Original Article

A four-gene signature predicts overall survival of patients with esophageal adenocarcinoma

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Background: Esophageal adenocarcinoma (EAC) is an aggressive cancer with poor prognosis. Thus, this study aimed to identify a prognostic molecular signature to predict the overall survival (OS) of patients with EAC.

Methods: The mRNA microarray data sets GSE13898 and GSE26886 were downloaded from the Gene Expression Omnibus (GEO) database. RNA sequencing profile and clinical data of EAC patients were downloaded from The Cancer Genome Atlas (TCGA) database. Differentially expressed genes (DEGs) between EAC tissues and adjacent non-cancerous tissues were obtained using R software. DEGs associated with prognosis of OS were assessed by univariate Cox analysis, and a prognostic signature was built using stepwise multivariate Cox analysis. Time-dependent receiver operating characteristic (ROC) analysis and stratification analysis were conducted to evaluate its predictive performance. Functional enrichment analysis was performed for genes co-expressed with the signature to explore its biological functions in EAC.

Results: A total of 336 genes were identified to be differentially expressed between EAC tissues and adjacent non-cancerous tissues. After univariate and multivariate Cox regression analysis, four genes (*ALAD*, *ABLIM3*, *IL17RB* and *IFI6*) were screened out to construct a prognostic signature. According to this signature, patients could be assigned into high-risk and low-risk group with significantly different OS (P=4.92e−05<0.0001). Multivariate Cox regression analysis suggested that the four-gene signature served as an independent factor in OS prediction. In the time-dependent ROC analysis, the areas under the curves (AUCs) were 0.804, 0.792 and 0.695 for 1-, 3- and 5-year survival prediction, respectively, suggesting a good performance. Functional enrichment analysis showed that the signature was mainly clustered in cell proliferation related biological processes or pathways.

Conclusions: The four-gene signature identified in the current study may be a potential prognostic factor for predicting OS of EAC patients.

Keywords: Esophageal adenocarcinoma (EAC); overall survival (OS); high-throughput; prognostic signature

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Introduction

Among cancers, esophageal cancer ranks seventh in terms of incidence and sixth in mortality. It was estimated that there would be 604,100 new esophageal cancer cases and 544,076 deaths worldwide in 2020 (1). Esophageal cancer has two major histological subtypes: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). ESCC accounts for about 90% of esophageal cancer cases worldwide, while EAC cases have been progressively increasing and comprise the majority of esophageal cancer cases in the United States and some western European countries (2,3). Owing to the lack of adequate diagnostic methods, esophageal cancer is usually detected at an advanced stage. For patients diagnosed during 2006 to 2012 in the United States, the 5-year relative survival rate was only 18%, which was much lower than that of many other solid tumors (4). Thus, it is imperative to establish reliable and reproducible prognostic markers that can identify esophageal cancer patients at high risk of mortality.

Currently, the strongest clinical prognostic factor in patients with EAC is the tumor, node, metastasis (TNM) stage (5). The 5-year survival rates decrease substantially from 80.5% in the small proportion of EAC patients with stage I tumors, to 45.1%, 17.6% and, 2.1% for patients with stage II, III, and IV tumors, respectively (3). However, the adequacy for TNM stage to predict prognosis of EAC is questioned repeatedly with limited ability to stratify patients who are stage-matched and nonetheless present considerable variations in clinical outcomes (6). Cancers are heterogeneous at the molecular and genetic levels (7,8). Clearly, the ideal staging system would consider the unique molecular mechanisms underlying EAC development and progression and correlate prognosis with specific tumor biomarkers.

There have been some studies focused on finding molecular prognostic factors of EAC. A recent study, assessing the effect of human epidermal growth factor receptor 2 (HER2) heterogeneity on survival among *HER2*-amplified EACs, indicated that *HER2* heterogeneity was independently prognostic for both disease-free survival (DFS, P=0.025) and overall survival (OS, P=0.026) (9). Another study showed that low antigen-presenting molecule human leukocyte antigen DR (HLA-DR) expression in leading edge tumor epithelium was an independent predictor of poor survival, associated with a 2.8-fold increase in disease-associated death (P=0.023) (10). Using gene expression analysis and array-comparative genomic hybridization arrays, Goh *et al.* and Peters *et al.* have identified eight biologically relevant molecular targets associated with EAC prognosis, including TRIM44, SIRT2, EGFR, PAPSS2, NEIL2, WT1, MTMR9 and DCK (11,12). Further study has established and validated that the immunohistochemical panel consisting of EGFR, TRIM44 and SIRT2 was independently associated with OS and provided additional prognostic information to current survival predictors, such as TNM stage (13). Unfortunately, clinical useful molecular biomarkers for EAC prognosis are still scarce. Further study is needed to obtain accurate prognostic information for improving patient staging and management decisions.

With the development of microarray and sequencing technology, high-throughput data grow rapidly. Public databases such as Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database and The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) provide us enormous expression data of multiple cancer types including esophageal cancer (14-17). Thus, it appears reasonable and practicable to determine prognosis-related biomarkers through bioinformatical ways. Recently, a number of studies have successfully used the in silico data to identify potential prognostic signature in esophageal cancer. A previous study by Fan *et al.* identified from

Highlight box

Key findings

- We present a novel signature that incorporates four important prognostic genes (*ALAD*, *ABLIM3*, *IL17RB* and *IFI6*) to predict the overall survival (OS) for esophageal adenocarcinoma (EAC) patients.

What is known and what is new?

- Prognostic assessment is a crucial step in the management of patients with EAC, yet there are no globally reliable and reproducible prognostic markers that can identify esophageal cancer patients at high risk of mortality.
- The signature identified in this study exhibited a strong correlation with patients OS. Multivariate Cox regression analysis further indicated that its prognostic ability was independent of commonly used clinical features and may serve as an independent prognostic biomarker.

What is the implication, and what should change now?

- Our work is a supplement to the identification of molecular prognostic biomarkers in the field of EAC. Next, a large scale multicenter trial is necessary to validate its reliability and applicability in clinical practice.

TCGA an eight-long non-coding RNA signature for OS prediction of esophageal cancer (18). Another 8-long non-coding RNA signature from GSE53625 was identified to incorporate with age and pathologic stage for predicting 3- and 5-year survival probability of patients with ESCC (19). Furthermore, recent studies paid more attention to constructing prognostic signatures involved in specific biological process, such as autophagy (20), lactic acid metabolism (21), necroptosis (22,23) and ferroptosis (24), which explained their functional association with prognostic outcomes better. However, all the above analysis was conducted in single ESCC cohort or the total esophageal cohort regardless of subtypes, suggesting that the EAC public data still need a more sufficient utilization.

In this study, we used public high-throughput data from TCGA and GEO databases and identified a four-gene signature comprised of *ALAD*, *ABLIM3*, *IL17RB* and *IFI6* as a predictor of OS in EAC patients. This signature exhibited a strong correlation with patient OS and may serve as an independent prognostic biomarker. The results obtained provided a base for further validation of this molecular signature as a prognostic biomarker in EAC patients. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1798/rc).

Methods

Gene expression profiles of EAC

Gene expression profiles of GSE13898 and GSE26886 were downloaded from GEO. The GSE13898 data set included 64 primary EAC tissues and 28 surrounding non-cancerous fresh frozen tissues. The GSE26886 data set included 21 specimens of adenocarcinoma patients and 19 biopsies of normal esophageal squamous epithelium. The preprocessed level 3 RNA sequencing data and corresponding clinical information of esophageal cancer patients were obtained from TCGA database, which included 80 EAC tissues and 11 adjacent tissues. For GSE13898, normalized data were provided directly by the authors. For GSE26886, the analysis of raw probe-level data (.CEL file) was performed using the robust multiarray average algorithm RMA in the Affy package of R (25) after background correction and quantile normalization, and the expression values were then obtained. The averages of the probe sets of values were calculated as the expression values for the same gene with multiple probe sets (26). For TCGA profiles, row counts data were normalized by "edgeR" R package to obtain the normalized expression data (27). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differentially expressed genes (DEGs) screening between EAC tissues and non-cancerous tissues

For GEO profiles, DEGs between EAC tissues and non-cancerous tissues were identified through the "Limma" package in R software (28). Genes with $|\log_2 (\text{fold change})| > 1$ and $P<0.05$ were considered to be differentially expressed. For TCGA profiles, the R package of "edgeR" was employed to identify the DEGs following the same threshold (27). The DEGs from each data set were then intersected to obtain common DEGs for next analysis.

Identification of DEGs related prognostic signature and Kaplan-Meier analysis

The "survival" R package was used to evaluate the association between DEGs and patient OS by univariate Cox proportional hazards regression analysis. Only those DEGs with P value <0.05 were reserved for a stepwise multivariate Cox proportional hazards regression analysis tested by Akaike information criterion (AIC) to identify the prognostic model with the best performance. Then, the survival risk score (SRS) for each patient was calculated by summing the mRNA expression level of each gene multiplied with its corresponding coefficient from the multivariate Cox regression analysis. Using the median risk score as the cutoff value, patients were divided into high-risk and low-risk groups. Kaplan-Meier method was used to generate the OS curves, and log-rank test was employed to compare the differences between high-risk and low-risk patient groups. Time-dependent receiver operating characteristic (ROC) curves were generated to detect the prognostic power of the risk score model, using "survivalROC" R package.

Multivariate Cox regression analysis and stratification analysis

To assess whether the survival prediction ability of the DEGs signature was independent of other clinical or pathological factors of patients with EAC, multivariate Cox proportional hazards regression analysis was carried out. The included variates covered clinical or pathological

factors which had been identified to be significant in the univariate Cox regression analysis. For clinical features with P value <0.05 in multivariate Cox regression analysis, stratification analysis was further performed to determine whether the DEGs signature exhibit prognostic value within the same clinical feature.

Functional enrichment analysis

The Gene Ontology (GO) annotation analysis was conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (29) to obtain enriched GO terms of biological processes with P<0.05 as the threshold value. Pathway enrichment analysis was performed using KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/), which is the first hypergeometric distribution-based examination software to evaluate the significance of enrichment of pathways (30). P<0.05 was also set as the threshold value.

Statistical analysis

All statistical analyses were conducted using Graphpad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA), SPSS (IBM Statistics, Armonk, NY, USA) and R 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Identification of DEGs between EAC tissues and non-cancerous tissues

According to the cutoff criteria, 2,330 (861 upregulated, 1,469 downregulated), 3,545 (1,892 upregulated, 1,653 downregulated), and 3,740 (1,413 upregulated, 2,327 downregulated) differentially expressed genes were identified from GSE13898, GSE26886 and TCGA, respectively (available online: https://cdn.amegroups.cn/static/public/tcr-23-1798-1.xls). Among them, 336 genes showed the same trend of change in these three data sets, including 145 upregulated genes and 191 downregulated genes in EAC tissues compared with normal or paracancerous tissues.

Establishment of DEGs related prognostic model to predict OS of patients with EAC

By performing univariate Cox proportional hazards regression analysis using TCGA follow-up data, 14 OS related DEGs (available online: https://cdn.amegroups.cn/static/public/tcr-23-1798-1.xls) were identified with P value <0.05, and they were used for a stepwise multivariate Cox proportional hazards regression analysis. Finally, a four-gene predictive signature with the best explanatory and informative efficacy tested by AIC was established, which included *ALAD*, *ABLIM3*, *IL17RB* and *IFI6*. Kaplan-Meier curves of the four prognostic genes using median expression level as the cutoff value are shown in *Figure 1*. As described previously, the prognostic model was defined as the linear combination of the expression levels of these four genes weighted by their individual coefficient from the multivariate Cox regression analysis. The formula of SRS was as follows: SRS = (−0.0336 × *ALAD* mRNA expression value) + (−0.0485 × *ABLIM3* mRNA expression value) + (0.0101 × *IL17RB* mRNA expression value) + (−0.0004 × *IFI6* mRNA expression value). *ALAD*, *ABLIM3* and *IFI6* showed negative coefficients, indicating that they were protective prognostic factors, while *IL17RB* was considered to be an unfavorable prognostic factor with a positive coefficient.

Kaplan-Meier analysis and ROC curve indicated good performance of the four-gene signature in predicting the OS of EAC

According to the established prognostic model, SRS of each patient was calculated, and the distribution of SRSs and survival status are shown in *Figure 2A*,*2B*. Using a median risk score of −1.5425 as the cutoff value, a set of 40 patients with SRSs >−1.5425 were assigned into the high-risk group, while the other 40 patients were assigned into the low-risk group. The OS for patients in high-risk group was significantly poorer than those in low-risk group (P=4.92e−05<0.0001), with a much shorter median survival time (480 *vs.* 1599 days for high-risk *vs.* low-risk, *Figure 2C*). Time-dependent ROC analysis showed that the prognostic signature performed well for EAC OS prediction, as the areas under the curves (AUCs) of the time-dependent ROC curve were 0.804, 0.792 and 0.695 for 1-, 3- and 5-year survival prediction, respectively (*Figure 2D*).

Survival prediction of the four-gene signature was independent of other clinicopathological factors

The main clinical and pathological factors of the enrolled 80 EAC patients are listed in *Table 1*. Univariate Cox regression analysis identified N classification, metastasis and tumor stage as well as the four-gene-defined SRS group

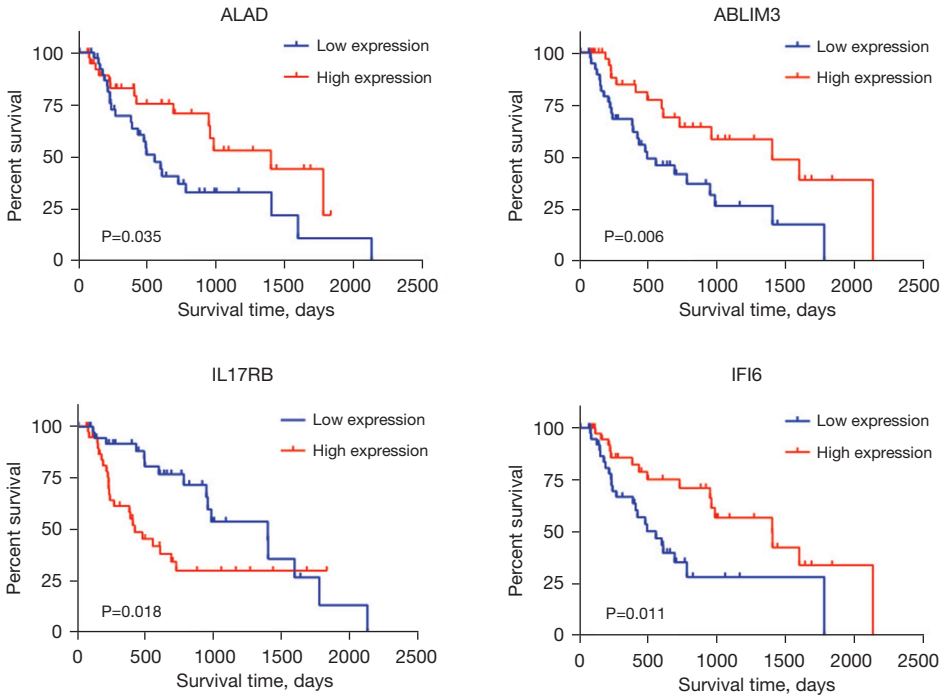

Figure 1 Kaplan-Meier curves of overall survival of four genes in the prognostic signature. Median expression level was set as the cutoff value for each gene.

as the candidate prognostic factors (*Table 2*). Multivariate Cox regression analysis demonstrated that only the four-gene signature maintained an independent prognostic factor after adjusting for the above factors (*Table 2*). Tumor stages was shown to be probably another independent prognostic factor with a P value of 0.07, which was close to 0.05. Therefore, stratification analysis was introduced to determine the independence of four-gene signature within the same tumor stage. Because of limited sample size of patients in stage I and IV (N=8 and 10), they were put together with stage II and III, respectively. For patients with stage I and II EAC, log-rank test showed that the four-gene signature could distinguish them with marginally significantly different survival (P=0.052, *Figure 3A*), and better predictive performance was observed for patients with stage III and IV EAC (P<0.001, *Figure 3B*). Thus, this four-gene signature might be able to help predict the survival of EAC patients independently.

Functional enrichment analysis of the four-gene signature related gene sets

To explore the potential biological function of the four-gene signature, we screened protein-coding genes co-expressed with the four genes in the TCGA cohort. A total of 161 genes were identified to be co-expressed with at least one of the four genes (spearman correlation coefficient >0.50, P<0.05). GO analysis identified 29 significant biological process terms (P<0.05), and the results showed that these genes mainly clustered in cell proliferation related GO biological process, such as DNA replication, cell division, cell proliferation and G1/S transition of mitotic cell cycle (*Figure 4A*). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis returned 14 significant terms (P<0.05), of which the top two were DNA replication and cell cycle (*Figure 4B*). These results indicated that the four genes might be involved in the regulation of cancer cell development, thus affecting patients' prognosis of OS.

Discussion

In this study, we established a four-gene signature with good performance for prediction of OS in patients with EAC. Multivariate Cox regression analysis further indicated that its prognostic ability was independent of commonly used clinical features. Our work helps to identify patients with high risk of mortality who may need individualized therapeutic interventions.

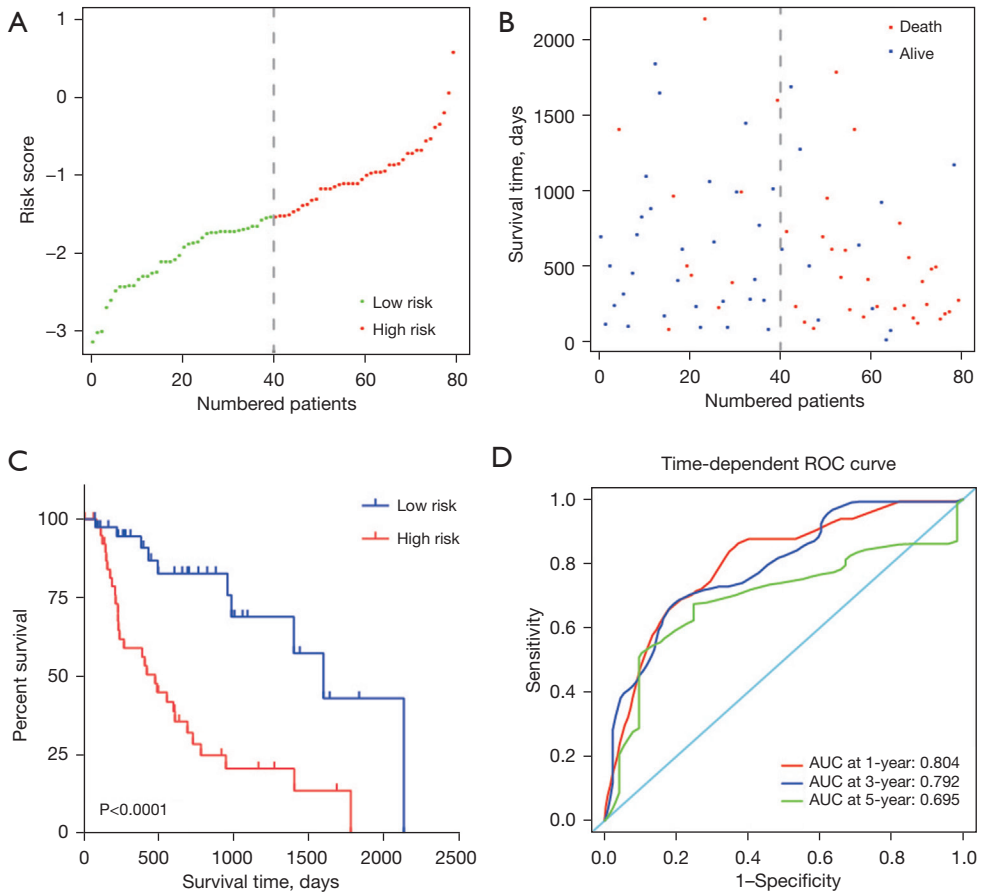

Figure 2 Prognostic evaluation of the four-gene signature. (A) Survival risk score of patients. (B) Distribution of patients' overall survival status. (C) Kaplan-Meier curve of overall survival for low- and high-risk groups. (D) Time-dependent ROC curve analysis of survival prediction according to the risk score. ROC, receiver operating characteristic; AUC, area under the curve.

Over the past couple of decades, substantial improvements of prognosis have been made in EAC, owing to several main reasons including earlier tumor diagnosis, better surgical and perioperative therapy and the application of neoadjuvant chemotherapy or chemoradiotherapy for locally advanced EAC (31-33). However, the above factors still contribute limitedly in improving prognosis with the current 5-year survival rate less than 20%. Achieving the goal of effective treatments remains challenging.

Tumor stage is the most important prognostic factor for EAC. Besides tumor stage, the tumor response to neoadjuvant chemotherapy or chemoradiotherapy is another important prognostic factor (34), which determines the tumor stage at the time of surgery. Other prognostic factors include patients' performance status, comorbidities and quality of life (35). However, they are still inadequate for EAC prognosis prediction, and other kinds of prognostic factors are being under development.

Gene mutation and dysregulation are the intrinsic causes of cancer occurrence and progression (36), so the feasibility of gene as a prognostic marker cannot be ignored. Although some studies had been devoted to identifying genes associated with EAC prognosis, they mainly focused on known biomarkers in cancer, such as HER2 and EGFR (9,13). Considering the complexity of molecular context underlying EAC biology, there must be quite a few remaining genes worth mining. Undoubtedly, public high-throughput expression data provide us an excellent opportunity to find them. As previously mentioned, EAC patients are in urgent need of effective treatment. Another immense benefit for identifying such prognostic genes is that they might provide new therapy targets and help largely improve EAC treatment as well as patient survival. For EAC, molecular target therapy is now still at the early

Table 1 Summary of clinical and pathological factors of EAC patients in TCGA cohort

Clinical and pathological factors	Number of cases (%)
Age (years)	
<60	26 (32.50)
≥60	54 (67.50)
Gender	
Male	69 (86.25)
Female	11 (13.75)
Reflux history	
Yes	39 (48.75)
No	30 (37.50)
Unavailable	11 (13.75)
Grade	
G1+G2	29 (36.25)
G3	25 (31.25)
Unavailable	26 (32.50)
Tumor classification	
T1+T2	30 (37.50)
T3+T4	48 (60.00)
Unavailable	2 (2.50)
Lymph node classification	
N0	22 (27.50)
N1+N2+N3	55 (68.75)
Unavailable	3 (3.75)
Metastasis	
M0	58 (72.50)
M1	10 (12.50)
Unavailable	12 (15.00)
Tumor stage	
I+II	35 (43.75)
III+IV	44 (55.00)
Unavailable	1 (1.25)
Vital status	
Alive	41 (51.25)
Dead	39 (48.75)

EAC, esophageal adenocarcinoma; TCGA, The Cancer Genome Atlas.

stage with limited options. Targeting HER2 has showed certain clinical efficacy in combination with chemotherapy, and more molecular targets and inhibitors are now being tested in clinical trials (37,38).

The bioinformatical analysis of EAC public data on prognosis is less advanced. A latest study identified *RORA*, *KAT2B*, *CDC25B* and *ECT2* as the hub genes in esophageal cancer progression, which may participate the modulation of immune cell infiltration (39). However, they were identified through transcriptomic analysis of GEO data sets including ESCC, ECA and small cell carcinoma and are not specific biomarkers for EAC. Noticeably, we also hope that the prognostic genes would ideally account for the biology of EAC to add its potency to be developed as therapy targets. Therefore, the prognostic signature was constructed based on DEGs between cancer tissues and adjacent tissues. We ultimately got a signature consisting of four deregulated genes, namely *ALAD*, *ABLIM3*, *IL17RB* and *IFI6*, all of which were protective prognostic factors except for *IL17RB*. ALAD is an enzyme of delta-aminolevulinic acid dehydratase, which catalyzes the second step of heme synthesis (40). Recent studies reveal that ALAD is a favorable prognostic factor in patients with hepatocellular carcinoma (41), breast cancer (42) and clear cell renal cell carcinoma (43). Cell experiment shows that overexpression of ALAD could inhibit the proliferation and invasion of breast cancer cells by regulating transforming growth factor-beta (TGF-β) mediated epithelial-mesenchymal transition. ABLIM3, a member of the actin-binding LIM (abLIM) protein family, is rarely reported in cancer research, whereas its homologous protein ABLIM1, is considered to be a tumor suppressor in melanoma (44). IL-17RB is a member of the interleukin (IL)-17 receptor family, which can be activated by IL-17B and has been proved to be involved in inflammatory diseases and cancers (45). It has been reported that IL-17RB expression is significantly increased in gastric cancer tissues, indicating a poor prognosis of patients (46). In pancreatic cancer, overexpression of IL-17RB promotes cancer cell invasion via the ERK1/2 pathway and inversely correlates with progression-free survival (47). Two bioinformatical studies using TCGA data also identifies IL17RB as a poor prognosis factor of OS in EAC (48) and ESCA (49), which further corroborates the reliability of our study. IFI6 is a glycosylated protein induced by interferon and localizes at mitochondria. It exerts an anti-apoptotic function through inhibition of the depolarization of mitochondrial membrane potential and release of

Table 2 Univariate and multivariate Cox proportional hazards regression analysis

Clinical and pathological factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (<60, ≥60 years)	0.708 (0.370–1.353)	0.296		
Gender (male, female)	1.366 (0.476–3.922)	0.562		
Reflux history (no, yes)	1.011 (0.468–2.181)	0.978		
Grade (G1+G2, G3)	2.006 (0.957–4.206)	0.065		
Tumor classification (T1+T2, T3+T4)	1.476 (0.731–2.980)	0.277		
Lymph node classification (N0, N1+N2+N3)	3.413 (1.307–8.908)	0.012	1.508 (0.479–4.745)	0.482
Metastasis (M0, M1)	2.860 (1.273–6.423)	0.011	1.335 (0.500–3.559)	0.564
Tumor stage (I+II, III+IV)	2.786 (1.371–5.660)	0.005	2.472 (0.923–6.621)	0.072
SRS group (low-risk, high-risk)	4.374 (2.061–9.282)	<0.001	3.071 (1.341–7.033)	0.008

HR, hazard ratio; CI, confidence interval; SRS, survival risk score.

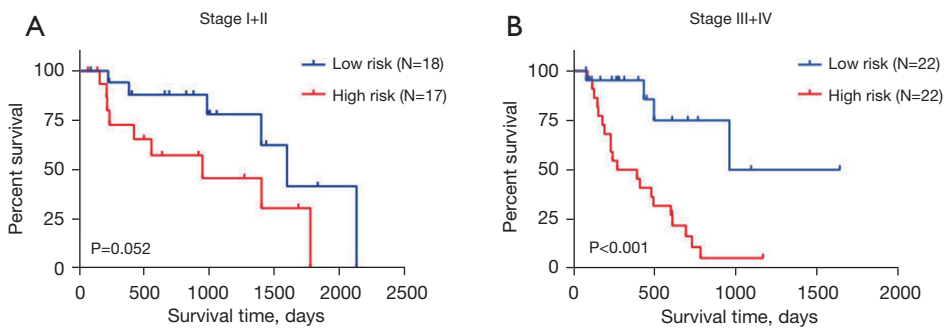

Figure 3 Kaplan-Meier curves analysis of overall survival stratified by tumor stage. (A) Stage I and II. (B) Stage III and IV.

cytochrome c (50). In ESCC, IFI6 is involved in cell senescence, apoptosis as well as mitochondrial dysfunction (51,52). Moreover, the overexpression of IFI6 is reported to be correlated with poor clinical prognosis in ESCC. However, our study indicates that elevated IFI6 indicated a beneficial clinical outcome in EAC, suggesting a different role of IFI6 in ESCC and EAC. Generally, all these genes are far from being fully elucidated in EAC, and are worthy of future investigation on their functions in the biology process during EAC occurrence and development.

Functional assessment of co-expressed genes of the prognostic signature showed that cell proliferation related biological processes and pathways were significantly enriched, including DNA replication, cell cycle and cell division, which were closely associated with tumor growth. Interestingly, type I interferon signaling pathway was most significantly clustered among GO biological processes, and we found that genes in this term were all co-expressed with IFI6. Type I interferon signaling pathway plays an essential role in tumor immune surveillance, and mediates antitumor effects against several tumor types (53). It is also essential for the full-blown efficacy of anticancer agents, with its activation reported to predict clinical responses to chemotherapy in patients with breast cancer (54). These might explain why the upregulation of IFI6 constitutes a positive prognostic factor in EAC patients.

There are some limitations in the present study. First, the results generated here were derived from a single cohort, of which the included patients could not represent all patient populations in terms of genetic diversity. In addition, many other elements add the heterogeneity of a population, such as treatments and dietary habits. It is therefore necessary to validate the present results in external cohorts. Second, the population is relatively small, and clinical information of some patients, including tumor grade and metastasis status, is incomplete, which further decrease the sample number in

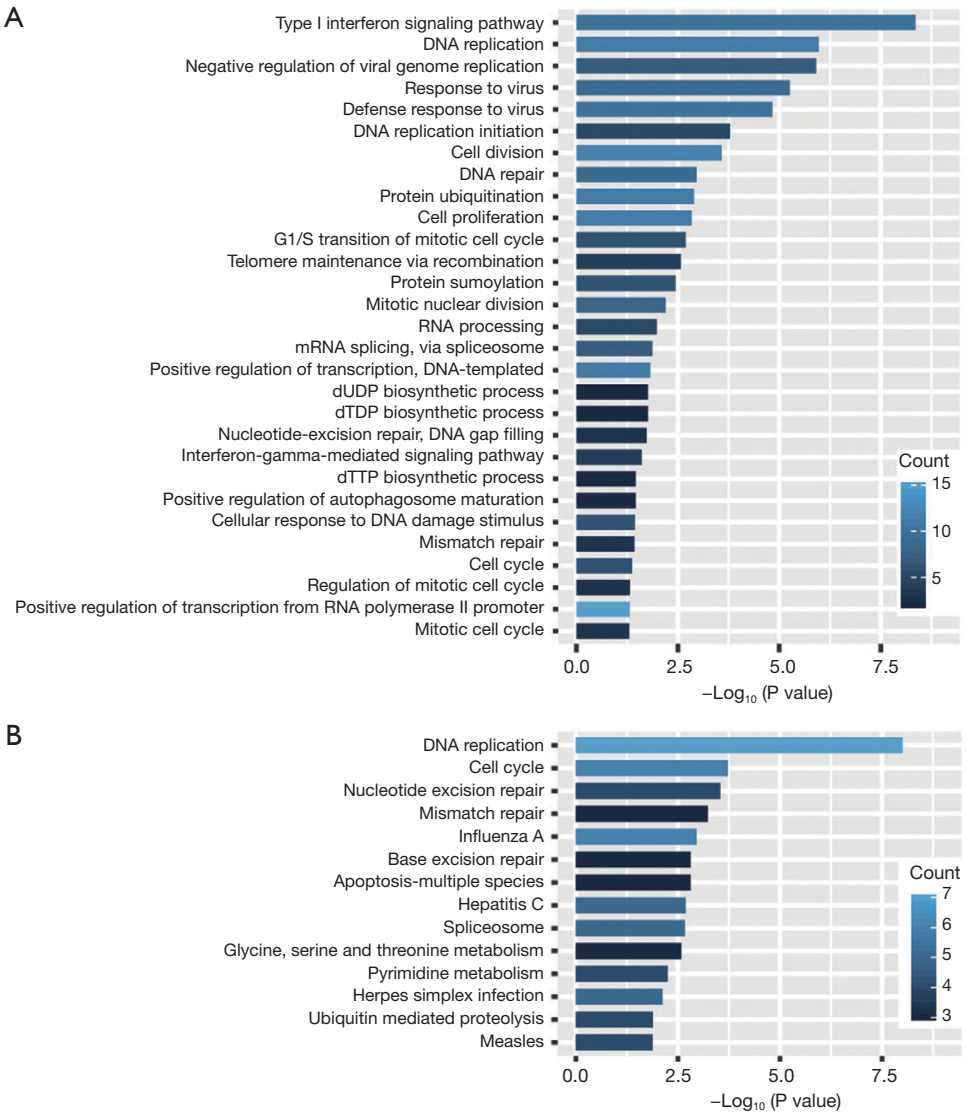

Figure 4 Enrichment analysis of four-gene signature-related gene set. (A) Significant GO biological process terms. (B) Significant KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

the Cox proportional hazard regression analysis for relevant clinical factors.

Conclusions

In conclusion, the current study has firstly employed the public TCGA database and identified a four-gene signature with potential prognostic value for OS of patients with EAC. Our work is a supplement to the identification of molecular prognostic biomarkers in the field of EAC. Next, more work is necessary to validate its reliability and applicability in clinical practice.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1798/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1798/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1798/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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