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A pan-cancer analysis of TNFAIP8L1 in human tumors

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Abstract

TNFAIP8L1, as a recently identified member in TNFAIP8 family, plays an important role in tumorigenesis. However, a pan-cancer analysis of TNFAIP8L1 in human tumors has not been conducted until now. The main purpose of study is to investigate TNFAIP8L1 during 33 different types of human tumors by using TCGA and GTEx. The pan-cancer analysis showed that TNFAIP8L1 was significantly over-expressed in 15 cancers and low-expressed in 9 cancers. There were distinct relations between TNFAIP8L1 expression and prognosis of patients with cancer. Furthermore, we also found that DNA methylation and RNA modification of TNFAIP8L1 were associated with many cancers. And then, we detected that TNFAIP8L1 level was positively associated with cancer-associated fibroblasts (CAFs) in many tumors. And, we obtained that TNFAIP8L1 expression was related with most of immune inhibitory and stimulatory genes in multiple types of tumors. We also found TNFAIP8L1 expression was correlated with most of chemokine, receptor, MHC, immunoinhibitor and immunostimulator gens in most of cancers. Moreover, we detected TNFAIP8L1 expression was associated with TMB and MSI in several tumors. Finally, TNFAIP8L1 gene had a significant positive association with 5 genes including BCL6B, DLL4, PCDH12, COL4A1 and DLL4 in the majority of tumors. GO enrichment and KEGG pathway analyses showed that TNFAIP8L1 in thepathogenesis of cancer may be related to "purine nucleoside binding," "purine ribonucleoside binding," "ECM-receptor interaction," etc. Our first pan-cancer study may provide a deep comprehending of TNFAIP8L1 in tumoeigenesis from different tumors.

Abbreviations: LAML = acute myeloid leukemia, ACC = adrenocortical cancer, BLCA = bladder cancer, BRCA = breast cancer, CESC = cervical cancer, CHOL = bile duct cancer, COAD = colon cancer, ESCA = esophageal cancer, GBM = glioblastoma, HNSC = head and neck cancer, KICH = kidney chromophobe, KIRC = kidney clear cell carcinoma, KIRP DLBC = kidney papillary cell carcinoma large B-cell lymphoma, LGG = lower grade glioma, LIHC = liver cancer, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, OV = ovarian cancer, PAAD = pancreatic cancer, PCPG PRAD = pheochromocytoma & paraganglioma prostate cancer, READ = rectal cancer, SARC = sarcoma, SKCM = melanoma, STAD = stomach cancer, TGCT = testicular cancer, THCA = thyroid cancer, THYM = thymoma, UCEC = endometrioid cancer, UCS = uterine carcinosarcoma, UVM = ocular melanomas.

Keywords: pan-cancer analysis, prognosis, TNFAIP8L1, tumors

1. Introduction

Because of the complexity of tumorigenesis, it is significant to conduct gene pan-cancer analyses and explore the relationship between clinical prognosis and possible molecular mechanisms.[1] Databases such as TCGA and GTEx could provide functional genomics data about a wide variety of tumors and enable us to conduct pan-cancer analyses.

Tumor necrosis factor (TNF)-α-induced protein 8 (TNFAIP8) like 1 (TNFAIP8L1, TIPE1), as a recently identified member in TNFAIP8 family, acts distinct roles in tumorigenesis and regulation of immune.[2,3] It has been reported that TNFAIP8L1 is implicated in many types of cancer. Several recently studies reported that the expression of TNFAIP8L1 was significant downregulation in breast cancer,[4,5] colorectal cancer,[6] gastric cancer,[7] hepatocellular carcinoma,[8] lung cancer,[9] osteosarcoma[10] and ovarian cancer.[11,12] Additionally, TNFAIP8L1 seems to have opposite functions in different types of cancers. Recent research about cervical cancer revealed that TNFAIP8L1 promoted the cancer progression through repression of p53 acetylation.[13] Nasopharyngeal carcinoma (NPC) study also found that TNFAIP8L1 was significantly upregulated in NPC.[14] These findings suggested that TNFAIP8L1 may play different

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The datasets generated during and/or analyzed during the current study are publicly available.

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roles in different cancers. However, studies of TNFAIP8L1 have been confined to a few types of cancer, and its role in other cancers remains unclear.

In the study, we investigated expression profiles of TNFAIP8L1 across different types of cancer in a pan-cancer analysis based on several databases. A number of factors, including gene expression, survival prognosis, genetic alteration, DNA methylation, immune infiltration, related immunological markers, and gene enrichment analysis, were considered to explore the potential molecular mechanism by which TNFAIP8L1 was involved in the pathogenesis or clinical prognosis of cancer.

2. Materials and methods

2.1. Gene expression analysis

TNFAIP8L1 expression of tumor tissues and normal tissues in TCGA and GTEx data were detected by the method of R package and R language software. The $\log_2$FC (fold change) cutoff was set 1, and a *P* value cutoff was 0.01. Gene Expression Profiling Interactive Analysis, vesion 2 (GEPIA2, web: http://gepia2.cancer-pku.cn/#analysis) tool was used to acquire the violin plots of TNFAIP8L1 expression in different types of pathological stages of TCGA tumors. The UALCAN website (http://ualcan.path.uab.edu/) can analyze protein expression utilizing data from the TCGA and CPTAC datasets.

2.2. Survival prognosis analysis

GEPIA2 tool was used to analyze overall survival (OS) and disease-free survival (DFS) significance map data (setting Group cutoff = median) of TNFAIP8L1 gene in all TCGA tumors. Log-Rank method was used for statistical hypothesis test.

2.3. Genetic alteration analysis

The cBioPortal tool (http://www.cbioportal.org/) was used to obtain genetic alteration characteristics of TNFAIP8L1.[15] Genetic alteration characteristics including alteration frequency, mutation type, mutated site information, copy number alteration (CNA) and 3-dimensional (3D) structure of protein were collected. Survival data including overall, progression-free, disease-free and disease-free survival were obtained in TCGA tumors with or without TNFAIP8L1 genetic alteration with "Comparison."

2.4. TNFAIP8L1 methylation analysis and RNA modification

The level of methylation of YKT6 in different tumors was obtained from TCGA dataset by using UCLCAN.[16] And multiple probes of TNFAIP8L1 associated with DNA methylation in diverse types of tumor of TCGA were detected by the method of R package and R language software. We went further detecting the relationship between TNFAIPL1 and RNA modification with tumors in TCGA by using R package and R language software.

2.5. Immune infiltration analysis

We then used TIMER2 online tool to acquire the data of association between TNFAIP8L1 level and immune infiltration. The gene of TNFAIP8L1 was entered into "gene name field" for analysis. The results of the selected immune cells were performed by the method of a heatmap and scatter plots. The TIMER algorithms, *EPIC*, *XCELL*, *MCPCOUNTER*, *CIBERSORT-ABS*, *QUANTISEQ*, and *CIBERSORT* were used to evaluate the degree of immune infiltrating situation.[17] We went further detecting the relationship between TNFAIP8L1 and tumor mutational burden (TMB), microsatellite instability (MSI) and m^6A regulators with tumors in TCGA by using R package and R language software.

2.6. TNFAIP8L1-related gene enrichment analysis

The STRING online tool (https://cn.string-db.org/) was used to detect the proteins which bind to TNFAIP8L1. The following main parameters were set: score for minimum interaction required ["low confidence (0.150)"], maximum number of interactors shown ("no more than 50"), examples of network edges ("evidence") and active interactions ("experiments"). Finally, the available experimentally determined TNFAIP8L1-binding proteins were detected.

Based on the datasets of all TCGA tumors and normal tissues, we identified the top 100 TNFAIP8L1-correlated targeting genes by using GEPIA2 platform. Analysis of gene Pearson correlation pairwise was conducted between TNFAIP8L1 and genes which were selected. The mean of log2 TPM was used to calculate the dot plot. The heatmap data was presented using TIMER2 "Gene_Corr" module. Spearman's rank correlation was calculated using purity-adjusted test.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were analyzed by using DAVID website and the "ggplot2" R packages. The results of KEGG were shown as bubble chart. Finally, the gene ontology (GO) enrichment was analyzed by the method of "cluster Profiler" R package and R language software. As a results of GO, bar plots were produced.

3. Results

3.1. Gene expression analysis data

We analyzed the expression of TNFAIP8L1 by using tumor tissues and normal tissues in TCGA and GTEx data to get detailed statistical calculations. We have provided information of the sample sizes in each cancer type in Table S1, Supplemental Digital Content, http://links.lww.com/MD/K832. As shown in Figure 1A, TNFAIP8L1 was significantly upregulated in BRCA, CESC, DLBC, ESCA, GBM, HNSC, KIRC, LAML, LIHC, OV, PAAD, SKCM, STAD, THYM and UCEC. By contrast, the expression of TNFAIP8L1 was low expressed in CHOL, COAD, KICH, KIRP, LUAD, PRAD, READ, TGCT and THCA. There were no statistical differences in ACC, BLCA, LGG, LUSC, PCPG and UCS. The protein level of TNFAIP8L1 was significantly lower in UCEC, LUAD, GBM and HCC than normal tissues by CPTAC dataset (Fig. 1B). Furthermore, we use GEPIA2 online tool to detect expression of TNFAIP8L1 in different tumor stages. The data of Figure 1C showed that there was a significant relation between level of TNFAIP8L1 and the pathological stages of several tumors, including HNSC ($P = .0044$), KIRP ($P = .0114$), LIHC ($P = .0497$), STAD ($P = .0029$), and THCA ($P = .000345$).

3.2. Survival prognosis analysis

To evaluate the relationship between TNFAIP8L1 and the prognosis of patients with diverse kinds of tumors, the tumors were dichotomized into 2 groups (high TNFAIP8L1 expression and low TNFAIP8L1 expression group) based on the level of TNFAIP8L1 in TCGA and GTEx datasets. As data displayed in Figure 2A, we found that high-expression of TNFAIP8L1 was positively related to poor prognosis of overall survival (OS) in several types of tumors including ACC ($P = .0072$), LGG ($P = 7.6e\text{-}05$) and MESO ($P = .00079$). While, low-expression of TNFAIP8L1 was positively related to poor prognosis of OS in KIRC ($P = .01$). As shown in Figure 2B, high-expression of TNFAIP8L1 was associated with poor disease-free survival (DFS) prognosis for ACC ($P = 9.9e\text{-}05$),

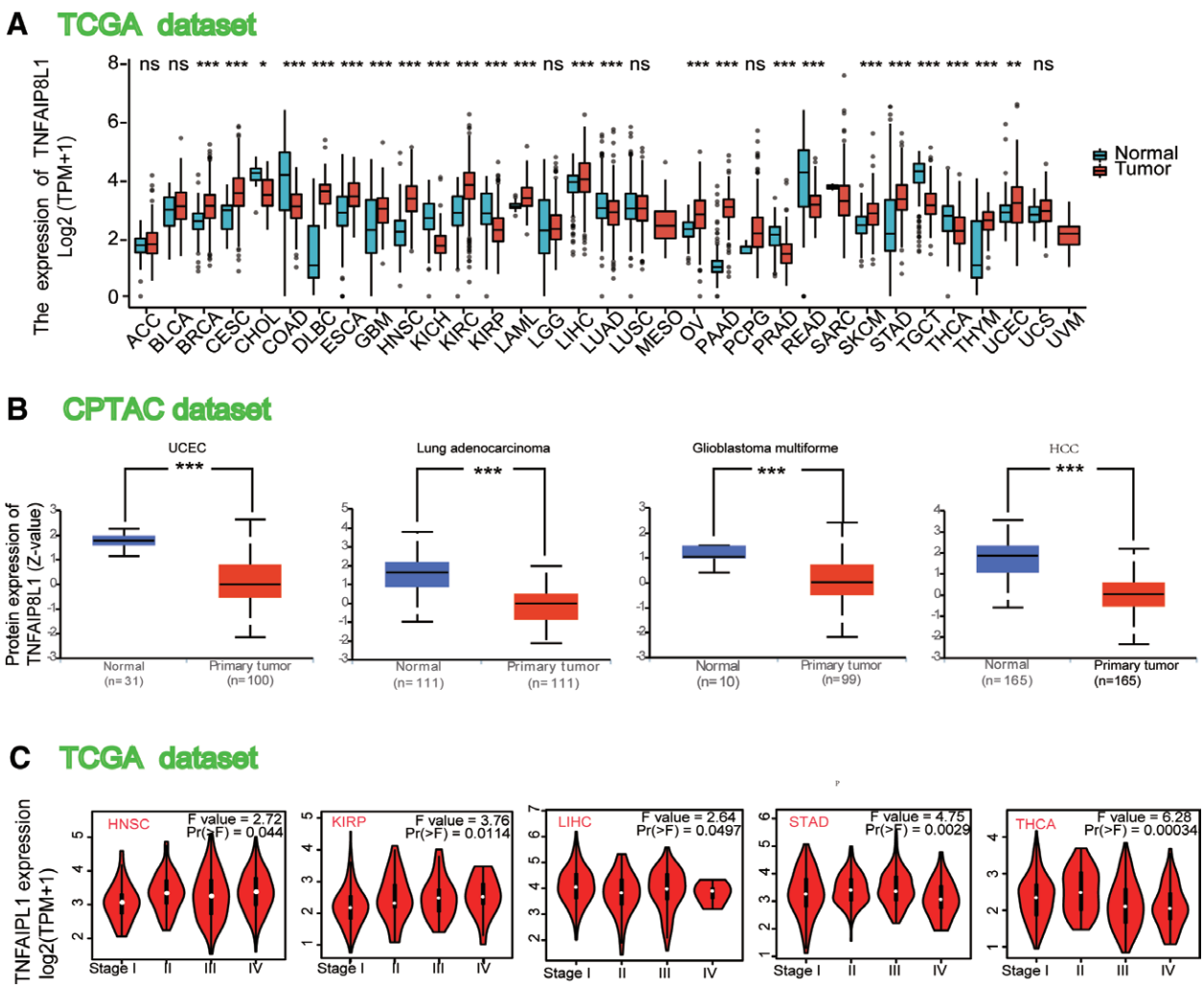

Figure 1. Expression of TNFAIP8L1 in different types of tumors and pathological stages (A) expression of TNFAIP8L1 was analyzed.(*$P < .05$, **$P < .01$, ***$P < .001$). (B) We analyzed the protein level of TNFAIP8L1 between normal tissue and primary tissue of was UCEC, LUAD, GBM and HCC by CPTAC dataset. (***$P < .001$) (C) We furthermore analyzed expression of TNFAIP8L1 in different tumor stages (stageI, stageII, stageIII, stageIV) of HNSC ($P = .0044$), KIRP ($P = .0114$), LIHC ($P = .0497$), STAD ($P = .0029$), and THCA ($P = .000345$).

KICH ($P = .005$), KIRP ($P = .046$), LGG ($P = .0026$), PRAD ($P = .048$), TGCT ($P = .045$), and UVM ($P = .03$).

3.3. Genetic alteration analysis

The genetic alteration of TNFAIP8L1 in different types of tumors from TCGA was analyzed. As data described in Figure 3A, TNFAIP8L1 alteration frequency (4.71%) is the highest in Sarcoma, with the primary alteration type being "Amplification." We obtained that the second-most frequency of TNFAIP8L1 alteration (3.03%) in cases with Cervical Squamous Cell Carcinoma with "Deep Deletion" as the main type. "Deep Deletion" was the sole form of variation in Diffuse LBC, Uterine C, Esophageal A and Uuveal M. "Amplification" was the sole form of variation in Brain L GG, Mesothelioma, Pheonchromocytoma and P and Adrenocortical C. The additional mutations and location of TNFAIP8L1 were shown on Figure 3B. Missense as the predominant genetic alterations was obtained. For example, a missense mutation of R91C/H alteration was detected in 1 case of THYMOMA, 1 case of UCEC and 1case of CESC (Fig. 3C). In UCEC case, we detected whether TNFAIP8L1 genetic alterations affect clinical survival prognosis. We found that prognosis in terms of OS ($P = .683$), DFS ($P = .311$), progression-free survival (PFS) ($P = .421$), and disease-specific (DS) ($P = .972$) were no significant difference between TNFAIP8L1 altered group and unaltered group. In CESC case, we also detected whether TNFAIP8L1 genetic alterations affect clinical survival prognosis. We found that prognosis in terms of OS ($P = .0109$) and DFS ($P = 3.729e\text{-}4$) were significant difference between TNFAIP8L1 altered group and unaltered group. This means that unaltered group of TNFAIP8L1 was positively related to better prognosis of OS and DFS in CESC tumor.

3.4. DNA methylation analysis and RNA modification

DNA methylation, as an important epigenetic regulator of post-replication, played a significant role in tumorigenesis.[18] Figure 4 showed that there was a hypermethylation status in promoter region of TNFAIP8L1 in COAD and PRAD. While, there was a hypomethylation level in the promoter region of TNFAIP8L1 in KIRC, BLCA, READ, TGCT, HNSC, THCA, GBM, KIRP, LIHC, CESC and UCEC. The occurrence and development of tumors was affected by up-regulated or down-regulated DNA methylation state of target gene.

As the data shown in Figure 5, we obtained a significant positive association between TNFAIP8L1 and RNA modification-related genes (including m1A, m5C and m6A). TNFAIP8L1 expression was significantly positively correlated with most of m1A, m5C and m6A genes in multiple types of tumors including GBM, WT, OV,

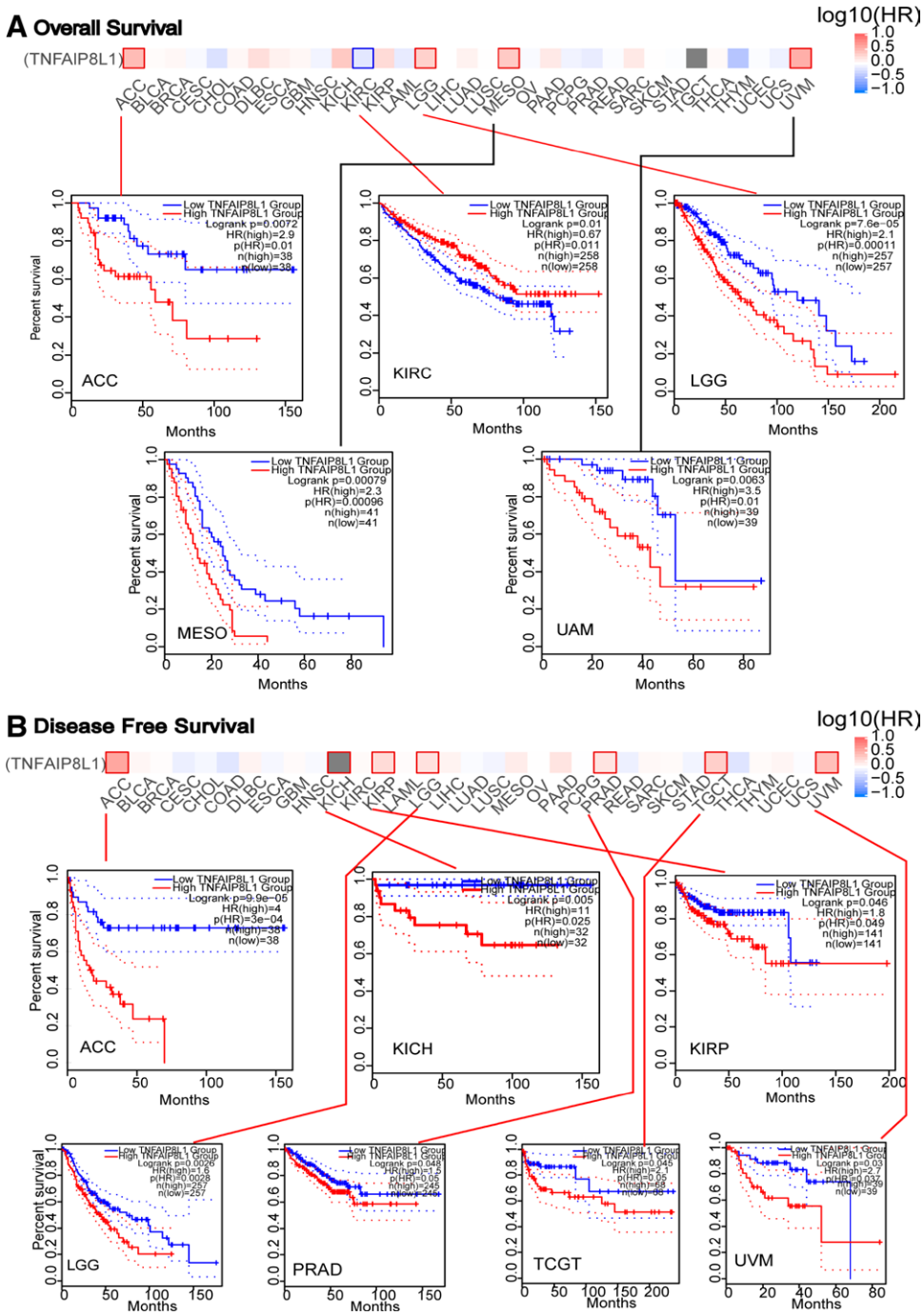

Figure 2. The correlation between TNFAIP8L1 expression and survival prognosis of tumors in TCGA. We used the GEPIA2 tool to perform overall survival (A) and diseasefree survival (B) analyses of different tumors in TCGA by TNFAIP8L1 gene expression. The survival map and Kaplan–Meier curves with positive results are shown.

ACC, PAAD, BRCA, UCEC, LIHC, ALL, THCA, UVM, HNSC, COAD, COADREAD, LAML, BLCA, ESCA, KIRP, DLBC, PRAD, LUAD, READ, STAD, STES, KIPAN, THYM, KICH and KIRC. We also found ELAVL1, as a reader of M6A, was significant positively associated with TNFAIP8L1 in all cancers except CHOL. These results revealed that expression of TNFAIP8L1 might be mainly regulated by posttranscriptional modification of RNA.

3.5. Immune infiltration analysis

Tumor-infiltrating immune cells, as an integral part of tumor microenvironment (TME), played a crucial role in tumor progression and development.[19,20] We then used the TIMER, CIBERSORT, CIBERSORT-ABS, TIDE, XCEL, MCPCOUNTER, QUANTISEQ and EPIC algorithms to detect the possible association between the different immune infiltration and immune cells and TNFAIP8L1 level in different types of tumors in TCGA. As data shown in Figure 6A, we discovered a significantly positively association between TNFAIP8L1 level and neutrophil cell in tumors of BLCA. We also detected that TNFAIP8L1 level was positively associated with cancer-associated fibroblasts (CAFs) for TCGA tumors of BRCA, COAD, HNSC, HNSC-HPV-, LUAD, PAAD, PARD, SKCM, SKCM-Metastasis, STAD and TGCT (Fig. 6B).

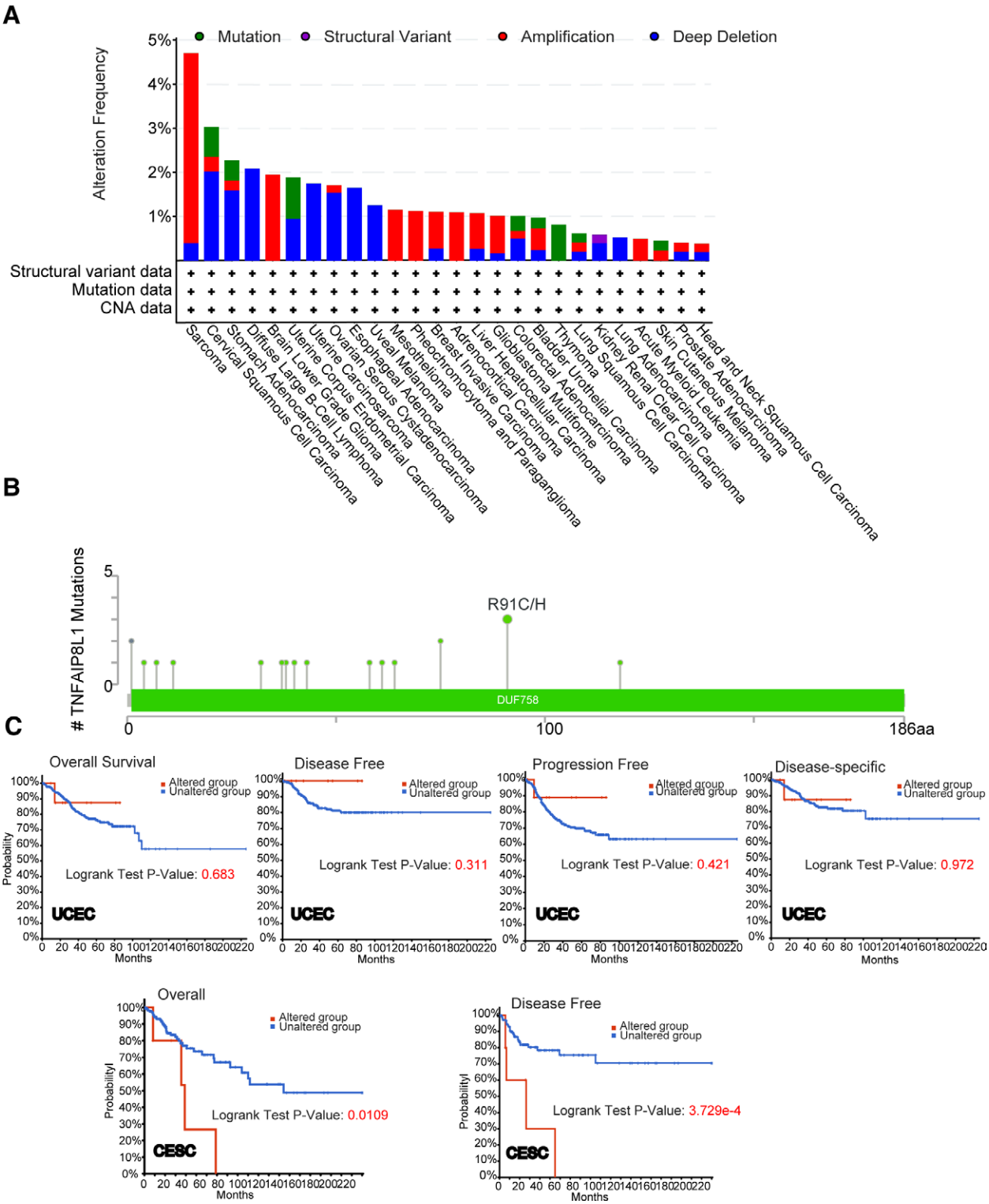

Figure 3. Mutation feature of TNFAIP8L1 in different types of cancers of TCGA. The mutation features of TNFAIP8L1 were analyzed by using the cBioPortal tool. (A) The alteration frequency of mutation type was displayed. (B) The alteration frequency of mutation site was displayed. (C) The potential correlation between mutation status and overall, disease-specific, disease-free and progression-free survival of UCEC and overall and disease-free of CESC were analyzed by using the cBioPortal tool.

3.6. The relationship between TNFAIP8L1 and immune checkpoints and immune moderator genes

To further investigate the relationship between TNFAIP8L1 expression and immune checkpoints and immune moderator genes, we carried out a comprehensive correlation analysis. As Figure 7 shows, expression of TNFAIP8L1 was significantly positively related with most of immune inhibitory and stimulatory genes in multiple types of tumors including KIPAN, UVM, PRAD, KICH, PAAD, PCPG, COAD, COADREAD, LUAD, OV, KIRP, KIRC, BLCA, GBMLGG, LGG, HNSC, LUSC, BRCA and STAD. While TNFAIP8L1 expression was negatively related with most of immune inhibitory and stimulatory genes in TGCT

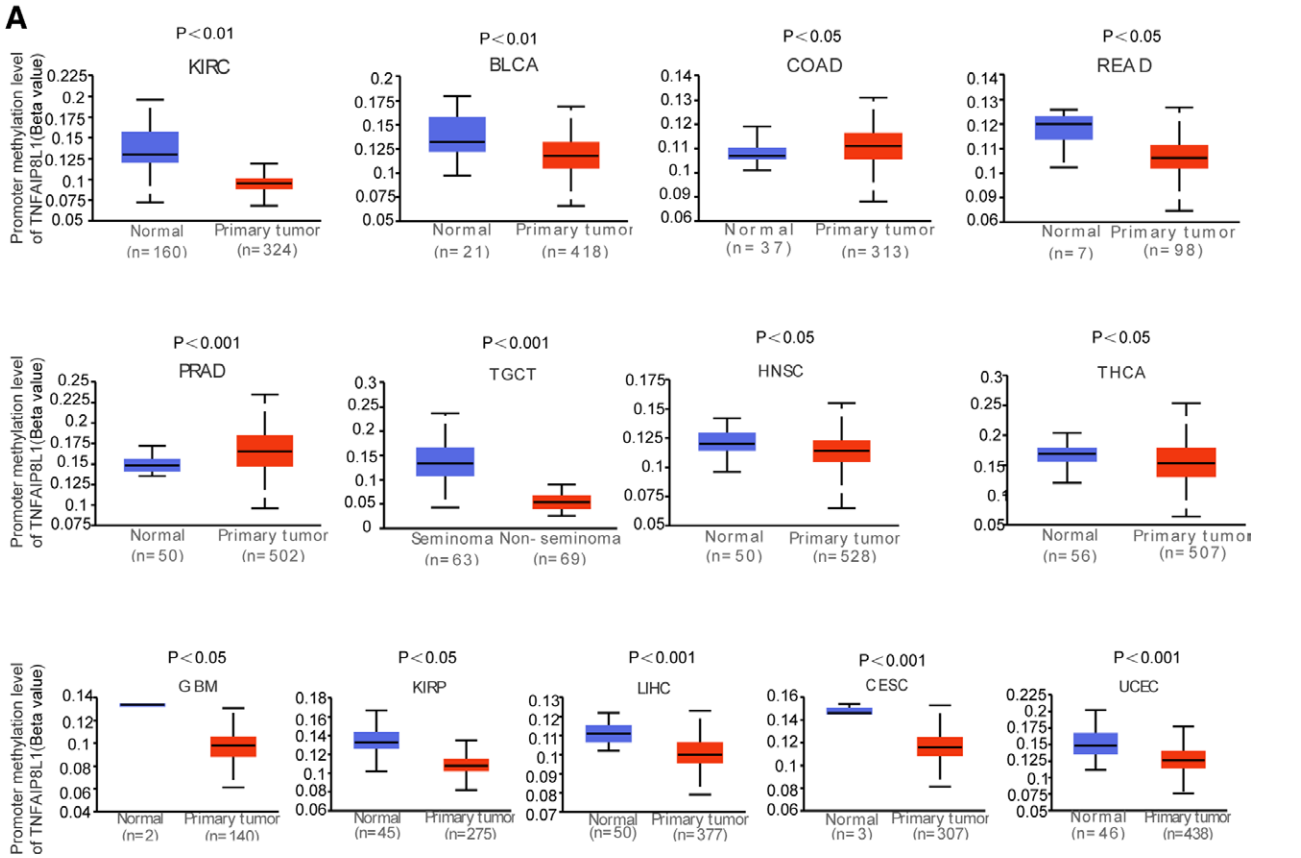

Figure 4. DNA methylation analysis of TNFAIP8L1 in different types of tumors. Based on UALCAN and TCGA databases, we found different methylation expression levels of TNFAIP8L1 in COAD, PRAD, KIRC, BLCA, READ, TGCT, HNSC, THCA, GBM, KIRP, LIHC, CESC and UCEC tumor tissues compared to normal tissues.

and THCA. As data shown in Figure 8, TNFAIP8L1 expression was notably positively correlated with most of chemokine, receptor, MHC, immunoinhibitor and immunostimulator gens in multiple kinds of cancers including KIPAN, UVM, PRAD, KICH, PAAD, PCPG, COAD, COADREAD, LUAD, OV, KIRP, KIRC, BLCA, HNSC, LUSC, BRCA, STAD, GBMLGG and LGG, but negatively correlated with most of these genes in TGCT and THCA.

3.7. The relationship between TNFAIP8L1 and tumor mutational burden (TMB) and microsatellite instability (MSI)

Moreover, we detected the relationship between TNFAIP8L1 and TMB and MSI with tumors. As described in Figure 9A, we obtained that the expression of TNFAIP8L1 was positively associated with TMB in ACC (P = .0046). While, the expression of TNFAIP8L1 was negative association with TMB in LAML (P = .031) and THCA (P = .026). As described in Figure 9B, we also explored that TNFAIP8L1 expression was positively correlated with MSI in SARC (P = .025) and LUSC (P = .0001). By contrast, the expression of TNFAIP8L1 was negatively correlated with MSI in BRCA (P = .041) and UCEC (P = .013). These results suggested the genetic alteration of TNFAIP8L1 may be considered as potential novel drivers of some tumors.

3.8. Enrichment analysis of TNFAIP8L1-related gene

To explore the mechanism of TNFAIP8L1 in tumorigenesis, we then sought to identify TNFAIP8L1-binding proteins and TNFAIP8L1 level-correlated genes for a variety of pathway enrichment analyses. In Figure 10A, we got 50 TNFAIP8L1-binding proteins by using STRING online tool. We also obtained the top 100 genes which associated with TNFAIP8L1 expression by using GEPIA2 online tool. As data shown in Figure 10B, we found that the expression of TNFAIP8L1 was positively related with BCL6B (R = 0.4), DLL4 (R = 0.4), PCDH12 (R = 0.4), COL4A1 (R = 0.38) and DLL4 (R = 0.38) genes (all P < .001). Heatmap data showed that TNFAIP8L1 gene had a significant positive association with the above 5 genes in the majority of tumors (Fig. 10C).

And then, we used KEGG pathway and GO enrichment analyses to explore the functions of TNFAIP8L1. In molecular function (MF) term, "purine nucleoside binding," "purine ribonucleoside binding," "GTP binding" were mainly involved in the influence of TNFAIP8L1 on tumor pathogenesis. In cellular component (CC) term, "host cell cytoplasm," "host cell cytoplasm part" and "collagen-containing extracellular matrix" were mainly associated with the influence of TNFAIP8L1 on tumor pathogenesis. In biological process (BP) term, "cell-substrate adhesion," "kidney vasculature development" and "renal system vasculature development" were mainly enriched in the influence of TNFAIP8L1 on tumor pathogenesis. In KEGG pathway, "ECM-receptor interaction," "complement and coagulation cascades" and "focal adhesion" were related to the influence of TNFAIP8L1 on tumor pathogenesis (Fig. 10D and E).

4. Discussion

In the TNFAIP8 family, TNFAIP8L1 contains a death effector domain and is located on chromosome 19p13.3. TNFAIP8L1 seems to play a more complex role in tumor progression

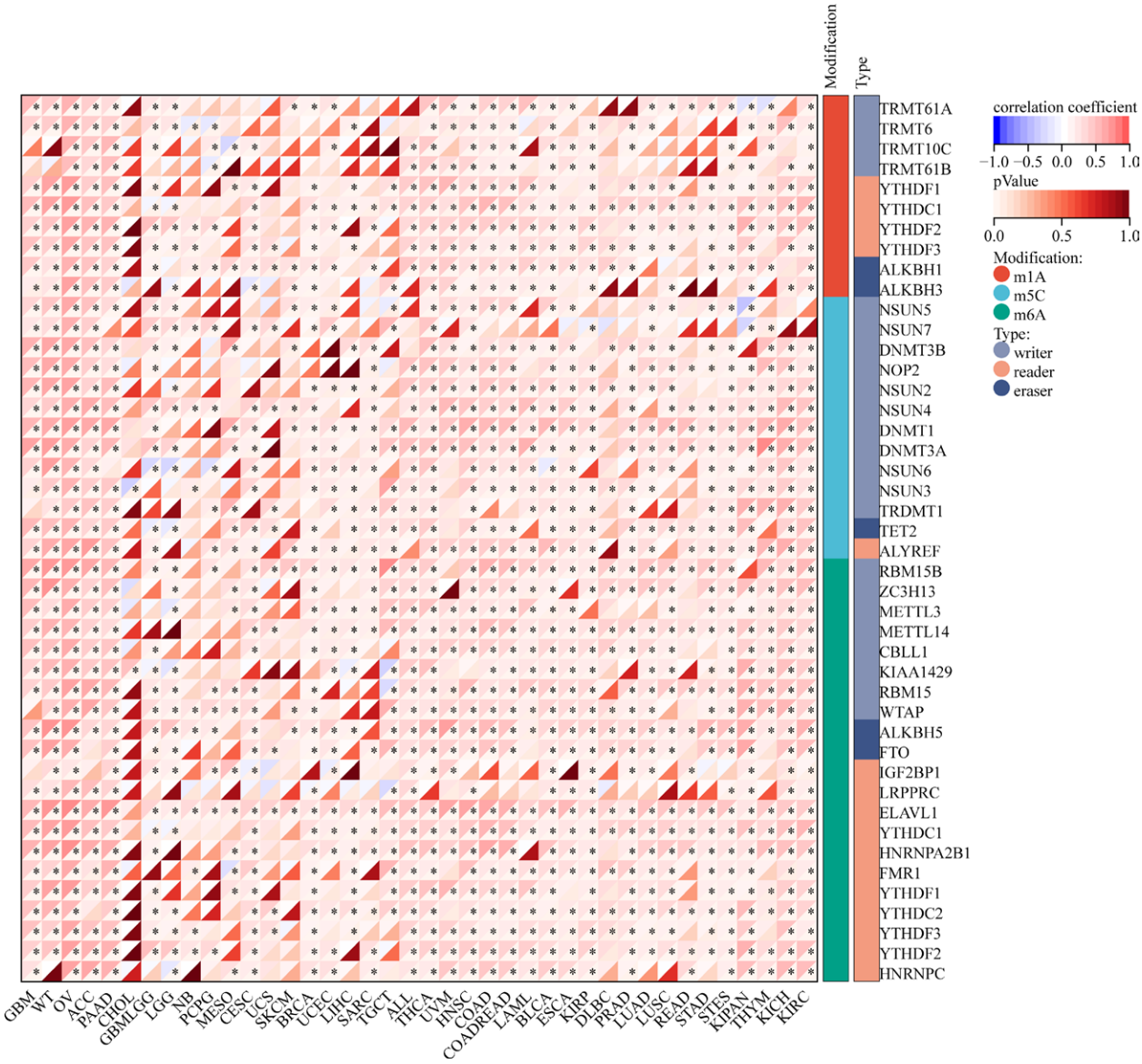

Figure 5. RNA modification analysis of TNFAIP8L1 in different types of tumors. TNFAIP8L1 expression was significantly positively correlated with most of m1A, m5C and m6A genes in multiple types of tumors including GBM, WT, OV, ACC, PAAD, BRCA, UCEC, LIHC, ALL, THCA, UVM, HNSC, COAD, COADREAD, LAML, BLCA, ESCA, KIRP, DLBC, PRAD, LUAD, READ, STAD, STES, KIPAN, THYM, KICH and KIRC.

than other TNFAIP8 family members including TNFAIP8, TNFAIP8L2 and TNFAIP8L3. Previous studies have reported that TNFAIP8L1 has emerged as a significant protein involved in immunity, inflammation and tumorigenesis. It remains to be answered that whether TNFAIP8L1 can play an important role in the progression of different types of cancers via certain common molecular mechanisms. In our result, the expression level of TNFAIP8L1 was significantly elevated in the tumor tissues of BRCA, CESC, DLBC, ESCA, GBM, HNSC, KIRC, LAML, LIHC, OV, PAAD, SKCM, STAD, THYM and UCEC than that in the corresponding control tissues. By contrast, the expression of TNFAIP8L1 was low expressed in CHOL, COAD, KICH, KIRP, LUAD, PRAD, READ, TGCT and THCA. Our these results were not exactly consistent with the recently researches which have shown that TNFAIP8L1 has been involved in the progression of several cancers, including breast cancer,[4,5] colorectal cancer,[6] gastric cancer,[7] hepatocellular carcinoma,[8] lung cancer,[9] osteosarcoma,[10] ovarian cancer[11,12] and nasopharyngeal carcinoma.[14] That is may be due to these results were mainly relay on bioinformatics and public databases. And our data about expression of TNFAIP8L1 were not verified by experiment. We also found that the expression of TNFAIP8L1 was over expression in CESC, which was in accordance with ZHAO report.[13]

In addition, we further detected the relationship between TNFAIP8L1 and the prognosis of patients with tumors. We found that high level of TNFAIP8L1 commonly predicted poor OS for patients with tumors including ACC, LGG and MESO with over-expression of TNFAIP8L1. By contrast, we detected low-expression of TNFAIP8L1 predicted poor OS for KIRC patients with low TNFAIP8L1 expression. And, we also found that high level of TNFAIP8L1 was associated with poor DFS prognosis for ACC, KIRC, KIRP, LGG, PRAD, TGCT and UVM. These results revealed that TNFAIP8L1 is a potential biomarker for the prognosis of patients with tumor.

DNA methylation, as an important epigenetic regulator of postreplication, played a significant role in tumorigenesis.[18] The occurrence and development of tumors was affected by up-regulated or down-regulated DNA methylation state of target gene. In our study, we firstly showed that there was a hypermethylation status in promoter region of TNFAIP8L1 in COAD and PRAD. While, there was a hypomethylation level

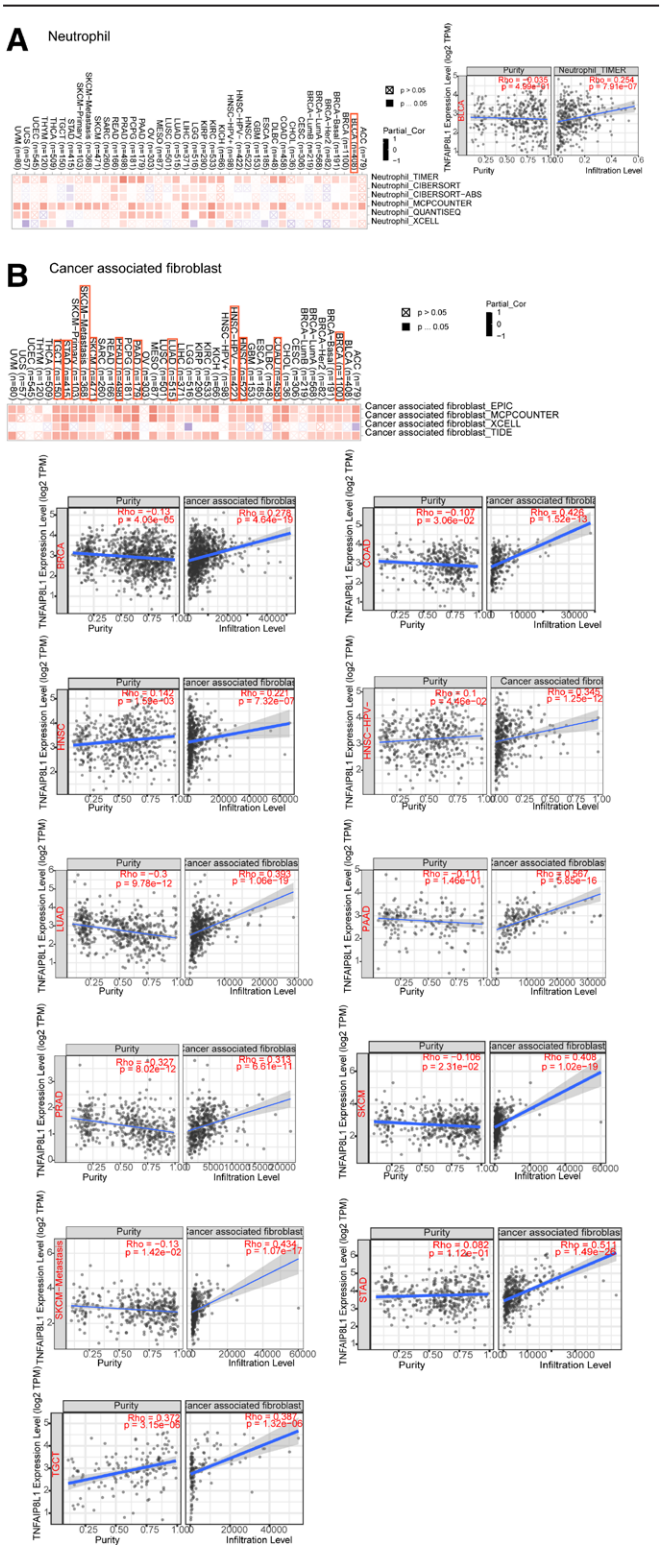

Figure 6. Correlation analysis between TNFAIP8L1 expression and neutrophil cell(A) and immune infiltration of cancer-associated fibroblasts(B). Different algorithms were used to explore the potential correlation between the expression level of the YKT6 gene and the infiltration level of cancer-associated fibroblasts across all types of cancer in TCGA.

in the promoter region of TNFAIP8L1 in KIRC, BLCA, READ, TGCT, HNSC, THCA, GBM, KIRP, LIHC, CESC and UCEC.

Increasing evidence suggested that RNA modification was implicated in human cancer and may be an ideal target for cancer therapy.[21] Adenosine at position 6 is methylated to give m6A, which is the most well-characterized RNA modification. In our study, we firstly obtained a significant positive association between TNFAIP8L1 and RNA modification-related genes (including m1A, m5C and m6A). We firstly showed that TNFAIP8L1 expression was significantly positively correlated with most of m1A, m5C and m6A genes in multiple types of human cancers. We also found ELAVL1, as a reader of M6A, was significant positively related to TNFAIP8L1 in all cancers except CHOL. These results need to be verified by experiments.

The degree of immune infiltration is closely related to the clinical outcomes of different types of cancer. Immune infiltration refers to the density and distribution of immune cells (such as lymphocytes, macrophages, etc) in tumor tissues. Higher levels of immune infiltration are generally associated with better prognosis (prolonged survival). Active involvement of immune cells may help control tumor growth and spread. In the research, we discovered a significantly positively association between TNFAIP8L1 level and neutrophil cell in tumors of BLCA. We also detected that TNFAIP8L1 level was positively associated with cancer-associated fibroblasts (CAFs) for TCGA tumors of BRCA, COAD, HNSC, HNSC-HPV-, LUAD, PAAD, PARD, SKCM, SKCM-Metastasis, STAD and TGCT. The degree of immune infiltration plays a significant role in the clinical outcomes of different types of cancer. However, specific impacts may vary depending on factors such as the type of tumor, individual patient differences, and treatment regimens. Confirmation through clinical research and experiments is necessary.

We also carried out a comprehensive correlation analysis to investigate the relationship between TNFAIP8L1 and immune checkpoints and immune moderator genes. We found that TNFAIP8L1 was significantly positively related with most of immune inhibitory and stimulatory genes in multiple types of tumors, while TNFAIP8L1 was negatively related with most of immune inhibitory and stimulatory genes in TGCT and THCA. TNFAIP8L1 was notably positively correlated with most of chemokine, receptor, MHC, immunoinhibitor and immunostimulator gens in multiple kinds of cancers, but negatively correlated with most of these genes in TGCT and THCA.

Throughout the genome, microsatellites are short stretches of DNA that are repeated, and MSI occurs when one or more repeats are added or removed. TMB is the total amount of mutations per DNA megabase.[22] Both TMB and MSI are 2 new biomarkers correlated with immunotherapy responses.[23] In our present study, we firstly demonstrated the potential relationship between TNFAIP8L1 and TMB or MSI. And the results suggested the genetic alteration of TNFAIP8L1 may be considered as potential novel drivers of some tumors.

Furthermore, we conducted a series of enrichment analyses using information on TNFAIP8L1-binding components. TNFAIP8L1 gene had a significant positive association with 5 genes including BCL6B, DLL4, PCDH12, COL4A1 and DLL4 in the majority of tumors. BCL6B is a gene encoding a transcription factor and belongs to the BCL6 gene family.[24] Under normal physiological conditions, BCL6B is involved in various biological processes, including cell differentiation and immune responses. Some studies suggested that BCL6B played a role in inhibiting tumor development in certain types of cancer, including gastric cancer,[25] colorectal cancer,[26] hepatocellular carcinoma[27] and lung cancer.[24] CDH5, also known as VE-cadherin, belongs to the superfamily of transmembrane cadherin (CDH) proteins.[28] CDH5 plays a crucial role in cell-cell adhesion, which is essential for maintaining the integrity of endothelial and vascular tissues. Previous studies have reported abnormal expression of CDH5 in various types of tumors.[28] CDH5 has been found to influence angiogenesis and thereby regulate tumor development. In glioma and melanoma, CDH5 promotes tumor progression by inducing the formation of blood vessels.[28] Moreover, elevated levels of CDH5 are associated with poorer clinical outcomes in patients with breast cancer[29] and gastric cancer.[30] COL4A1, as a member of the type IV collagen family and a crucial constituent of the basement membrane, has

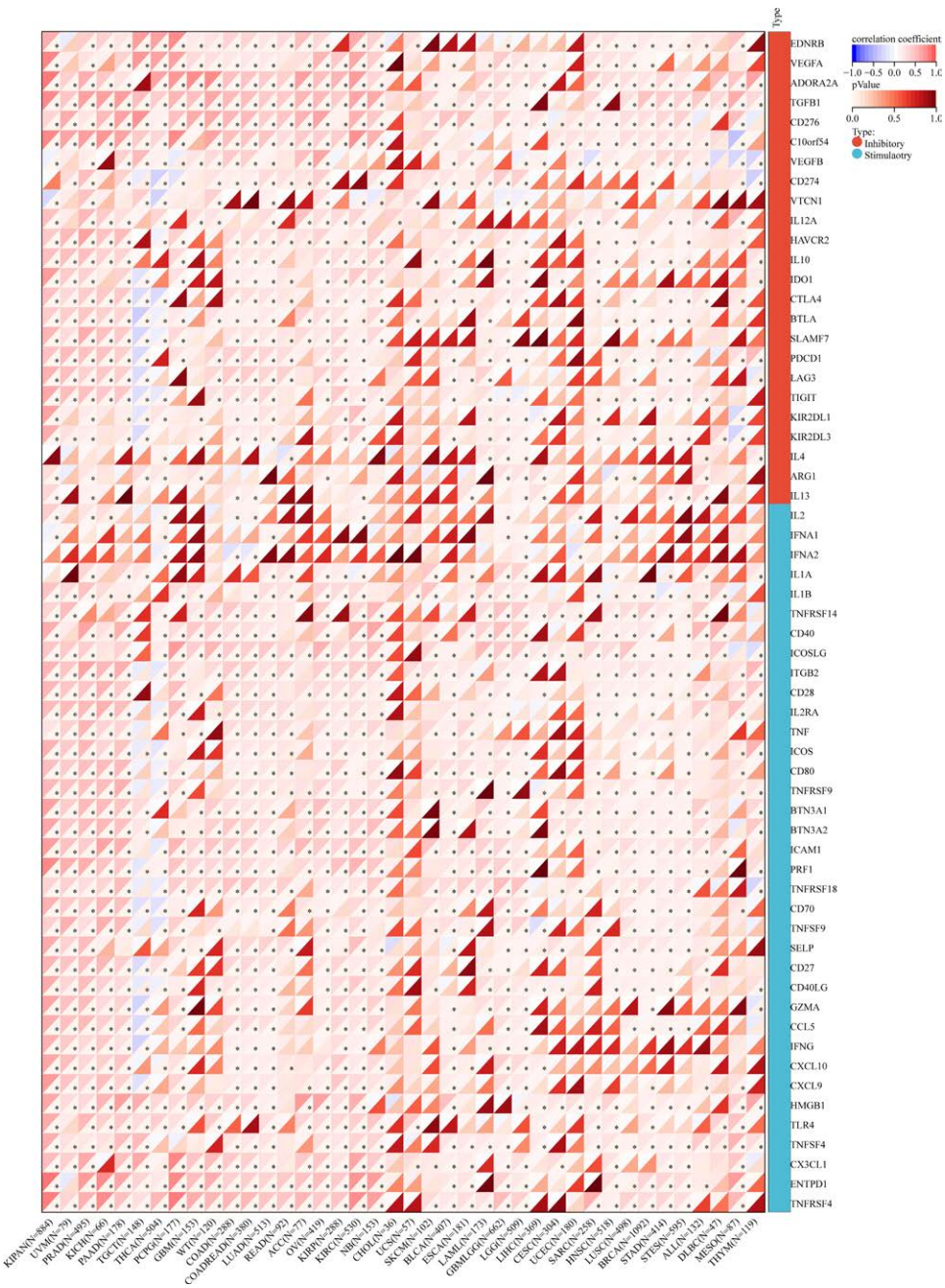

Figure 7. Correlation analysis between TNFAIP8L1 expression and immune checkpoints. TNFAIP8L1 was significantly positively related with most of immune inhibitory and stimulatory genes in multiple types of tumors including KIPAN, UVM, PRAD, KICH, PAAD, PCPG, COAD, COADREAD, LUAD, OV, KIRP, KIRC, BLCA, GBMLGG, LGG, HNSC, LUSC, BRCA and STAD. While TNFAIP8L1 was negatively related with most of immune inhibitory and stimulatory genes in TGCT and THCA.

been identified as a significant contributor in various types of cancer.[31] Recent investigations have revealed abnormal expression of COL4A1 in invasive ductal carcinoma of the breast cancer[32] and bladder tumor,[33] linking it to tumor invasion and metastasis. Moreover, upregulation of COL4A1 has been reported in patients with Oral Squamous Cell Carcinoma.[34] Elevated levels of COL4A1 contribute to enhanced migration, invasion, and proliferation in various cancers, including breast cancer and bladder cancer. Prior investigations have also recognized COL4A1 as a pivotal gene implicated in the initiation, advancement, and recurrence of gastric cancer,[35] and have associated its overexpression with unfavorable prognosis. DLL4 is a ligand associated with the Notch signaling pathway, playing crucial roles in carcinogenesis, angiogenesis, tumor progression, and chemoresistance.[36] DLL4 is often found to be overexpressed in tumor vasculature, and several agents have been identified to hinder DLL4-induced angiogenesis in tumors. DLL4 expression has been documented in various types of carcinomas, including endometrial,[37] nasopharyngeal,[38] pancreatic,[39] and colorectal carcinoma.[40] PCDH12 is a gene that encodes a protein, belonging to the protocadherin family.[41] Protocadherins are a type of protein that play a significant role in cell adhesion and signaling between cells. In papillary renal cell carcinoma patients, PCDH12 was related to the survival status of patients, and the expression of PCDH12 was positively associated with pathological grade.[41]

We also found that "purine nucleoside binding," "purine ribonucleoside binding," "GTP binding," "host cell cytoplasm," "host cell cytoplasm part," "collagen-containing

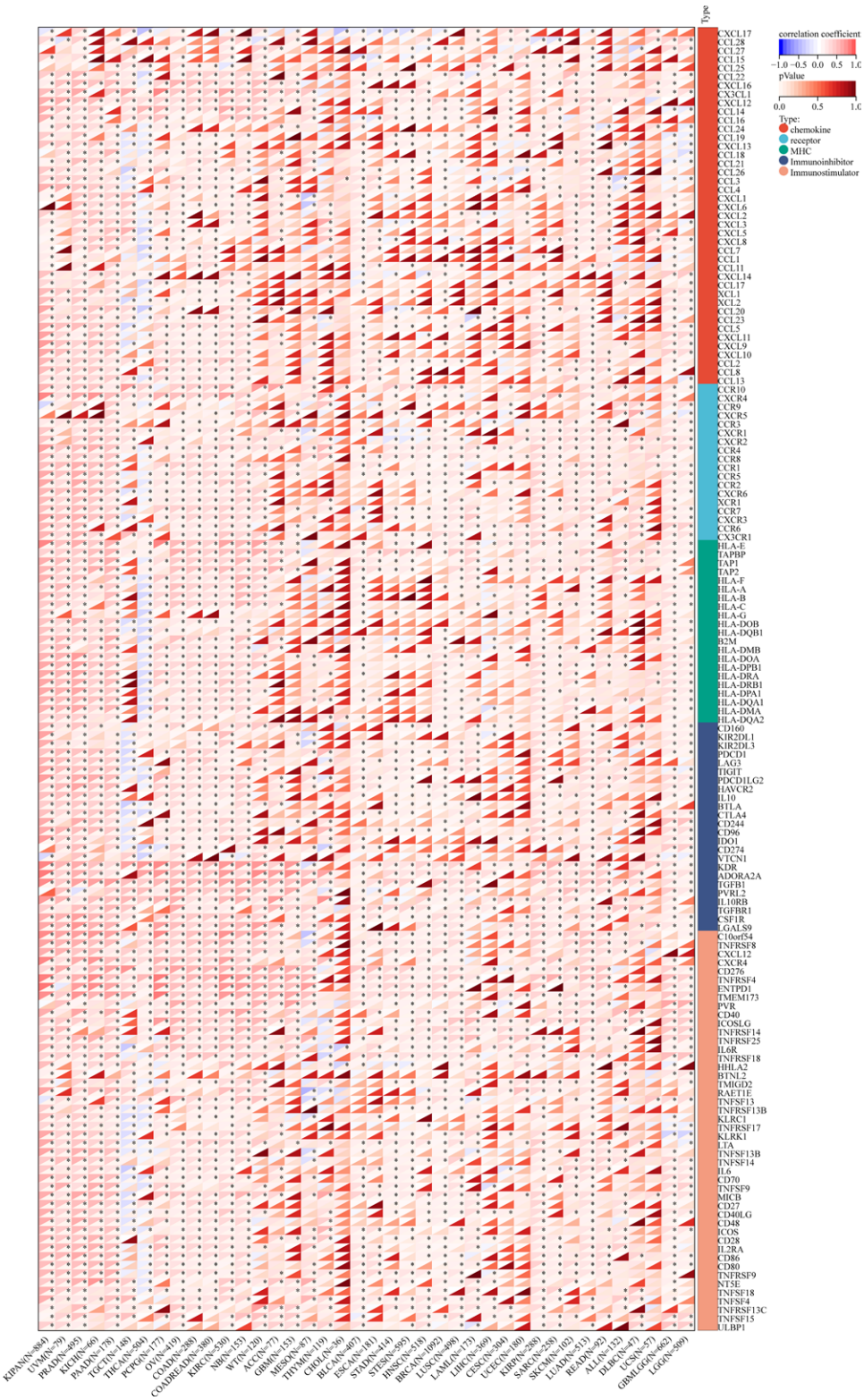

Figure 8. Correlation analysis between TNFAIP8L1 expression and Immune Moderator genes. TNFAIP8L1 was notably positively correlated with most of chemokine, receptor, MHC, immunoinhibitor and immunostimulator gens in multiple kinds of cancers including KIPAN, UVM, PRAD, KICH, PAAD, PCPG, COAD, COADREAD, LUAD, OV, KIRP, KIRC, BLCA, HNSC, LUSC, BRCA, STAD, GBMLGG and LGG, but negatively correlated with most of these genes in TGCT and THCA.

extracellular matrix," "cell-substrate adhesion," "kidney vasculature development" and "renal system vasculature development" were mainly involved in the influence of TNFAIP8L1 on tumor pathogenesis. In KEGG pathway, "ECM-receptor interaction," "complement and coagulation cascades" and "focal adhesion" were associate with the influence of TNFAIP8L1 on tumor pathogenesis. The pathway of ECM-receptor interaction is a critical process in cell migration and invasion. By interacting with the extracellular matrix, cancer cells acquire the ability to penetrate through blood vessel

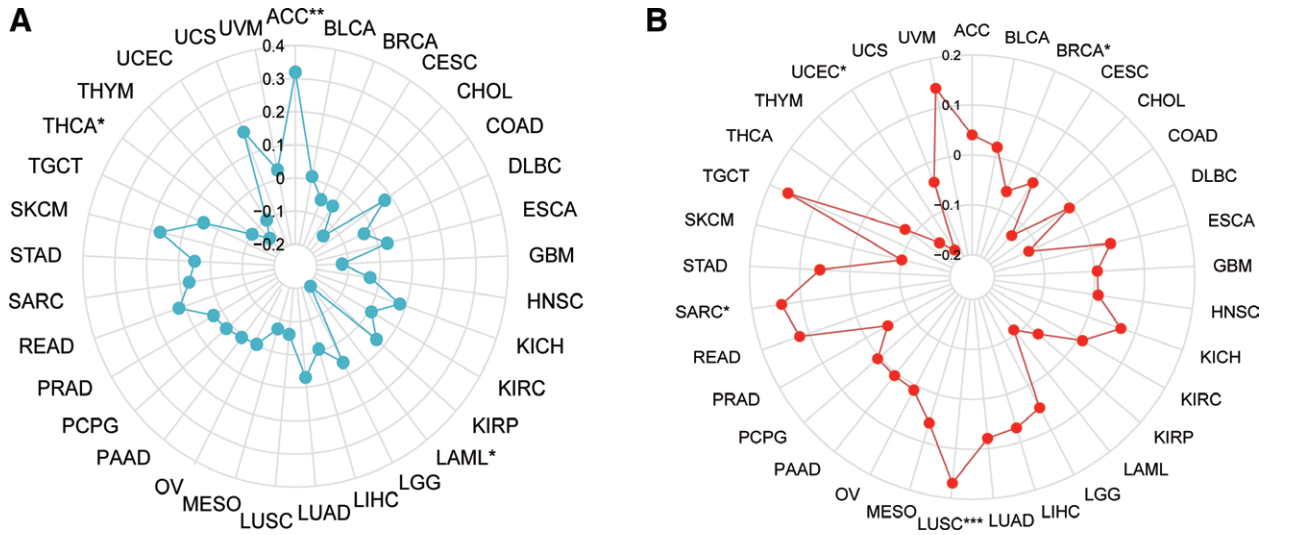

Figure 9. Correlation analysis TNFAIP8L1 and tumor mutational burden (TMB) and microsatellite instability (MSI). (A)TNFAIP8L1 was positively associated with TMB in ACC. And, TNFAIP8L1 was negative association with TMB in LAML and THCA. (B) TNFAIP8L1 was positively correlated with MSI in SARC and LUSC. By contrast, the expression of TNFAIP8L1 was negatively correlated with MSI in BRCA and UCEC. (*$P < .05$, **$P < .01$, ***$P < .001$).

walls and infiltrate surrounding tissues.[42] Additionally, cancer cells can promote the formation of new blood vessels by interacting with matrix proteins, thereby providing the tumor with an ample supply of nutrients and oxygen.[43] TNFAIP8L1 gene may also regulate tumor metastasis by affecting the pathway of ECM-receptor interaction. The complement and coagulation cascades pathway has a multifaceted impact on tumorigenesis, influencing both positive and negative outcomes, and plays a role in shaping the components of the tumor microenvironment.[44,45] Complement, a crucial component of the innate immune system, culminates in the cleavage of C3 and C5 upon activation, resulting in the release of anaphylatoxins like C3a, C4a, and C5a. This activation process can lead to the lysis of target cells through the membrane attack complex. Cancer cells can manipulate the complement and coagulation cascades pathway to influence and mold the tumor microenvironment. The focal adhesion pathway of a cell is one of the crucial pathways for the interaction between the cell and its surrounding extracellular matrix.[46] It involves a multitude of protein-protein interactions and signaling molecules that regulate various biological processes including cell adhesion, migration, and morphological changes.[47] The focal adhesion pathway of a cell participates in the occurrence and development of cancer through its influence on various aspects such as cell migration, signal transduction, and the tumor microenvironment.

Different types of cancers originate from different tissues and have distinct differentiation states. This may lead to varying roles of TNFAIP8L1 in different types of cancer. TNFAIP8L1 may be involved in multiple signaling pathways, and these pathways may produce opposing effects in different cell types or types of cancer. Recently researches indicated a significant decrease in TNFAIP8L1 expression in Ewing sarcoma cell lines when compared to normal osseous cells. TNFAIP8L1 exerted its inhibitory effects on Ewing sarcoma growth, motility, and survival through the regulation of the Wnt/β-catenin signaling pathway.[48] A study about osteosarcoma reported that the level of TNFAIP8L1 was down-regulated in osteosarcoma tissues than that in normal and adjacent nontumor tissues. TNFAIP8L1 inhibited osteosarcoma tumorigenesis and progression by regulating PRMT1 mediated STAT3 arginine methylation.[49] The expression of TNFAIP8L1 was significant downregulated in lung cancer tissues than that in normal lung tissues. TIPE1 regulated proliferation, survival, migration and invasion of lung cancer cells through Akt/mTOR/STAT-3 signaling pathway.[50] The expression of TNFAIP8L1 was downregulated in oral cancer tissues compared with normal tissues. TNFAIP8L1 regulated oral cancer progression through Akt/mTOR signaling pathway.[51] In colon cancer, TNFAIP8L1 was downregulated in cancer tissues and positively associated with prognosis of colon cancer patients. TNFAIP8L1 regulated colon cancer progression by directly targeting β-catenin.[6] In nasopharyngeal carcinoma, TNFAIP8L1 expression was up-regulated in cancer tissue compared with adjacent normal tissue. TNFAIP8L1 promoted nasopharyngeal carcinoma cell proliferation through the AMPK/mTOR signaling pathway.[14] In cervical cancer, TNFAIP8L1 expression was up-regulated in cancer tissue compared to adjacent normal tissue. TNFAIP8L1 prompted cervical cancer progression via the p53 pathway.[13] In breast cancer, TNFAIP8L1 was downregulated in cancer tissues, and TNFAIP8L1 suppressed the invasion and migration by the ERK signaling pathway.[5]

The pan-cancer analysis is based on existing public data and previous research findings.[52] If there is missing data or incomplete information for certain cancer types, it may affect the comprehensiveness of the analysis. The reliability of the analysis results depends on the quality of the original data. If there are errors, noise, or biases in the original data, it may also affect my analysis results. There is sample heterogeneity among different cancer types, including differences in cell origin and tissue characteristics. This may lead to differences in the analysis results between different cancer types. Cancer is an extremely complex disease, and different types of cancer have significant differences in pathological physiology, molecular characteristics, and other aspects. My analysis may not be able to capture all of these differences. My analysis may be limited by clinical data, such as incomplete or missing clinical information for certain cancer types. We will also update and revise the research based on the latest research findings and data to ensure the accuracy and comprehensiveness of the study. The results of pan-cancer analysis need to be validated through experiments to confirm their accuracy and reliability. Experimental validation is a crucial step in scientific research as it helps confirm the analysis results and provide substantial evidence. This ensures the scientific validity of the study and establishes a solid foundation for further research.

In summary, our pan-cancer analysis of TNFAIP8L1 showed statistical association of TNFAIP8L1 level with survival prognosis, genetic alteration, DNA methylation, RNA modification and

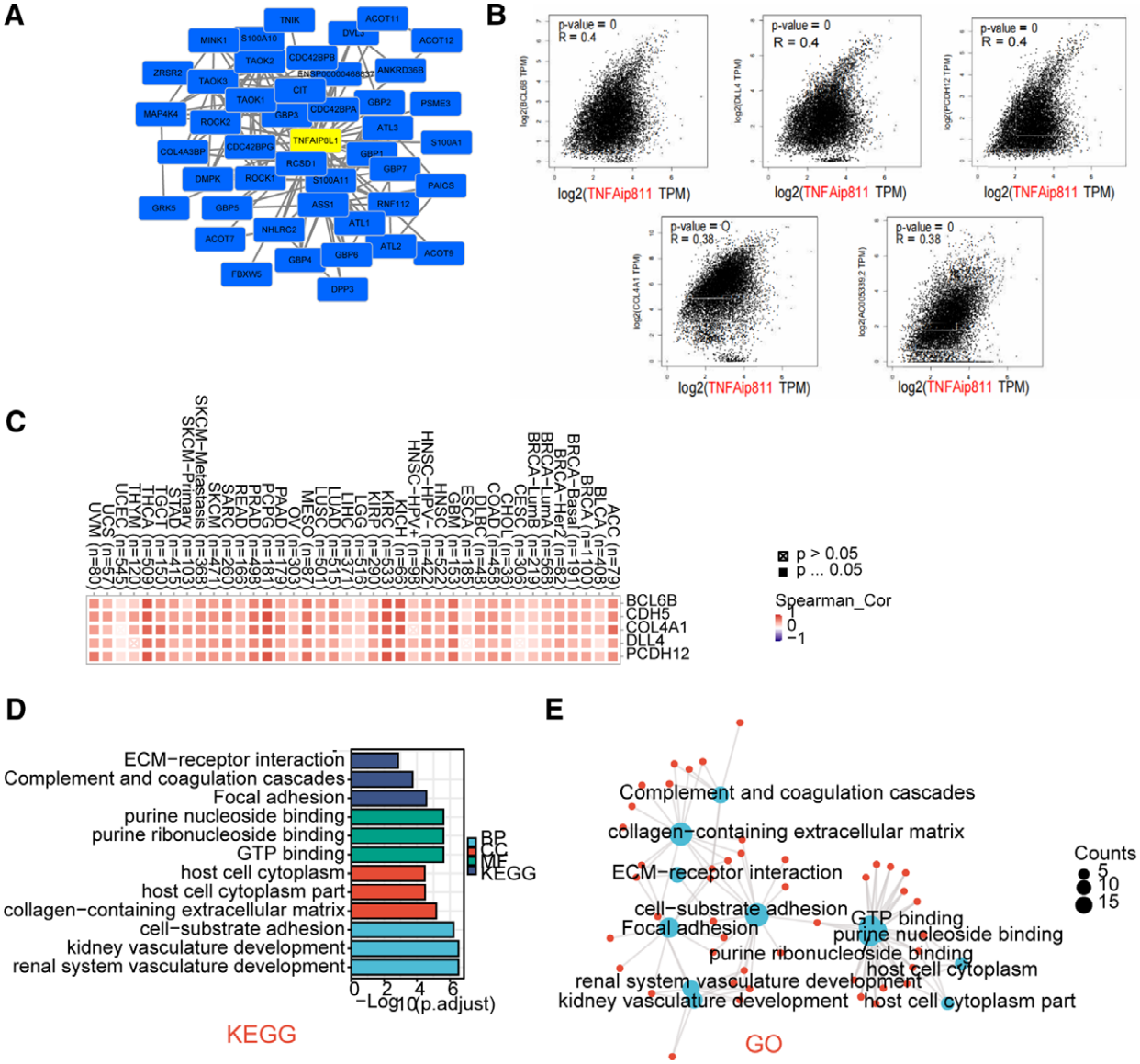

Figure 10. TNFAIP8L1-related gene enrichment analysis.(A)We first obtained the available experimentally determined TNFAIP8L1-binding proteins using the STRING tool.(B)Using the GEPIA2 approach, we also obtained the top 100 TNFAIP8L1-correlated genes in TCGA projects and analyzed the expression correlation between TNFAIP8L1 and selected targeting genes including BCL6B, DLL4, PCDH12, COL4A1 and AC005339.2 genes.(C)The corresponding heatmap data in the detailed cancer types are displayed. (D)Based on the TNFAIP8L1-binding and interacted genes, KEGG pathway analysis was performed. (E)Go enrichment analysis of TNFAIP8L1-binding and interacted genes in molecular is also shown.

immune infiltration across most tumors, which can contribute to showing the role of TNFAIP8L1 in tumoeigenesis from perspective of clinical tumor samples.

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Writing – review & editing: Xuezhong Zhang.

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