Transcription Elongation Factor A (SII)-Like (TCEAL) Gene Family Member-TCEAL2: A Novel Prognostic Marker in Pan-Cancer

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ABSTRACT

BACKGROUND: Cancer is the leading cause of death in the world. The mechanism is not fully elucidated and the therapeutic effect is also unsatisfactory. In our study, we aim to find new target gene in pan-cancer.

METHODS: Differently expressed genes (DEGs) was screened out in various types of cancers from GEO database. The expression of DEG (TCEAL2) in tumor cell lines, normal tissues and tumor tissues was calculated. Then the clinical characteristics, DNA methylation, tumor infiltration and gene enrichment of TCEAL2 was studied.

RESULTS: TCEAL2 expressions were down-regulated in most cancers. Its expression and methylation were positively or negatively associated with prognosis in different cancers. The tumor infiltration results revealed that TCEAL2 was significantly related with many immune cells especially NK cells and immune-related genes in majority cancers. Furthermore, tau protein and tubulin binding were involved in the molecular function mechanisms of TCEAL2.

CONCLUSION: TCEAL2 may be a novel prognostic marker in different cancers and may affect tumor through immune infiltration.

KEYWORDS: TCEAL2, pan-cancer, prognosis, immune infiltration

Introduction

Cancer is the leading cause of death in the world. The International Agency for Research on Cancer released that there was about 19.29 million new cancer cases and 9.96 million cancer deaths worldwide in 2020. Cancer patients not only have to bear great physical pain, but also a heavy financial burden. Elucidating the pathogenesis and finding a cure are very important and urgent. As known to all, cancer arises through a multistep, mutagenic process. A number of oncogenes and tumor suppressors was found to promote and inhibit the development of tumors, and many drugs based on these genes was designed to diagnose and treat tumors.[1] Even though so many genes were discovered, the mechanisms are not fully elucidated and the therapeutic effect is also unsatisfactory. It is necessary to find new oncogenes or tumor suppressors.

The Gene Expression Omnibus (GEO) database is an international public repository which collects microarray, next-generation sequencing and other forms of high-throughput functional genomics data submitted by the research community. It contains a large number of genomics data of pan-cancer. We analyzed GEO dataset of different types of cancers and screened out differentially expressed genes (DEGs). Among the DEGs, transcription elongation factor A (SII) -like 2 (TCEAL2, also known as WEX1, my048 and MY0876G05) was the only DEG in all 9 different kinds of cancers. TCEAL2

is a nuclear phosphoprotein that modulates transcription in a promoter context-dependent manner and has been recognized as the important nuclear target for intracellular signal transduction. It belongs to the transcription elongation factor A (SII) -like (TCEAL) gene family which includes 9 members (TCEAL1-9) and contains common TFA domains.[2] They are all located on the X chromosome and TCEAL2 located on the Xq22.1 chromosome.[3,4] Human TCEAL2 mRNA is 1100 bp long and encodes a protein (227 amino acids) with a relative molecular mass of 26 kDa. Recent studies had shown that TCEAL2 was down-regulated in ovarian cancer, clear cell renal cell carcinoma (ccRCC), transitional cell carcinoma (TCC), the testicular germ cell tumors (TGCT) and oral cancer.[5-8] The up-related of TCEAL2 expression was associated with poor prognosis patients with ovarian cancer.[5] In ccRCC, TCEAL2, as a tumor suppressor, could inhibit cell proliferation and induced cell cycle arrest into S phase, and the low expression was related with higher tumor stage.[6] TCEAL2 is rarely reported and studied. Its expression, relationship with clinical prognosis and function in other cancers are unknown. So, in our study, we studied TCEAL2 expression in 33 cancer types and its correlations with clinical prognosis and immunity based on TCGA database. The deoxyribonucleic acid (DNA) methylation and its relationship with prognosis were also studied. Relevant genes were enriched to investigate the
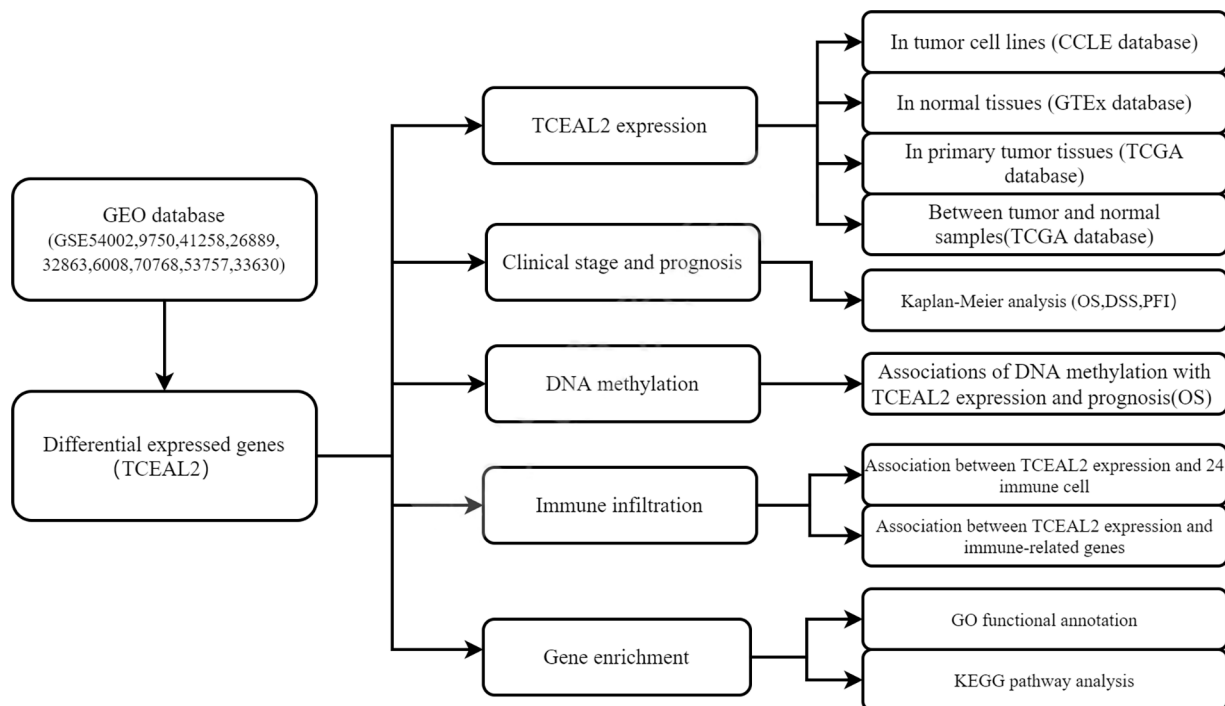
Figure 1. The flow chart of our research.

function and pathway involved. The flow chart was shown in Figure 1.

Materials and Methods

DEGs from GEO database

Nine different GSE datasets (GSE54002, 9750, 41258, 26899, 32863, 6008, 70768, 53757, and 33630) of breast, cervical, colorectal, gastric, lung, ovarian, prostate, renal and thyroid cancer were chosen from GEO database. Data were analyzed using GEO2R. The adjusted $P < .05$ and $|logFC| > 1$ was set as the cut-off criteria for DEG screening. The Venn diagram of 9 cancer types and Volcano plot of every cancer type were drawn using R software (version 4.1.0; https://www.r-project.org) with R package "ggplot2." The structure of TCEAL2 was downloaded from Universal Protein database and the location in the cell was found in THE HUMAN PROTEIN ATLAS. The basic information of GEO datasets was shown in Supplemental Table S1.

TCEAL2 expression analysis

RNA sequencing data of 33 types of cancer were downloaded from TCGA. Gene expression data of each tumor cell line of 22 tumor cell lines and 31 normal tissues were downloaded from CCLE database and GTEx. The expression of TCEAL2 were calculated in 22 tumor cell lines, 31 normal tissues, 33 primary tumor tissues and its matched control tissues in 33 cancers. Expression data were Log2 transformed and Mann-Whitney U test was used to analyze the expression difference between tumor and normal tissues. R software was used to analyze the expression data and R package "ggplot2" used to draw box plots.

Correlations between TCEAL2, tumor stage and prognosis

Clinical data of 33 types of cancer were downloaded from TCGA. The correlation between TCEAL2 expression and the tumor stage (Stage I, II, III and IV) was analyzed using Kruskal-Wallis Test which was drawn with R package "ggplot2." Three prognosis indicators including overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) were selected to study the relationships between TCEAL2 expression and clinical prognosis in every cancer type. Forest plots and Kaplan-Meier curve was shown using R packages "survival" and "survminer."

The DNA methylation of TCEAL2

The data of DNA methylation detected by Illumina Human Methylation 450 BeadChip and gene expression of TCEAL2 of each type of cancer were downloaded from the UCSC Xena database. The correlation of TCEAL2 expression with DNA methylation was conducted using Spearman's correlation test with R software. R packages "ggplot2" was used for visualization. The relationship of TCEAL2 methylation with clinical prognosis was performed using Kaplan-Meier analysis which was analyzed and drawn by R packages "survival" and "survminer."

Immune infiltration analysis

Data of 24 immune cells were downloaded from TCGA. The R package "GSVA" and the ssGSEA algorithm were used to explore the relationship between TCEAL2 expression and immunocytes in every cancer type. Bubble chart was used to demonstrate the correlation. Relationship between TCEAL2 expression and immune-related genes, including major histocompatibility complex (MHC), immune activation, immunosuppressive, chemokine and chemokine receptor genes were also analyzed using Pearson's test.

Gene enrichment analysis

TCEAL2-related genes were downloaded from STRING and GEPIA2 website. Fifty interacted genes from STRING and 100 correlated genes from GEPIA2 were screened. Venn diagram was used to conduct the intersection analysis among the 150 genes. Then the correlation between TCEAL2 and the related genes in 32 types of cancer analyzed by Spearman's test using TIMER 2.0 database. Gene enrichment analysis including GO and KEGG analysis of the 150 genes was analyzed using R package "clusterProfiler." The data of biological process (BP), cellular component (CC) and molecular function (MF) of GO analysis and KEGG analysis were shown as bubble, bar chart or network analysis view.

Statistical analysis

The data of TCEAL2 expression were all Log2 transformed. The correlation analyses were all performed using Pearson's or Spearman's test. The Kaplan-Meier analysis with log-rank test was used for all survival analyzes. $P < .05$ were defined statistical significance for all study. All statistical analyses were carried out by R software.

Results

DEGs from different cancer types

TCEAL2 was the only DEG and its expression was downregulated in all 9 cancer types (Figure 2A-J, $P < .05$). The other co-expressed differential genes partially showed in Supplemental Table S2. We searched TCEAL2 location in the cell from THE HUMAN PROTEIN ATLAS website and found that it mainly localized on the nuclear speckles and cytosol shown in Figure 2L. The protein structure of TCEAL2 was shown in Figure 2K.

TCEAL2 expression in pan cancer

Relative TCEAL2 expressions in 22 tumor cell lines were shown in Figure 3A. The expressions in autonomic ganglia, lung and thyroid cell lines were relatively higher. We also analyzed TCEAL2 expression levels in 31 normal tissues and found that the expression level was highest in pituitary. In blood and bone marrow, TCEAL2 expression was relatively low (Figure 3B). Furthermore, we analyzed the expression in various tumors. In 33 types of cancers, brain lower grade

glioma (LGG), pheochromocytoma & paraganglioma (PCPG) and glioblastoma multiforme (GBM) has the higher expression level (Figure 3C). In the last, we compared the TCEAL2 expression between various cancers and matched normal samples (Figure 3D). In addition to those which the normal tissues data were not available, data of 21 cancers and normal tissues were further analyzed. The results showed that except cholangiocarcinoma (CHOL) and pancreatic adenocarcinoma (PAAD), there were significant differences in 19 cancer types ($P < .05$). TCEAL2 expression was markedly decreased in tumor tissues in 18 cancer types included bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical & endocervical cancer (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), GBM, head & neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney clear cell carcinoma (KIRC), kidney papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), uterine corpus endometrioid carcinoma (UCEC). In PCPG, the expression was obviously upregulated in tumor tissues.

Correlations of TCEAL2 expression with clinical stage and prognosis

We analyzed the relationships between TCEAL2 expression and clinical stage in 21 types of cancers which can acquire the stage data. We found that TCEAL2 expression was significantly related with clinical stage in BLCA, BRCA, PAAD, READ, STAD, TGCT, and THCA (Figure 4, $P < .05$). And the main difference was between stage I and the other stages (stage II, III, and IV). The expression in stage I was higher in BRCA, PAAD, and THCA, but lower in BLCA, READ, STAD, and TGCT ($P < .05$). In THCA, the expression in stage II and III was also higher than stage IV ($P < .05$).

To investigate the prognostic value of TCEAL2, the survival analysis contained OS, DSS and PFI was studied. The Kaplan-Meier analysis revealed that TCEAL2 was markedly associated with OS in BLCA, BRCA, CESC, LGG, LIHC, LUAD, mesothelioma (MESO) and PAAD (Figure 5). The high expression of TCEAL2 in BRCA, CESC, LGG, LIHC, MESO, and PAAD was related with relative prolonged OS. But in BLCA and LUAD, the higher expression was related with shorter OS. DSS analysis showed that patients with high expression had long survival time in BRCA, CESC, LGG, and MESO (Figure 6). In BRCA, LGG, and MESO, the high expression was also associated with longer PFI. However, in COAD, patients with high levels had shorter PFI (Figure 6).

Correlation of TCEAL2 expression with DNA methylation

TCEAL2 expression was markedly correlated with DNA methylation in 17 types of tumors. We presented 6 strongest relationships included 5 negative correlations in COAD,
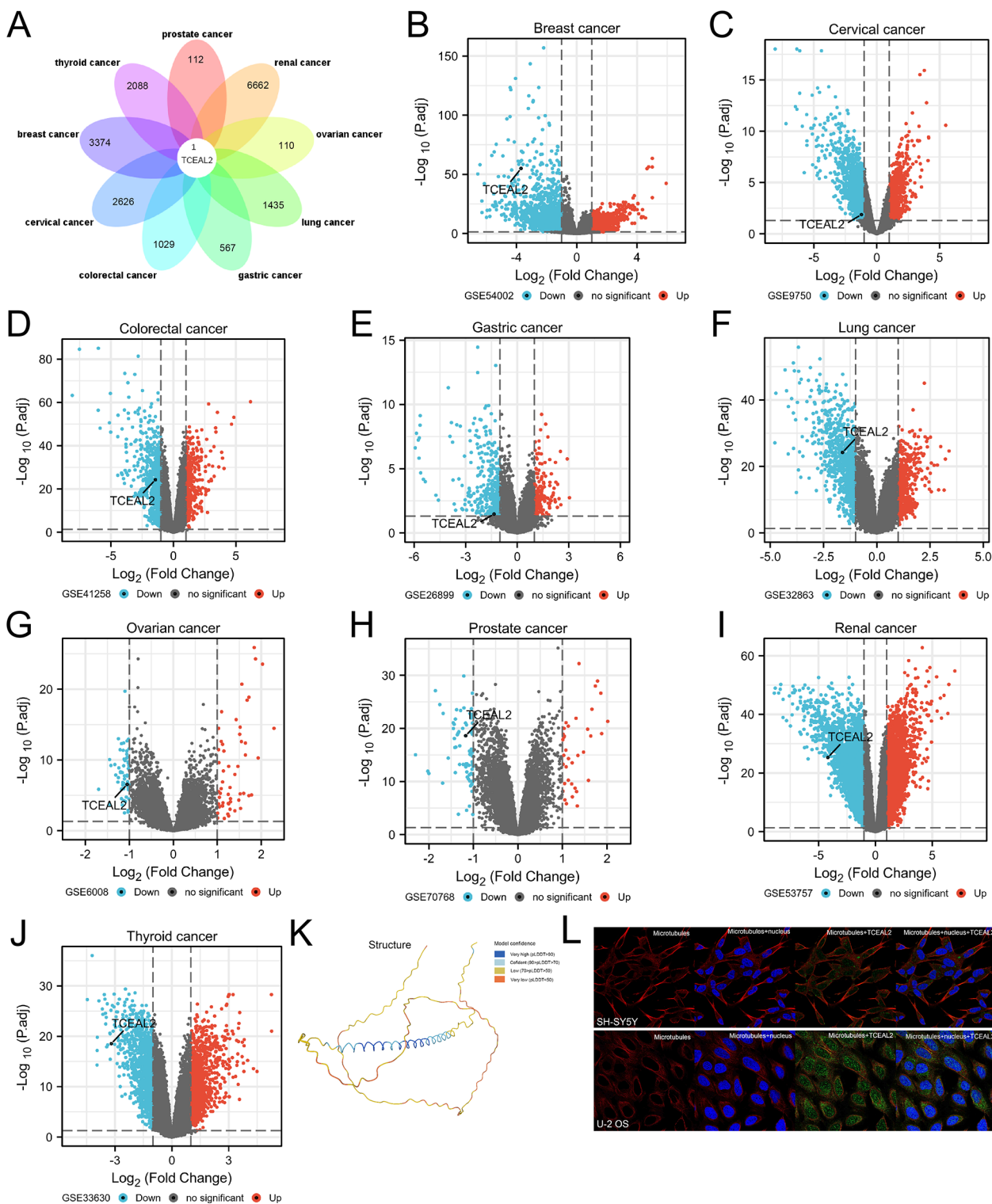
Figure 2. The expression of TCEAL2 in 9 types of cancers from GEO database: (A) the Venn diagrams of various cancers, (B-J) the Volcano plot of genes in breast, cervical, colorectal, gastric, lung, ovarian, prostate, renal and thyroid cancer, (K) the molecular structure of TCEAL2, and (L) the location of TCEAL2 in cell.

HNSC, MESO, PAAD, TGCT and 1 positive correlations in PCPG in Figure 7A. The other 11 types of tumors were shown in Supplemental Figure S1. Then we analyzed correlations between methylation and OS in pan cancer. In STAD, UCEC, and UVM, TCEAL2 methylation prolonged the overall survival time. However, in MESO and PAAD, it shortened the OS time (Figure 7B). In other cancers, there was no statistically significant difference.
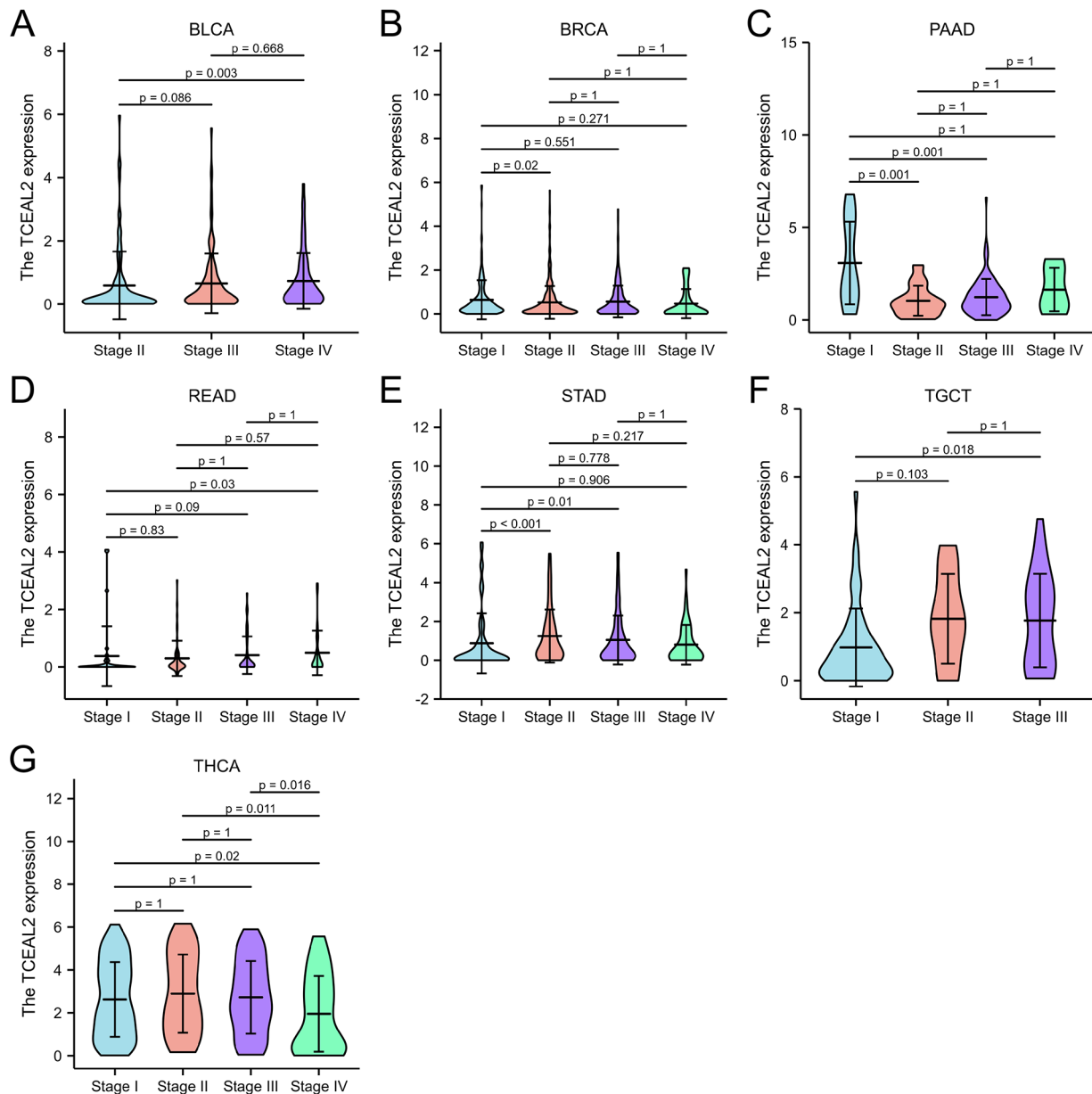
Figure 3. Differential expression of TCEAL2: (A) TCEAL2 expression in 22 tumor cell lines, (B) TCEAL2 expression in normal tissues, (C) TCEAL2 expression in 33 types of cancers, and (D) comparison of TCEAL2 expression between tumor and normal samples. $**P < .01$; $***P < .001$; ns means no significant.

Figure 4. Association between TCEAL2 expression and clinical stage in (A) bladder urothelial carcinoma (BLCA), (B) breast invasive carcinoma (BRCA), (C) prostate adenocarcinoma (PRAD), (D) rectum adenocarcinoma (READ), (E) stomach adenocarcinoma (STAD), (F) testicular germ cell tumors (TGCT), and (G) thyroid carcinoma (THCA).

Relationship between TCEAL2 and immunity

TCEAL2 expression was significantly related with the levels of immune cell in mostly cancers. Ten types of cancer (BLCA, CHOL, ESCA, LAML, PAAD, PRAD, READ, SARC, STAD, and TGCT) with relative higher correlation coefficient were showed in Figure 8A. The other types of cancers were showed in Supplemental Figure S2. To explore which immune cells were most likely to be involved, we further calculated the sum of correlation coefficients (the absolute value) of all tumors in each immune cell. The results showed that Natural Killer (NK) cells had the highest sum value and followed by Mast cells and TFH cell. Then, correlation between NK cells and TCEAL2 expression in 33 cancers was further studied.

Significant associations were found in 24 cancers. Among them, TCEAL2 expression was positively correlated with NK cells in 20 cancers except ACC, GBM, LGG and PCPG. Ten cancers include BLCA, CESC, COAD, ESCA, PRAD, READ, SARC, STAD, TGCT, and UCS which has the strongest correlations were showed in Figure 8B.

Furthermore, we analyzed the relationships between TCEAL2 and immune-related genes in pan cancer. The related genes included 21 MHC, 41 chemokine, 18 chemokines receptors, 46 immune activation and 24 immunosuppressive genes. TCEAL2 were mostly related with immune-related genes in most types of cancers in addition to ACC, KICH, UCS and UVM (Figure 9). Moreover, the correlations were positive in most various cancers except LGG.
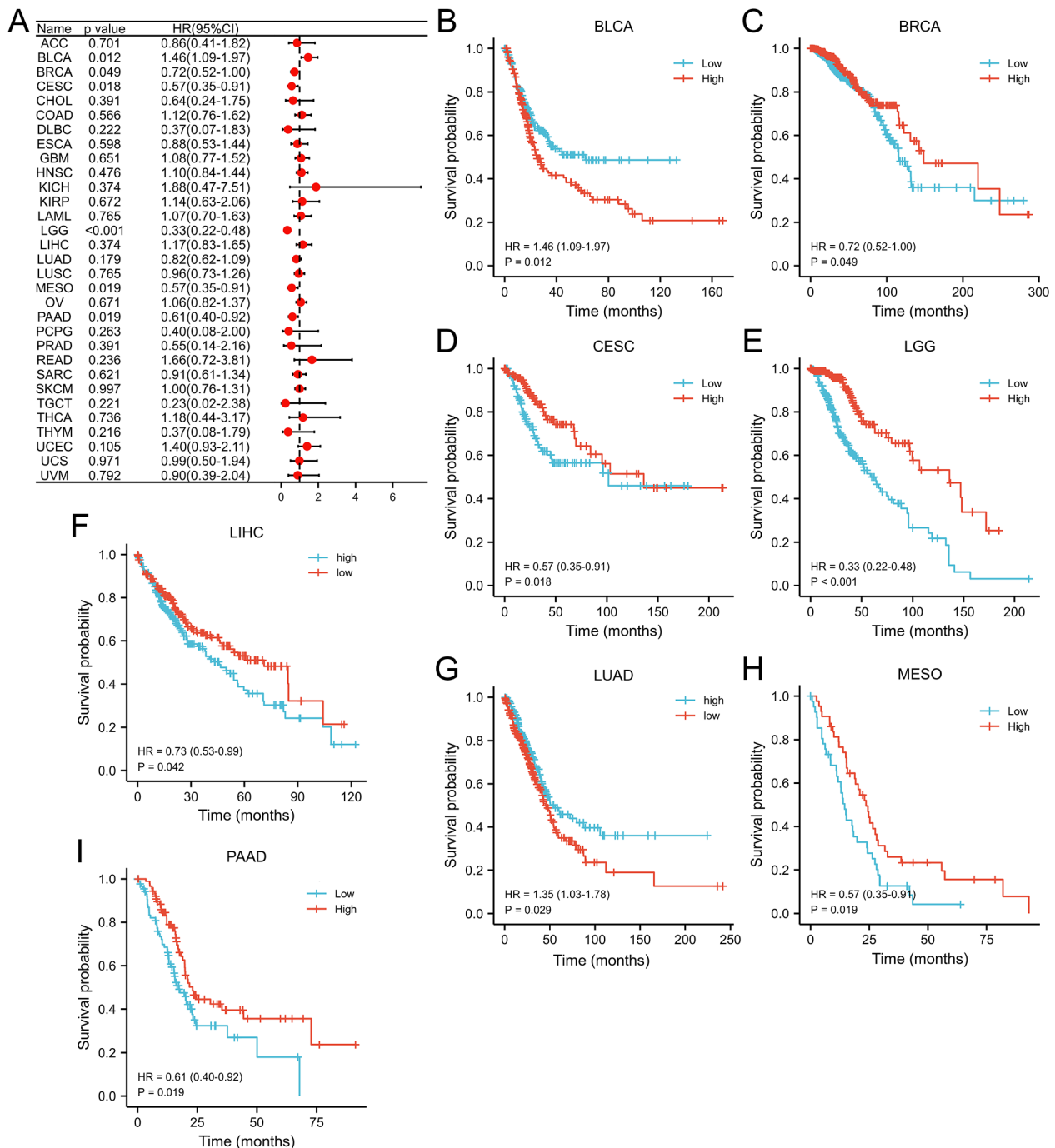
Figure 5. Association between TCEAL2 expression and overall survival time (OS): (A) Forest plot analysis of OS in 33 types of cancers and (B-I) Kaplan-Meier analysis of associations between TCEAL2 expression and OS.

Enrichment analysis of TCEAL2

To investigate the role of TCEAL2 in tumorigenesis, we screened out relevant genes for enrichment analysis using STRING and GEPIA2 website. Fifty interacted genes from STRING website (Figure 10A) and 100 top correlated genes from GEPIA2 were selected. Then we calculated the intersection of the 2 datasets using Venn diagram and obtained 3 related genes (BEX1, CNRIP1, and USP11, Figure 10B). Further, the correlations between TCEAL2 and 3 related genes in 32 types of cancers were explored. BEX1 was significantly

and positively correlated with TCEAL2 in all various cancers (Figure 10C). CNRIP1 and USP11 had remarkable positive correlations with TCEAL2 in most types of cancers. The negative correlation was just observed between USP11 and TCEAL2 in MESO (Figure 10C). The 150 related genes (50 from STRING and 100 from GEPIA2) were used to explore the GO and KEGG enrichment analyses. Through GO analysis, we found that TCEAL2 was mainly localized in axon and synaptic vesicle, and the involved biological process (BP) was mainly about synaptic vesicle localization, cognition and axonal
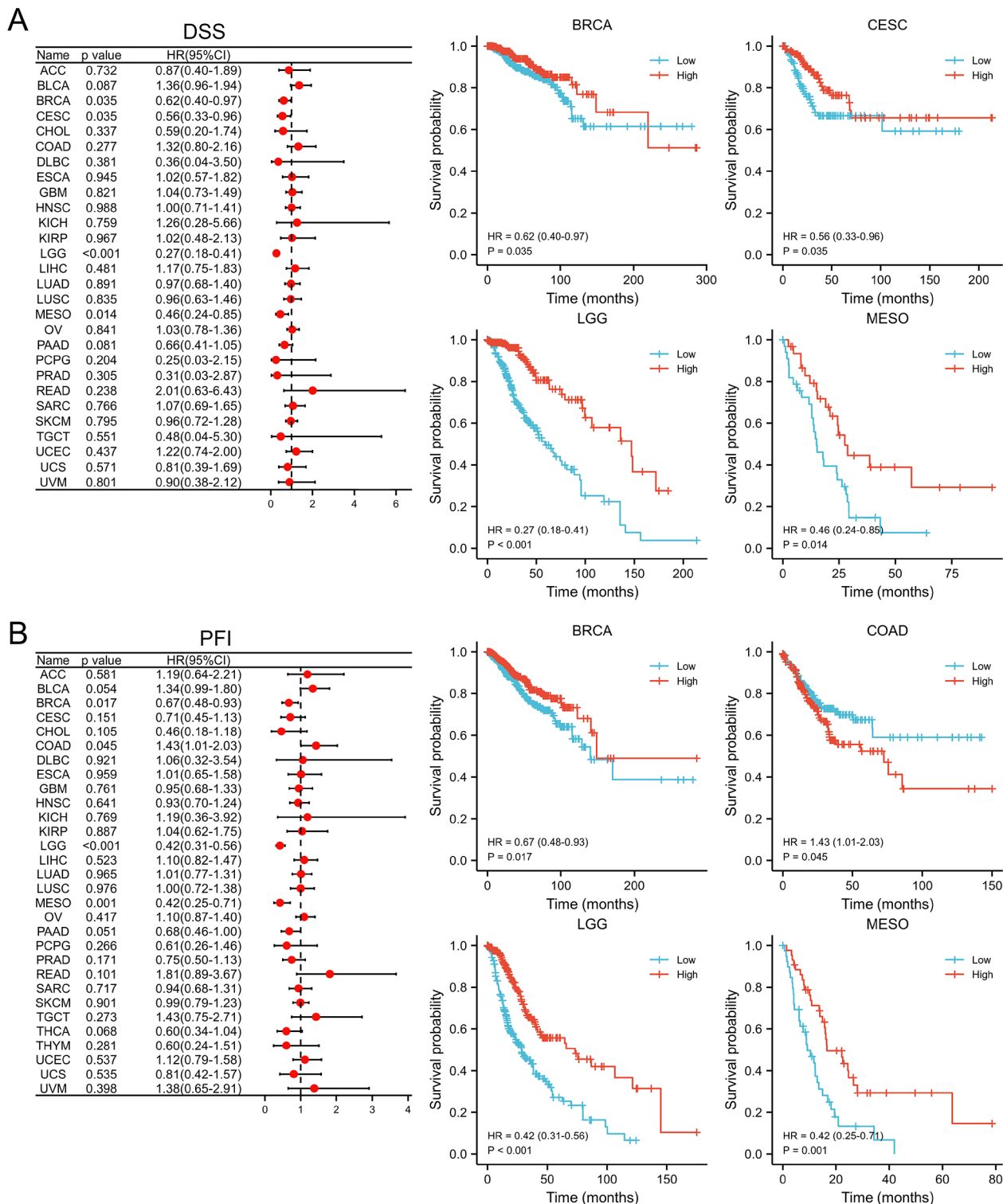
Figure 6. Association of TCEAL2 expression with disease-specific survival (DSS) and progression-free interval (PFI). (A) Forest plot of DSS in 33 types of cancers and Kaplan-Meier analysis of associations between TCEAL2 expression and DSS. (B) Forest plot of PFI in 33 types of cancers and Kaplan-Meier analysis of associations between TCEAL2 expression and PFI.

transport (Figure 10D). Two molecular functions were enriched. One is tau protein binding, and the other one is WW domain binding (Figure 10E). The related genes involved in WW domain binding were all TCEAL gene family (TCEAL 1,4,5, and 8, Figure 10E). Through KEGG analysis, 4 pathways were enriched. But only 2 (mRNA surveillance pathway and Ribosome biogenesis in eukaryotes) was significant

($P < .05$, Figure 10F). Figure 10G displayed the related genes of each pathway.

Discussion

Based on the gene expression data of 9 types of cancers from GEO dataset, we screened out one differentially expressed gene—TCEAL2 which belongs to the TCEAL family. In our
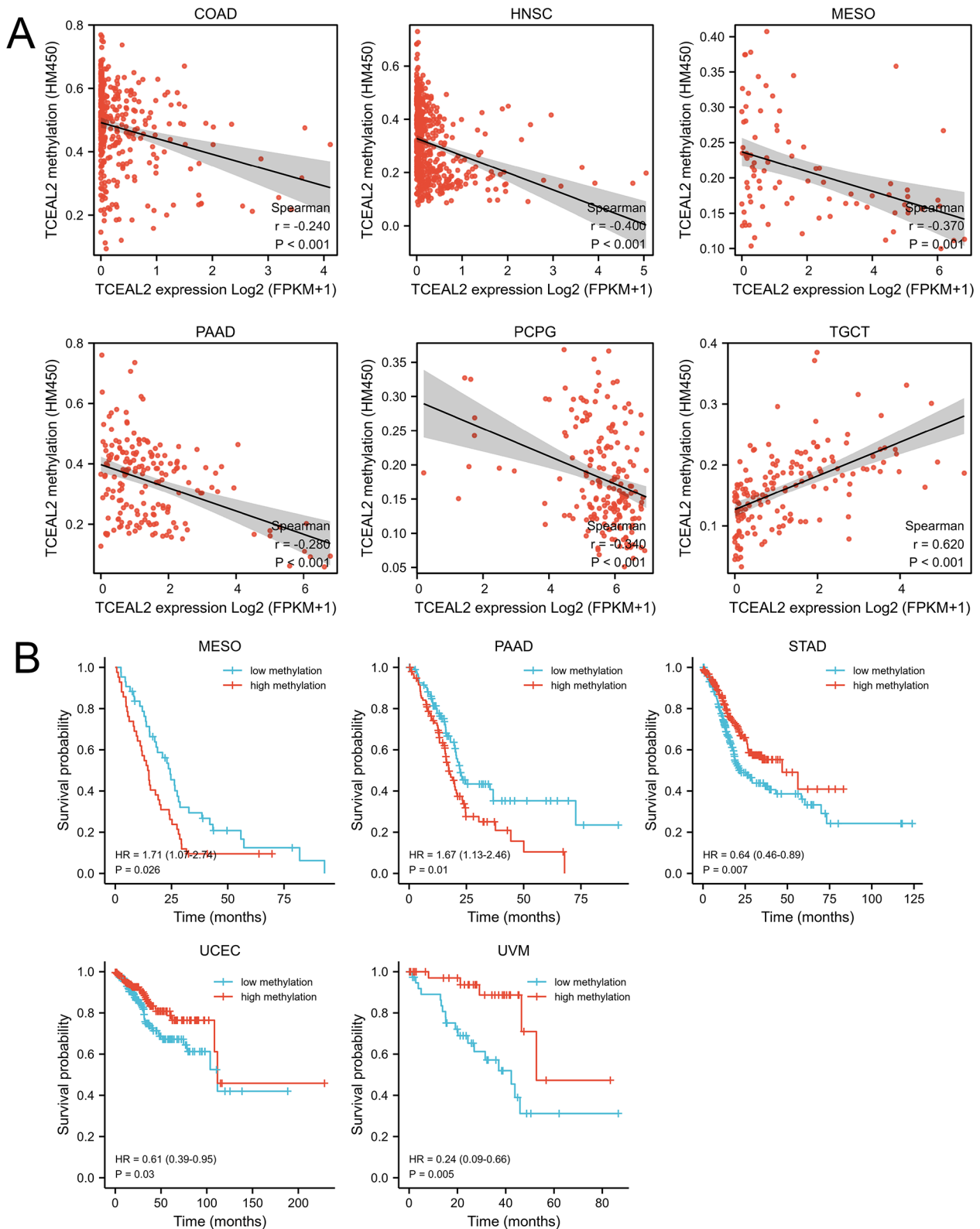
Figure 7. Associations of TCEAL2 methylation with TCEAL2 expression and clinical prognosis in cancers. (A) Correlation analysis between TCEAL2 expression and DNA methylation in 6 strongest correlated cancers. (B) Kaplan-Meier analysis of associations between TCEAL2 methylation and overall survival time (OS).

study, we found that TCEAL2 was down-regulated in all 9 types of cancer from GEO dataset and majority types of cancers from TCGA dataset. In other studies, the decreased expression of TCEAL2 was also verified in ovarian cancer, ccRCC, TCC, TGCT and oral cancer.[5-8] As the member of TCEAL family, TCEAL1, TCEAL4, and TCEAL7 also had the declined expression in various cancers. Through analyzing the cDNA arrays in 12 matched normal mucosa and
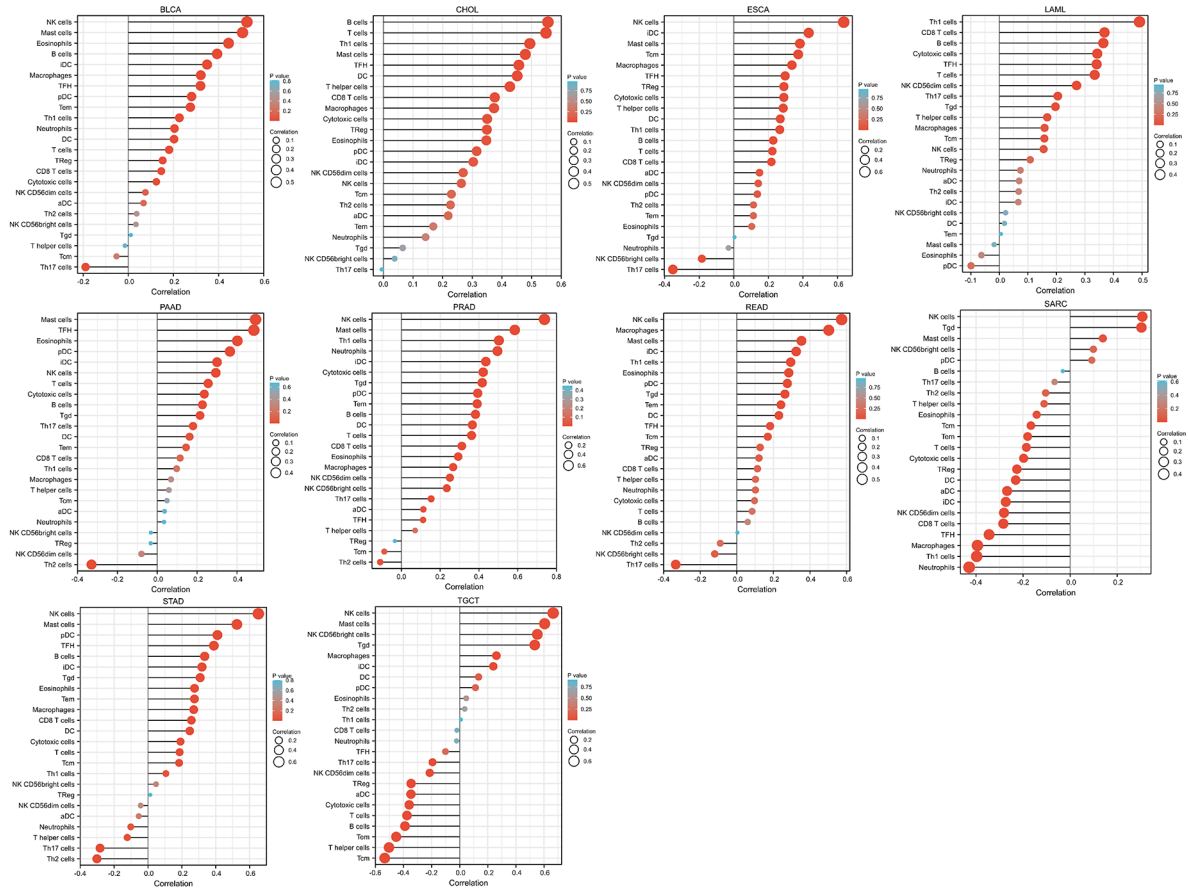
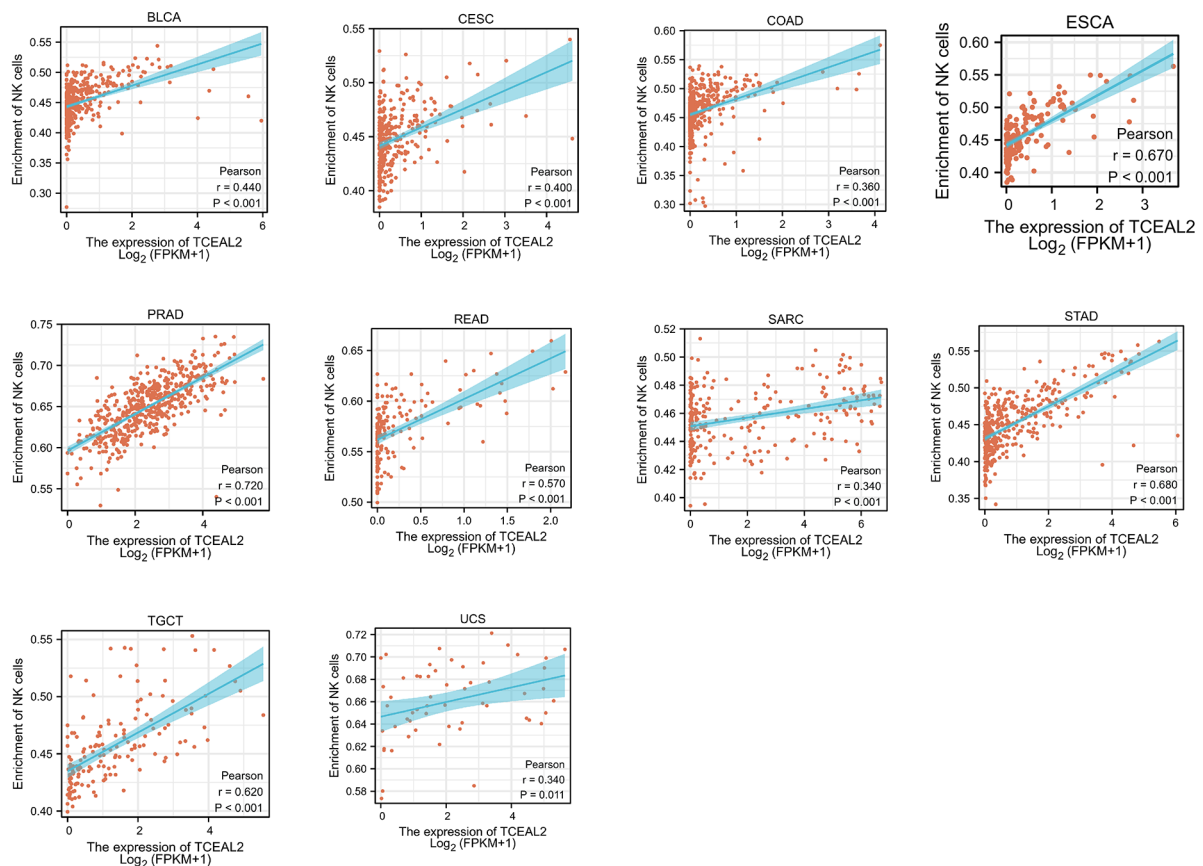**Figure 8.** Associations between TCEAL2 expression and immune cells. (A) Bubble chart of associations between TCEAL2 expression and 24 immune cells in 10 types of cancers. (B) Pearson's correlation test between TCEAL2 expression and NK cells.
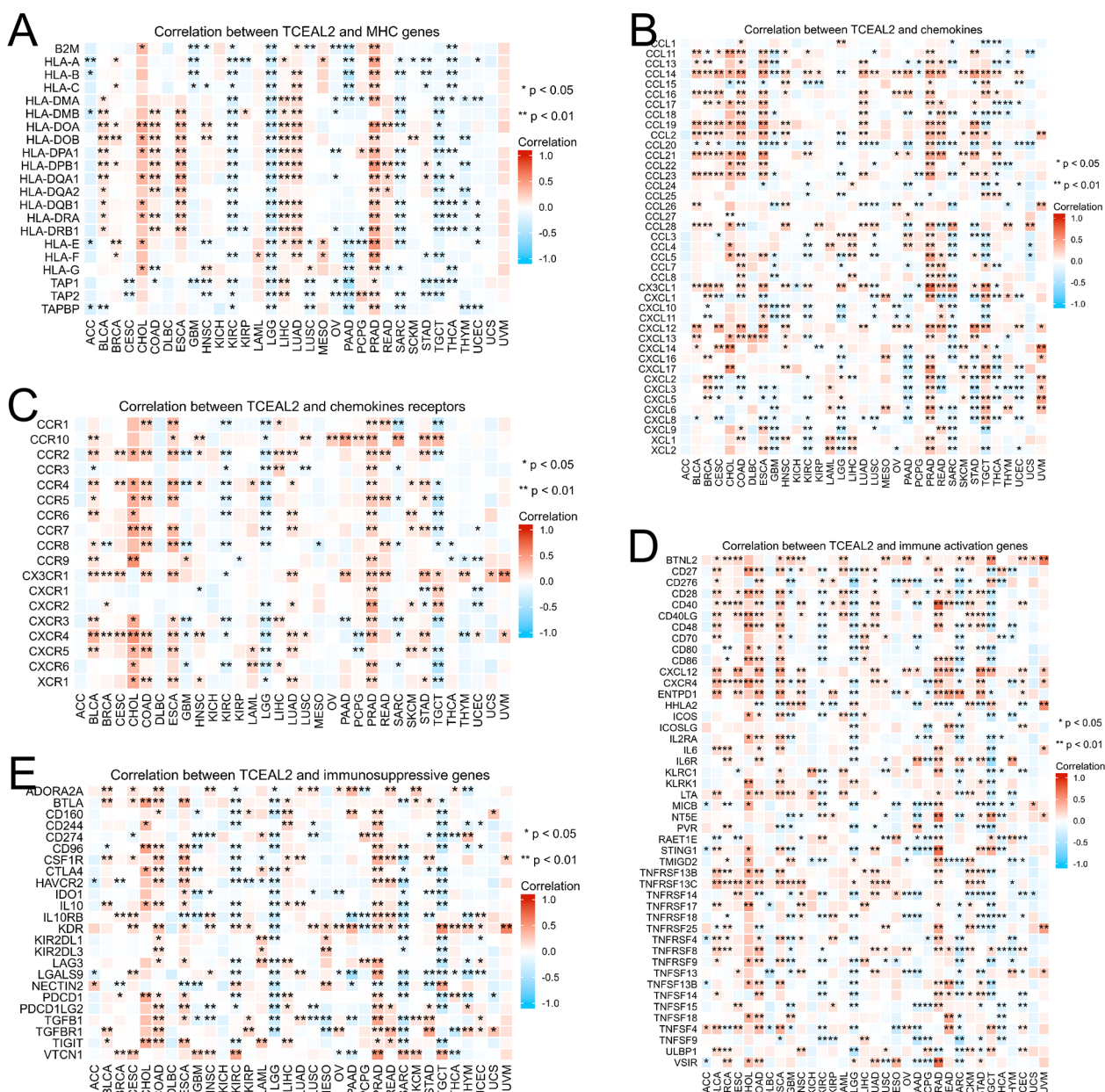
Figure 9. Associations of TCEAL2 expression with immune-related genes included genes of (A) major histocompatibility complex (MHC), (B) chemokine, (C) chemokine receptor, (D) immune activation, and (E) immunosuppressive.

esophageal cancers, TCEAL1 had reduced expression in many tumor samples.[9] Akaishi et al's study showed that TCEAL4 was absent or under-expressed in hepatic cell carcinomas, gastric cancers, colon cancers, anaplastic thyroid cancers, ovarian cancers, renal cell carcinomas and neuroblastomas.[10] TCEAL7 was down-regulated in many various cancers contained of breast, brain, prostate, gastric, ovarian, cervical and lung cancer.[11-13] We wonder whether the other members of TCEAL family were down-regulated in most cancers or not. Therefore, we compared the expressions of other TCEAL members between tumor and normal tissues in various cancers from TCGA dataset which showed in Supplemental Figure S3. Interestingly, we found that the expressions of TCEAL gene family were reduced in majority types of cancers. Researchers

had reported that TCEAL1, TCEAL2, and TCEAL7 inhibited tumor growth as a tumor suppressor. TCEAL1 was involved in the apoptosis of human cancer cells.[2] TCEAL2 inhibited cell proliferation and induced cell cycle arrest in S phase of ccRCC cells.[6] TCEAL7 induces ovarian cancer cell death, reduces colony formation efficiency and restricts ovarian epithelial cell transformation.[11,12,14] These results indicated that TCEAL gene family may function as tumor suppressors in multi cancers which need further studied.

We also investigated the association of TCEAL2 with clinical stage and survival. TCEAL2 expression was associated with clinical stage and the expression difference was mainly existed in stage I and other stages. The decrease of TCEAL2 expression affected the clinical prognosis in various cancers. Low
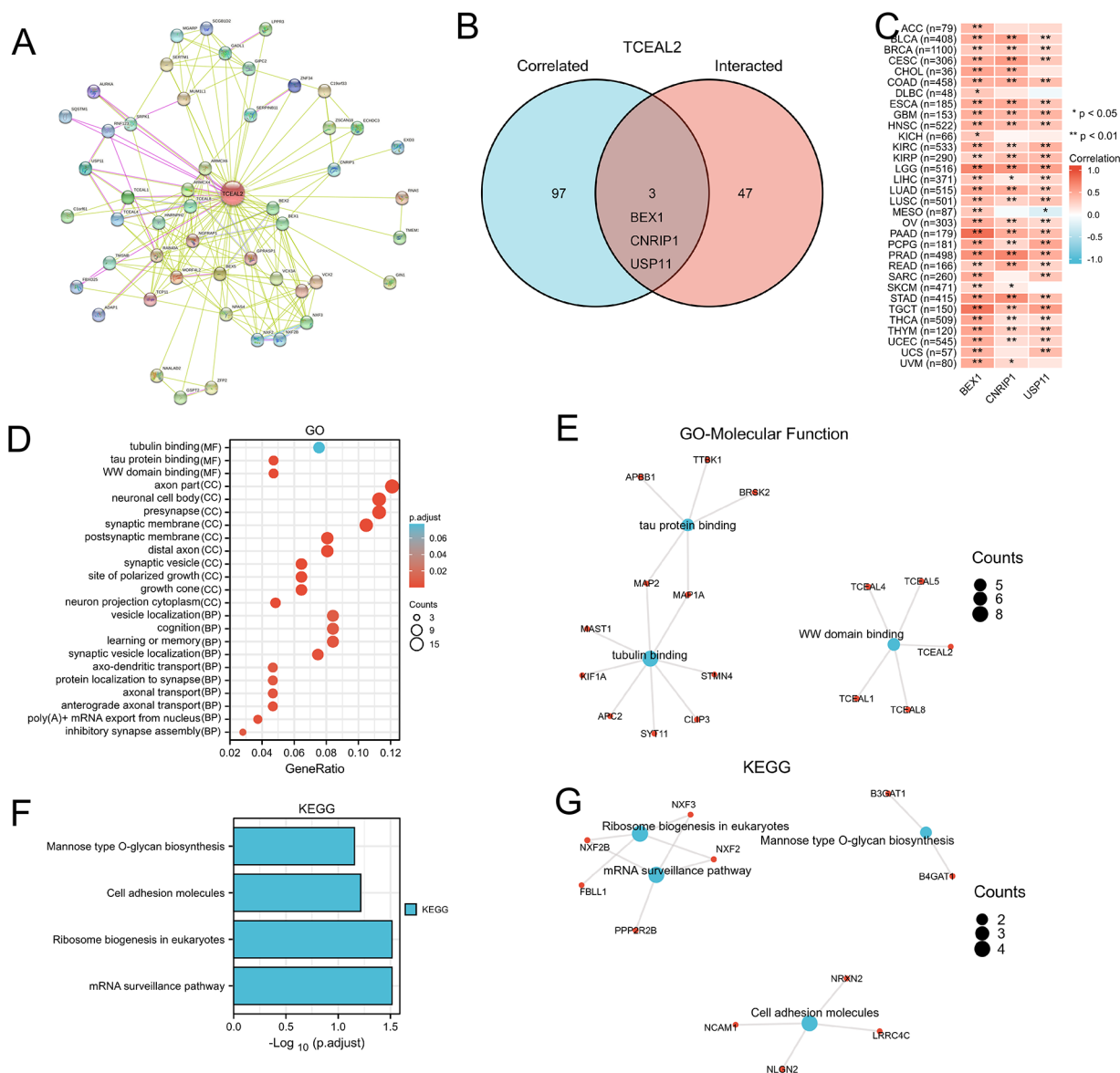
Figure 10. Enrichment analysis of TCEAL2: (A) 50 interacted genes with TCEAL2 from STRING website, (B) Venn diagram between 50 interacted genes from STRING website and 100 correlated genes from GEPIA2 database, (C) Associations of TCEAL2 with BEX1, CNRIP1, and USP11 in pan cancers, (D) GO enrichment analysis of 150 related genes, (E) Network enrichment analysis of Molecular function, (F) KEGG enrichment analysis of 150 related genes, and (G) Network enrichment analysis of KEGG pathway.

expression level of TCEAL2 was associated with shorter OS in BRCA, CESC, LGG, LIHC, MESO, and PAAD, shorter DSS in BRCA, CESC, LGG, and MESO, and shorter PFI in BRCA, LGG, and MESO. On the contrary, low expression led to longer OS in BLCA and LUAD, and longer PFI in COAD. Kim et al's study showed that ovarian cancer patients with low expression of TCEAL2 had longer OS time which was not observed in our study.[5] In the present study, we further explored the relationship between TCEAL2 promoter methylation and cancers. We found that TCEAL2 expression was related with DNA methylation and the methylation level could determine the prognosis of various cancers. These findings demonstrated that TCEAL2 may be a novel biomarker to predict clinical prognosis in various cancers.

Tumor microenvironment (TME) played crucial roles in tumor immune suppression, distant metastasis and the targeted therapy response.[15-17] As one of the important components of TME, immune-infiltration cells had crucial roles in occurrence and development of tumors.[18,19] We analyzed the relationship of TCEAL2 with immune-infiltration cells and found that TCEAL2 was significantly associated with immune cells in pan-cancer. Among them, NK cells were markedly and positively related with TCEAL2 in majority cancers. NK cells are innate immune cells which play important roles in immune responses to cancer.[20,21] It can recognize malignant cells through an array of germline-encoded receptors on their surface, and rapidly kill tumor cells through targeted cytotoxicity to control tumor growth and metastasis.[22,23] Our study also

revealed the obviously relationship between TCEAL2 and immune-related genes. These results indicate that TCEAL2 may function with immune cells especially NK cells to affect immune infiltration of tumor cells.

Furthermore, the role of TCEAL2 in tumor was investigated. We found that TCEAL2 was significantly associated with one member of the brain expressed X-Linked (BEX)- BEX1. BEX family, containing BEX1, BEX2, BEX3, BEX4, and BEX5, had similar sequence and adjacent location with TCEAL family, which formed the BEX/TCEAL transcriptional regulator cluster.[24] BEX family proteins are known to play a role in neuronal development and recent studies suggest a role in cancers.[25-29] BEX1 suppressed tumor cell growth of esophageal squamous cell cancer, colorectal cancer, acute myeloid leukemia, oral squamous cell carcinoma, malignant glioma.[30-34] But in lung adenocarcinoma, malignant pleural mesothelioma and GBM, BEX1 promoted tumor cell growth.[35,36] TCEAL2 may be worked with BEX1 to control tumor growth. BEX/TCEAL gene display neural-enriched patterns and integrated into existing signaling pathways in the development, maintenance, and function of the CNS. Our GO enrichment analysis proved it. The CC analysis revealed that TCEAL2 was mainly localized in axon and synaptic vesicle, and its biological process was mainly about synaptic vesicle localization, cognition and axonal transport. The tau protein binding is one molecular function of TCEAL2. Tau protein is microtubule-associated protein, predominantly expressed in neurons and played important roles in microtubule assembly and stabilization, nerve growth and development, and axons transport.[37,38] Recent studies revealed that tau protein is a predictive marker of survival and taxane resistance of cancer patients.[39-41] WW domain binding is another molecular function enriched. WW domains are involved in very critical cellular processes including transcription, splicing, ubiquitination, cell growth, proliferation, differentiation and apoptosis.[42] In cancer, WW domain proteins function as tumor suppressor or oncogene.[42-44] TCEAL1, TCEAL4, TCEAL5, and TCEAL8 were enriched genes of WW domain binding. Taken together, TCEAL family may be influence tumor growth through WW domain which need further studied. KEGG analysis revealed that TCEAL2 involved in mRNA surveillance pathway and Ribosome biogenesis in eukaryotes pathway which also need further research.

Conclusion

In conclusion, our first pan-cancer analyses of TCEAL2 indicates that TCEAL2 was down-regulated in majority types of cancers. And it significantly related with clinical prognosis and DNA methylation indicating that TCEAL2 may be a novel prognostic factor of multi tumors. Moreover, TCEAL2 may affect tumor growth through tumor immunity and its molecular function including tau protein and WW domain binding.

Author Contributions

YS downloaded, analyzed the data and drafted the paper. JZ reviewed the manuscript. All authors reviewed the final manuscript.

Availability of Data and Materials

The raw data this study is used derived from the GEO (https://www.ncbi.nlm.nih.gov/geo/), TCGA(http://portal. gdc.cancer.gov), CCLE (https://portals.broadinstitute.org/ ccle/), GTEx (https://commonfund.nih.gov/GTEx), UCSC Xena (https://xena.ucsc.edu/), STRING (https://www.string-db.org/), GEPIA2 database(http://gepia2.cancer-pku. cn/#index), and TIMER 2.0 database (http://timer.cistrome. org/), which are publicly available databases.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Supplemental Material

Supplemental material for this article is available online.

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