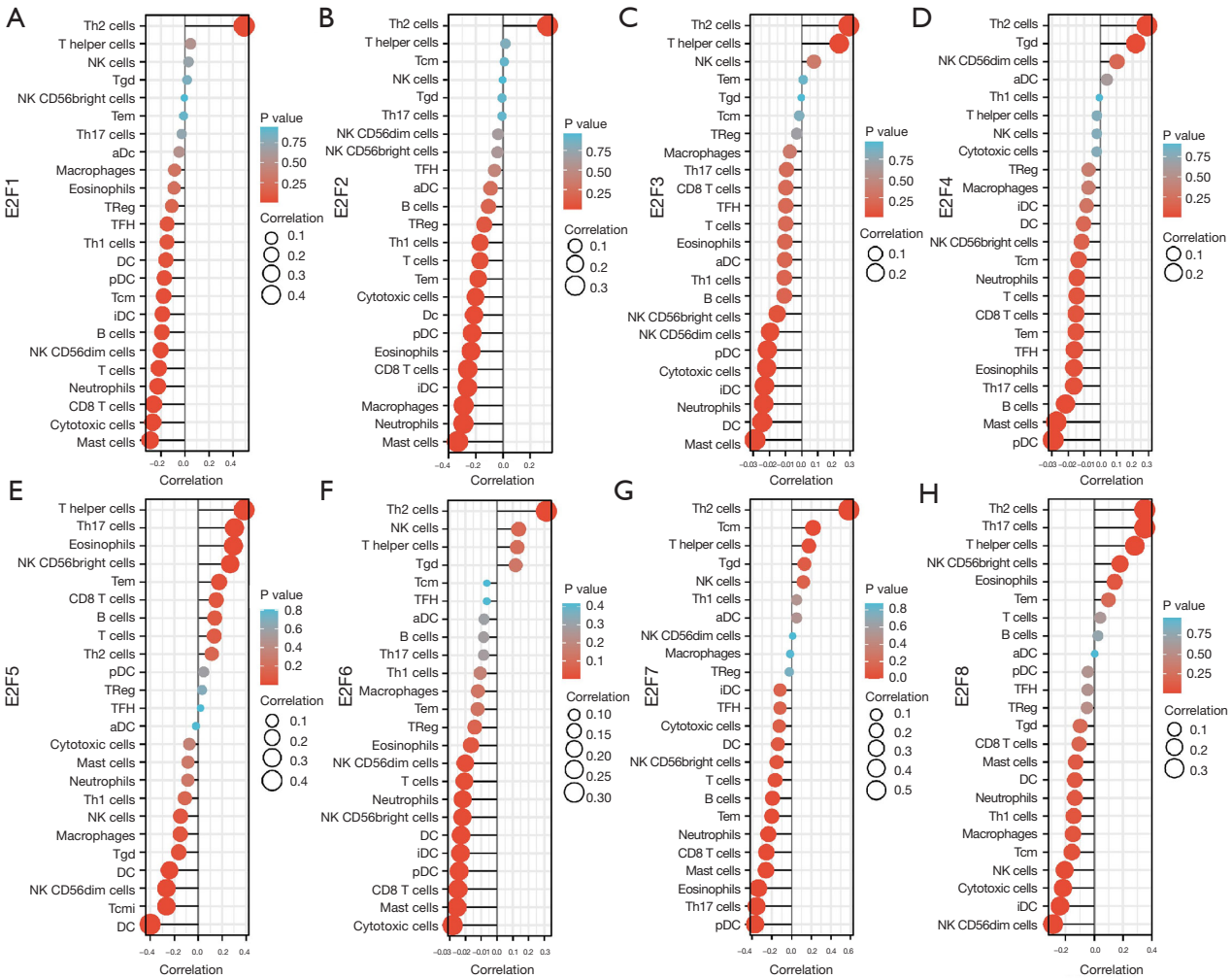

Figure 10 Correlations between 24 kinds of tumor-infiltrating immune cells and the E2F family.

suggested that *E2F1* is the direct target gene of *miR-622*, which plays a functional tumor suppressor role in ESCC (8). A recent result demonstrated that *E2F1* inhibits *miR-375* expression in ESCC and promotes *SESN3* expression, thereby activating the *PI3K/AKT* pathway (20). Another paper reported that the abnormal expression of *NSUN2* is positively regulated by *E2F1*; higher *NSUN2* levels predict poorer survival in ESCC patients (21). However, the overexpression of *miR-25* promotes the invasion and metastasis of ESCC cells (22). In our study, *E2F1* was found to be differentially expressed but had no prognostic significance for OS and PFI. Compared to the previous different conclusions, maybe different pathological types and samples cause the opposite results.

At present, research about *E2F2* has rarely been reported. A 2020's study points out that the *miR-17-92a* cluster and *E2Fs* (*E2F1*, *E2F2*, *E2F3*) create a cellular balance between apoptosis and proliferation. *MiR-18a-5p*, which belongs to the *miR-17-92a* cluster, is a poor prognostic biomarker in ESCA (23). *MiR-98* and *miR-363* are associated with esophageal cancer and regulate a sequence of cell cycle-related genes, including *E2F1* and *E2F2* (10). In ESCC patients, a previous RT-PCR analysis revealed that expression of *E2F2*, which is a known *miR-31* target oncogene, was negatively connected with the expression of *miR-31* in a *p21*-dependent manner, and overexpression of *miR-31* resulted in a better OS in ESCC patients (24). In our study, low expression was only observed in *E2F2*, but no significant differences were found in OS and PFI.

The overexpression of *E2F3* is associated with worse

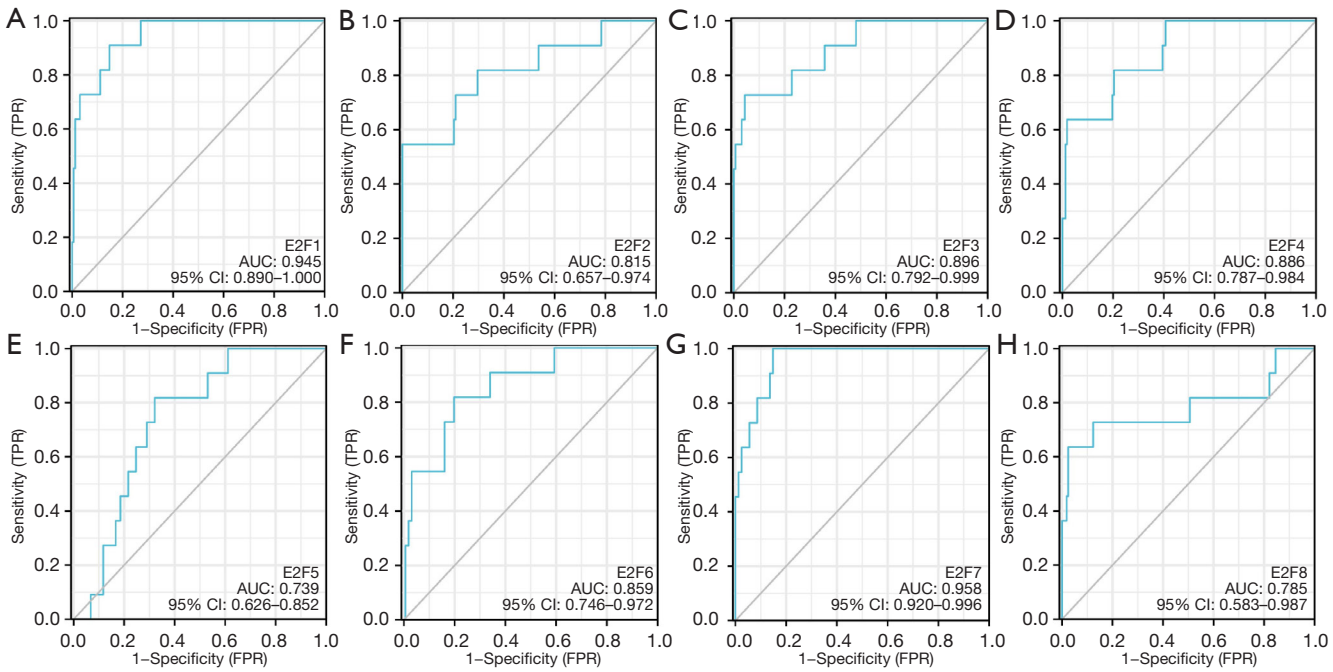

Figure 11 ROC curves were used to assess the diagnostic accuracy of esophageal cancer based on the E2Fs. ROC, receiver operating characteristic; AUC, area under the curve; FPR, false discovery rate; TPR, true positive rate.

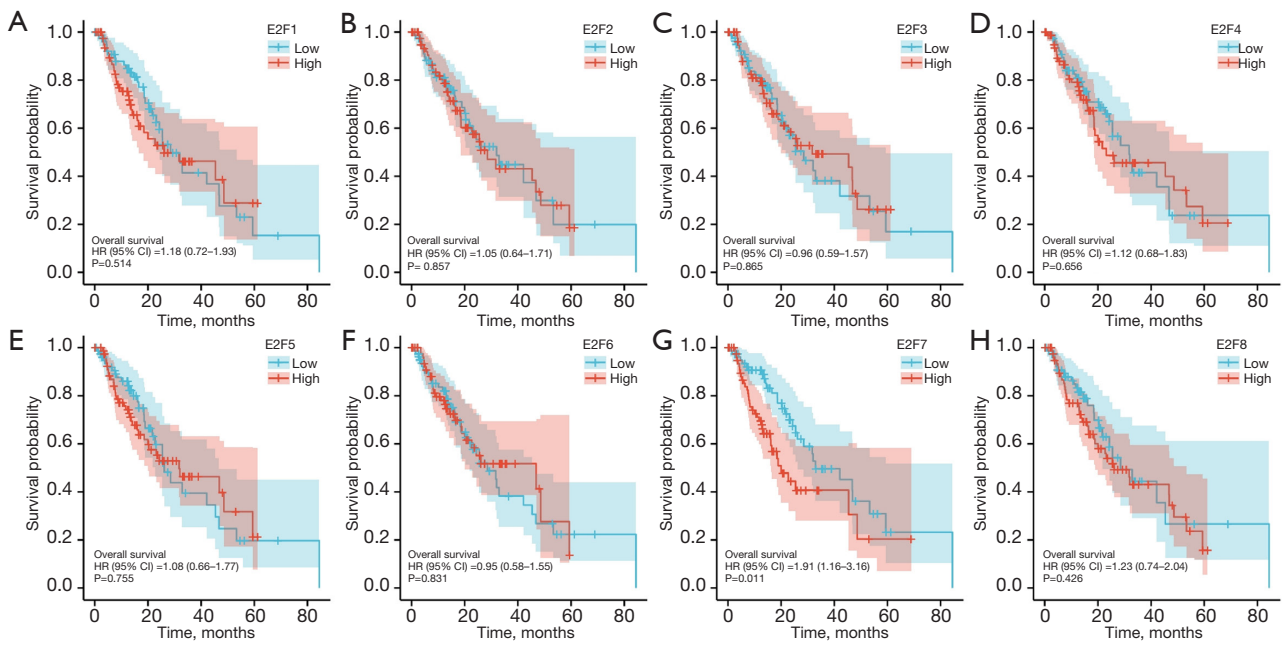

Figure 12 Prognostic analysis of E2Fs in esophageal cancer. HR, hazard ratio.

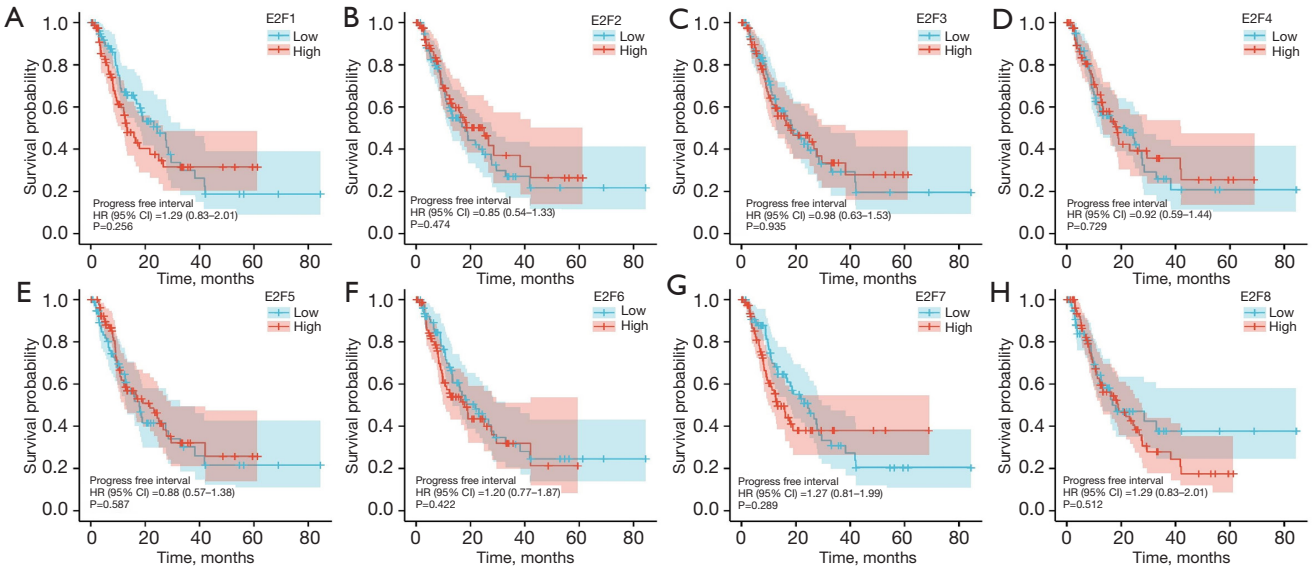

Figure 13 Prognostic analysis of E2Fs in esophageal cancer. HR, hazard ratio.

esophageal cancer outcomes. *RACGAP1* is a transcription target of *E2F3*; *E2F3* inhibition can down-regulate the mRNA expression of *RACGAP1*, leading to extensive apoptosis (11). In addition to the *miR-17-92a* cluster mentioned above, high levels of β-catenin have been found to activate the expression of *E2F3*. Moreover, *DHODH* (dihydroorotate dehydrogenase) is positively correlated to β-catenin, and high *DHODH* expression induces a worse prognosis (25). The new *circ_0087378* in ESCC can bind to *miR-140-3p* to up-regulate the expression of *E2F3* and eventually induces poorer outcomes in ESCC (26). In a 2019 study, Lv *et al.* showed that *E2F3* is up-regulated in esophageal adenocarcinoma but was not related to prognosis (27). Another study conducted in 2021 reported new crosstalk of the oncogenic role of *circ_0000654/mir-375/E2F3* ceRNA (competing endogenous RNA) in ESCC and established the concept that targeting *circ_0000654* and its pathways may improve the prognosis of ESCC (28). Another report found that silencing *circFIG 4* can mitigate EC malignant progression by mediating the *miR-493-5p/E2F3* pathway and may become a new biomarker and therapeutic target for EC treatment (29). Our results illustrated that there were no statistical differences in the OS and PFI of *E2F3*. However, *E2F3* was differentially expressed in both tumor and normal tissues.

E2F4 has been reported to play a role in the functional proliferation of gastric cancers. *LINC00337* can recruit *E2F4* to enhance the transcription of *TPX2*(a microtubule nucleation factor), thus promoting cell autophagy. Silencing of *LINC00337* enhances apoptosis in ESCC (12). A previous study indicated that the *E2F4* methylation exists in the promoter regions of EAC (esophageal adenocarcinoma) and ESCC, which leads to the inhibition of *LTBP4* (latent transforming growth factor beta binding protein 4) and promotes migration (30). In this paper, the expression of *E2F4* was higher in tumor tissues compared to normal tissues, but there were no differences in the stages and survival analysis.

E2F5 enables increased cell proliferation and migration and inhibits apoptosis. In ESCC, *miR-34a* has a direct effect on *E2F5*, and low *miR-34a* expression promotes apoptosis in ESCC (31). In contrast, *miR-544* is negatively correlated with *E2F5*; its overexpression induces ESCC proliferation (32). A previous study pointed out that *E2F5* was a biomarker for poor prognosis in ESCC patients (13). Our results showed that *E2F5* was not related to the prognosis of EC.

At present, there are no studies about *E2F6* and esophageal cancer. Yang *et al.* demonstrated that *E2F6* inhibits the trans-activation and apoptosis of *E2F1* by competing with *E2F1* for DNA binding sites, and *E2F6* plays a role in hypoxia-induced apoptosis by regulating *E2F1* (33). Silencing *E2F7* reduces the proliferation, migration, and invasion of EC cells, and induces apoptosis. The effects of lncRNA (long non-coding RNA) *DLEU2* on the proliferation, migration, and invasion of EC cells are reversed by *miR-30E-5P* inhibitors or the up-regulation of

Table 1 Cox proportional-hazards model about different variables of ESCA patients

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (years)	162				
≤60	83	Reference			
>60	79	0.831 (0.506–1.365)	0.466		
Gender	162				
Female	23	Reference			
Male	139	2.306 (0.922–5.770)	0.074	2.124 (0.601–7.506)	0.242
Race	144				
Asian	38	Reference			
Black or African American	6	4.286 (1.249–14.717)	0.021	1.240 (0.146–10.537)	0.844
White	100	1.408 (0.616–3.217)	0.417	1.023 (0.402–2.604)	0.962
Pathologic stage	142				
Stage I	16	Reference			
Stage II	69	1.969 (0.581–6.676)	0.277	1.653 (0.364–7.501)	0.515
Stage III	49	4.730 (1.365–16.391)	0.014	2.893 (0.620–13.503)	0.177
Stage IV	8	13.716 (3.479–54.079)	<0.001	16.947 (3.036–94.583)	0.001
Histological type	162				
Adenocarcinoma	80	Reference			
Squamous cell carcinoma	82	0.875 (0.526–1.455)	0.607		
Smoker	144				
No	47	Reference			
Yes	97	1.539 (0.799–2.966)	0.197		
Alcohol history	159				
No	46	Reference			
Yes	113	0.738 (0.442–1.231)	0.245		
E2F1	162				
Low	81	Reference			
High	81	1.178 (0.720–1.928)	0.514		
E2F2	162				
Low	81	Reference			
High	81	1.046 (0.640–1.711)	0.857		
E2F3	162				
Low	81	Reference			
High	81	0.958 (0.586–1.567)	0.865		

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
E2F4	162				
Low	81	Reference			
High	81	1.119 (0.683–1.833)	0.656		
E2F5	162				
Low	81	Reference			
High	81	1.082 (0.660–1.773)	0.755		
E2F6	162				
Low	81	Reference			
High	81	0.947 (0.577–1.555)	0.831		
E2F7	162				
Low	81	Reference			
High	81	1.912 (1.157–3.161)	0.011	1.611 (0.782–3.317)	0.196
E2F8	162				
Low	81	Reference			
High	81	1.228 (0.740–2.039)	0.426		

ESCA, esophageal carcinoma.

E2F7 (14). *E2F8* is up-regulated to promote cell proliferation and affect cyclin D1 (*CCND1)/P21* expression in ESCC. Down-regulation of *E2F8* expression inhibits cell proliferation *in vivo* (15). Our study confirmed that *E2F7* is correlated with OS and PFI (P<0.05). *E2F7* was the only transcription factor that we validated as statistically significant.

Our results also illustrated that esophageal cancer is closely related to lipid metabolism, especially the *PPAR* signaling pathway. According to the studies mentioned above, *E2F*s are related to esophageal cancer through various miRNA signaling pathways. MiRNA is a key regulator of lipid metabolism (34), which is involved in the regulation of numerous cellular processes, including cell growth, proliferation, differentiation, survival, apoptosis, inflammation, motility, membrane homeostasis, chemotherapy response, and drug resistance (35). A previous animal experiment showed that cholesterol stimulates the proliferation of ECA109 cells both *in vivo* and *in vitro* (36). Several reports have confirmed that the *PPAR*-γ pathway is correlated with the development of esophageal cancer. A 2020 study demonstrated that miRNA-mediated *PPAR*-γ pathway inhibition enhances the proliferation of ESCC (37). Nevertheless, the increased expression of *PPAR*-γ may play an important role in the development and progression of normal cell transformation to Barrett's esophagus and esophageal adenocarcinoma (38). Combined with the above studies, we speculate that lipid metabolism plays an important role in the generation of EC.

In summary, our study provides some possible prognostic biomarkers and mechanisms, which could lead to the development of novel targeted drugs. Through molecular typing of esophageal cancer patients, accurate prognostic prediction in individual patients will gain further improvement. Therefore, more meaningful approaches will be explored.

Conclusions

This study demonstrated that *E2F7* is a prognostic biomarker. Also, the *E2F* family plays a role in esophageal cancer formation through lipid metabolism pathways.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-855/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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