Human pan-cancer analysis of the predictive biomarker for the tumor necrosis factor-alpha-induced protein 8-like 2 (TNFAIP8L2)

Yingjun Chen
Binzhou Medical University Hospital

Xuezhong Zhang
Zibo Central Hospital

Dai Li
The Fourth Affiliated Hospital of China Medical University

Kaihui Sha
Binzhou Medical University School of Nursing

Tonggang Liu (liutonggang123@126.com)
Binzhou Medical University Hospital

Research Article

Keywords: TNFAIP8L2, predictive biomarker, pan-cancer

Posted Date: January 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2461425/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** TNFAIP8L2 is a member of the tumor necrosis factor-alpha-inducible protein 8 (TNFAIP8) family shown to have oncogenic effects. However, no pan-cancer analysis has shown an association between TNFAIP8L2 and various tumor types.

**Methods:** Using the Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and other bioinformatics tools, we performed a pan-cancer analysis of the TNFAIP8L2 gene. We investigated TNFAIP8L2’s function in 33 tumor types, exploring its gene expression, survival status, DNA methylation, genetic alterations, immune infiltration, and associated cellular pathways.

**Results:** TNFAIP8L2 was strongly expressed in most malignancies and linked with patients' overall survival (OS) and disease-free survival (DFS). Missense mutations are the main type of mutation in the TNFAIP8L2 gene. TNFAIP8L2’s methylation levels were significantly different between tumors and normal tissues. In addition, infiltration of CD8+ T cells, cancer-associated fibroblasts, and macrophages has been linked to TNFAIP8L2 expression in various malignancies, including cervical cancer, breast-infiltrating cancer, and renal clear cell carcinoma. Mechanistically, Rac2, actin filament, and Fc gamma R-mediated phagocytosis are all implicated.

**Conclusion:** TNFAIP8L2 may be a biomarker or treatment target for predicting the prognosis of cancer victims. In addition, it might interfere with Rac2-mediated pathways regulating macrophage M1 to M2 differentiation and metastasis. This could provide a new direction for tumor therapy.

Introduction

Malignant tumors are one of the hazards to the health of humans. The development of tumors is complex and unclear. Pan-cancer analyses are necessary to investigate the potential molecular mechanisms underlying the association between genes and numerous types of malignancies. To this end, we can use a large amount of genetic sequencing data and significant clinical datasets from various types of cancer due to the public availability of whole genome sequencing technology (e.g., TCGA, GEO).

TNFAIP8L2, also called TIPE2, belongs to the tumor necrosis factor-alpha-induced protein 8 (TNFAIP8) family, involved in immunological homeostasis (1). TNFAIP8L2 is an aberrantly expressed gene first identified in mice with experimental autoimmune encephalomyelitis (2). TNFAIP8L2 has a putative death effector domain (DED)-like structural domain (3) with a large hydrophobic capsule, almost a mirror version of the DED structural domain of caspase-8 or c-FLIP in terms of geometry, in contrast to the classical DED structural domain. Thus, TNFAIP8L2’s function may be different from that of other DED proteins. TNFAIP8L2 has been implicated in human inflammatory reactions and immunological disorders such as multiple sclerosis and silvery psoriasis(4). Moreover, TNFAIP8L2 interrupted the tumor factor Ras by blocking Ras binding to the Ras-interaction domain site of RGL (5). This suggested that TNFAIP8L2 is a crucial gene associated with inflammation and cancer. Additionally, previous research has indicated that TNFAIP8L2 and carcinogenesis are involved in breast cancer(6), hepatocellular cancer(7), gastric
cancer(8), breast cancer(9), and skin squamous cell carcinoma(10). However, no pan-cancer investigation has shown a link between TNFAIP8L2 and other kinds of tumors.

Given the unclear mechanism of TNFAIP8L2-regulated tumors, we conducted a pan-cancer study to investigate expression levels, survival quality, DNA methylation, gene alterations, immune invasion, and related cellular networks. In addition, we investigated the probable molecular involvement of TNFAIP8L2 in the development of various carcinoma and clinical prognosis via TCGA, the Clinical Proteomic Tumor Analysis Consortium (CPTAC), and GEO databases to explore the potential link between TNFAIP8L2 and cancer.

**Materials And Methods**

**Differential expression analysis**

The TIMER2 dataset (http://timer.cistrome.org/) is utilized for TNFAIP8L2 expression profiling and to investigate the quantity of immune infiltrates in diverse cancer types. In the TCGA study, the “Gene DE” section of the TIMER2 database was utilized to study variations in TNFAIP8L2 expression between tumor tissues and the surrounding normal tissues of various cancers or tumor subtypes. The GEPIA2 database (http://gepia2.cancer-pku.cn/) is a web-based tool for evaluating tissue samples from the TCGA and the Genotype-Tissue Expression (GTEx) systems. The “Expression Analysis-Box Plot” module of the GEPIA2(11) was used to generate TNFAIP8L2 expression box plots based on the GTEx database for certain tumors lacking or having a small amount [e.g., Lower Grade Brain Glioma (TCGA-LGG), acute myeloid leukemia (TCGA-LAML), etc.]. P-value threshold = 0.01, log2FC (fold change) cutoff = 1, and “Match TCGA normal and GTEx data” were the parameters used. Moreover, applying the “Pathological Stage Plot” module of GEPIA2, we generated violin plots of TNFAIP8L2 levels in distinct pathological cancer stages in the TCGA database. Expression data from Log2 [TPM (Transcripts per million) + 1] served to make the box and violin plots.

The UALCAN website (http://ualcan.path.uab.edu/) can analyze protein expression based on data from the TCGA and CPTAC datasets (12). Using the “CPTAC” module of UALCAN, TNFAIP8L2 expression was examined in tumor tissue and neighboring normal tissue. The P-value threshold was 0.05.

**Survival prognosis analysis**

The GEPIA2 database(11) is used for patient survival analyses as well. Using GEPIA2’s “Survival Map” module, we created overall survival (OS) and disease-free survival (DFS) relevance map data for all TCGA cancers. To classify patients into the high- and low-expression groups, we selected high-cutoff (50%) and low-cutoff (50%) values. The survival plots were obtained with GEPIA2’s “Survival Analysis” module. The log-rank test is employed in the hypothesis test.

**Genetic alteration analysis**
The cBioPortal website (https://www.cbioportal.org/)(13) offers online tools for examining, visualizing, and interpreting multimodal cancer genomics data. We gathered information on alteration frequency, mutation type, mutant site information, copy number alteration (CNA), and three-dimensional (3D) structure of the protein structure for TCGA tumors using the “Cancer Types Summary” and the “Mutations” modules of the cBioPortal tool. Furthermore, data on the correlation between TNFAIP8L2 genetic changes and prognoses [OS, disease-specific survival (DSS), progression-free survival (PFS), and DFS] was acquired via cBioPortal’s “Comparison/Survival” module. Log-rank P-values were used to depict the survival data.

**Methylation analysis**

The methylation expression levels in tumors and normal tissues were evaluated using the ‘Methylation’ module on the UALCAN website and generated using TCGA datasets.

**Immune infiltration analysis**

Using the “Immune-Gene” board of TIMER2 online, we investigated the connection between TNFAIP8L2 expression and immune infiltrates in the TCGA data. Then we chose the cancer-associated fibroblasts, CD8+ T-cells, and macrophages as the reference objects. Immune infiltration was estimated using the TIDE, TIMER, XCELL, EPIC, MCPCOUNTER, CIBERSORT, and QUANTISEQ methods. A purity-adjusted Spearman's correlation test was performed to calculate P-values and correlation (cor) results. This data is presented in the form of heat and scatter plots.

**TNFAIP8L2-related gene enrichment analysis**

We used the STRING database (https://string-db.org/) to investigate whether proteins interacted with one another, combining their scores (14). In our research, we searched for a particular protein name (“TNFAIP8L2”) and organism (“Homo sapiens”) in particular. Afterwards, we set the minimum required interaction score to “Low confidence (0.150),” the implication of network edges to “evidence,” the maximum number of interactors to show to “<20 interactors” in the first shell, and the active interaction sources to “experiments.” Last, pressing the “Update” button displays the 18 relevant proteins.

The “Similar Genes Detection” board of GEPIA2 was used to find the top 100 TNFAIP8L2-associated targeted genes in the TCGA. Additionally, we used GEPIA2’s “Correlation Analysis” board to conduct paired gene Pearson relevance analysis on TNFAIP8L2 and some other genes. The box plots were created using log2 [transcripts per million (TPM) +1] expression data. Both the P-value and correlation coefficient (R) were included. When using the “Gene Corr” board of TIMER2 to prepare heatmaps for each of our chosen genes, showing the partial correlation (cor) and P-value for the purity adjusted Spearman's rank correlation assessment, our results were easy to see at a glance.

In this study, an interactive Venn diagram viewer, Jvenn(15) was used to conduct an intersecting analysis of TNFAIP8L2-binding and its interacting genes. Additionally, we used the merged data sets to conduct the Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis. The “tidyr” and “ggplot2” R
packages were utilized to illustrate the enriched routes. Additionally, the "cnetplots" R package was utilized to depict the GO enrichment analysis results for biological mechanisms, cellular components, and molecular function. This research was conducted using the R language software [R- 4.0.4, 64-bit] (https://www.r-project.org/). P < 0.05 indicated statistical significance.

Results

TNFAIP8L2- associated gene expression analysis data

The TIMER2 database was used to evaluate TNFAIP8L2 expression in TCGA samples from various kinds of cancers. As shown in Figure 1a, TNFAIP8L2 was expressed higher in breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA, P < 0.001), glioblastoma multiforme (GBM, P < 0.01), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), gastric carcinoma (STAD), and thyroid carcinoma (THCA) than in the corresponding control organizations. In contrast, colon adenocarcinoma (COAD, P < 0.001), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD, P < 0.01), and rectal adenocarcinoma (READ) were all lower than the corresponding control organizations. As some TCGA databases lack control data for normal tissues, we further assessed differences in TNFAIP8L2 expression between tumor and normal tissues after integrating normal tissues from the GTEx dataset. The expression levels of acute myeloid leukemia (LAML), LGG, ovarian serous cystadenocarcinoma (OV), skin cutaneous melanoma (SKCM), and testicular germ cell tumor (TGCT) tissues were greater than those of normal tissues (Figure 1b, p < 0.05). However, we found no substantial alterations in other tumors, such as adrenocortical carcinoma (ACC), lymphoid neoplasm diffuse large B cell lymphoma (DLBC), sarcoma (SARC) and uterine carcinosarcoma(UCS).

In addition, the CPTAC dataset was used to examine TNFAIP8L2 protein levels in different tumors and normal tissues. There was no difference in TNFAIP8L2 total protein levels between breast, ovarian, colon, and lung adenocarcinomas and normal tissues. Protein expression was considerably higher in advanced tumor tissues of clear cells such as renal clear cell carcinoma (RCC), endometrial carcinoma (UCEC), HNSC, PAAD, and GBM (Figure 1c, all P < 0.001,) but significantly lower in hepatocellular carcinoma (HCC) tumor tissues (Figure 1c, P < 0.001) than in normal tissues.

In addition, the GEPIA2 tool indicated a correlation only between TNFAIP8L2 expression and the pathological classification of carcinomas such as SKCM, THCA, STAD, KIRC, kidney chromophobe (KICH), ESCA (Figure 1d, all P < 0.05), and not seen otherwise.

Survival analysis data

Using the TCGA and GEO databases, cancer cases were divided into low- and high-expression groups according to their TNFAIP8L2 expression profiles, and the relationship between TNFAIP8L2 expression and prognosis was explored in patients with diverse tumors. In the TCGA dataset, increased TNFAIP8L2
expression was connected with a poor prognosis in terms of total OS for LGG (P = 0.0036) and UVM (P = 0.0068) tumors, as shown in Figure 2a. The DFS analysis results (Figure 2b) revealed a link between high TNFAIP8L2 expression and poor prognosis in PRAD (P = 0.0074) among TCGA cases. Notably, decreased TNFAIP8L2 gene expression was correlated with a poor OS prognosis in BRCA (P = 0.0027), CESC (P = 0.0095), DLBC (P = 0.049), LUAD (P = 0.01), SARC (sarcoma) (P = 0.023), and SKCM (P = 0.0015) (Figure 2a), as well as with a poor DFS prognosis in ACC (P = 0.023) and CHOL (P = 0.025). (Figure 2b).

Genetic alteration analysis data

The cBioPortal tool was used to determine the status of TNFAIP8L2 gene alterations in different tumor tissues from the TCGA dataset. According to Figure 3a, the greatest prevalence of TNFAIP8L2 variations (about 11%) was observed in individuals with a predominance of LIHC “amplification.” All information about the kinds, locations, and case numbers of TNFAIP8L2 genetic alteration—including details regarding missense, truncating, and fusion mutations—is presented in Figure 3b. Two instances of stomach cancer and one of colorectal cancer were found at the R53Q/Q55Pfs*29 locus (Figure 3b). Missense mutations were the main type of TNFAIP8L2 gene mutations. The R53Q/Q55Pfs*29 site was observed in the 3D structure of the TNFAIP8L2 protein (Figure 3c). Furthermore, we investigated a possible link between TNFAIP8L2 gene variations and survival prognosis in several cancer types. In BRCA, patients with TNFAIP8L2 gene alterations had DFS than those without gene alterations (P = 0.0291), but without significant differences in OS (P = 0.570), DSS (P = 0.905), or PFS (P = 0.381, Figure 3d).

DNA methylation analysis

Abnormal DNA methylation is closely correlated with tumorigenesis, development, and cellular carcinogenesis. Moreover, alterations in DNA methylation levels and the degree of methylation of specific genes can be used as diagnostic indicators of tumors (16). The DNA methylation levels of TNFAIP8L2 were compared between normal and primary tumor tissues using the UALCAN and TCGA databases. According to the UALCAN database, TNFAIP8L2 methylation expression levels were considerably lower in BLCA, BRCA, CHOL, KIRP, KIRC, LIHC, LUAD, LUSC, PCPG, PAAD, PRAD, READ, and UCEC tumor tissues than in normal tissues (Figure 4a). Moreover, TNFAIP8L2 methylation expression levels were significantly increased in HNSC and SKCM tumor tissues (Figure 4a).

Immune infiltration analysis data

TNFAIP8L2 is an immune checkpoint regulator of inflammation and metabolism closely connected to tumorigenesis, progression, or metastasis(17-19). Therefore, in this investigation, we used various algorithms to investigate the possible connection between various degrees of immune cell infiltration, CD8+ T- cell, macrophage, and TNFAIP8L2 gene expression in various kinds of TCGA tumors. We observed a statistically significant positive correlation between TNFAIP8L2 expression and infiltrative levels of cancer-associated fibroblasts in TCGA tumors of BLCA, BRCA, BRCA-LumA, COAD, ESCA, HNSC, HNSC-HPV-, KIRP, LUAD, LUSC, PAAD, PCPG, PRAD, READ, STAD, THCA, THCA tumors in TCGA (Figure 5a).
In addition, the algorithm was used to obtain scatter data for the above tumors (Figure 5b). For example, based on the MCPCOUNTER algorithm, the TNFAIP8L2 expression level in BLCA was positively correlated with the infiltration level of cancer-associated fibroblasts (Figure 5b, cor = 0.404, P = 7.32e-16). Figure 5c shows a positive association between TNFAIP8L2 expression and macrophages in ACC, COAD, KIRP, KIRC, KICH, HNSC-HPV, LUAD, MESO, OV, READ, PRAD, PCPG, and UCEC. Furthermore, we noticed a positive correlation between TNFAIP8L2 expression and CD8+ T cells in CESC, KIRC, SKCM, and UVM tumor tissues (Figure 5d).

**Enrichment analysis of TNFAIP8L2-related partners**

To gain deeper insights into the underlying molecular processes through which the TNFAIP8L2 gene contributes to tumor growth, we performed KEGG and GO enrichment analyses to search for TNFAIP8L2 target-binding proteins and genes associated with TNFAIP8L2 expression. The STRING tool revealed 18 TNFAIP8L2 binding proteins, as corroborated by experimental data. Figure 6a shows the proteins' interaction network. Next, we used the GEPIA2 method in combination with TCGA data to identify the top 100 genes related to TNFAIP8L2 expression. Figure 6b shows that TNFAIP8L2 expression levels were positively associated with ARHGDIB (R = 0.62), IGSF6 (R = 0.65), RGS19 (R = 0.64), S1PR4 (R = 0.67), and GMFG (R = 0.71) gene expression levels. The corresponding heatmap data also showed that TNFAIP8L2 was positively linked with the above five genes in most cancer types (Figure 6c). A Venn diagram was further used to analyze the interrelationships between the two groups, which had a common member: RAC2 (Figure 6d).

In this research, we integrated the two databases and conducted KEGG and GO enrichment studies on the combined results. In Figure 6e, the KEGG analysis revealed that osteoclast differentiation and the Fc gamma R−mediated phagocytosis may involve in the connection between TNFAIP8L2 and tumor progression. Furthermore, the GO enrichment study revealed that most of these genes correlate with neutrophil-mediated immunity in the BP category, actin filament in the CC category, and superoxide-generating NADPH oxidase activator activity binding in the MF category (Figure 6f).

**Discussion**

TNFAIP8L2 has been implicated in tumor development and migration, as well as inflammatory response and immunological modulation, and been linked to a poor clinical prognosis in cancer patients(20, 21). TNFAIP8L2 can prevent Ras from binding to the Ras-Interacting Domain of RGL. This further prevents Ras from binding to other signaling molecules such as Ral and AKT, preventing the activation of downstream signaling cascades(5). TNFAIP8L2 also affects regulatory T cells via its interaction with the pAKT and F-actin proteins(22). In addition, abnormal TNFAIP8L2 expression is often related to the occurrence, advancement, or migration of human malignancies(23, 24). Given the crucial role of TNFAIP8L2 in carcinogenesis and tumor formation, it is vital to research the function of TNFAIP8L2 in diverse types of malignancies. However, there is a lack of comprehensive research on TNFAIP8L2’s role in
many types of cancer. As a result, we used the TCGA, CPTAC, and GEO datasets to undertake a comprehensive investigation of the TNFAIP8L2 gene in 33 distinct malignancies.

Based on our results, we found higher TNFAIP8L2 expression levels in the tumor tissues of BRCA, ESCA, GBM, HNSC, KIRC, KIRP, STAD, THCA, LAML, LGG, OV, SKCM, and TGCT than in matching control tissues; in contrast, poor expression was found in COAD, LUAD, LUSC, PAAD, and READ tumor tissues. This demonstrates that TNFAIP8L2 may have an influence on the progression of these tumors.

TNFAIP8L2 expression levels may indicate changes in its biological activity and implicated mechanisms in the affected tumors. Accordingly, we discovered that elevated TNFAIP8L2 expression was positively correlated with OS and DFS in BRCA, LUAD, and SKCM tumors, indicating that it might be a novel prognostic factor and therapeutic target for BRCA, LUAD, and SKCM patients. Previous research indicated that TNFAIP8L2 inhibits breast cancer(25) and lung cancer(26) formation, growth, and metastasis. We also discovered that patients with LGG and UVM, which display high TNFAIP8L2 levels, had a poor OS prognosis. The function of TNFAIP8L2 in LGG in the brain has not been verified. Our results might provide a novel clinical biomarker for forecasting the OS of LGG patients. According to these findings, TNFAIP8L2 may be both a prognostic biomarker and a treatment target for cancer patients.

Gene mutations play a crucial part in the etiology of several malignancies (27). According to present research, missense mutations were the most commonly occurring DNA modifications to the TNFAIP8L2 gene in the TCGA dataset. Certain genetic variants may predict tumor development in certain people. In the CPTAC database, the overall TNFAIP8L2 protein levels in HCC were considerably lower than in normal tissue. One HCC study (5) revealed that 80% of cancer cells had significantly lower TNFAIP8L2 expression levels than neighboring hepatocytes, as validated by our results. In addition, Cao, et al. (28) found that metastasis was substantially related to the lack of TNFAIP8L2 expression in primary HCC tissues. TNFAIP8L2 inhibited migration and invasion by intervening Rac1, further reduced F-actin polymerization, matrix metalloproteinase 9, and urokinase plasminogen activator expression.

Based on BRCA tumor data from the TCGA, we discovered that BRCA patients with TNFAIP8L2 gene mutations had a poor prognosis, suggesting that TNFAIP8L2 might be a possible therapeutic target for BRCA. This conclusion has been supported by previous research. Namely, Zhang, et al. (25) discovered that TNFAIP8L2 may have a role in breast cancer development by reducing breast cancer cell growth by decreasing ATK and p38 phosphorylation. Additionally, Wang, et al. (9) discovered that ectopic TNFAIP8L2 expression suppresses the Wnt/b-catenin signaling pathway, preventing breast cancer cell motility and invasion.

In the TCGA database, we discovered a possible association between elevated TNFAIP8L2 gene levels in tumor patients and low DNA methylation status in non-promoter regions. Additionally, the relationship between TNFAIP8L2 and DNA methylation has not been studied, and whether DNA methylation is involved in the development of TGCT tumors needs to be investigated experimentally. Furthermore, this study revealed significantly increased TNFAIP8L2 methylation levels in HNSC and SKCM tumors, suggesting that TNFAIP8L2 promoter methylation may be involved in HNSC and SKCM emergence and
growth, which suggests that TNFAIP8L2 may be a promising biomarker for early clinical diagnosis of HNSC and SKCM.

Tumor-associated macrophages (TAM) (29) mostly include a polarized M2 macrophage population formed from the infiltration of circulating bone marrow-derived monocytes. TNFAIP8L2 may have a significant impact on the TAM. This research explored the correlation between the TNFAIP8L2 gene and immune cells. In numerous cancers, TNFAIP8L2 expression is linked to CD8+ T-cell infiltration, cancer-associated fibroblasts, and macrophages. Protumoral actions of the TAM include angiogenesis promotion, adaptive immunity inhibition, and the production of growth factors and matrix proteases in the tumor microenvironment (30). Li (10) experimentally demonstrated that TNFAIP8L2 controls TAM in skin SCC. TNFAIP8L2 acts as a tandem relationship between skin SCC and TAMs and is a novel target for the treatment of skin SCC. Zhao (31) also found that TNFAIP8L2 regulates FoxP3(+) regulatory T cells, reducing the development and carcinogenesis of tongue SCC. The relationship between TNFAIP8L2 and TAM may explain how TNFAIP8L2 mediates the development of certain cancers, providing a new direction for tumor therapy.

The protein-protein interaction study indicated that RAC2 has a positive correlation with the TNFAIP8L2 gene. Rac2 belongs to three families of highly conserved Rac proteins: Rac 1–3 (32). Rac2 was initially discovered as a GTPase involved in NADPH oxidase activation (33) and actin cytoskeleton remodeling. Rac2 is also implicated in membrane ruffling, Fcγ receptor-mediated phagocytosis, and the production of phagocytic cups and macropinosomes (34). Deficiencies in Rac2 have been demonstrated to have an impact on B- and T-cell migration, activation, and differentiation. There is compelling evidence that Ras2 influences tumor development, metastasis, and macrophage differentiation in vitro (35). Li, et al. (26) discovered that TNFAIP8L2 inhibits Rac1 activation and VEGF expression in non-small cell lung cancer to reduce angiogenesis and invasiveness. We speculate that TNFAIP8L2 might interfere with Rac2-mediated pathways regulating macrophage M1 to M2 differentiation and metastasis.

GO enrichment analysis and KEGG pathway enrichment analysis are used to define the function of differentially expressed TNFAIP8L2. According to our findings, differentially expressed TNFAIP8L2 was linked to neutrophil-mediated immunity, actin filament, osteoclast development, and Fc gamma R mediated phagocytosis. Fan, et al. (22) revealed that TNFAIP8L2 affects regulatory T cells through interactions with pAKT and F-actin proteins. TNFAIP8L2 inhibits the migration and proliferation of endometrial cells by reversing the epithelial-mesenchymal transition by targeting- catenin (36). Similarly, TNFAIP8L2 overexpression suppresses gastric cancer spread by reversing the epithelial mesenchymal transition (37). The mechanism by which TNFAIP8L2 mediates tumor development is currently unknown. However, we can investigate if the aforementioned pathway is also implicated in the advancement of certain tumors by utilizing KEGG GO enrichment analysis.

Conclusions
The research explored the expression of TNFAIP8L2 in a variety of cancer types, as well as its possible involvement in cancer pathways. The study has several limitations. Firstly, this research used the TCGA, GTEx, and CPTAC databases; however, data for some forms of cancer were inadequate, and patient numbers for certain cancer types were limited. Secondly, due to the large individual differences between cancer patients in each country, it is hard to encompass all potential variations in this research. To identify the expression and function of TNFAIP8L2 in cancer, more experimental validation is necessary since this study relies only on bioinformatics and publically available information. Lastly, this research relies only on bioinformatics and publicly accessible datasets, more experimental validation is required to verify the expression and function of TNFAIP8L2 in the tumor.

In conclusion, we observed substantially significant connections between TNFAIP8L2 expression and clinical prognosis, gene mutations, DNA methylation, immune cell infiltration, and tumor mutations in a variety of human malignancies through a comprehensive pan-cancer investigation of TNFAIP8L2, attempting to aid in understanding the function of TNFAIP8L2 in tumors from a number of viewpoints.

### Abbreviations

- Tumor necrosis factor-alpha-induced protein 8 (TNFAIP8)
- Death effector domain (DED)
- Genotype-Tissue Expression (GTEx)
- Copy number alteration (CNA)
- Disease-specific survival (DSS)
- Breast invasive carcinoma (BRCA)
- Esophageal carcinoma (ESCA)
- Glioblastoma multiforme (GBM)
- Kidney renal clear cell carcinoma (KIRC)
- Kidney renal papillary cell carcinoma (KIRP)
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>gastric carcinoma</td>
<td>STAD</td>
</tr>
<tr>
<td>thyroid carcinoma</td>
<td>THCA</td>
</tr>
<tr>
<td>colon adenocarcinoma</td>
<td>COAD</td>
</tr>
<tr>
<td>lung adenocarcinoma</td>
<td>LUAD</td>
</tr>
<tr>
<td>lung squamous cell carcinoma</td>
<td>LUSC</td>
</tr>
<tr>
<td>pancreatic adenocarcinoma</td>
<td>PAAD</td>
</tr>
<tr>
<td>rectal adenocarcinoma</td>
<td>READ</td>
</tr>
<tr>
<td>skin cutaneous melanoma</td>
<td>SKCM</td>
</tr>
<tr>
<td>testicular germ cell tumor</td>
<td>TGCT</td>
</tr>
<tr>
<td>adrenocortical carcinoma</td>
<td>ACC</td>
</tr>
<tr>
<td>diffuse large B cell lymphoma</td>
<td>DLBC</td>
</tr>
<tr>
<td>sarcoma</td>
<td>SARC</td>
</tr>
</tbody>
</table>
uterine carcinosarcoma  
renal clear cell carcinoma  
endometrial carcinoma  
hepatocellular carcinoma  
kidney chromophobe  
Tumor-associated macrophages

**Declarations**

**Ethical Approval**

Our study do not contain any animal and patients.

**Competing interests**

All the authors declare no conflicts of interest.

**Authors' contributions**

YJC and XzZ proposed the study idea. YjC collected and analysed the data and drafted the manuscript. XzZ, TgL and KhS critically revised the manuscript. All authors contributed to the article and approved the submitted version.

**Funding**

This study is supported by grants from the Natural Science Foundation of Shandong Province. (Grant No. ZR2020MH322)

**Availability of data and materials**

None
Acknowledgements

None

Authors and Affiliations

Yingjun Chen†, Xuezhong Zhang‡, Dai Li3, Kaihui Sha4*Tonggang Liu1*

1 Department of Infectious Diseases, Binzhou Medical University Hospital, Binzhou, 256600, Shandong, China

2 Department of Laboratory Medicine, Zibo Central Hospital, Zibo, 255000, Shandong, China

3 Department of General Surgery, The Fourth Affiliated Hospital of China Medical University, Shenyang, 110000, Liaoning, China

4 Binzhou Medical University School of Nursing, Binzhou, 256600, Shandong, China

Correspondence should be addressed to Tonggang Liu; liutonggang123@126.com

References


**Figures**
Figure 1

TNFAIP8L2 gene expression level in different tumors and pathological stages. (a) Expression status of the TNFAIP8L2 gene in different cancers or specific cancer subtypes analyzed in TIMER2. * P < .05; ** P < .01; *** P < .001. (b) For LAML, LGG, OV, TGCT, and SKCM in the TCGA project, the corresponding normal tissues of the GTEx database were included as controls. Box plot data are indicated. ** P < .01. (c) Based on the CPTAC dataset, we also analyzed the expression level of SND1 total protein between normal tissue
and primary tissue of clear cell RCC, UCEC, HNSC, PAAD, GBM, and HCC. *** P < .001. (d) Based on TCGA data, the expression levels of the SND1 gene were analyzed according to the main pathological stages (stage I, stage II, stage III, and stage IV) of SKCM, THCA, STAD, KIRC, KICH, and ESCA. Log2 (TPM + 1) was applied for log-scale.

Figure 2

Correlation between TNFAIP8L2 gene expression and survival prognosis of different cancers in TCGA. We used the GEPIA2 tool to analyze overall survival (a) and disease free survival (b) analyses of different tumors in TCGA according to TNFAIP8L2 gene expression. The survival map and Kaplan-Meier curves with related results are provided in the figure.
Figure 3

TNFAIP8L2 mutation features in different TCGA tumors. We analyzed TNFAIP8L2 mutation features for TCGA tumors using the cBioPortal tool. The alteration frequency is displayed according to mutation type (a) and mutation site (b). The mutation site with the highest alteration frequency (R53Q/Q55Pfs*29) is overlaid in TNFAIP8L2's 3D structure (c). We analyzed the potential correlation between mutation status and overall, disease-specific, disease-free, and PFS of UCEC (d) using the cBioPortal tool.
Figure 4

TNFAIP8L2 DNA methylation levels in different tumors in the UALCAN and TCGA databases. We observed different methylation expression levels of TNFAIP8L2 in BLCA, BRCA, CHOL, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PCPG, PRAD, READ, UCEC, HNSC, and SKCM tumor tissues compared to normal tissues (a).
Figure 5

Correlation analysis between TNFAIP8L2 expression and immune infiltration of cancer-associated fibroblasts (a), macrophages (c), and CD8+ T cells (d). Different algorithms were used to explore the potential correlation between TNFAIP8L2 gene expression level and the infiltration level of cancer-associated fibroblasts across all types of cancer in TCGA (b).
Figure 6

TNFAIP8L2-related gene enrichment analysis. (a) We first obtained the available experimentally determined TNFAIP8L2-binding proteins using the STRING tool. (b) Using the GEPIA2 approach, we also obtained the top 100 SND1-correlated genes in TCGA projects and analyzed the expression correlation between TNFAIP8L2 and selected targeting genes including ARHGDIB, IGSF6, RGS19, S1PR4, and GMFG. (c) The corresponding heatmap data in the detailed cancer types are displayed. (d) An intersection analysis of TNFAIP8L2-binding and correlated genes was conducted. (e) KEGG pathway analysis based on TNFAIP8L2 binding and interacted genes. (f) Cnetplot for the molecular function data in GO analysis.