Development of a prognostic model for esophageal cancer based on nine immune related genes

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Abstract

Background: Function of the immune system is correlated with the prognosis of the tumor. The effect of immune microenvironment on esophageal cancer (EC) development has not been fully investigated. This study aimed to explore a prognostic model based on immune-related genes (IRGs) for EC.

Methods: We obtained the RNA-seq dataset and clinical information of EC from the Cancer Genome Atlas (TCGA). We identified 247 upregulated IRGs and 56 downregulated IRGs. Pathway analysis revealed that the most differentially expressed IRGs were enriched in Cytokine-cytokine receptor interaction. We further screened 13 survival-related IRGs and constructed regulatory networks involving related transcription factors (TFs). Finally, a prognostic model was constructed with 9 IRGs (HSPA6, S100A12, CACYBP, NOS2, DKK1, OSM ,STC2 ,NGPTL3 and NR2F2) by multivariate Cox regression analysis.

Results: The patients were classified into two subgroups with different outcomes. When adjusted with clinical factors, this model was verified as an independent predictor, which performed accurately in prognostic prediction. Next, M0 and M2 macrophages and activated mast cells were significantly enriched in high-risk group, while CD8 T cells and regulatory T cells (Tregs) were significantly enriched in low-risk group.

Conclusion: These molecules may serve as potential therapeutic targets and biomarkers for the new-immunotherapy of EC.

1. Background

Esophageal cancer (EC) is the eighth commonest cancer worldwide. The National Cancer Institute estimated 16,910 new cases and 15,910 deaths from esophageal cancer in the United States in 2016 [1]. Its incidence has risen by more than six times(1999-2008) [2]. The overall five-year survival of EC and that after esophagectomy are still poor, although great improvements have been made in treatment[3]. Squamous cell carcinoma is the most common histological type of EC [4]. Tobacco, alcohol, and malnutrition are the most associated risk factors in the development of EC[5]. Once diagnosed, EC must be accurately staged prior to the initiation of treatment. TNM (tumor, lymph node, metastasis) is a staging system based on the status of tumor invasion, lymph node, and metastasis [6]. Early-stage EC is usually treated with endoscopic surgery, advanced EC with surgery with or without chemoradiation [7].

Certain specific genes and biomarkers are needed to predict the patient's therapeutic response and increase their survival[3]. Immune responses is critical in the tumor microenvironment. Tumor cells with genomic alterations can produce new antigens that can be recognized by the immune cells[8]. Expression of IRGs can serve as efficient biomarkers. Previous research have explored the IRGs-based prognostic features in patients with non-squamous non-small cell lung cancer[9] and papillary thyroid carcinoma[10]. However, prognostic models based on IRGs for EC remain to be elucidated.

This study investigated the clinical significance of a prognostic model based on immunogenomics.

2. Methods

2.1 Data collection

The mRNA profiles and corresponding clinical information of 11 normal tissues and 160 EC samples were downloaded from TCGA (https://www.cancer.gov/) [11]. A set of IRGs were obtained through the Immunology Database and Analysis Portal (ImmPort) database (https://www.immport.org) [12]. A set of tumor-related TFs were obtained from Cistrome Cancer(http://cistrome.org/CistromeCancer/) [13]. CIBERSORT (https://cibersort.stanford.edu/index.php) is based on a gene expression deconvolution algorithm[14] for obtaining immune cells with differences between cancer and normal tissues.

2.2 Identification of differentially expressed genes (DEGs)

DEGs between EC and normal tissues were identified via R software (version: x64 3.2.1) and package Limma. The p value was adjusted into the false discovery rate (FDR). A value of FDR less than 0.05 and |log2(FC)| higher than 1 were considered significant.

2.3 Identification of immune-related genes (IRGs)

DEGs overlapped with immune-related genes were obtained as the differentially expressed IRGs. Based on these IRGs, Gene Ontology (GO)[15] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[16] analyses were performed with the clusterprofiler R package to explore the underlying mechanisms of these IRGs.

2.4 Identification of prognosis-related IRGs and construction of regulatory network

Prognosis-related IRGs were identified using univariate COX regression analysis. We analyzed these prognosis-related IRGs using the package R. Then, we investigated the interaction of these IRGs and differentially expressed TFs with a threshold of $P<0.05$. Coefficient >0.3 was considered as positive regulation, otherwise as negative regulation. Subsequently, we constructed a regulatory network with relevant TFs and prognosis-related IRGs by using cytoscape software 3.7.1[17].

2.5 Construction of a prognostic model in EC based on IRGs

We constructed a prognostic model based on the results of a multivariate Cox regression analysis. Based on the median risk score, EC patients were divided into high-risk and low-risk groups. The performance of prognostic model was validated by survival analysis between groups with thresholds of $p<0.05$ using the survival and survminer package of R. Receiver operating characteristic (ROC) analysis was performed via the survivalROC package, and the area under curve (AUC) was calculated to evaluate the efficiency of the model in predicting disease onset [18]. Association between IRG expression and clinical parameters was tested using independent t-tests, and $p<0.05$ were considered statistically significant. Clinical survival analysis in subgroups was also conducted, and $p<0.05$ was considered statistically significant.

2.6 Verification of the prognosis-related IRGs in this model

We used the online software Oncomine (https://www.oncomine.org) to verify the IRGs. For screening, we set the following criteria: 1 "Gene: IRGs in this model"; 2 "Analysis Type: Cancer vs. Normal Analysis"; 3 "Cancer Type: Esophageal Cancer"; 4" Clinical Outcome: Survival Status "; 5 "Data Type: mRNA". Based on the specific binding between antibodies and antigens, immunohistochemistry can reveal the relative distribution and abundance of proteins. Using The Human Protein Atlas (THPA) (https://www.proteinatlas.org) [19], we observed the differences in key gene expression between normal and EC tissues.

2.7 Building a predictive nomogram

To investigate the possibility of EC 1-OS and 3-OS, we established nomograms by including all independent prognostic factors identified by multivariate Cox regression analysis. The effectiveness of the nomogram was evaluated by discrimination and calibration. Finally, we plotted the curve of the nomogram by package rms of R to observe the relationship between the predicted rate of nomogram and the observed rate.

2.8 Functional enrichment analysis

We used Gene Set Enrichment Analysis (GSEA)[20] to identify consistent differences between high-risk and low-risk groups and the associated biological processes. In screening the gene list of KEGG, $p<0.05$ was considered statistically significant.

2.9 Differential expression of tumor-infiltrating immune cells between high-risk and low-risk groups

Status of immune infiltration in EC patients was achieved from the dataset of CIBERSORT. Subsequently, we tested the abundance of immune cells, and its difference between high-risk and low-risk groups by using two-sample T-test.

3. Results

3.1 DEGs between EC and normal samples

The RNAseq tertiary data set of EC from TCGA included the biological information of 11 normal tissue and 160 EC samples. We identified 4094 DEGs, including 3272 upregulated DEGs and 822 downregulated DEGs. (Figure 1A)

3.2 Identification of IRGs

By overlapping the immune-related genes and DEGs of EC, we identified 247 upregulated and 56 downregulated IRGs, as shown in Figure 1B. Figure 2 shows the results of functional enrichment analysis. GO analysis (Figure 2A) demonstrated that these IRGs were most involved in leukocyte migration in Biological Process（BP）, vesicle lumen in Cellular Component(CC) and receptor ligand activity in Molecular Function(MF). KEGG analysis indicated that these genes were most involved in the interaction of cytokines with cytokine receptors. (Figure 2B).

3.3 Survival analysis and construction of regulatory network

A total of 13 survival-associated IRGs were identified after integrating clinical information from TCGA via univariate COX regression, as shown in Figure 3. After examining the expression of 318 transcription factors (TF), we found 61 with differential expressions between EC and normal samples, as shown in Figure 4A-B. Finally a regulatory network was constructed using these survival-associated IRGs with differently expressed TFs（Figure 4C）.

3.4 Construction of a prognostic model based on prognosis-related IRGs and external validation

We constructed a prognostic model with nine prognostic IRGs based on the results of multivariate Cox regression analysis (Table 1). The formula was as follows: Risk score = expression level of HSPA6*0.006713979+S100A12*0.003828117+CACYBP*0.042341765+NOS2*0.02490294+DKK1*0.015602891+OSM*0.207589957+STC2*0.075574581+AN
We further explored the protein expression of these nine prognosis-related IRGs in THPA (Figure 5). Consistent with our results, THPA database showed that HSPA6, S100A12, CACYBP, NOS2, and STC2 in EC tissues were up-regulated, and ANGPTL3 was down-regulated compared with those in normal tissues. However, we did not find expression of DKK1, OSM and NR2F2 proteins in the database.

3.5 Validation of the prognosis-related IRGs in the Oncomine database

We validated the reliability of the prognosis-related IRGs by using Oncomine. The databases showed that the IRGs were differentially expressed in EC and normal tissues. As shown in Supplementary Figure 1, HSPA6, S100A12, CACYBP, NOS2, DKK1, OSM and STC2 were up-regulated, and ANGPTL3 and NR2F2 were down-regulated in EC tissues compared with those in normal tissues.We found that the results were almost consistent with our predictions.

3.6 Validation of the prognostic capacity of the model

Patients were separated into the high-risk group and the low-risk group based on the median risk score (Figure 6A-C). Survival analysis showed that the survival rate in the high-risk group was remarkably lower than those in the low-risk group (p==2.366e−06, Figure 6D). The area under curve (AUC) of the receiver operating characteristic (ROC) curve was 0.826 (Figure 6E). Compared with clinical factors (including age, gender, grade, stage and TMN), this signature showed a greater performance in predicting the prognosis of EC. At the same time, univariate and multiple regression analysis(Figure 7A-B) showed that when other clinical parameters were adjusted, the prognostic signature may become an independent predictor. The clinical significance of included genes was also explored in this study (Figure 8A-J). In order to assess the prognostic capacity of the model, we conducted a stratified analysis of clinical factors. Interestingly, we found that nearly the high-risk patients in subgroups of age ≤ 65(Figure 9A), male(Figure 9B), G1 & G2(Figure 9C) , stage III & IV(Figure 9D) ,T-3-4(Figure 9E),MO(Figure 9F),N1-3(Figure 9G) and EAC (Figure 9H)were inclined to unfavorable overall survival.

3.7 Construction and validation of predictive nomogram

Using a number of independent prognostic factors (including age, gender, grade, stage, TMN, histology, and risk scores), we established a nomogram to predict 1-year and 3-year OS in 100 EC patients. The calibration chart showed that the nomogram might overestimate or underestimate the mortality (Figure 10). These results suggested that the nomogram based on multiple factors can better predict short-term survival (1 year and 3 years) compared to the nomogram based on a single factor.

3.8 Identification of related biological processes and pathways

We employed risk score to classify the entire data set and determine the related pathways with these nine genes by using the Java software GSEA. The results showed that "one carbon pool by folate", "proteasome", "spliceosome" and "RNA degradation" were more abundant in the high-risk group than in the low-risk group. This suggests that in high-risk patients, the nine genes were most involved in pathways of protein degradation, RNA degradation and splicing. That is to say, patients with protein degradation, RNA degradation and splicing effects were more inclined to a poor prognosis (Figure 11).

3.9 Level difference of tumor-infiltrating immune cells between the two risk groups

We compared the infiltration of immune cells in different risk groups. The results showed that Macrophages M0, Macrophages M2 and activated mast cells were significantly enriched in high-risk group, while CD8 T cells and regulatory T cells (Tregs) were significantly enriched in the low-risk group (Figure 12).

4. Discussion

Esophageal cancer has a large number of new cases every year, and it has historically been regarded as an uncontrollable disease process. The etiology of esophageal cancer may be multifactorial, but part of it is due to the unique manifestation of this cancer[21]. At present, for the treatment of esophageal cancer, attention has shifted to the development of immunotherapy with novel immune biomarkers [22]. Somatic cells acquire malignancy through genetic alterations. Cancer cells usually evade the recognition of the immune system and develop into clinically meaningful masses [23]. Compared with conventional therapies, cancer immunotherapy shows long-lasting response with fewer adverse reactions [24]. This provides a new option for the treatment of EC.

The prognostic model for EC has been continuously updated[25-27]. In this study, we identified 247 up-regulated and 56 down-regulated IRGs in EC and screened out survival-related IRGs. Based on these data, we established a prognostic model that divided EC patients into high-risk and low-risk groups. This model showed a good predictive performance (AUC 0.826). The model was also an independent prognostic indicator by multivariate analysis incorporating other clinical factors. KEGG analysis indicated that the main pathway was enriched in cytokine-cytokine receptor interaction. Many biological processes are regulated by cytokines, including cell growth, differentiation, immunity, inflammation, and metabolism[28]. Tumor progression can be promoted by cytokines

that affect the tumor microenvironment and directly act on cancer cells[29]. Moreover, cytokines participate in the immune response of cytotoxic T lymphocytes (CTLs) by modulating the differentiation of Th1 and Th2 cells[30]. Kita Y et al found that STC2 may be involved in lymph node metastasis, making it a potential prognostic marker for patients with EC[31]. Studies also demonstrated that STC2 may play an important role in ESCC tumorigenesis[32]. Abnormal expression of DKK1, which is regulated by DKK1-CKAP4 pathway, predicts the poor prognosis of esophageal squamous cell carcinoma (ESCC) [33]. These results are consistent with our findings. CacyBP regulates cell proliferation, tumorigenesis, differentiation or gene expression[34]. In colon cancer, CacyBP can promote the growth of cancer cells by enhancing the ubiquitin-mediated degradation of p27kip1[35]. In addition, studies have confirmed that CacyBP level increased in gastric, nasopharyngeal carcinoma, osteogenic sarcoma and melanoma[36, 37].

In our prognostic model, the IRGs showing prognostic values included HSPA6, S100A12, CACYBP, NOS2, DKK1, OSM, STC2, ANGPTL3 and NR2F2. Among the, HSPA6 may be associated with early recurrence of HCC[38]. In ESCC, S100A12 is downregulated at the protein level[39]. In Barrett's esophagus and related adenocarcinoma, expression of inducible nitric oxide synthase (NOS-2) is increased, and NOS-2 also plays a role in inflammation and epithelial cell growth[40]. OSM has been identified as an inhibitor of tumor cell growth in a variety of cancers, including melanoma, ovarian cancer, and glioblastoma carcinomas[41-43]. The splice variant of oncostatin M receptor β is overexpressed in human esophageal squamous cell carcinoma[44]. Angiopoietin-like protein 3(ANGPTL3) is indicative of EC prognosis [45]. NR2F2 is involved in the progression of prostate adenocarcinoma[46], and NR2F2 expression is a prognostic factor for breast neoplasms[47]. High expression of NR2F2 in certain gastric and esophageal adenocarcinomas is associated with abnormal expression of cadherin 11, suggesting that the NR2F2-related embryonic pathways in these tumors are reactivated[48]. Proteasome dysregulation is implicated in the development of many types of cancer[49]. The proteasome is involved in cell cycle and transcription, two processes indispensable for cancer development[50]. The spliceosome catalyzes pre-mRNA splicing, a key regulatory step in gene expression[51, 52]. Mutations in genes encoding splice proteins are frequently found in cancer[53]. Small molecule inhibitors that target splice components can be used to create anti-cancer drugs [52]. RNA degradation is a key post-transcriptional regulatory checkpoint to maintain proper functions of organisms. Ribonuclease, a key enzyme responsible for RNA stability, can be used alone for RNA degradation, and can bind to other proteins in the RNA degradation complex[54].

Previous immunotherapies mainly rely on T cells in tumor immune defense[55, 56]. Evidence has verified the role of B cells in tumor immunology[57, 58]. In the present research, the abundance of CD8 T cells and regulatory T cells in the low-risk group increased.

T cells are critical in host defense against cancer [59]. The value of CD8 T cells for cancer prognosis has been assessed [60-64]. In addition, CD8 T cells also play a role in the progression of EC [65, 66]. The abundance of CD8 T cells and regulatory T cells(Treg) in the low-risk group was higher than in the high-risk group, suggesting that the infiltration of both types of cells may be a good prognostic signal for EC patients. Tregs are divided into two major subpopulations: thymus-derived Tregs (nTregs) and inducible Tregs (iTregs)[67]. Tregs show significant versatility in their inhibitory mechanisms by releasing cytokines to directly inhibit signal transduction of effector T cells[68]. Tregs can also inhibit and kill B cells by inducing programmed cell death [69]. In ESCC, Tregs expression is positively correlated with tumor invasion, suggesting that Tregs could be taken as a useful prognostic marker for ESCC[70]. The number of Treg cells in patients with advanced EC is significantly reduced after chemotherapy[71]. Tregs can also weaken anti-tumor immunity, making them potential indicators of poor prognosis[72].

It is the first time that a prognostic nomogram is developed with nine immune related genes. This nomogram can be routinely applied and is cost-effective in practice, as it does not need whole-genome sequencing for EC patients. When combined with clinical parameters like TNM stage, the nomogram can show a greater prognostic performance.

Our research still has some shortcomings. First, clinical data should be introduced to validate our findings. Second, the inequality between the samples may lead to potential bias. Third, the underlying molecular mechanisms needs to be explored through in vitro and in vivo studies.

5. Conclusion

The model based on nine IRGs is accurate to predict EC prognosis. Infiltration of immune cells is related with EC risk. These molecules and immunobiomarkers provide a possibility of developing new target therapies.

Abbreviations

EC: Sophageal cancer

IRGs: Immune-related genes

TCGA: The Cancer Genome Atlas

TFs: Transcription factors

FDR: False discovery rate

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

ROC: Receiver operating characteristic

AUC: Area under curve

THPA: The Human Protein Atlas

GSEA: Gene Set Enrichment Analysis

Declarations

Availability of data and materials

The raw datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate.

Not applicable.

Consent for publication.

Not applicable.

Availability of data and materials.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests.

The authors declare that they have no competing interests.

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Authors' contributions.

Zhi Zhang and Cheng Chen contributed equally to this study.

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References

1. Short MW, Burgers KG, Fry VT: **Esophageal Cancer**. *Am Fam Physician* 2017, **95**(1):22-28.
2. Simard EP, Ward EM, Siegel R, Jemal A: **Cancers with increasing incidence trends in the United States: 1999 through 2008**. *CA Cancer J Clin* 2012, **62**(2):118-128.
3. Huang FL, Yu SJ: **Esophageal cancer: Risk factors, genetic association, and treatment**. *Asian J Surg* 2018, **41**(3):210-215.
4. Xu L, Li Y, Sun H, Zheng Y, Wang Z, Chen X: **[Impact of postoperative pathological features of esophageal squamous cell carcinoma on the prognosis]**. *Zhonghua Wei Chang Wai Ke Za Zhi* 2017, **20**(12):1448-1451.
5. Prabhu A, Obi K, Lieberman D, Rubenstein JH: **The Race-Specific Incidence of Esophageal Squamous Cell Carcinoma in Individuals With Exposure to Tobacco and Alcohol**. *Am J Gastroenterol* 2016, **111**(12):1718-1725.
6. Pennathur A, Gibson MK, Jobe BA, Luketich JD: **Oesophageal carcinoma**. *Lancet* 2013, **381**(9864):400-412.

7. Bollschweiler E, Plum P, Monig SP, Holscher AH: **Current and future treatment options for esophageal cancer in the elderly**. *Expert Opin Pharmacother* 2017, **18**(10):1001-1010.
8. Cerezo-Wallis D, Soengas MS: **Understanding Tumor-Antigen Presentation in the New Era of Cancer Immunotherapy**. *Curr Pharm Des* 2016, **22**(41):6234-6250.
9. Li B, Cui Y, Diehn M, Li R: **Development and Validation of an Individualized Immune Prognostic Signature in Early-Stage Nonsquamous Non-Small Cell Lung Cancer**. *JAMA Oncol* 2017, **3**(11):1529-1537.
10. Lin P, Guo YN, Shi L, Li XJ, Yang H, He Y, Li Q, Dang YW, Wei KL, Chen G: **Development of a prognostic index based on an immunogenomic landscape analysis of papillary thyroid cancer**. *Aging (Albany NY)* 2019, **11**(2):480-500.
11. Lee JS: **Exploring cancer genomic data from the cancer genome atlas project**. *BMB Rep* 2016, **49**(11):607-611.
12. Bhattacharya S, Dunn P, Thomas CG, Smith B, Schaefer H, Chen J, Hu Z, Zalocusky KA, Shankar RD, Shen-Orr SS *et al*: **ImmPort, toward repurposing of open access immunological assay data for translational and clinical research**. *Sci Data* 2018, **5**:180015.
13. Mei S, Meyer CA, Zheng R, Qin Q, Wu Q, Jiang P, Li B, Shi X, Wang B, Fan J *et al*: **Cistrome Cancer: A Web Resource for Integrative Gene Regulation Modeling in Cancer**. *Cancer Res* 2017, **77**(21):e19-e22.
14. Ali HR, Chlon L, Pharoah PD, Markowetz F, Caldas C: **Patterns of Immune Infiltration in Breast Cancer and Their Clinical Implications: A Gene-Expression-Based Retrospective Study**. *PLoS Med* 2016, **13**(12):e1002194.
15. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT *et al*: **Gene ontology: tool for the unification of biology. The Gene Ontology Consortium**. *Nat Genet* 2000, **25**(1):25-29.
16. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M: **KEGG as a reference resource for gene and protein annotation**. *Nucleic Acids Res* 2016, **44**(D1):D457-462.
17. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: **Cytoscape: a software environment for integrated models of biomolecular interaction networks**. *Genome Res* 2003, **13**(11):2498-2504.
18. Kamarudin AN, Cox T, Kolamunnage-Dona R: **Time-dependent ROC curve analysis in medical research: current methods and applications**. *BMC Med Res Methodol* 2017, **17**(1):53.
19. Almdahl SM, Jenssen TG, Samdal FA, Burhol PG: **The effect of pancreatectomy and gastroenterectomy on the release of somatostatin and vasoactive intestinal polypeptide in experimental fecal peritonitis**. *Scand J Gastroenterol* 1988, **23**(1):31-34.
20. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles**. *Proc Natl Acad Sci U S A* 2005, **102**(43):15545-15550.
21. Vaghjiani RG, Molena D: **Surgical management of esophageal cancer**. *Chin Clin Oncol* 2017, **6**(5):47.
22. Ku GY: **Systemic therapy for esophageal cancer: chemotherapy**. *Chin Clin Oncol* 2017, **6**(5):49.
23. Sugie T: **Immunotherapy for metastatic breast cancer**. *Chin Clin Oncol* 2018, **7**(3):28.
24. Moy JD, Moskovitz JM, Ferris RL: **Biological mechanisms of immune escape and implications for immunotherapy in head and neck squamous cell carcinoma**. *Eur J Cancer* 2017, **76**:152-166.
25. Xi M, Liao Z, Deng W, Xu C, Komaki R, Blum M, Hofstetter WL, Ho L, Lin SH: **A Prognostic Scoring Model for the Utility of Induction Chemotherapy Prior to Neoadjuvant Chemoradiotherapy in Esophageal Cancer**. *J Thorac Oncol* 2017, **12**(6):1001-1010.
26. Winther M, Alsner J, Tramm T, Baeksgaard L, Holtved E, Nordsmark M: **Evaluation of miR-21 and miR-375 as prognostic biomarkers in esophageal cancer**. *Acta Oncol* 2015, **54**(9):1582-1591.
27. Cao HH, Zheng CP, Wang SH, Wu JY, Shen JH, Xu XE, Fu JH, Wu ZY, Li EM, Xu LY: **A molecular prognostic model predicts esophageal squamous cell carcinoma prognosis**. *PLoS One* 2014, **9**(8):e106007.
28. O'Shea JJ, Holland SM, Staudt LM: **JAKs and STATs in immunity, immunodeficiency, and cancer**. *N Engl J Med* 2013, **368**(2):161-170.
29. Roshani R, McCarthy F, Hagemann T: **Inflammatory cytokines in human pancreatic cancer**. *Cancer Lett* 2014, **345**(2):157-163.
30. Agarwal A, Verma S, Burra U, Murthy NS, Mohanty NK, Saxena S: **Flow cytometric analysis of Th1 and Th2 cytokines in PBMCs as a parameter of immunological dysfunction in patients of superficial transitional cell carcinoma of bladder**. *Cancer Immunol Immunother* 2006, **55**(6):734-743.
31. Kita Y, Mimori K, Iwatsuki M, Yokobori T, Ieta K, Tanaka F, Ishii H, Okumura H, Natsugoe S, Mori M: **STC2: a predictive marker for lymph node metastasis in esophageal squamous-cell carcinoma**. *Ann Surg Oncol* 2011, **18**(1):261-272.
32. Kashyap MK, Pawar HA, Keerthikumar S, Sharma J, Goel R, Mahmood R, Kumar MV, Kumar KV, Pandey A, Kumar RV *et al*: **Evaluation of protein expression pattern of stanniocalcin 2, insulin-like growth factor-binding protein 7, inhibin beta A and four and a half LIM domains 1 in esophageal squamous cell carcinoma**. *Cancer Biomark* 2012, **12**(1):1-9.
33. Shinno N, Kimura H, Sada R, Takiguchi S, Mori M, Fumoto K, Doki Y, Kikuchi A: **Activation of the Dickkopf1-CKAP4 pathway is associated with poor prognosis of esophageal cancer and anti-CKAP4 antibody may be a new therapeutic drug**. *Oncogene* 2018, **37**(26):3471-3484.
34. Topolska-Wos AM, Chazin WJ, Filipek A: **CacyBP/SIP--Structure and variety of functions**. *Biochim Biophys Acta* 2016, **1860**(1 Pt A):79-85.
35. Zhai H, Shi Y, Chen X, Wang J, Lu Y, Zhang F, Liu Z, Lei T, Fan D: **CacyBP/SIP promotes the proliferation of colon cancer cells**. *PLoS One* 2017, **12**(2):e0169959.
36. Zhai H, Shi Y, Jin H, Li Y, Lu Y, Chen X, Wang J, Ding L, Wang X, Fan D: **Expression of calcyclin-binding protein/Siah-1 interacting protein in normal and malignant human tissues: an immunohistochemical survey**. *J Histochem Cytochem* 2008, **56**(8):765-772.

37. Zhu L, Miake S, Ijichi A, Kawahara S, Kohno M, Sonoyama H, Mitamura Y, Kaku Y, Tsuru H, Tu Y *et al*: **Upregulated expression of calcyclin-binding protein/siah-1 interacting protein in malignant melanoma**. *Ann Dermatol* 2014, **26**(5):670-673.
38. Yang Z, Zhuang L, Szatmary P, Wen L, Sun H, Lu Y, Xu Q, Chen X: **Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma**. *Int J Med Sci* 2015, **12**(3):256-263.
39. Ji J, Zhao L, Wang X, Zhou C, Ding F, Su L, Zhang C, Mao X, Wu M, Liu Z: **Differential expression of S100 gene family in human esophageal squamous cell carcinoma**. *J Cancer Res Clin Oncol* 2004, **130**(8):480-486.
40. Wilson KT, Fu S, Ramanujam KS, Meltzer SJ: **Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas**. *Cancer Res* 1998, **58**(14):2929-2934.
41. Brown TJ, Lioubin MN, Marquardt H: **Purification and characterization of cytostatic lymphokines produced by activated human T lymphocytes. Synergistic antiproliferative activity of transforming growth factor beta 1, interferon-gamma, and oncostatin M for human melanoma cells**. *J Immunol* 1987, **139**(9):2977-2983.
42. Ohata Y, Harada T, Fujii A, Yoshida S, Iwabe T, Terakawa N: **Menstrual cycle-specific inhibition of endometrial stromal cell proliferation by oncostatin M**. *Mol Hum Reprod* 2001, **7**(7):665-670.
43. Friedrich M, Hoss N, Stogbauer F, Senner V, Paulus W, Ringelstein EB, Halfter H: **Complete inhibition of in vivo glioma growth by oncostatin M**. *J Neurochem* 2001, **76**(5):1589-1592.
44. Kausar T, Sharma R, Hasan MR, Saraya A, Chattopadhyay TK, Gupta SD, Ralhan R: **Overexpression of a splice variant of oncostatin M receptor beta in human esophageal squamous carcinoma**. *Cell Oncol (Dordr)* 2011, **34**(3):177-187.
45. Zhu L, Jiang L, Wang W, Jia W, Liu F, Jiao X, Zhu X, Bao J, Yu H: **Angiopoietin-like protein 3 is an indicator of prognosis in esophageal cancer patients**. *Int J Clin Exp Med* 2015, **8**(9):16101-16106.
46. Qin J, Wu SP, Creighton CJ, Dai F, Xie X, Cheng CM, Frolov A, Ayala G, Lin X, Feng XH *et al*: **COUP-TFII inhibits TGF-beta-induced growth barrier to promote prostate tumorigenesis**. *Nature* 2013, **493**(7431):236-240.
47. Nagasaki S, Suzuki T, Miki Y, Akahira J, Shibata H, Ishida T, Ohuchi N, Sasano H: **Chicken ovalbumin upstream promoter transcription factor II in human breast carcinoma: possible regulator of lymphangiogenesis via vascular endothelial growth factor-C expression**. *Cancer Sci* 2009, **100**(4):639-645.
48. Bringuier PP, Schalken JA, Hervieu V, Giroldi LA: **Involvement of orphan nuclear receptor COUP-TFII in cadherin-6 and cadherin-11 regulation: implications in development and cancer**. *Mech Dev* 2015, **136**:64-72.
49. Chen Y, Zhang Y, Guo X: **Proteasome dysregulation in human cancer: implications for clinical therapies**. *Cancer Metastasis Rev* 2017, **36**(4):703-716.
50. Catalgol B: **Proteasome and cancer**. *Prog Mol Biol Transl Sci* 2012, **109**:277-293.
51. Will CL, Luhrmann R: **Spliceosome structure and function**. *Cold Spring Harb Perspect Biol* 2011, **3**(7).
52. Effenberger KA, Urabe VK, Jurica MS: **Modulating splicing with small molecular inhibitors of the spliceosome**. *Wiley Interdiscip Rev RNA* 2017, **8**(2).
53. Lee SC, Abdel-Wahab O: **Therapeutic targeting of splicing in cancer**. *Nat Med* 2016, **22**(9):976-986.
54. Saramago M, da Costa PJ, Viegas SC, Arraiano CM: **The Implication of mRNA Degradation Disorders on Human DISease: Focus on DIS3 and DIS3-Like Enzymes**. *Adv Exp Med Biol* 2019, **1157**:85-98.
55. McGray AJ, Hallett R, Bernard D, Swift SL, Zhu Z, Teoderascu F, Vanseggelen H, Hassell JA, Hurwitz AA, Wan Y *et al*: **Immunotherapy-induced CD8+ T cells instigate immune suppression in the tumor**. *Mol Ther* 2014, **22**(1):206-218.
56. Traversari C, Russo V: **T Cells as Antigen Carriers for Anti-tumor Vaccination**. *Methods Mol Biol* 2016, **1393**:97-104.
57. Tsou P, Katayama H, Ostrin EJ, Hanash SM: **The Emerging Role of B Cells in Tumor Immunity**. *Cancer Res* 2016, **76**(19):5597-5601.
58. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A *et al*: **Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer**. *Immunity* 2013, **39**(4):782-795.
59. Jiang S, Yan W: **T-cell immunometabolism against cancer**. *Cancer Lett* 2016, **382**(2):255-258.
60. Mahmoud S, Lee A, Ellis I, Green A: **CD8(+) T lymphocytes infiltrating breast cancer: A promising new prognostic marker?** *Oncoimmunology* 2012, **1**(3):364-365.
61. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO: **CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer**. *Breast Cancer Res* 2012, **14**(2):R48.
62. Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, Lagorce C, Wind P, Marliot F, Bruneval P *et al*: **In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer**. *J Clin Oncol* 2009, **27**(35):5944-5951.
63. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C *et al*: **Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer**. *Proc Natl Acad Sci U S A* 2005, **102**(51):18538-18543.
64. Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, Kato K, Kurokawa T, Suzuoki M, Nakakubo Y *et al*: **CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma**. *Pancreas* 2004, **28**(1):e26-31.
65. Kato T, Noma K, Ohara T, Kashima H, Katsura Y, Sato H, Komoto S, Katsube R, Ninomiya T, Tazawa H *et al*: **Cancer-Associated Fibroblasts Affect Intratumoral CD8(+) and FoxP3(+) T Cells Via IL6 in the Tumor Microenvironment**. *Clin Cancer Res* 2018, **24**(19):4820-4833.
66. Li J, Qiu G, Fang B, Dai X, Cai J: **Deficiency of IL-18 Aggravates Esophageal Carcinoma Through Inhibiting IFN-gamma Production by CD8(+)T Cells and NK Cells**. *Inflammation* 2018, **41**(2):667-676.

67. Bilate AM, Lafaille JJ: **Induced CD4+Foxp3+ regulatory T cells in immune tolerance**. *Annu Rev Immunol* 2012, **30**:733-758.
68. Schmidt A, Oberle N, Krammer PH: **Molecular mechanisms of treg-mediated T cell suppression**. *Front Immunol* 2012, **3**:51.
69. Gondek DC, Devries V, Nowak EC, Lu LF, Bennett KA, Scott ZA, Noelle RJ: **Transplantation survival is maintained by granzyme B+ regulatory cells and adaptive regulatory T cells**. *J Immunol* 2008, **181**(7):4752-4760.
70. Nabeki B, Ishigami S, Uchikado Y, Sasaki K, Kita Y, Okumura H, Arigami T, Kijima Y, Kurahara H, Maemura K *et al*: **Interleukin-32 expression and Treg infiltration in esophageal squamous cell carcinoma**. *Anticancer Res* 2015, **35**(5):2941-2947.
71. Xu T, Duan Q, Wang G, Hu B: **CD4 + CD25high regulatory T cell numbers and FOXP3 mRNA expression in patients with advanced esophageal cancer before and after chemotherapy**. *Cell Biochem Biophys* 2011, **61**(2):389-392.
72. Lin EW, Karakasheva TA, Hicks PD, Bass AJ, Rustgi AK: **The tumor microenvironment in esophageal cancer**. *Oncogene* 2016, **35**(41):5337-5349.

Table

Table 1:The immune-based prognostic index model of EC.

id	coef	HR	HR.95L	HR.95H	pvalue
HSPA6	0.006713979	1.006736568	1.000468103	1.013044308	0.035133319
S100A12	0.003828117	1.003835454	1.002153991	1.005519738	7.62E-06
CACYBP	0.042341765	1.043250965	0.992392076	1.09671631	0.096819638
NOS2	0.024902941	1.02521561	1.009716012	1.040953134	0.001355421
DKK1	0.015602891	1.015725251	1.005677036	1.025873864	0.002098124
OSM	0.207589957	1.230708423	1.044607882	1.449963424	0.013076143
STC2	0.075574581	1.07850366	1.018550661	1.141985558	0.009601837
ANGPTL3	0.645334283	1.906624275	1.249904495	2.908395112	0.002741427
NR2F2	0.015710952	1.015835018	1.005558018	1.026217051	0.002459204

Description Of Supplementary Materials

Supplementary Figure 1. Analysis of the prognosis-related IRGs in box plots by Oncomine. A: HSPA6 mRNA expression (P-value:1.10E-10, t-Test:7.750,Fold Change:2.314). B: S100A12 mRNA expression (P-value:0.007, t-Test:2.888,Fold Change:6.630). C: CACYBP mRNA expression (P-value:0.004, t-Test:3.328,Fold Change:1.430). D: NOS2 mRNA expression (P-value:0.010, t-Test:2.485,Fold Change:1.566). E: DKK1 mRNA expression (P-value:0.038, t-Test:1.985,Fold Change:2.128). F: OSM mRNA expression (P-value:3.90E-8, t-Test:5.887,Fold Change: 2.045). G: STC2 mRNA expression (P-value:4.18E-7, t-Test:7.176,Fold Change: 2.293). H: ANGPTL3 mRNA expression (P-value:0.663, t-Test:-0.432,Fold Change:-1.169). I: NR2F2 mRNA expression (P-value:0.974, t-Test:-1.972,Fold Change: -1.318).(1: Barrett's Esophagus;2: Esophageal Carcinoma;***P<0.001; **P<0.01;*P<0.05;ns:no significance).

Figures

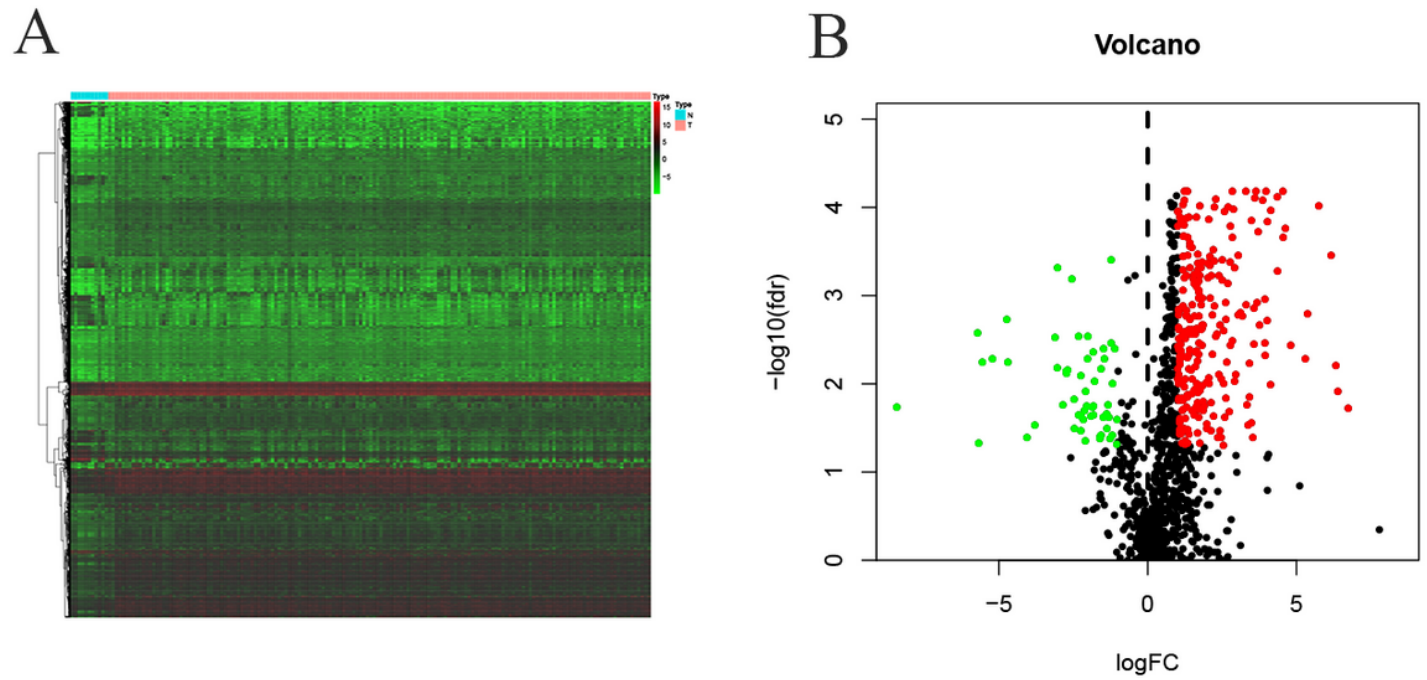

Figure 1

Differential expression analysis of DEGs and IRGs.A: Heatmap of DEGs;Red plots: upregulation; Green plots: downregulation;Black plots: normally expressed mRNAs.B: Volcano plot of IRGs;Red ,green and black plots: differentially expressed mRNAs as indicated in A.

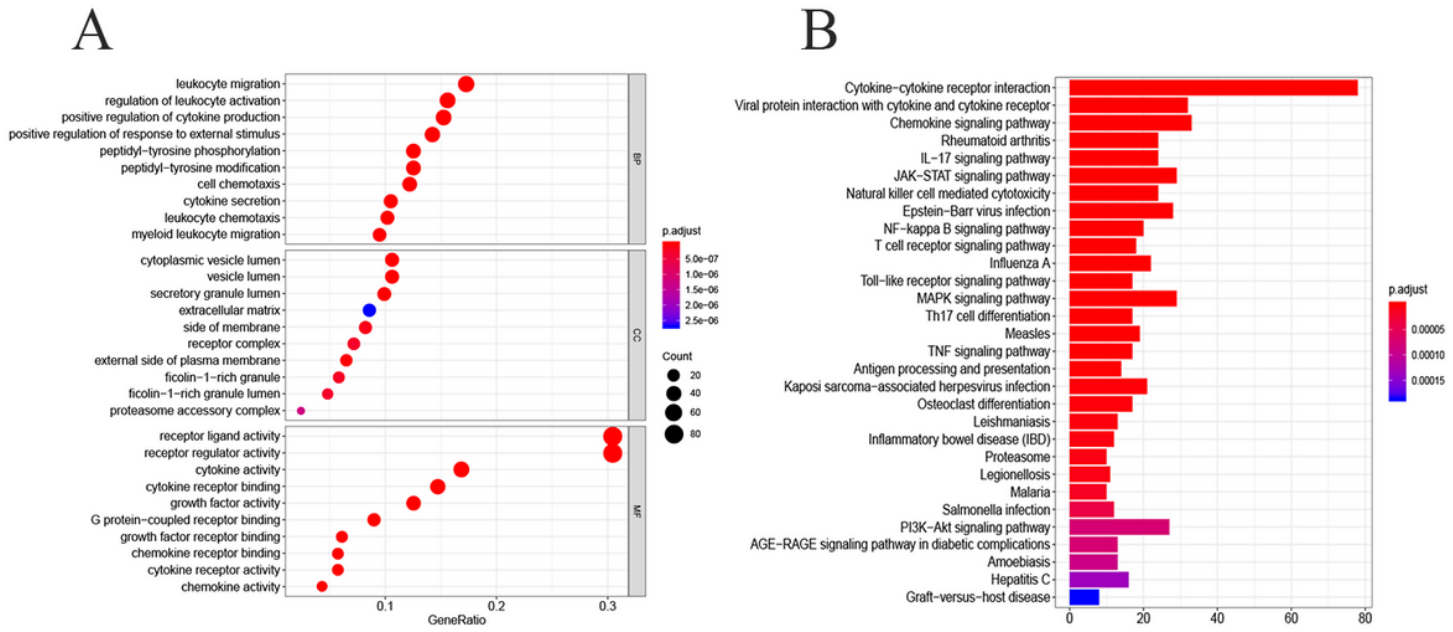

Figure 2

Functional enrichment analysis of differentially expressed IRGs. A: Gene ontology analysis;the ball in the three rectangles represent biological process, cellular component and molecular function, respectively.B: The significant KEGG pathways of IRGs.

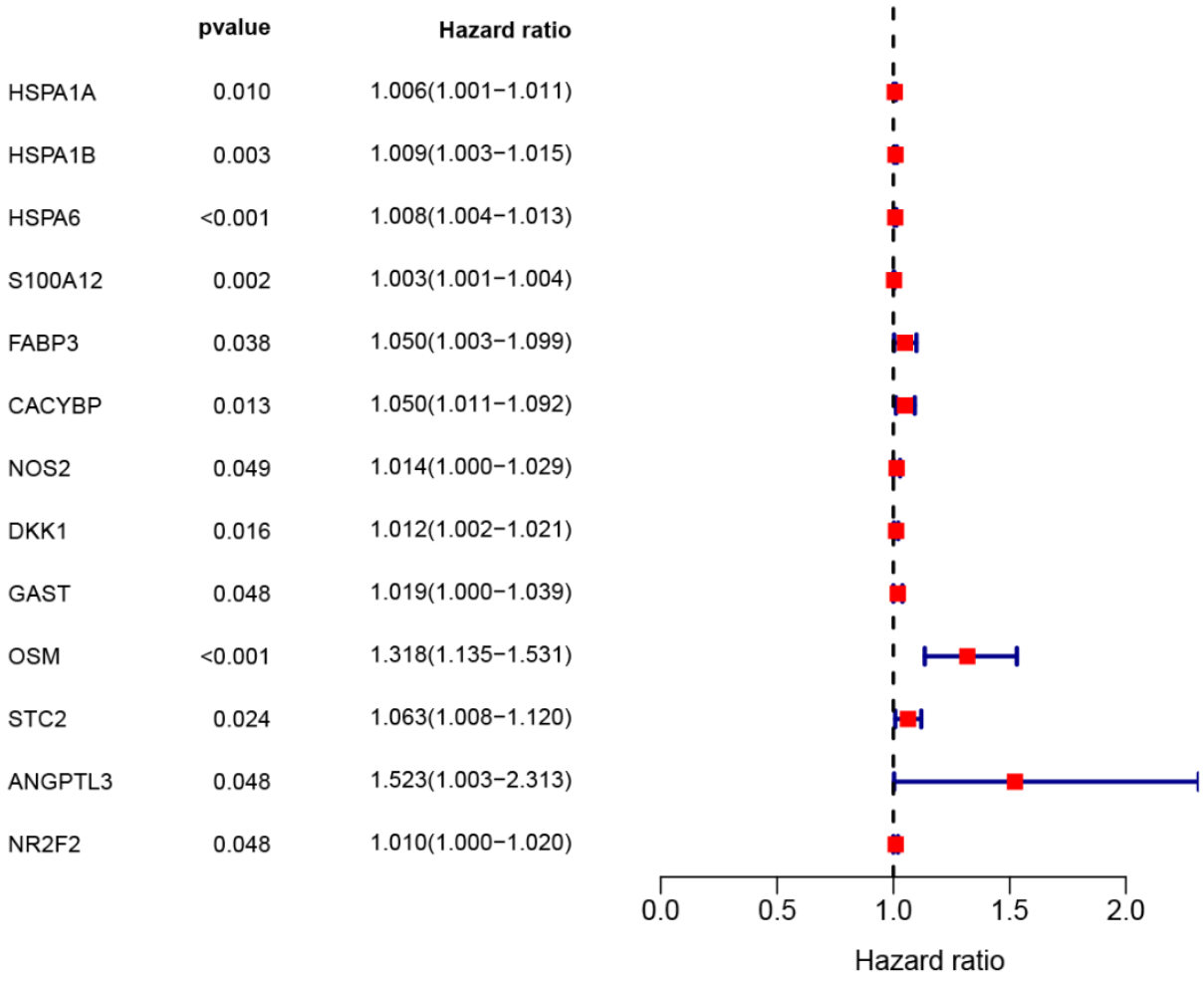

Figure 3

Forest plot of hazard ratios showing the prognostic values of genes,in which the unadjusted hazard ratios as well as the corresponding 95% confidence intervals are displayed.

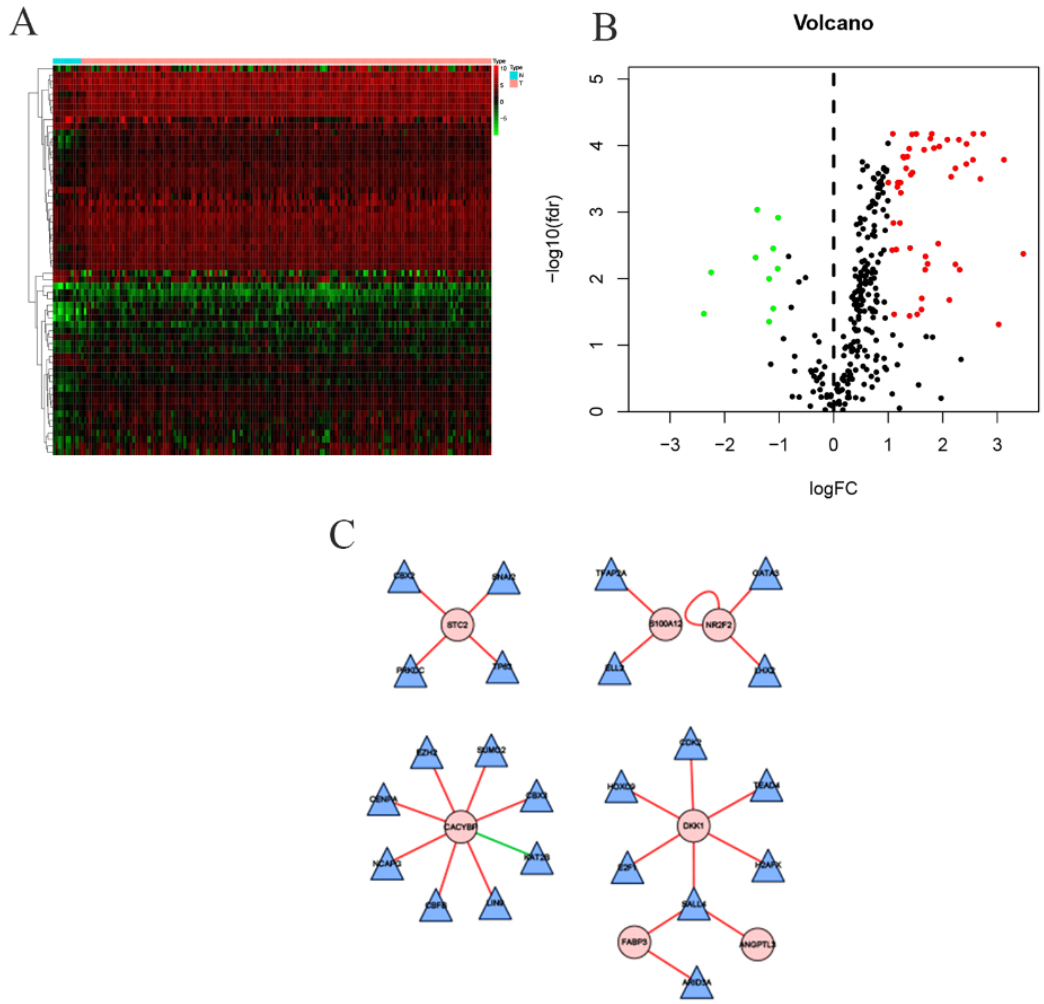

Figure 4

Differential expression analysis of TFs and the regulatory network. A: Heatmap of TFs, red: upregulation; green: downregulation; black: normally expressed mRNAs.B: Volcanic maps of TFs;red,green and black plots: differentially expressed mRNAs as indicated in A. C: Regulatory network integrated the survival associated IRGs and differentially expressed TFs;the circles filled with pink represent the survival associated IRGs and the triangles filled with blue represent TFs.

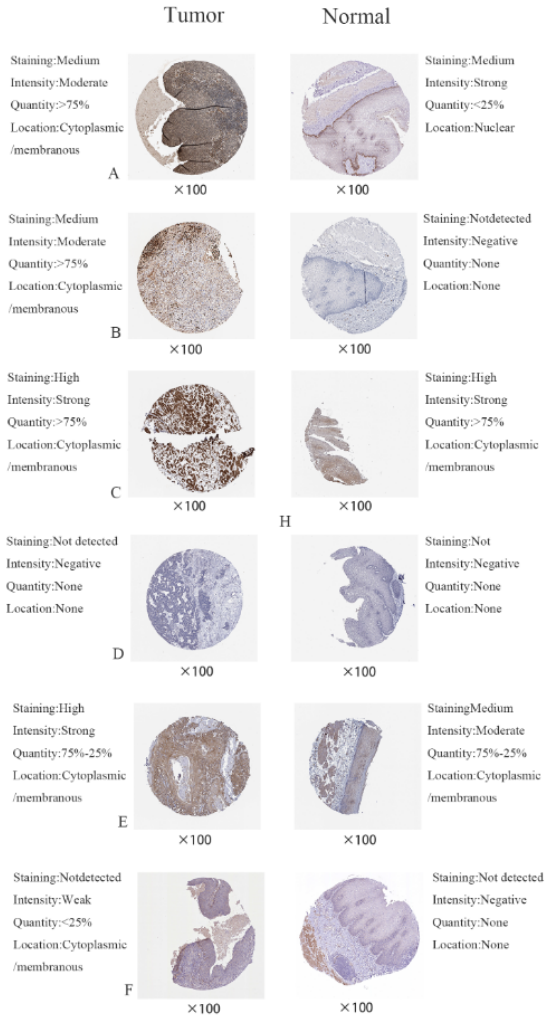

Figure 5

Protein expression of genes in the model. A:protein expression of HSPA6. B:protein expression of S100A12. C:protein expression of CACYBP. D:protein expression of NOS2. E:protein expression of STC2. F:protein expression of ANGPTL3.

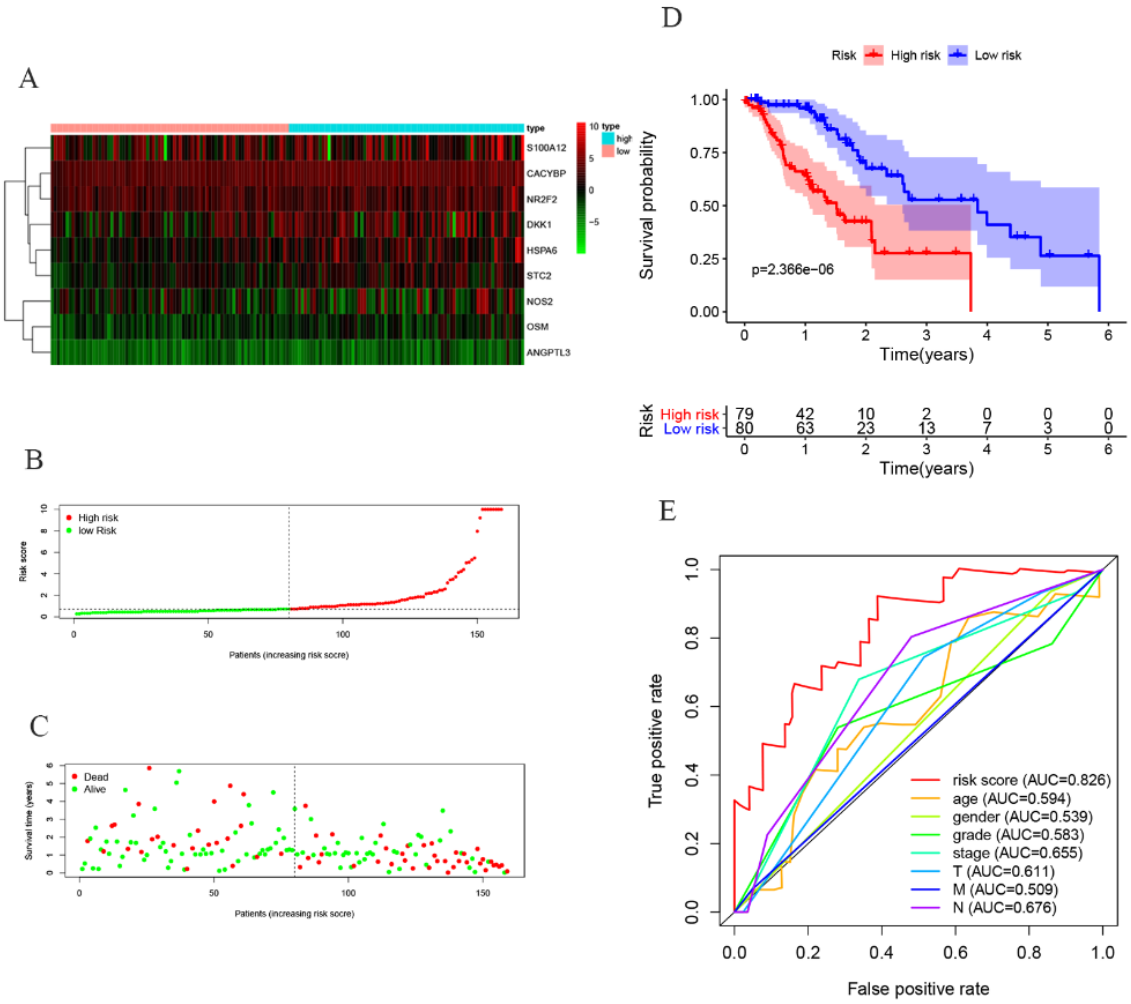

Figure 6

The prognostic value of prognostic index developed based on IRGs. A:Heatmap of expression profiles of included IRGs. B: Survival status of patients in different groups. C:Rank of prognostic index and distribution of groups.D: Survival analysis between the two groups. E: ROC curve of the prognostic index model .

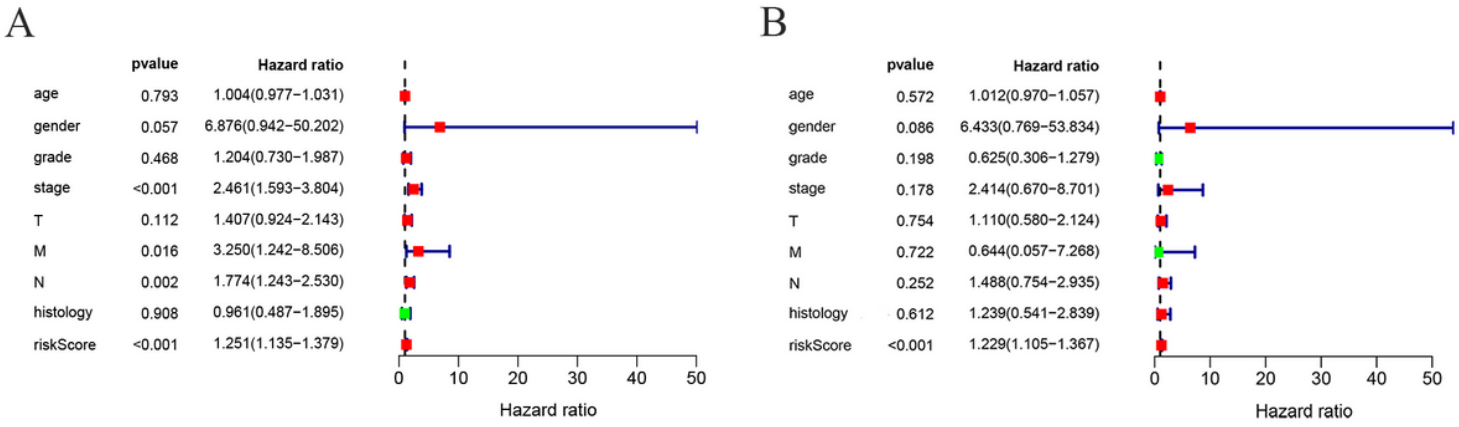

Figure 7

Forest plots including the risk score and other clinical parameters by univariate(A) and multiple regression analysis(B),in which the unadjusted hazard ratios as well as the corresponding 95% confidence intervals are displayed .

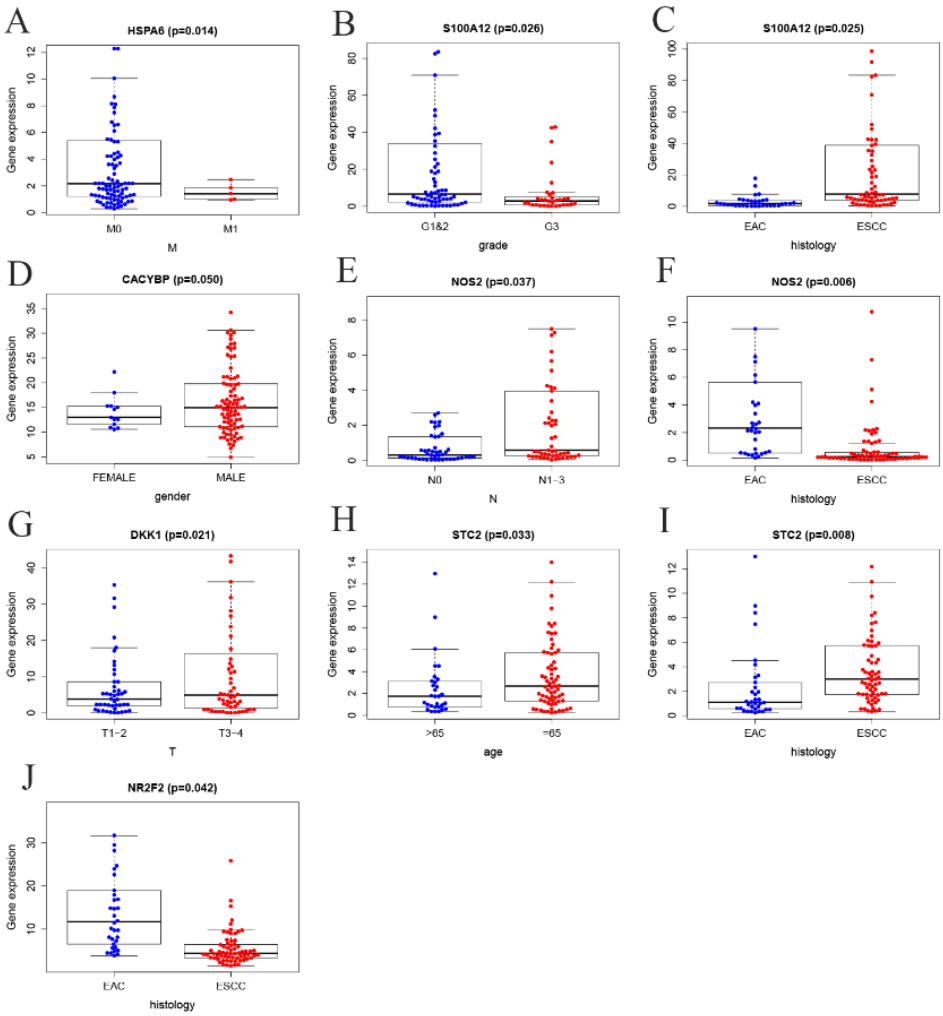

Figure 8

Relationships between the expressions of the immune-related genes and the clinicopathological factors.(A) HSPA6 and M.(B) S100A12 and grade.(C) S100A12 and histology.(D) CACYBP and gender.(E) NOS2 and N.(F) NOS2 and histology.(G) DKK1 and T.(H) STC2 and age.(I) STC2 and histology.(J) NR2F2 and histology.

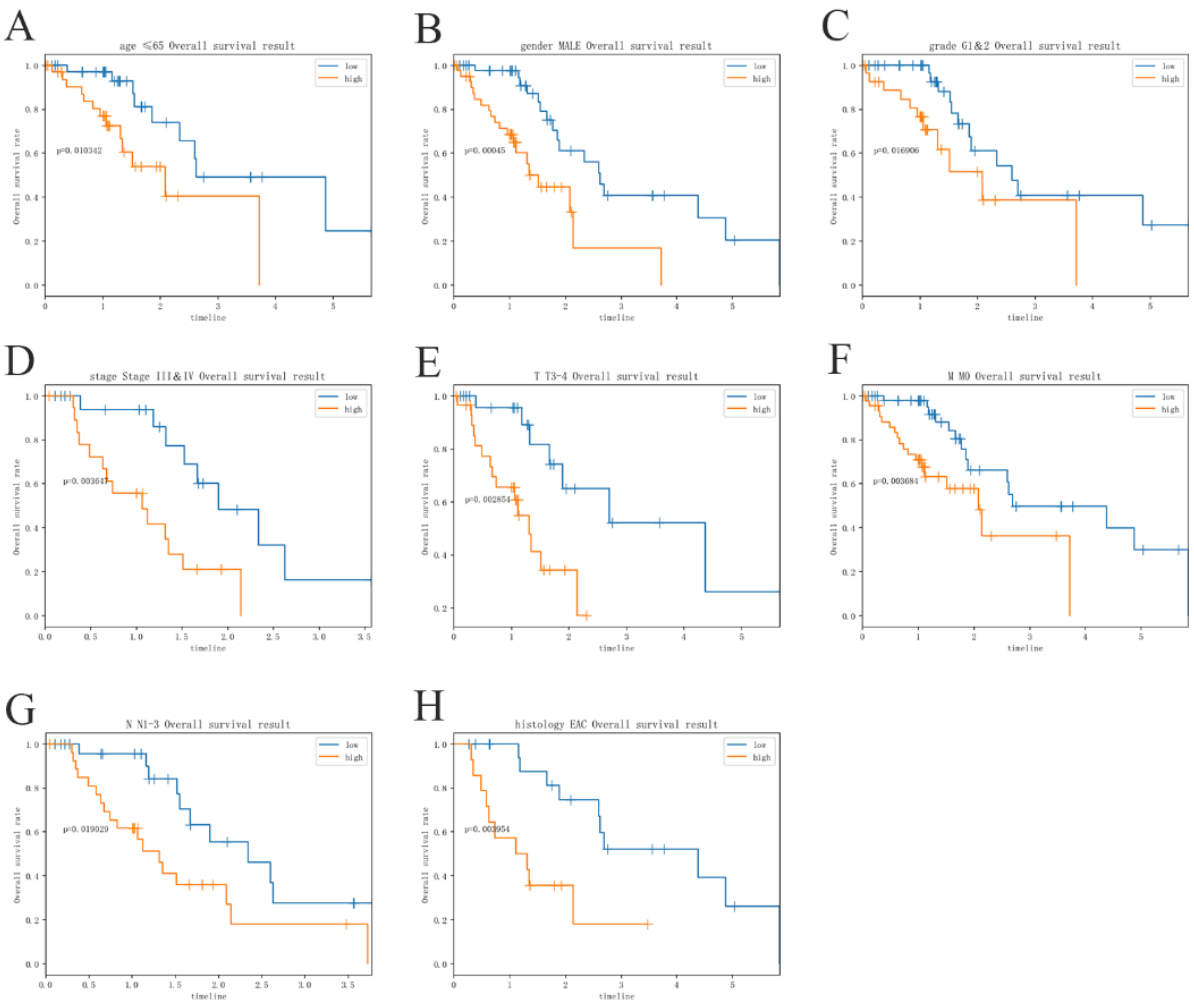

Figure 9

Subgroup survival analysis for patients with EC according to the prognostic index stratified by clinical factors.(A) age ≤65. (B) gender MALE. (C) grade G1–2. (D) stage III–IV. (E) T3-4. (F) M0. (G) N1-3. (H) histology EAC. EAC: esophageal adenocarcinoma.

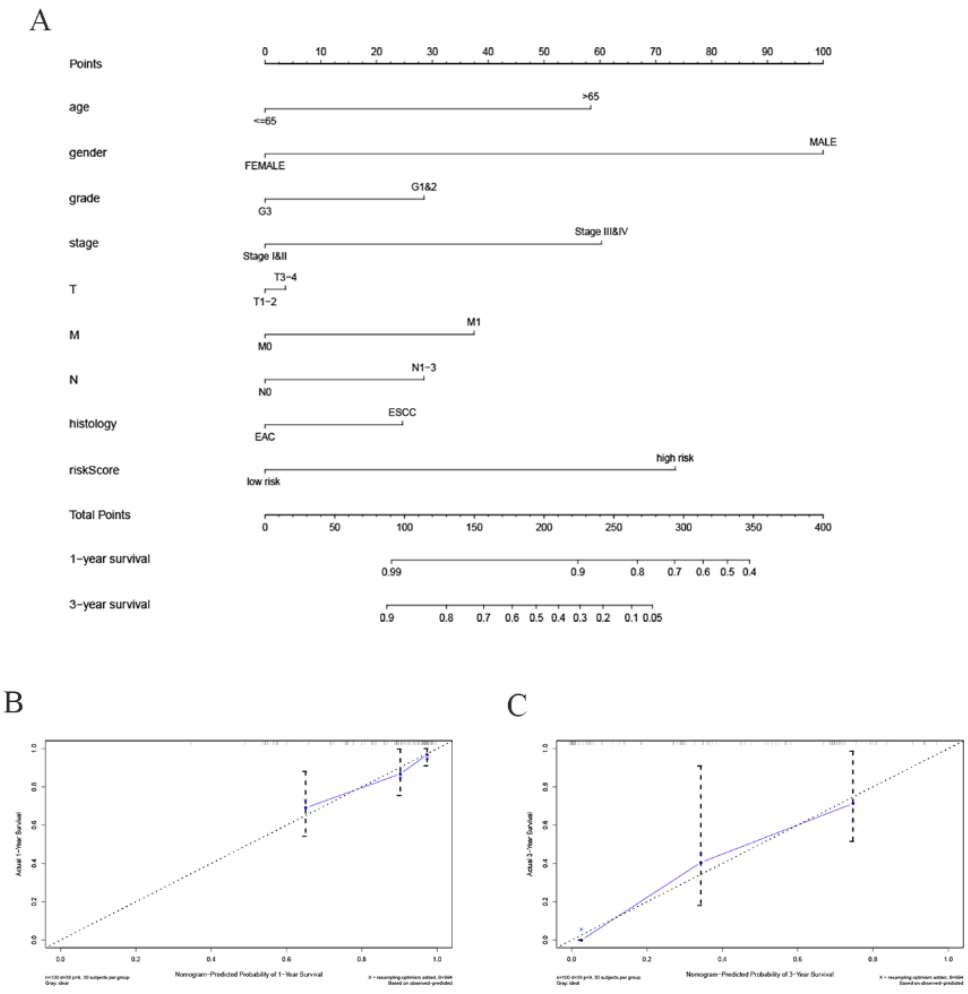

Figure 10

Nomogram predicting overall survival for EC patients. A: For each patient, several lines are drawn upward to determine the points received from the predictors in the nomogram. The sum of these points is on the "total point" axis. Then a line is drawn downward to determine the possibility of 1- and 3-year overall survival of EC. B,C:The calibration plot for internal validation of the nomogram. The Y-axis represents actual survival, and the X-axis represents nomogram-predicted survival.

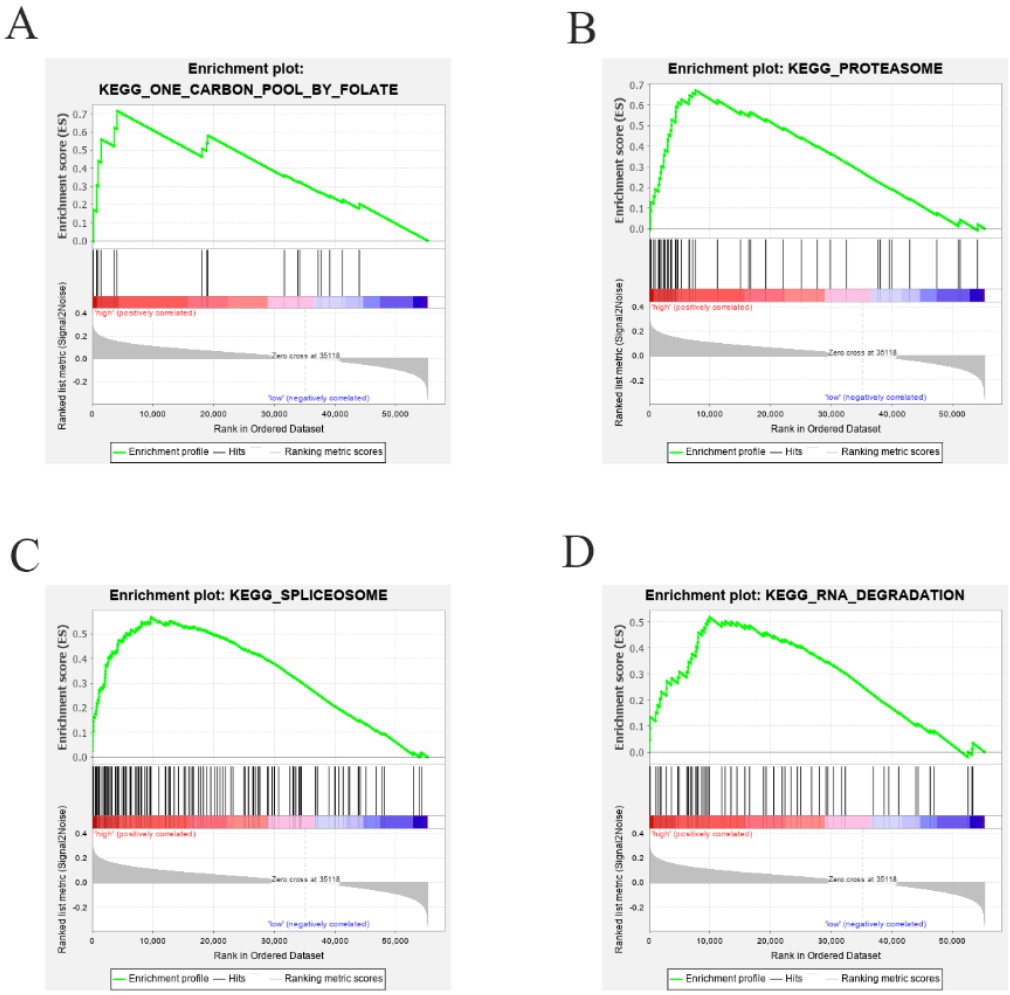

Figure 11

GSEA results for high and low risk differentially expressed genes in TCGA for (A) one carbon pool by folate, (B) proteasome, (C) spliceosome, and (D) RNA degradation.

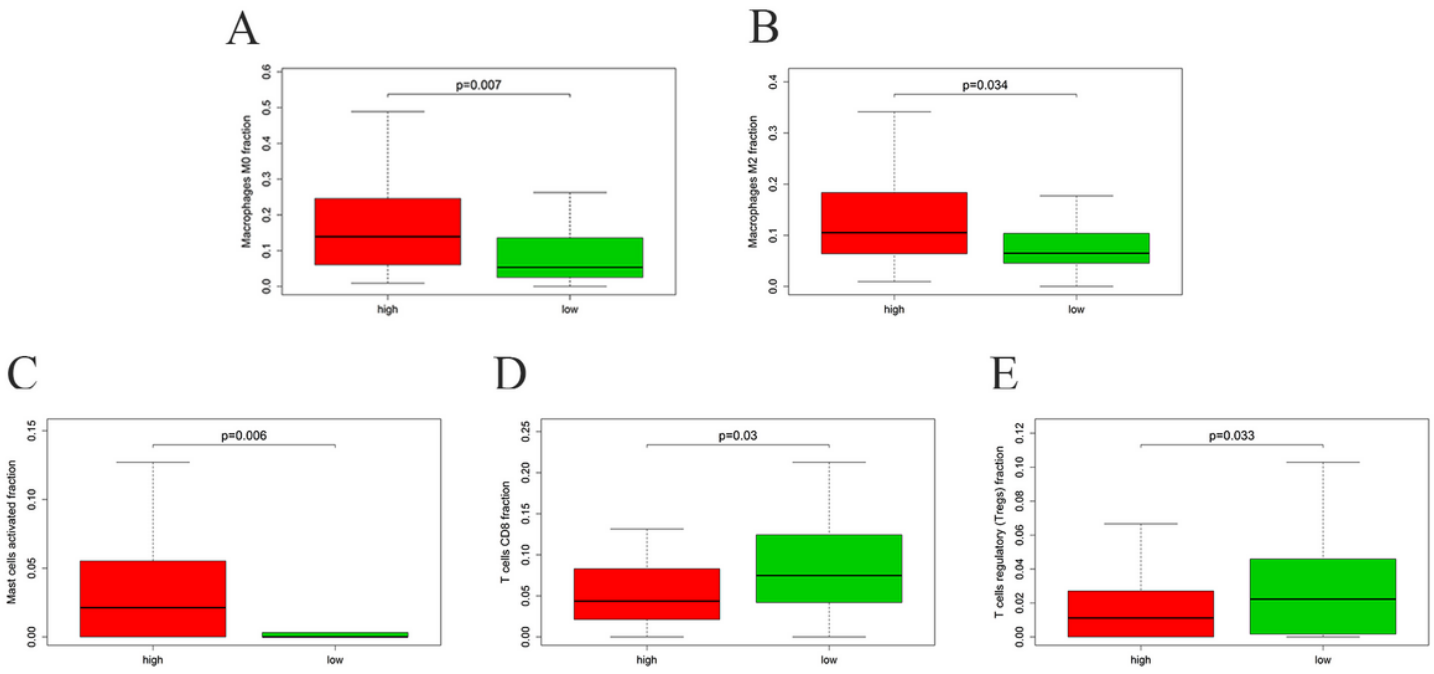

Figure 12

Relationships between the immune-related prognostic index and infiltration abundances of five types of immune cells. A: Macrophages M0.B:Macrophages M2.C: activated mast cells.D: T cells CD8.E: T cells regulatory(Tregs);the red parts present high risk group and the green parts represent low risk group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.tif