Original Article

Identification of enhancer RNA *AC005515.1* as a novel biomarker for prognosis in esophageal cancer and predictors of immunotherapy response

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Background: The enhancer RNA (eRNA) signature shows excellent promise in the prognostic role of many malignancies, but its value has not been fully explored in esophageal cancer (ESCA).

Methods: We comprehensively analyzed 33 oncogene expression matrices and clinical data from The Cancer Genome Atlas (TCGA) and identified ESCA prognostic-related key eRNAs by Kaplan-Meier and co-expression analysis. We also investigated the prognostic role of the key eRNA using a series of bioinformatics approaches, including immune infiltration, immune function, immune subtypes, and the tumor microenvironment. Finally, the tumor immune dysfunction and exclusion (TIDE) score was used to predict the immune response to immune checkpoint blockade (ICB) therapy.

Results: We identified eRNA AC005515.1, AC012368.1, AP003469.2, Clorf61, and WDFY3-AS2 were associated with the prognosis of ESCA. AC005515.1 is a critical prognostic-related eRNA in ESCA and was significantly co-expressed with immune checkpoint genes (*CTLA4*, *CD274*, etc.). In the pan-cancer analysis, AC005515.1 was also associated with the prognosis of seven cancers, including kidney renal papillary cell carcinoma and low-grade brain glioma. It was also found to be co-expressed with immune checkpoint genes in these tumors. Moreover, high expression of AC005515.1 was associated with $CD8^+$ T cells and M1 macrophages infiltration, and the AC005515.1 high-expression group had a higher TIDE score in ESCA.

Conclusions: Overall, eRNA AC005515.1 is associated with the local immune environment of ESCA and may become a new biomarker of ESCA prognosis and immunotherapy response.

Keywords: Esophageal cancer (ESCA); enhancer RNA (eRNA); *AC005515.1*; immune checkpoint; pan-cancer analysis

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Introduction

Esophageal cancer (ESCA) originates from the esophageal mucosa and is one of the most common malignant tumors in the digestive tract (1). Esophageal adenocarcinoma and esophageal squamous cell carcinoma are ESCA's two main histological categories. In 2018, ESCA's incidence and mortality rates ranked 7th and 6th among all malignant tumors worldwide, respectively (2). The prognosis of patients with ESCA is extremely poor with a 5-year survival rate of less than 20% (2,3). Early ESCA has no obvious symptoms, and most patients are diagnosed in advanced stages. Patients with advanced ESCA who are not eligible for radical treatment could choose to receive chemotherapy or immune checkpoint inhibitor (ICI) therapy. Importantly, it has been demonstrated that ICI is more effective and safer than chemotherapy for patients with advanced ESCA (4,5). ESCA is a highly heterogeneous disease with a widely varying prognosis among patients, with no available prognostic markers and limited clinically targeted therapies (6,7). Thus, exploring the effective prognostic biomarkers of ESCA remains of great importance.

Enhancer RNA (eRNA) is a type of long non-coding RNA (lncRNA) transcribed from enhancers and exists widely in most human cells and tissues (8). Recent studies have shown that eRNAs can contribute to the enhancer activity or bind to numerous proteins and perform some biological functions in an independent form (9-11). The expression of eRNA regulated the development of tumors (10,12,13). Meanwhile, eRNAs may influence drug action through pathways or cross-pathways and may serve as potential targets for clinical treatment and prognosis prediction (11,14). In hepatocellular carcinoma, high expression of eRNA DCP1A is associated with poor prognosis (15). In clear cell renal cell carcinoma, downregulation of EMX2OS was significantly associated with a higher histological grade, an advanced stage, and a poorer prognosis (16). In addition, eRNA plays an important role in regulating immune responses by modulating their target genes involved in immune-related pathways (17). Previous research has found that eRNA can also regulate immune checkpoint-related genes (11). These previous findings suggested that eRNAs as biomarkers or therapeutic targets have essential research value in tumor prognosis and targeted therapy. A previous study has indicated that lncRNA dysregulation promotes ESCA cell proliferation and metastasis and is associated with prognosis (8). However, comprehensive studies analyzing eRNAs in ESCA are still limited, especially exploring the role of eRNAs in the prognosis of ESCA. It has been suggested that eRNAs may be powerful diagnostic and/or prognostic markers in cancer therapy (11). Therefore, the identification of biomarkers that can predict the prognosis of ESCA remains relevant and urgently needed.

This study aimed to investigate the expression profile and prognostic value of eRNA in ESCA and to analyze the immunological functions of key prognostic-related eRNAs. eRNA *AC005515.1* shows promise as a novel biomarker for prognosis and immunotherapy of ESCA and is expected to be a novel therapeutic target in the future. This will not only help in clinical prognostic monitoring but also in immunotherapy treatment decisions. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-777/rc).

Highlight box

Key findings

- Enhancer RNA (eRNA) *AC005515.1* was identified as a novel prognostic biomarker for esophageal cancer (ESCA) and co-expressed with immune checkpoint genes.

What is known and what is new?

- Current studies have confirmed the excellent potential of eRNA in the prognosis of a variety of cancers. However, the role of eRNA in ESCA has not been fully elucidated.
- In this study, *WDFY3-AS2*, *AC012368.1*, *AC005515.1*, *AP003469.2*, and Clorf61 were identified as ESCA prognostic-related eRNAs. These newly predicted *eRNAs*, except *WDFY3-AS2*, have been reported for the first time in oncology studies.

What is the implication, and what should change now?

- eRNA *AC005515.1* is of great value in the treatment and prognosis of ESCA.

Methods

Data downloading and preparing

The Cancer Genome Atlas (TCGA) pan-cancer data, including gene expression matrix, clinical data, survival data, immune subtypes, cell stemness scores, and somatic mutation data of 32 tumors, were downloaded from UCSC Xena (https://xena.ucsc.edu) (18). The gene expression matrix was classified into mRNA and eRNA expression profiles based on the gene type and eRNA source literature in the human gene expression RNA-sequencing (RNAseq)

profile (19). The eRNA expression profiles of ESCA patients and their survival times were merged. A total of 161 ESCA and 11 control samples were ultimately included in this study, and patient information is shown in Table S1. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of differentially expressed mRNA (DEmRNA) and eRNA (DEeRNA) in ESCA

DEmRNA and DEeRNA in ESCA were identified between tumor and normal samples using the "limma" R package, followed by volcano plot visualization. The differentially expressed genes were defined as |fold change (FC)| >1 and false discovery rate (FDR) <0.05.

Identification of prognostic-related eRNAs of ESCA

The patients were subdivided into low- and high-expression groups according to each DEmRNA median expression value. Kaplan-Meier survival curves were constructed using the R package "survival" and the log-rank test. P<0.05 was considered statistically significant.

Identification of co-expressed genes and gene set enrichment analysis of prognostic-related eRNAs in ESCA

Spearman correlation analysis was performed to analyze the *correlation* of prognostic-related eRNAs and DEmRNAs, and genes with R>0.4 and P<0.001 were considered to be co-expressed genes of prognostic-related eRNAs. Gene function enrichment analysis was designed to explore the biologically significant processes of co-expressed genes in ESCA. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) statistical analyses and visualization of co-expressed genes were performed using the cluster Profiler package of the R software. P<0.05 was considered a statistically significant.

AC005515.1 survival analysis and its co-expression gene validation in pan-cancer

Patients with 32 other cancers were classified into low- and high-expression groups according to the median expression value of *AC005515.1*. The Kaplan-Meier method was used to investigate the prognostic value of *AC005515.1* in pan-cancer. The Spearman correlation was applied to verify the correlation between *AC005515.1* and its co-expressed genes in ESCA.

Evaluation of immune cell infiltration and tumor microenvironment (TME) in ESCA

The "CIBERSORT" R package was used to obtain data of 22 immune cell infiltration (20). We also assessed the immune function score of ESCA patients with the Gene Set Variation Analysis (GSVA), as this can be estimated from transcriptomic data. This is accomplished with the R package, including "limma", "GSVA", "GSEABase", "ggpubr", and "reshape2". The "ESTIMATE" R package is designed to calculate TME scores, including immune scores, stromal scores, and estimated scores (21). The Wilcoxon test was used to analyze the differences in infiltrating immune cells and immune function scores in the *AC005515.1* high- and low-expression groups. The correlation between *AC005515.1* and the TME and tumor stem cell score was performed using Spearman correlation analysis.

Analysis of tumor mutational burden (TMB) and prediction of immune checkpoint blockade (ICB) therapy

The somatic mutation data of the ESCA patients were obtained using the Perl script. The correlation between *AC005515.1* expression levels and TMB was assessed by Spearman correlation analysis. The tumor immune dysfunction and exclusion (TIDE) algorithm was used to predict the potential response to *ICB* therapy of AC005515.1 high- and low-expression groups (http://tide.dfci.harvard.edu) (22).

Statistical analysis

All statistical analyses in this study were performed using R software (v4.1.1). Spearman correlation was applied to estimate the correlation strength. The Kaplan-Meier method was used for survival analysis and tested by the log-rank test. Wilcoxon test was used to compare different expression levels of infiltrating immune cells in different groups. P<0.05 was considered statistically significant.

Results

Identification of prognostic-related eRNAs from ESCA

The mRNA and eRNA expression profiles of ESCA

patients were analyzed by the "limma" R package. A total of 1,681 DEmRNA and 156 DEeRNA were detected (*Figure 1A*,*1B*; FDR <0.05, |logFC| >1). Thirty-four eRNAs associated with prognosis were identified (*Table 1*). Meanwhile, there were significant differences in the expression of five prognostic-related eRNAs (*Figure 1C*). The Kaplan-Meier plots of the five prognostic-related eRNAs were demonstrated in *Figure 1D-1H*. The results showed that low *WDFY3-AS2* and *AC012368.1* (P=0.008 and P=0.014) expression and high *AC005515.1*, *Clorf61*, and *AP003469.2* (P=0.022, P=0.029, and P=0.034, respectively) expression significantly correlated with worse survival in survival in ESCA patients (*Figure 1D-1H*).

eRNAs AC005515.1 co-expressed with immune checkpoint genes and involved in ESCA prognosis

To explore the co-expressed genes of prognostic-related eRNA, we analyzed the correlation between five prognostic-related eRNAs in DEeRNA and 1,681 DEmRNAs. A total of 171 potential target genes were identified [R>0.4, P<0.001; online table (available at https://cdn.amegroups.cn/static/public/tcr-23-777-1.pdf)]. Subsequently, we performed GO and KEGG enrichment analyses of the co-expressed genes. The GO enrichment results are shown in *Figure 2A*, and the top three most significantly enriched in biological processes were negative regulation of leukocyte proliferation, negative regulation of lymphocyte proliferation, and negative regulation of mononuclear cell proliferation. The top three terms enriched in cellular components were external side of plasma membrane, cation channel complex, and I band. The top three terms enriched in molecular functions were cation channel activity, gated channel activity, and channel activity. Meanwhile, the top three pathways enriched by KEGG were cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, and cell adhesion molecules (*Figure 2B*). Notably, negative regulatory genes involved in immune cell proliferation and activation, including immune checkpoint genes (*CTLA4*, *CD274*, and *IDO1*, etc.), were significantly correlated with AC005515.1 (*Figure 2C*). The expression of immune checkpoint-related genes was increased in the high-expression group compared to the low-expression group of AC005515.1 (*Figure 2D*).

Relationship between AC005515.1 and clinical features in ESCA

We analyzed the relationship between *AC005515.1* and clinical features, including age (≤60 years, >60 years), gender, pathological tumor grade, pathological tumor stage, and tumor status. We found that *AC005515.1* expression correlated with the tumor stage. The expression level of *AC005515.1* significantly increased in stage II and stage IV compared with stage I (*Figure 3*). We next performed a multivariate Cox regression analysis, the results showed that ESCA patients with high expression of *AC005515.1* had a higher risk of death than those with low expression level, after adjusting for age, sex, grade, stage, and tumor status [hazard ratio (HR) =4.94, 95% confidence interval (CI): 1.21–20.13, P=0.03, Table S2). eRNA *AC005515.1* was identified as an independent prognostic factor for ESCA. Since *AC005515.1* was significantly co-expressed with immune checkpoint genes and related to ESCA survival, we propose a hypothetical to explain our findings that eRNA *AC005515.1* might influence the ESCA patients' prognosis by affecting the proliferation and activity of immune cells through co-expressed immune checkpoint genes.

Prognostic values of eRNA AC005515.1 and co-expression with immune checkpoint genes were validated in pan-cancer

We extracted the expression of *AC005515.1* from the 32 tumors and divided patients into low- and high-expression groups based on their median expression values. Our study found that *AC005515.1* was associated with the prognosis of seven tumors (*Figure 4*). Specifically, high expression of *AC005515.1* had worse overall survival (OS) in the prognosis of kidney renal papillary cell carcinoma (KIRP) (P=0.045, *Figure 4A*), brain lower grade glioma (LGG) (P<0.001, *Figure 4B*), and uveal melanoma (UVM) (P=0.015, *Figure 4G*). Conversely, low expression of *AC005515.1* predicted a worse prognosis in mesothelioma (MESO) (P=0.012, *Figure 4C*), ovarian serous cystadenocarcinoma (OV) (P=0.021, *Figure 4D*), skin cutaneous melanoma (SKCM) (P<0.001, *Figure 4E*), and thyroid carcinoma (THCA) (P=0.004, *Figure 4F*). In addition, we analyzed correlations between *AC005515.1* and its co-expressed

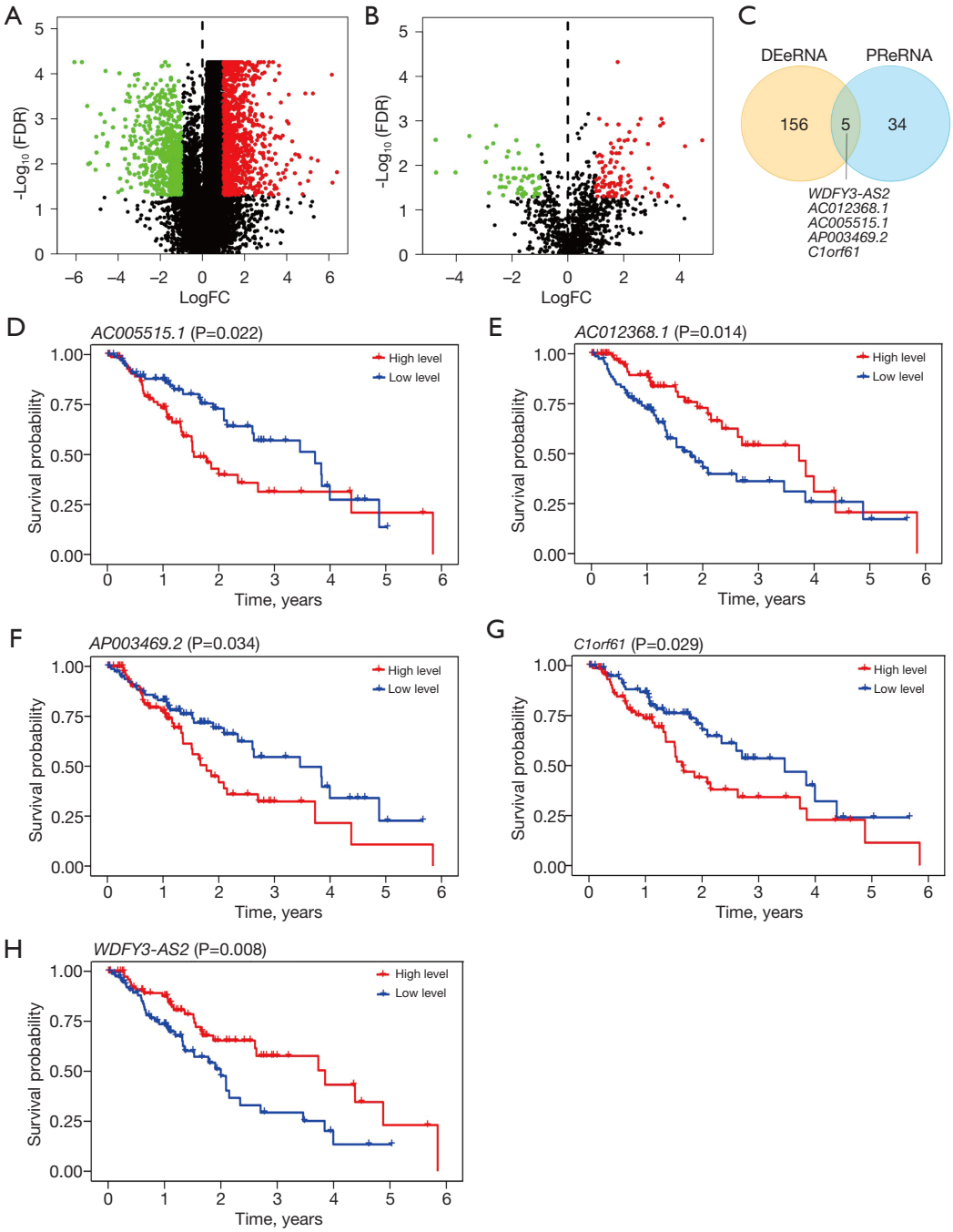

Figure 1 Identification of prognostic eRNAs of ESCA. Volcano plots showing differentially expressed mRNA (A) and eRNA (B) in ESCA. Red indicated up-regulated genes, and green indicated down-regulated genes. (C) Five eRNAs were identified to be associated with the prognosis of ESCA. (D-H) The Kaplan-Meier survival analysis showed a prognostic relationship between the five eRNAs and ESCA. FC, fold change; FDR, false discovery rate; DEeRNA, differentially expressed eRNA; PReRNA, prognostic related eRNA; eRNA, enhancer RNA; ESCA, esophageal cancer.

Table 1 The list of 34 eRNAs associated with prognosis in ESCA

Gene	KM P value
AL390879.1	0.0021
LINC01006	0.0031
KCP	0.0075
AP000943.1	0.008
WDFY3-AS2	0.0085
LINC01714	0.0137
AC012368.1	0.0143
AC025871.2	0.0179
AP000688.3	0.0201
AC098828.2	0.0204
AC098934.1	0.0214
AF241728.1	0.0217
AC005515.1	0.0218
AC245140.1	0.023
TMEM225B	0.0232
AP001781.1	0.025
AC092490.1	0.0259
AL021391.1	0.0274
AP001596.1	0.0286
C1orf61	0.0288
AP003469.2	0.0344
JPX	0.0349
FTX	0.0381
AC007255.1	0.0395
AP000696.1	0.0411
SLC44A3-AS1	0.0415
SPAAR	0.0415
AC022613.1	0.0415
AC108749.1	0.0419
TMEM88B	0.045
AC021028.1	0.0451
LINC01271	0.0453
CCDC18-AS1	0.0493
FOXP4-AS1	0.0493

eRNA, enhancer RNA; ESCA, esophageal cancer; KM, Kaplan-Meier.

genes in pan-cancer. Our results showed that *AC005515.1* has potential prognostic value in multiple tumors and a co-expression relationship with immune checkpoint genes (*Figure 5*, Table S3).

The high AC005515.1 expression group showed higher CD8⁺ T cells and M1 macrophages infiltration

To analyze the composition of immune cells in *AC005515.1* low- and high-expression groups, we used CIBERSORT to obtain immune cell infiltration data and analyzed the correlation between *AC005515.1* expression and immune cell infiltration. In ESCA, we found that $CD8^+$ T cells and M1 macrophages were more abundant in the high-expression group and positively correlated with *AC005515.1* (*Figure 6A*); however, M0 macrophages were more abundant in the low-expression group and negatively correlated with *AC005515.1* (*Figure 6A*). Interestingly, we found the same results in KIRP, LGG, MESO, OV, SKCM, THCA, and UVM (*Figure 6B-6H*). Moreover, we found that M2 macrophages were increased in the low-expression group compared with the high-expression group in KIRP, MESO, OV, SKCM, THCA, and UVM (*Figure 6B,6D-6H*).

Association of AC005515.1 expression with immune function, immune subtypes, and TME of ESCA

We found an important immunological role of *AC005515.1* in pan-cancer. To further understand the prognostic role of *AC005515.1* in ESCA, we next analyzed the association of *AC005515.1* expression with immune function, immune subtypes, TME, characteristics of tumor stem cells, and TMB. Firstly, the results of the immune function analysis suggested that all immune functions except type 2 interferon response, scored higher in the *AC005515.1* high-expression group (*Figure 7A*). Secondly, we found that the tumor immune subtype in ESCA patients was mainly C2 (IFN-γ dominant, 76/151, *Figure 7B*). *AC005515.1* was expressed at a higher level in C2 compared with other immune subtypes. As is well known, *TME* plays a crucial role in the occurrence and development of tumors. Therefore, we also explored the relationship between *AC005515.1* and TME. Notably, *AC005515.1* showed a positive correlation with the stromal score, immune score, and ESTIMATE score (*Figure 7C*). Our findings suggested that high expression levels of *AC005515.1* were significantly associated with lower tumor purity. In addition, we further explored the

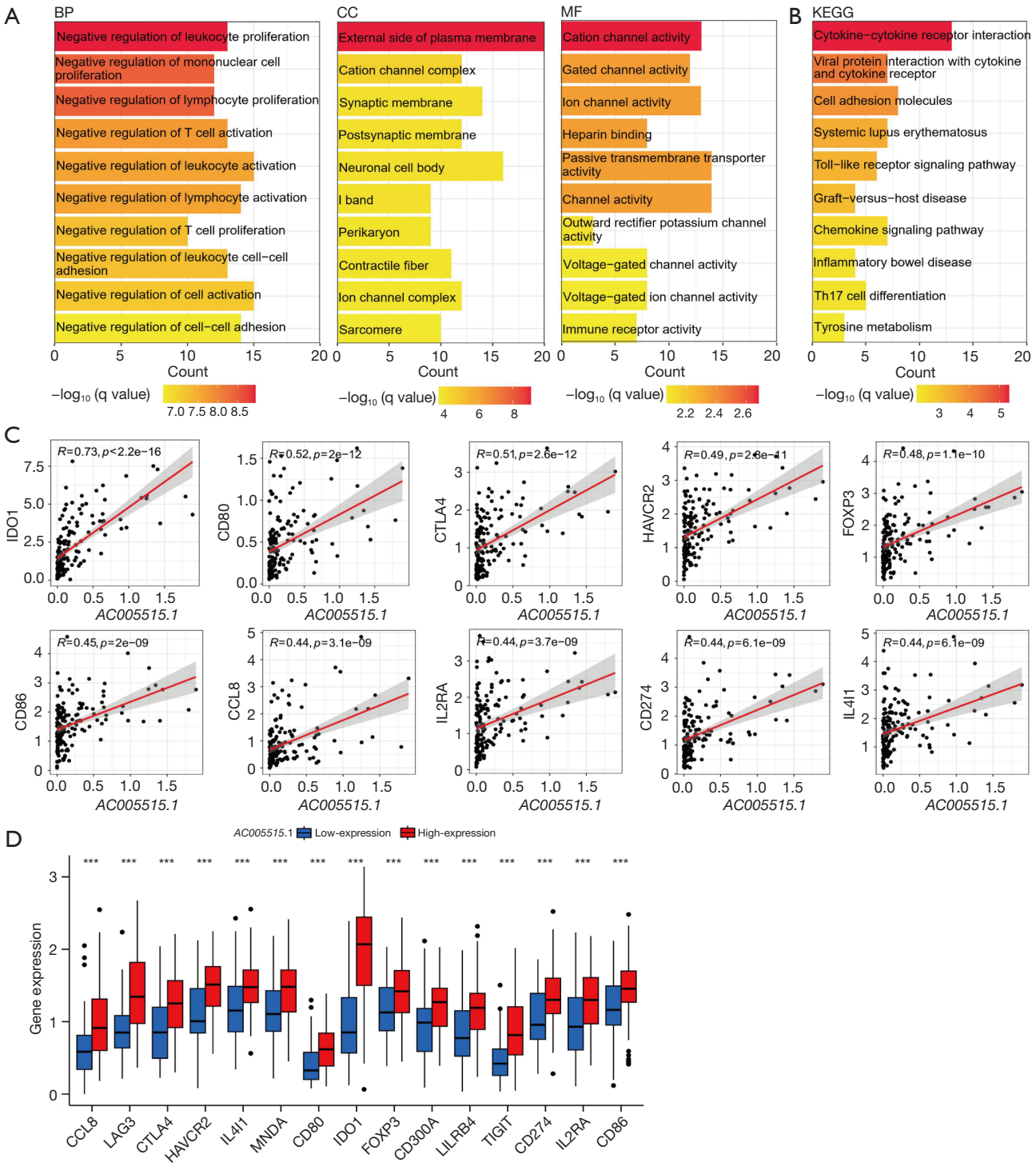

Figure 2 Identification of co-expressed genes of prognostic-related eRNAs in ESCA. Gene ontology term (A) and KEGG (B) enrichment analysis results of co-expressed genes. The top ten significant pathways were shown based on the P value of enrichment analysis. (C) *AC005515.1* was significantly correlated with immune checkpoint genes. (D) Immune checkpoint genes were increased in the high-expression group of *AC005515.1*. ***, P<0.001. BP, biological processes; CC, cellular components; MF, molecular functions; KEGG, Kyoto Encyclopedia of Genes and Genomes; eRNA, enhancer RNA; ESCA, esophageal cancer.

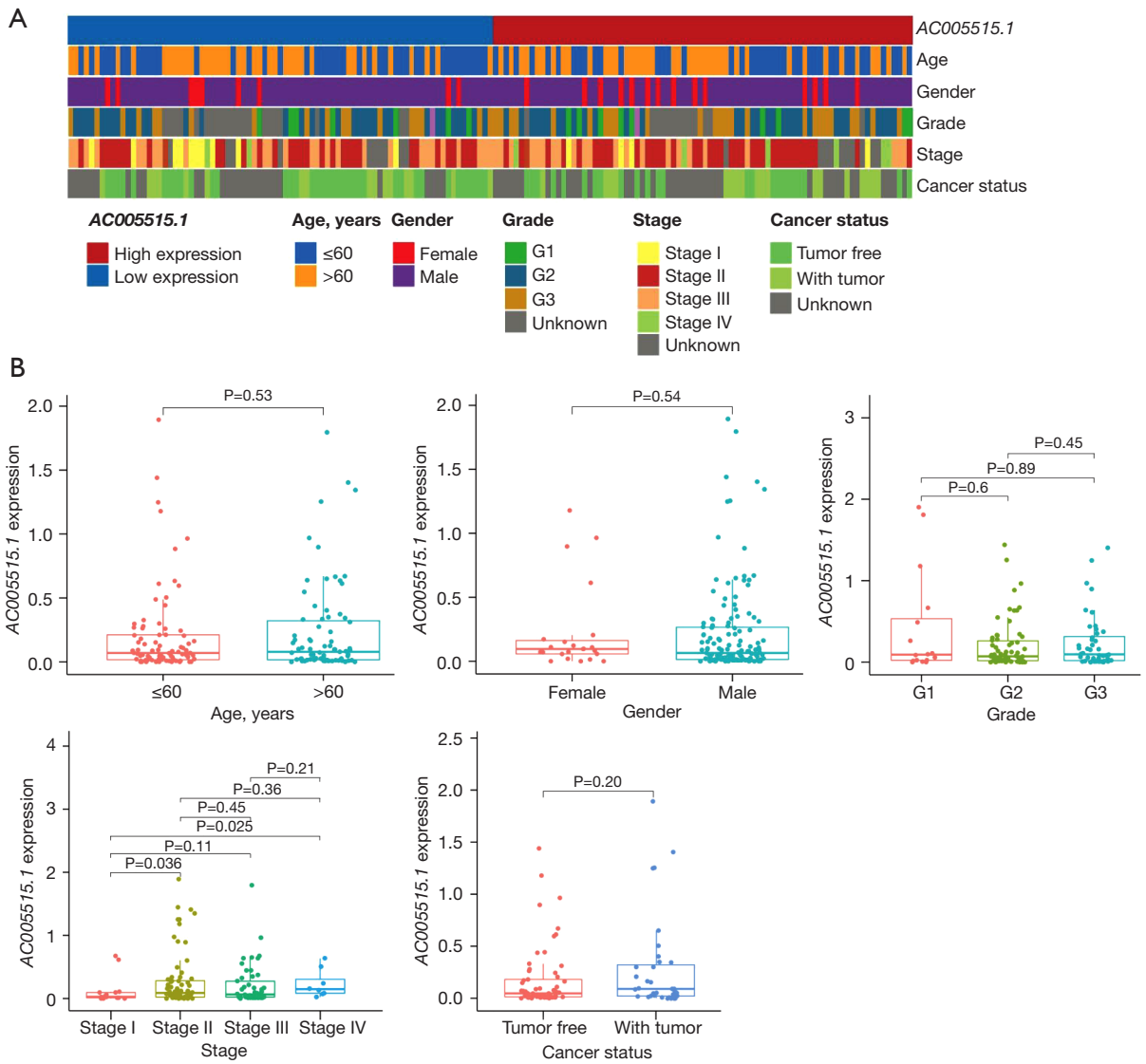

Figure 3 Relationship between *AC005515.1* and clinical features in ESCA. *AC005515.1* was associated with the tumor stage. ESCA, esophageal cancer.

relationship between *AC005515.1* and stem cell properties. We found that *AC005515.1* had a significant negative correlation with DNA stemness score, whereas RNA stemness score did not (*Figure 7C*). We did not find an association between *AC005515.1* expression and TMB (*Figure 7C*).

ESCA patients with high AC005515.1 expression had higher TIDE scores

We used the TIDE score to predict the immune response of ICB therapy in *AC005515.1* low- and high-expression groups. We found that the high-expression group had higher TIDE scores in ESCA, MESO, OV, SKCM, and THCA compared with the low-expression group (*Figure 8*). Furthermore, we found that in these tumors, the *AC005515.1* high-expression group had higher dysfunction scores (*Figure 8*). The results suggested that patients with high expression of *AC005515.1* are immune dysfunctional and may not be conducive to benefit from ICB therapy.

Discussion

ESCA is the sixth leading cause of cancer death, and its

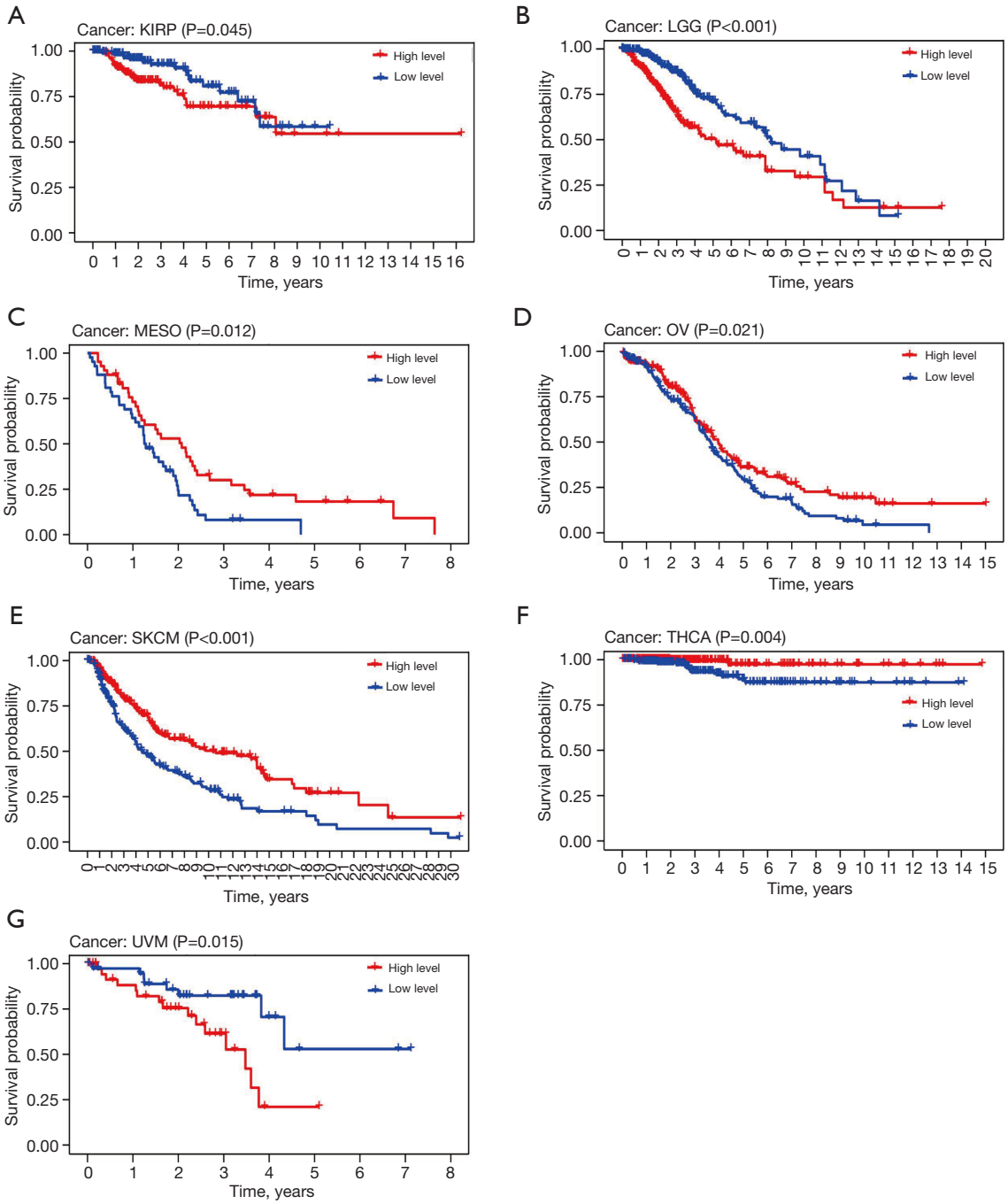

Figure 4 To explore the prognostic value of *AC005515.1* in pan-cancer. Survival analysis of *AC005515.1* in pan-cancer suggested that *AC005515.1* was also associated with tumor prognosis including KIRP (A), LGG (B), MESO (C), OV (D), SKCM (E), THCA (F), and UVM (G). KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; UVM, uveal melanoma.

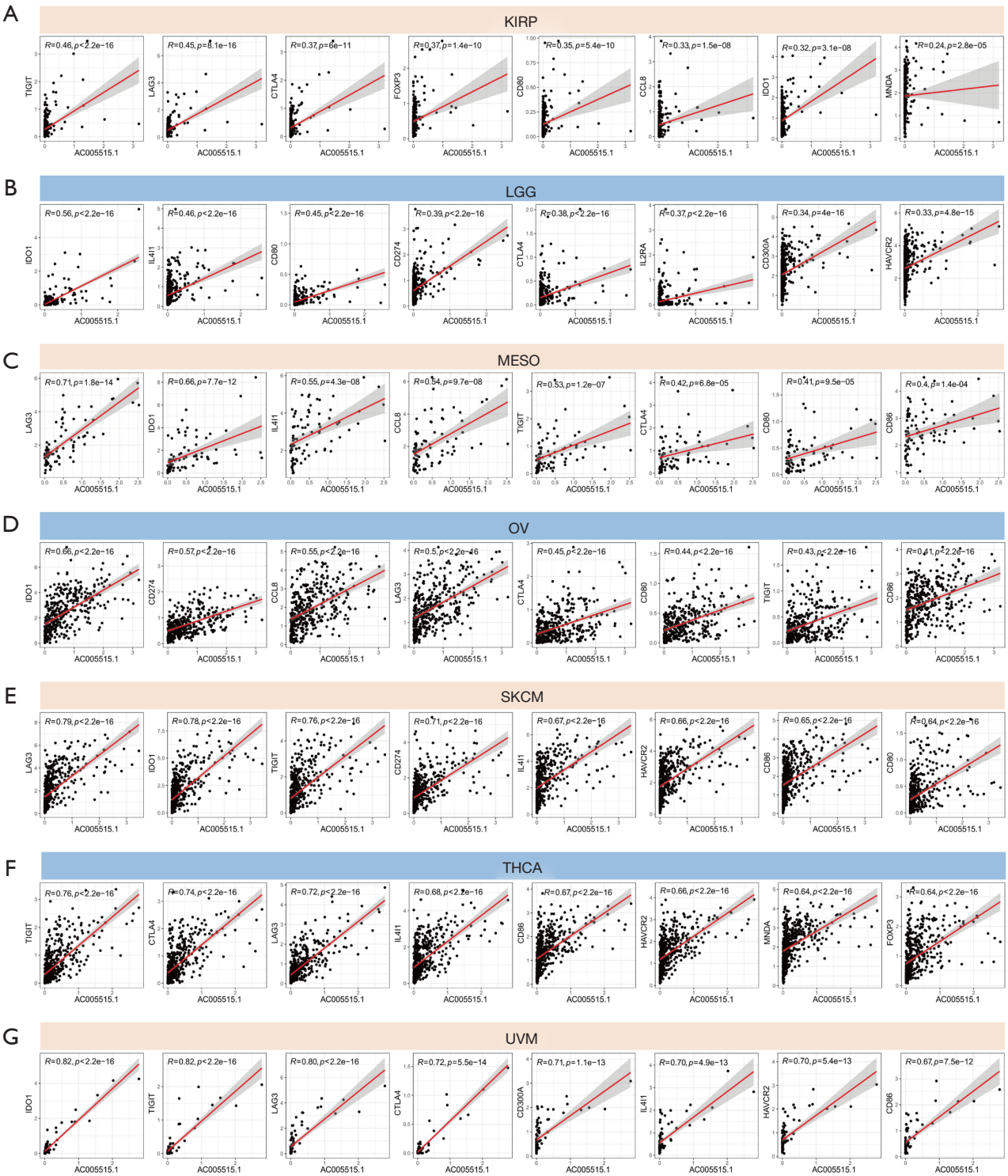

Figure 5 Validation of the correlation between *AC005515.1* and co-expressed genes. The correlation of *AC005515.1* with its co-expressed genes was verified in pan-cancer, including KIRP (A), LGG (B), MESO (C), OV (D), SKCM (E), THCA (F), and UVM (G). The top eight significantly co-expressed genes were shown based on the P value of Spearman's correlation analysis. KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; UVM, uveal melanoma.

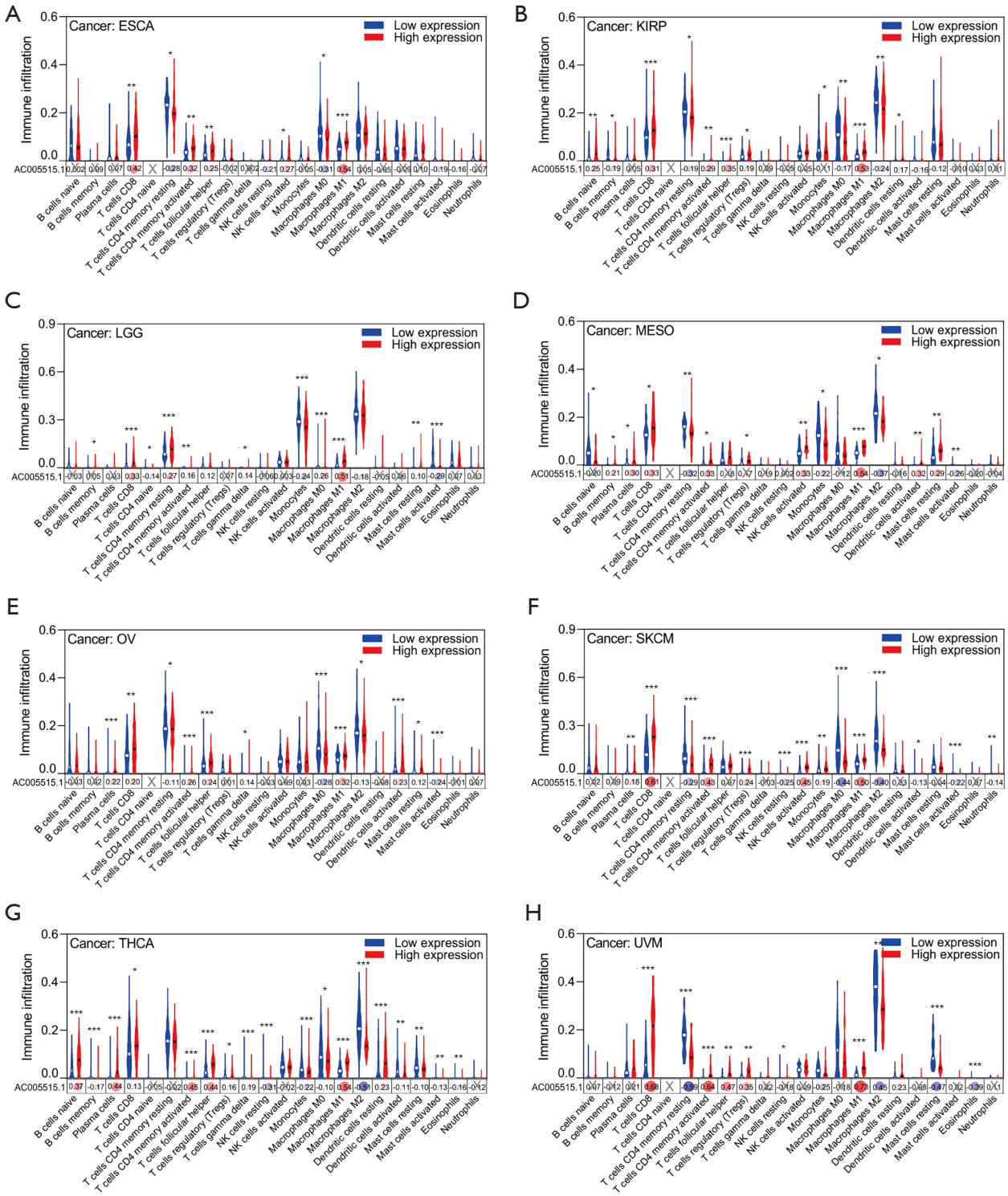

Figure 6 Correlation between *AC005515.1* expression and immune cell infiltration. Violin plot showing the difference in immune cell infiltration between *AC005515.1* high- and low-expression groups in ESCA (A), KIRP (B), LGG (C), MESO (D), OV (E), SKCM (F), THCA (G), and UVM (H). *, P<0.05; **, P<0.01; ***, P<0.001. NK, natural killer. ESCA, esophageal cancer; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; UVM, uveal melanoma.

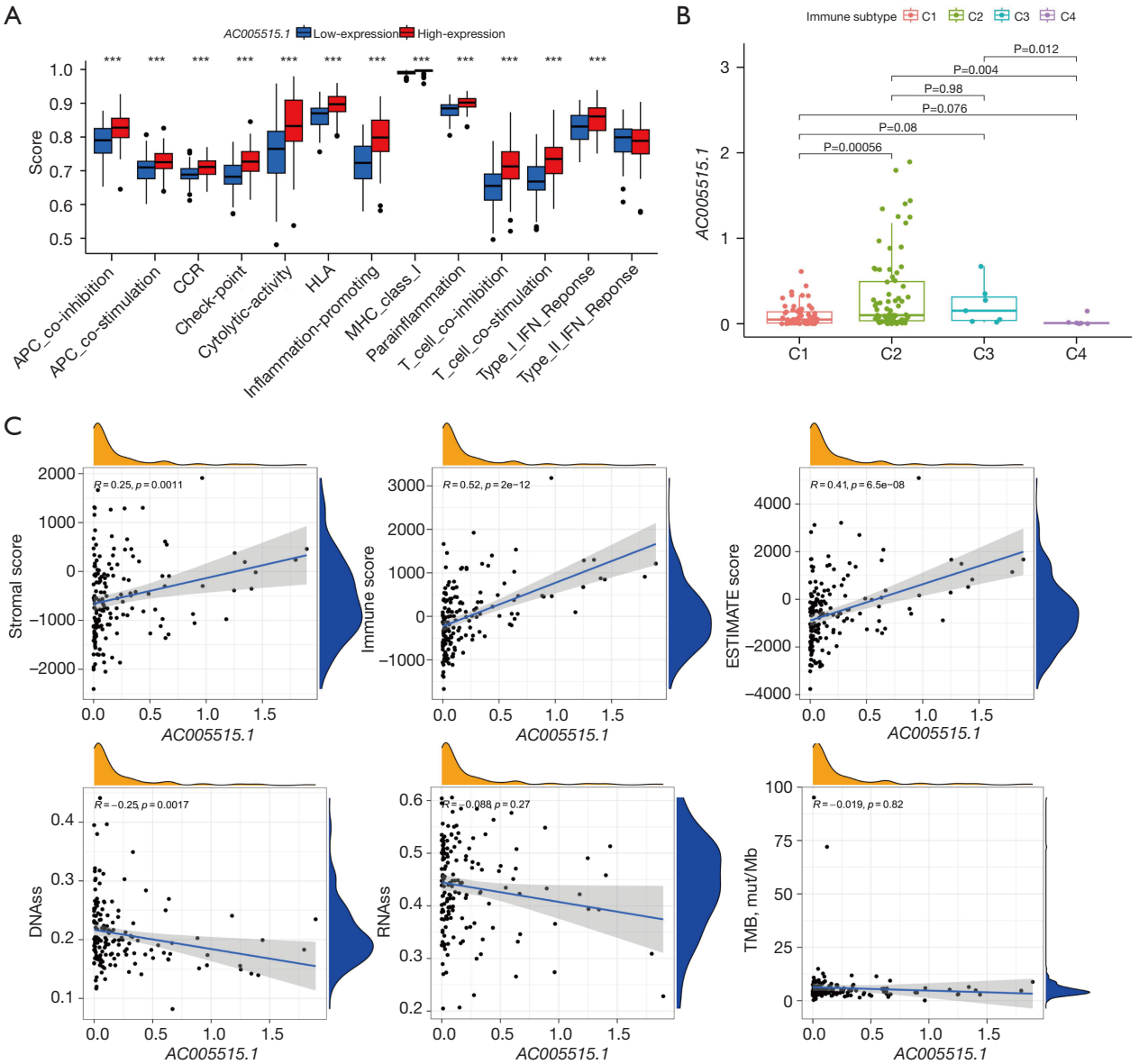

Figure 7 Analyzation of the immune landscape of the ESCA microenvironment. (A) Immune function was increased in the *AC005515.1* high-expression group. (B) *AC005515.1* was related to tumor immune subtypes. (C) Scatter plots show the correlation of *AC005515.1* with the stromal score, immune score, ESTIMATE score, cancer stemness DNAss, RNAss, and TMB, respectively. ***, P<0.001. APC, antigen-presenting cell; CCR, CC chemokine receptor; HLA, human leukocyte antigen; MHC, major histocompatibility complex; IFN, interferon; DNAss, DNA stemness score; RNAss, RNA stemness score; TMB, tumor mutational burden; mut/Mb, mutations per mega base; ESCA, esophageal cancer.

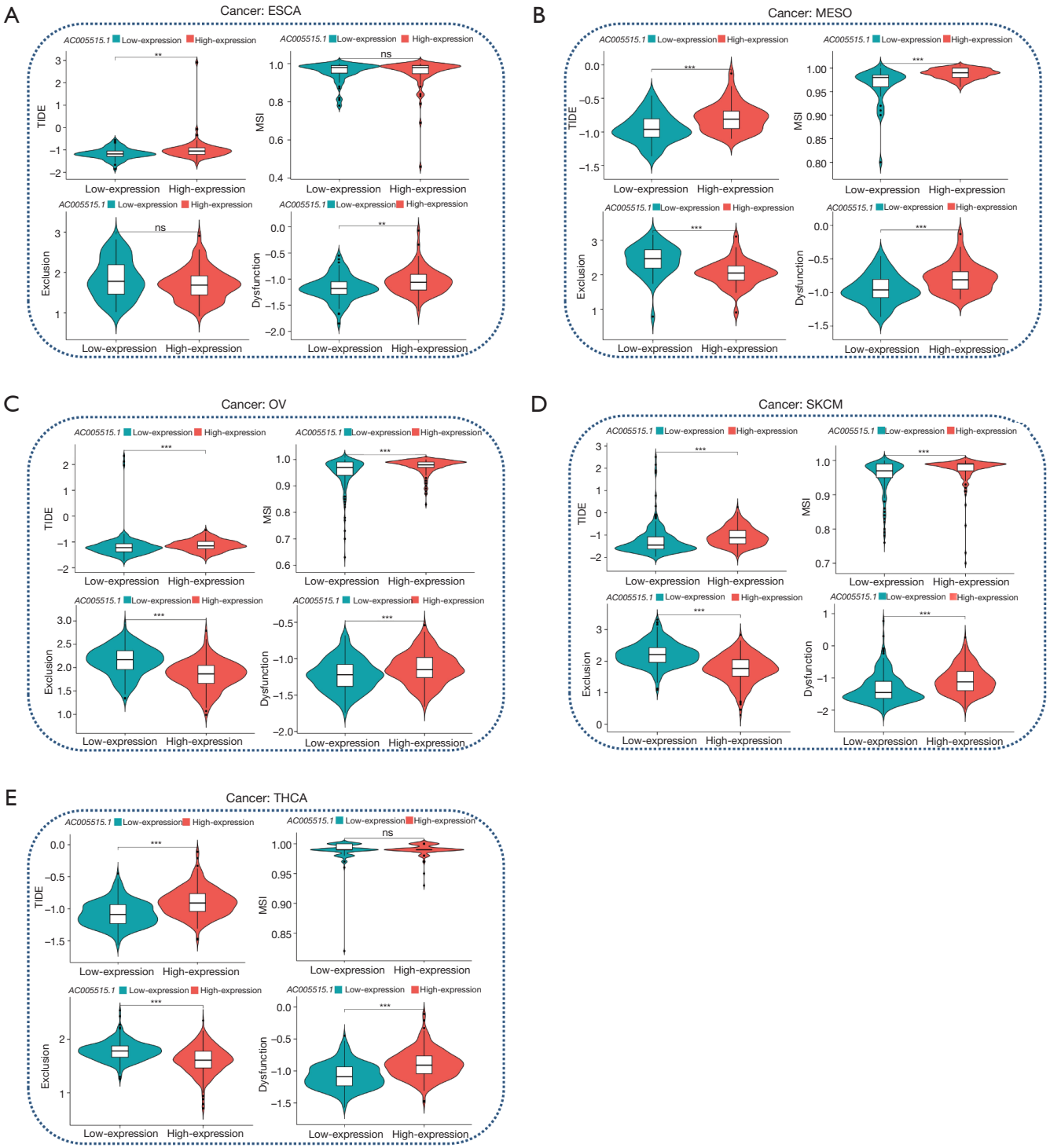

Figure 8 *AC005515.1* predicting response to ICB therapy. Violin plots depict differences in TIDE scores, MSI, exclusion, and T cell dysfunction between *AC005515.1* high- and low-expression groups in ESCA (A), MESO (B), OV (C), SKCM (D), and THCA (E), respectively. **, P<0.01; ***, P<0.001. ESCA, esophageal cancer; TIDE, tumor immune dysfunction and exclusion; ns, not significant; MSI, microsatellite instability; ICB, immune checkpoint blockade; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma.

high mortality rate poses a challenge for health systems worldwide (1). ESCA is highly heterogeneous, with two biologically distinct subtypes (23). While early surgery can improve patient survival, ESCA is often diagnosed late and the best time for surgery is missed (24). For those patients not amenable to surgery, chemotherapy or immunosuppressive therapy offers an improvement in quality of life. However, their 5-year survival rate remains very low (25). Therefore, the study of biomarkers for early disease screening and prognosis is desperately needed. Currently, eRNA signature-based research is receiving a lot of attention (10,13,26,27). Previous studies have shown that eRNA has a high potential for cancer prognosis (28-30).

Transcription is a critical step in the regulation of gene expression. eRNAs are a group of noncoding RNAs that are transcribed from enhancer elements on the genome by RNA polymerase II (31). eRNAs can be involved in the regulation of transcription by a variety of mechanisms, including interaction with DNA or RNA through nucleic acid base pairing, or interaction with proteins through advanced RNA structures (32-34). The most frequently described mechanism of action of eRNAs is the regulation of enhancer-promoter loops, in addition to the regulation of chromatin accessibility and transcriptional elongation (9,35). Recent studies have also shown that eRNAs can control gene expression patterns by regulating acetylation and can be chemically modified to recruit read proteins, regulating transcriptional condensates (36,37). Despite these studies provide new directions for exploring the mechanistic functions of eRNAs, there are still many challenges: the functions and potential mechanisms of eRNAs in gene regulation and chromosomal interactions need to be further understood experimentally; eRNAs' biological activities and relevance to diseases are not fully elucidated; more importantly, eRNAs are less abundant and unstable in vivo, and there is a need to develop more sensitive methods to recognize eRNAs.

In this study, we used bioinformatics to identify eRNAs as potential prognostic-related biomarkers in ESCA. *WDFY3-AS2*, *AC012368.1*, *AC005515.1*, *AP003469.2*, and *Clorf61* were identified as ESCA prognostic-related eRNAs in our study. These newly predicted eRNAs, except *WDFY3-AS2*, have been reported for the first time in oncology study (38). Analysis of gene co-expression revealed that *AC005515.1* was significantly associated with immune checkpoint genes. Based on our analysis, *AC005515.1* was found to be the key prognostic-related eRNA for ESCA and to be correlated with $CD8^+$ T cells and M1 macrophages infiltration. In addition, we found that *AC005515.1* correlated with the prognosis of multiple tumors in a pan-cancer analysis, such as KIRP, LGG, and MESO, etc. *AC005515.1* was found to be a potential biomarker for predicting ICB response through the TIDE score.

The production of eRNA is a true manifestation of enhancer activation. eRNAs are also involved in immune checkpoint-related pathways to regulate immune responses and influence tumor development (39). In this study, *AC005515.1* was found to be co-expressed with immune checkpoint-related genes such as *CTLA4*, *PD-L1*, and *IDO1* in ESCA, and in a pan-cancer analysis, *AC005515.1* was also found to be co-expressed with immune checkpoint-related genes in multiple cancers. Pathway enrichment analysis of these co-expressed genes enriched in negative regulatory pathways for immune cell proliferation and activation. The immune cell of TME acts as a double-edged sword that have been shown to harbor either tumor-promoting or tumor-suppressing activities (40). It was found that reactive oxygen species and reactive nitrogen species produced by neutrophils could involve in a pro-tumor or anti-tumor process by modulating phagocytosis, secretion, gene expression and apoptosis (41). Likewise, different immune cells can also have varying effects on tumor growth in different cancer types (40). Our research discovered that high levels of eRNA *AC005515.1* led to worse OS rates. This suggests that *AC005515.1* may have accelerated tumor progression by regulating genes that suppressed the growth and activation of immune cells. Furthermore, ICB therapy with anti-PD-L1 and anti-CTLA4 are increasingly common standards of care for certain forms of cancer (42). Since the efficacy of ICIs is affected by various factors, the results of therapy are often unsatisfactory in most cancers (43). We applied TIDE to predict the therapeutic effect of *AC005515.1* expression levels on ICB. TIDE is an algorithm developed to predict the efficacy of ICB based on two immune escape mechanisms from tumors (T cell dysfunction and immune exclusion) (22). We found that patients with high *AC005515.1* expression levels had higher TIDE scores, suggesting that some patients with high *AC005515.1* expression levels may not be conducive to benefit from ICB therapy. Notably, we found that in ESCA, MESO, OV, SKCM, and THCA, high levels of *AC005515.1* dysfunction were also scored higher. This may be an important reason why *AC005515.1* affects tumor prognosis.

Based on the expression of *AC005515.1*, we further analyzed the immune landscape of the ESCA

microenvironment. A single-cell analysis shows that ESCA was enriched in immune-suppressive cell populations, including exhausted $CD8^+$ T cells, which may contribute to immune escape and promote tumor progression (44). These immune cells can act as potential markers for predicting prognosis and immunotherapy responsiveness in the ESCA immune landscape (45). Our study has revealed that the eRNA *AC005515.1* high-expression group has higher immune cell infiltration, including $CD8^+$ T cells and M1 macrophages. In our results, the group with poor OS had high levels of $CD8^+$ T cells, this suggests that ESCA with high expression of *AC005515.1* have poor OS may be associated with having higher immune cell infiltration. Furthermore, ESCA is a tumor with high mutational burden and TMB may be a potential prognostic marker (46). Previous study has suggested that higher TMB may contain more mutation-associated neoantigens present and is also associated with increased immune cell infiltration in the TME (47). However, we did not observe an association between eRNA *AC005515.1* and TMB in our study. Some studies have found that immune gene expression and immune cell infiltration are associated with tumor immune subtypes (48,49). Thorsson *et al.* identified six immune subtypes in human tumors, including wound healing (C1), IFN-γ dominant (C2), inflammatory (C3), lymphocyte depleted (C4), immunologically quiet (C5), and TGF-β dominant (C6) (50). C2 is considered to have abundant $CD8^+$ T cells and M1 macrophages infiltration, which is consistent with our results (50). Immune subtypes were associated with OS and progression-free survival, C3 had the best prognosis, while C2 and C1 had a less favorable prognosis despite having a large immune component (51). The majority of ESCA patients with high *AC005515.1* expression were of type C2. This may be one reason for the poor OS in patients with high *AC005515.1* expression.

Numerous studies have demonstrated that TME is essential in tumor development and metastasis (52,53). TME comprises cancer and non-cancer cells (immune cells, stromal cells, etc.), which play an important role in tumor growth and aggressiveness (54). TME with a higher number of stromal cells and immune cells suggested low tumor purity. According to previous researchers, lower tumor purity has been linked to a worse prognosis and increased aggressiveness in gliomas (55). In gastric cancer, Lou *et al.* also discovered similar outcomes and found tumor purity can serve as the predicting factor for adjuvant chemotherapy efficacy (56). In the present study, the ESTIMATE score suggested that ESCA patients with high expression of *AC005515.1* had a lower tumor purity and a poorer prognosis.

This study has several limitations. Firstly, our study only analyzed the publicly available dataset of TCGA and lacked a validation cohort to confirm the association of eRNA *AC005515.1* with the prognosis of ESCA. Secondly, although the correlation of eRNA *AC005515.1* with immune checkpoint-related genes was validated in pan-cancer, basic experiments are needed to confirm the relationship, which is the focus of our subsequent studies. Finally, we predicted the relationship between eRNA *AC005515.1* and immunotherapy efficacy from RNA-Seq data, but only clinical trials can confirm its true relationship.

Conclusions

In summary, we identified eRNA *AC005515.1* as a key prognostic-related marker in ESCA and also elucidated the immunological function of *AC005515.1* in ESCA. This study demonstrated an association between eRNA *AC005515.1* and the local immune environment and provided a potential predictive biomarker for the prognosis of ESCA.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-777/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-777/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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