Computational and Structural Biotechnology Journal 23 (2024) 234–250

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Research article

Comprehensive pan-cancer analysis unveils the significant prognostic value and potential role in immune microenvironment modulation of TRIB3

Chao Hu [a], Qingzhou Li [a], Lei Xiang [b], Yan Luo [b], Shengrong Li [a], Jun An [b], Xiankuo Yu [b], Guochen Zhang [b], Yuhui Chen [b], Yumei Wang [b,*], Dong Wang [a,b,**]

[a] State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China
[b] School of Basic Medical Sciences, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

ARTICLE INFO

Keywords:
TRIB3
Pan-cancer analysis
Tumor immune microenvironment
Prognostic biomarker

ABSTRACT

TRIB3, a pseudokinase, was previously studied within only some specific cancer types, leaving its comprehensive functions in pan-cancer contexts largely unexplored. Here, we performed an integrated analysis of *TRIB3* expression, prognosis, genetic alterations, functional enrichment and tumor immune-related characteristics in 33 cancer types. Our results showed that *TRIB3* exhibits high expression levels across 24 different cancer types and correlates closely with unfavorable prognoses. Meanwhile, *TRIB3* shows mutations in a wide spectrum of 22 distinct cancer types, with the predominant mutation types being missense mutations and gene amplifications, and significant changes in DNA methylation levels in 14 types of cancer. We further discovered that *TRIB3* expression is significantly associated with cancer immune-related genome mutations, such as tumor mutational burden (TMB), microsatellite instability (MSI) and DNA mismatch repair (MMR), and infiltration of immunosuppressive cells, such as $CD4^+$ Th2 cells and myeloid-derived suppressor cells (MDSCs), into the tumor microenvironment. These results indicated that the expression of *TRIB3* might reshape the tumor immune microenvironment (TIME) and lead to immunosuppressive "cold" tumors. In addition, our results confirmed that the loss of function of *TRIB3* inhibits cell proliferation, promotes apoptosis, and leads to significant enrichment of "hot" tumor-related immune pathways, at least in breast cancer cells, which further supports the important role of *TRIB3* in cancer prognosis and TIME regulation. Together, this pan-cancer investigation provided a comprehensive understanding of the critical role of *TRIB3* in human cancers, and suggested that *TRIB3* might be a promising prognostic biomarker and a potential target for cancer immunotherapy.

1. Introduction

According to data reported by the World Health Organization, cancer is the second leading cause of death globally and poses a serious threat to human health. Unfortunately, the data indicates that cancer cases will continue to increase rapidly until 2040 [1,2]. Currently, cancer treatment methods primarily include surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy [3,4]. Despite the constant improvement of cancer treatment strategies and the increased understanding of the complex pathogenesis of tumors, issues such as drug resistance, side effects, and differences in the tumor immune microenvironment (TIME) of different patients still result in unsatisfactory patient prognosis and survival rates [5]. Therefore, further research into the potential molecular mechanisms of cancer occurrence and development may reveal new cancer treatment methods. In recent years, immunotherapy has significantly changed the landscape of tumor treatment, particularly immune checkpoint inhibitors (ICIs), which have achieved great success in clinical practice. However, in many patients receiving the same treatment, the difference in the TIME of "cold" and "hot" tumors results in a very low objective response rate in "cold" tumor patients [6,7]. Hence, it is urgent to discover new immune-related treatment targets and develop new drugs for tumors.

TRIB3 is a member of the pseudokinase family, comprises 358 amino acids, and possesses three distinct functional domains. These domains include the central kinase domain, which lacks ATP-specific binding sites and a catalytic core, rendering it devoid of kinase activity; the N-

* Corresponding author.
** Corresponding author at: State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China.
E-mail addresses: yumeiwang@cdutcm.edu.cn (Y. Wang), dwang@cdutcm.edu.cn (D. Wang).

https://doi.org/10.1016/j.csbj.2023.11.043
Received 9 July 2023; Received in revised form 7 November 2023; Accepted 22 November 2023
Available online 30 November 2023

terminal domain, which is primarily involved in binding to transcription factors; and the C-terminal domain, which interacts mainly with ubiquitin ligases [8]. Although TRIB3 lacks kinase activity, it acts as an adaptor/scaffold protein and directly interacts with various proteins, including ATF4 [9], CHOP [10], COP1 [11], MEK1 [12], MKK7 [13], AKT1/2 [14], PPARγ [15], SIAH1 [16], EGFR [17], β-catenin, and TCF4 [18], and regulates their activity. Therefore, *TRIB3* is a key "stress regulator" in cancer. Recent research suggests that *TRIB3* may act as both a tumor suppressor gene and an oncogene, and the mechanisms underlying its dual role in cancer are not yet clear [19,20]. Several studies have found that *TRIB3* is upregulated in various types of cancer, including breast cancer [21], liver cancer [22], lung cancer [23], glioblastoma [24], ovarian cancer [25], oral squamous cell carcinoma [26], gastric cancer [27], colorectal cancer [28], and bladder cancer [29], and is closely associated with cancer development and poor prognosis. However, some studies have found that higher levels of TRIB3 protein in breast cancer are associated with better prognosis [30], and knocking down *TRIB3* in liver cancer cells increases the survival rate of MHCC02H liver cancer cells [31]. It is worth noting that an increasing number of studies suggest that *TRIB3* plays a more prominent role as an oncogene in cancer.

Here, through the implementation of an extensive pan-cancer analysis, this study revealed the abnormal expression, DNA mutations and DNA methylation patterns associated with the *TRIB3* gene. Importantly, we also uncovered the pivotal impact of *TRIB3* on tumor prognosis and its ability to modulate the immune response within tumors. Our study emphasizes the prognostic significance of *TRIB3* in pan-cancer scenarios and its potential as a target for advancing cancer immunotherapy.

2. Materials and methods

2.1. Cancer data collection

We downloaded gene expression data, clinical data, and mutation data for 33 cancers and their corresponding paracancerous tissues in TCGA from the UCSC Xena database (https://xena.ucsc.edu/) [32]. Furthermore, we obtained data from the METABRIC study, which includes both gene expression and clinical information from breast cancer patients, via the cBioPortal database (http://www.cbioportal.org/) [33]. We obtained the GSE116671 dataset from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) [34], which comprises RNA microarray data of control or *TRIB3*-silenced MCF7 cells samples. The full names and abbreviations of the 33 cancer types we studied can be found in Table 1.

2.2. Differentially expressed analysis

First, we performed differentially expressed analysis for the *TRIB3* gene between tumors and adjacent normal tissues in the TCGA database using the "Exploration-Gene DE" module of the TIMER2.0 network server (http://timer.comp-genomics.org/) [35] and calculated the statistical significance using the Wilcoxon test.

Furthermore, to analyze the expression of *TRIB3* in cancers without corresponding normal tissues in the TCGA database (e.g., ACC, DLBC, LAML, LGG, OV, SARC, SKCM, TGCT, THYM, UCS), we utilized the "Expression Analysis" module of the GEPIA 2 network server (http://gepia2.cancer-pku.cn/#analysis) to examine the expression differences between normal tissues in the GTEx database and the corresponding tumor tissues in the TCGA database [36]. We obtained the differentially expressed significance with p value < 0.05 using one-way ANOVA.

Finally, to investigate the differential protein expression of TRIB3 in cancer samples and normal tissues, immunohistochemical images based on the TRIB3 antibody (catalog number: HPA055442; Atlas Antibodies, Sigma—Aldrich) were obtained from the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) [37].

Table 1
Full names and abbreviations of the 33 cancer types in TCGA.

Serial number	Cancer Types	Abbreviations
1	Adrenocortical carcinoma	ACC
2	Bladder Urothelial Carcinoma	BLCA
3	Breast invasive carcinoma	BRCA
4	Cervical squamous cell carcinoma and endocervical adenocarcinoma	CESC
5	Cholangiocarcinoma	CHOL
6	Colon adenocarcinoma	COAD
7	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	DLBC
8	Esophageal carcinoma	ESCA
9	Glioblastoma multiforme	GBM
10	Head and Neck squamous cell carcinoma	HNSC
11	Kidney Chromophobe	KICH
12	Kidney renal clear cell carcinoma	KIRC
13	Kidney renal papillary cell carcinoma	KIRP
14	Acute Myeloid Leukemia	LAML
15	Brain Lower Grade Glioma	LGG
16	Liver hepatocellular carcinoma	LIHC
17	Lung adenocarcinoma	LUAD
18	Lung squamous cell carcinoma	LUSC
19	Mesothelioma	MESO
20	Ovarian serous cystadenocarcinoma	OV
21	Pancreatic adenocarcinoma	PAAD
22	Pheochromocytoma and Paraganglioma	PCPG
23	Prostate adenocarcinoma	PRAD
24	Rectum adenocarcinoma	READ
25	Sarcoma	SARC
26	Skin Cutaneous Melanoma	SKCM
27	Stomach adenocarcinoma	STAD
28	Testicular Germ Cell Tumors	TGCT
29	Thyroid carcinoma	THCA
30	Thymoma	THYM
31	Uterine Corpus Endometrial Carcinoma	UCEC
32	Uterine Carcinosarcoma	UCS
33	Uveal Melanoma	UVM

2.3. Correlation of TRIB3 expression with clinical tumor stage

The correlation between *TRIB3* expression and tumor stage for 33 types of cancers was analyzed by the R packages "limma" and "ggpubr". The results were then visualized as box plots.

2.4. Expression and survival prognosis analysis of TRIB3

We utilized three survival prognostic indicators, overall survival (OS), disease-specific survival (DSS), and progression-free period (PFI), to examine the correlation between *TRIB3* expression and cancer patient prognosis. Additionally, we also analyzed the prognostic correlation between *TRIB3* expression in METABRIC data and OS in breast cancer patients. The median of *TRIB3* expression were used as the expression threshold to classify low and high expression subgroups. Kaplan—Meier survival curves were plotted using the R packages "survminer" and "survivor", and statistical significance was determined using the log-rank test. Univariate Cox analysis was performed using the R packages "survival" and "forestplot", and forest plots were created to display the risk ratio (HR), 95% confidence interval, and p value. A risk ratio (HR) less than 1 indicated that *TRIB3* was a protective factor for cancer, while an HR greater than 1 indicated that *TRIB3* was a risk factor for cancer.

2.5. TRIB3 mutation characterization

To examine the mutational signature of *TRIB3* in various cancers, we utilized the cBioPortal tool. First, we selected the TCGA Pan-cancer Atlas Studies cohort. Next, we entered "*TRIB3*" in the "Query" module, and the tool provided information on *TRIB3* change sites, types, and numbers in both the "Cancer Types Summary" and "Mutations" modules.

2.6. DNA methylation analysis for TRIB3

To assess the relationship between *TRIB3* expression and DNA methylation in 33 different types of cancers found within the TCGA database, we utilized the SMART platform (http://www.bioinfo-zs.com/smartapp/), which is an interactive web server that provides a comprehensive analysis of DNA methylation in the TCGA project [38]. Specifically, we entered "*TRIB3*" into the "Quick Start" module. Then, we used the "CpG-aggregated methylation" module to calculate the pan-cancer *TRIB3* methylation level and displayed the resulting data in a box plot.

2.7. Functional enrichment analysis of TRIB3

To explore the biological function of *TRIB3*, we conducted functional enrichment analyses of *TRIB3* using the LinkedOmics (www.linkedomics.org/login.php), which is a powerful platform designed for acquiring, analyzing, and comparing multi-omics cancer data across various tumor types, providing a unique resource for cancer research [39]. To be more precise, we employed the Pearson correlation test to establish the correlation between *TRIB3* and co-expressed genes. Subsequently, we generated an enriched result bar graph by selecting the "GSEA Enrichment Analysis" and "KEGG pathway" options using the "LinkInterpreter" module.

2.8. Correlation analysis of TRIB3 expression with the tumor microenvironment (TME)

ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) is a sophisticated scoring system designed to evaluate the tumor purity, stromal cell presence, and immune cell infiltration in tumor tissue by analyzing expression data [40]. Hence, this scoring system has been used to calculate the ImmuneScore, StromalScore, and ESTIMATEScore for 33 different types of cancer in TCGA. The scores indicate the proportion of corresponding components present in the TME. Higher scores correspond to a greater proportion of the corresponding component in the TME. The ESTIMATEScore is the sum of the ImmuneScore and StromalScore, which reflects the combined proportion of the two components in the TME. The "estimate" R package and Spearman correlation test were used to estimate the ImmuneScore and StromalScore as well as tumor purity for each tumor. The resulting data were then visualized using the "ggplot2", "ggpubr", "heatmap" and "ggExtra" R packages.

2.9. Correlation analysis of TRIB3 expression with immune infiltration

We utilized the TIMER 2.0 database (http://timer.comp-genomics.org/) [35] "immune-gene" module, with purity adjustment applied, to investigate the association between *TRIB3* expression and tumor-infiltrating immune cells (TIICs) across various cancer types. The analysis incorporated TIMER, EPIC, MCPCOUNTER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, and TIDE algorithms. The Spearman correlation test was utilized to determine p values and partial correlation values, with the results presented as a heatmap. Additionally, the R package "limma" was utilized to identify the correlation between *TRIB3* expression and immune-related genes, including immunostimulator genes, immunoinhibitor genes, chemokine receptors genes, chemokines genes, and major histocompatibility complex (MHC) genes, which were also visualized as a heatmap using the R package "heatmap".

2.10. Correlation analysis of TRIB3 expression with tumor mutational burden (TMB), microsatellite instability (MSI), and mismatch repair (MMR) genes

Previous studies have demonstrated that tumor mutational burden-high (TMB-H), mismatch repair deficiency (dMMR), and high microsatellite instability (MSI-H) are predictive biomarkers that have gained significant attention in recent years for assessing the response to immune checkpoint blockade (ICB) therapy [41,42]. To explore the correlation between *TRIB3* expression and TMB or MSI, Spearman's rank correlation coefficient was employed, and the results were presented using a radar chart generated by the "fmsb" package in R. The correlation between *TRIB3* and MMR gene expression was determined based on gene expression profiling data from the TCGA cohort using the "limma" package in R, and the results were visualized as a heatmap using the "heatmap" package in R.

2.11. RNA microarray data analysis upon TRIB3 silencing in MCF-7 breast cancer cells

Differentially expressed analysis for the GSE116671 expression matrix was conducted using the "limma" R package. The result was visualized as a volcano plot using the "ggplot" R package. Subsequently, the "clusterProfiler" R package was employed to conduct KEGG and GSEA enrichment analyses. The enriched KEGG pathways were visualized as bar plots using the "ggplot" R package, while the GSEA enrichment results were visualized using the "GseaVis" R package.

2.12. Cell lines and cultures

Triple-negative breast cancer (TNBC) MDA-MB-231 cells were obtained from ATCC (Manassas, USA). Additionally, TNBC HCC1806 cells were acquired from Zhejiang Mason Cell Technology Co. (Zhejiang, China). The MDA-MB-231 and HCC1806 cell lines were maintained in RPMI 1640 medium (Gibco, Thermo Fisher, China) supplemented with 10% fetal bovine serum (FBS, ExCell, China) and 100 U/ml penicillin-streptomycin (HyClone™, Utah, USA), at 37 ℃ in a 5% CO2 incubator.

2.13. siRNA reverse transfection assay

Following the transfection reagent kit protocol, we used 40 pmol of siRNA per well for the 12-well plate containing 1 ml of culture medium and 100 pmol of siRNA per well for the 6-well plate containing 2 ml of cell culture medium. Opti-MEM™(Gibco, Thermo Fisher, China), *TRIB3*-siRNA (si*TRIB3*), and NC-siRNA (siNC) were mixed with the transfection reagent (Lipo8000, Beyotime, China) and incubated. Subsequently, the resulting mixture was evenly distributed at the bottom of the plates: 7×10^4 cells were seeded in each well of the 12-well plate, and 2×10^5 cells were seeded in each well of the 6-well plate. After 48 h of transfection, we extracted total mRNA or protein to assess the transfection efficiency of the siRNA. The sequence of *TRIB3* siRNA is as follows:

TRIB3 siRNA-1: GGUGUACCCCGUCCAGGAATT;
TRIB3 siRNA-2: GGACCUGAGAUACUCAGCUTT;
TRIB3 siRNA-3: GAUGAUUCCCUGUGGGACA;
TRIB3 siRNA-4: UCGCUGACCGUGAGAGGAA;
TRIB3 siRNA-5: ACAGAGAAGUGGUUCUGUA;

Negative control siRNA: UUCUCCGAACGUGUCACGUTT. These siRNAs were purchased from GenePharma (Shanghai, China).

2.14. Cloning formation experiment

Based on the transfection reagent kit protocol, Opti-MEM™ (Gibco, Thermo Fisher, China), *siTRIB3*/siNC and the transfection reagent (Lipo8000, Beyotime, China) were mixed and left to stand. The resulting mixture was then evenly added to the bottom of a 12-well plate, and cells were seeded into each well. Once cell colonies formed, they were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. Finally, the cell colonies were imaged and counted for statistical analysis.

2.15. RNA extraction and quantitative real-time fluorescence quantitative PCR

Total mRNA was extracted using the RNA isolater kit (Vazyme Biotech Co., Ltd, China) following the manufacturer's protocol. The extracted total mRNA was then reversely transcribed into cDNA using the HiScript II Q RT SuperMix for qPCR kit (Vazyme, China), followed by RT-PCR amplification using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) with GAPDH serving as the reference gene. Data analysis was performed using the $2^{-\Delta\Delta CT}$ method. The forward and reverse primers used are as follows:

TRIB3-F: TGCGTGATCTCAAGCTGTG;
TRIB3-R: CTTGTCCCACAGGGAATCA.
GAPDH-F: GTCTCCTCTGACTTCAACAGCG;
GAPDH-R: ACCACCCTGTTGCTGTAGCCAA.

2.16. Western blotting

After 48 h of cell transfection in a 6-well plate, the cells were lysed on ice for 30 min using RIPA buffer (Beyotime, Shanghai, China), which included protease inhibitors and phosphatase inhibitors (BOSTER, Wuhan, China). The lysate was then centrifuged to obtain the supernatant. Subsequently, the protein concentration in the samples was determined using a BCA protein assay kit (CWBIO, Jiangsu, China). The protein samples were mixed with loading buffer (Epizyme, Shanghai, China), heated at 95 °C for 10 min to denature the proteins, and then loaded onto a 10% SDS-polyacrylamide gel for electrophoresis. The proteins were subsequently transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). After a 20 min incubation in a QuickBlock™ Western blocking solution (Beyotime, Shanghai, China), primary antibodies and HRP-conjugated secondary antibodies were added sequentially to incubate the membrane. Finally, the target proteins were visualized using the ECL High Sensitivity Chemiluminescent Substrate (4 A BIOTECH, Suzhou, China). GAPDH (1:20,000, 60004–1-Ig), TRIB3 (1:500, 13300–1-AP), HRP-conjugated anti mouse IgG (1:5000, SA00001–1) and anti-rabbit IgG (1:5000, SA00001–2) were obtained from Proteintech (Wuhan, Hubei, China).

2.17. Apoptosis assay

After 48 h of cell transfection in a 6-well plate, following the protocol of the Annexin V-FITC Cell Apoptosis Detection Kit (Beyotime, Shanghai, China), the cell culture medium was removed, and the cells were washed once with PBS. Subsequently, Annexin V-FITC binding solution was added, followed by the addition of Annexin V-FITC and propidium iodide staining solution. The mixture was gently mixed and then incubated in a light-avoiding environment at room temperature for 15 min. This was followed by capturing images using a fluorescence microscope (Olympus, IX73, Japan) and subsequent statistical analysis.

2.18. Statistical analysis

For this study, all data were calculated, graphed, and statistically analyzed using GraphPad Prism 8.3.0 and R software (version 4.1.1). The differentially expressed analysis was performed using either the Wilcoxon test or one-way ANOVA, while the log-rank test was used to determine statistically significance in prognosis analysis. The correlation between the two groups was calculated using either Spearman or Pearson correlation analysis. Statistical analysis of qPCR and cloning formation data was conducted using Student's t-test or one-way ANOVA. Significance levels are defined as $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

3. Results

3.1. TRIB3 is significantly highly expressed in 24 types of cancers

To investigate the differential expression of *TRIB3* in various human cancers and their corresponding adjacent tissues, we utilized the TIMER2.0 platform to analyze RNA sequencing data of 33 types of cancer in TCGA (Fig. 1A). Simultaneously, we used the GEPIA2 platform to analyze various cancers in TCGA without normal samples by combining the GTEx and TCGA databases (Fig. 1B-C). Our findings indicated that *TRIB3* expression was statistically significant in 25 types of cancer, except for tumors without available normal tissue data (MESO and UVM). Among these, *TRIB3* was highly expressed in 24 types of cancer, including BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, OV, PRAD, READ, SKCM, STAD, TGCT, THYM, UCEC, and UCS. Notably, READ and COAD exhibited the highest upregulation of *TRIB3* expression compared to normal tissue, while *TRIB3* expression was significantly decreased in LAML.

We carried out further investigation into the protein expression levels of TRIB3 in both normal tissues and tumors. Immunohistochemical images were obtained from the HPA database. Our findings indicate that TRIB3 expression may be higher in BRCA, COAD, HNSC, LIHC, and SKCM than that in the corresponding normal tissues (Fig. S1). These results seemed to align with the mRNA expression results obtained from our analysis.

3.2. TRIB3 is significantly highly expressed in the late stages of 8 cancer types

As pathological staging is a critical prognostic indicator for cancer patients, the study evaluated the mRNA expression of *TRIB3* in stages I, II, III, IV, and X of cancer patients. The findings demonstrated a significant correlation between *TRIB3* expression and tumor stage across eight types of cancer (Fig. S2), namely, ACC, BLCA, BRCA, COAD, HNSC, KIRC, KIRP, and LIHC. Additionally, *TRIB3* expression was found to be higher in the later stages of most cancers. While *TRIB3* expression in ESCA, LUAD, MESO, and UVM did not show a significant association with tumor staging, its expression increased along with higher stages.

3.3. High TRIB3 expression indicates poor prognosis in multiple cancer types

After conducting research previously on the expression level of *TRIB3* and tumor pathological staging, we performed further analysis using single-factor Cox regression analysis and Kaplan—Meier survival analysis to determine the correlation between the *TRIB3* expression and the prognosis of various cancer patients. Notably, this study investigated three survival indicators: OS, DSS, and PFI.

Initially, the relationship between *TRIB3* expression and cancer patient OS was studied, and single-factor Cox regression analysis indicated that *TRIB3* is a hazard factor for BLCA, HNSC, KIRC, KIRP, LGG, LIHC, MESO, PCPG, SARC, UCEC, and UVM patients (Fig. 2A). Kaplan—Meier survival analysis also established that high *TRIB3* expression is significantly correlated with poor prognosis in BLCA, HNSC, KIRC, KIRP, LIHC, LUAD, MESO, PCPG, SARC, and UCEC patients (Fig. 2B-K).

Subsequently, a single-factor Cox regression analysis of DSS data found a significant positive correlation between high *TRIB3* expression and poor prognosis in ACC, HNSC, KIRC, KIRP, LGG, LIHC, MESO, PCPG, UCEC, and UVM patients (Fig. S3A). Additionally, Kaplan—Meier survival analysis of DSS data showed that high *TRIB3* expression is significantly correlated with poor prognosis in ESCA, HNSC, KIRC, KIRP, MESO, PCPG, UCEC, and UVM patients (Fig. S3B-I).

Finally, investigating the association between *TRIB3* expression and cancer patient PFI, the study discovered that *TRIB3* expression is a hazard factor in CESC, HNSC, KICH, KIRC, KIRP, MESO, PCPG, PRAD,

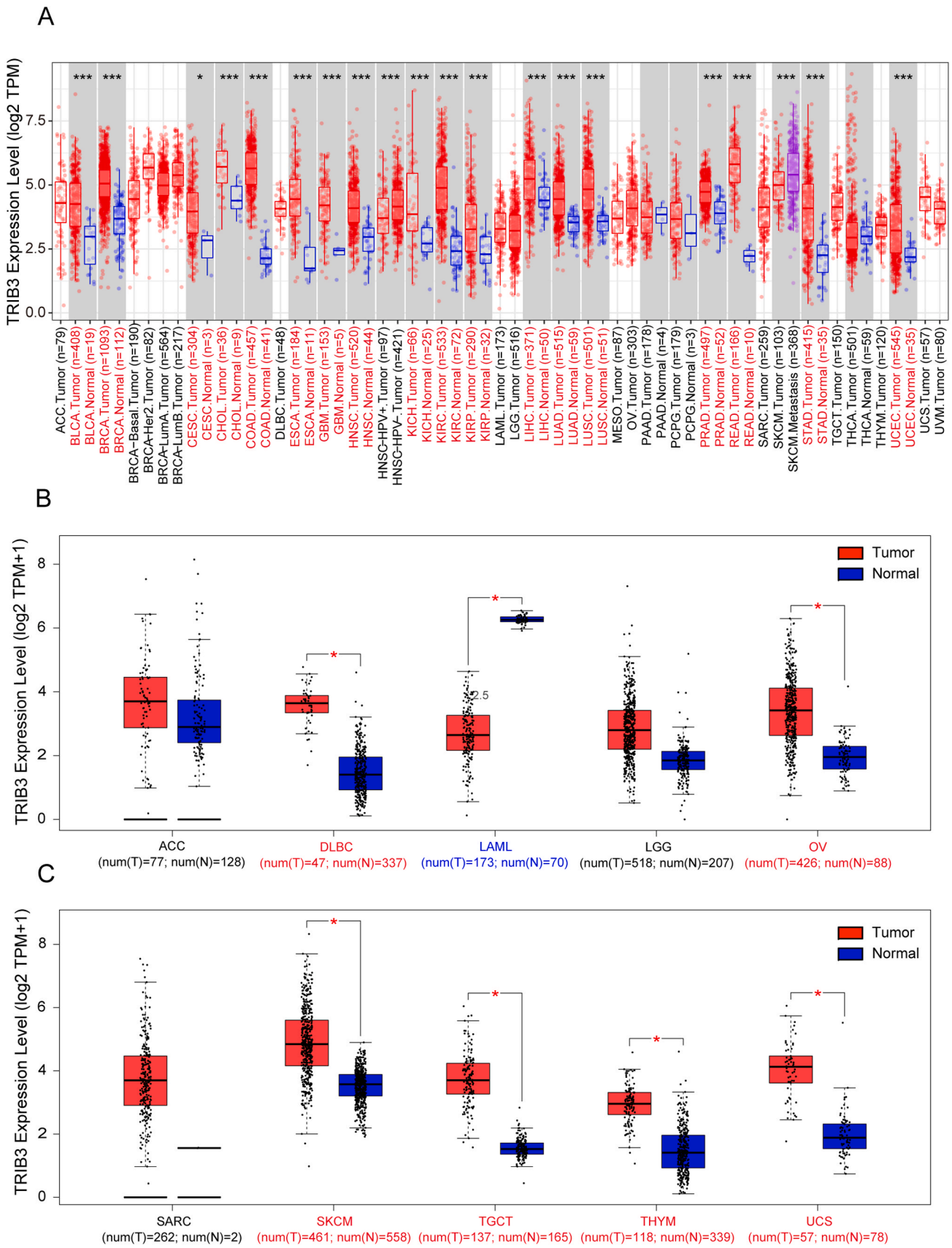

Fig. 1. *TRIB3* exhibits high expression levels in 24 types of tumors, but demonstrates low expression levels specifically in LAML. (A) Differences in *TRIB3* expression between 33 normal and tumor tissues in TCGA from the TIMER2.0 database (*P < 0.05, **P < 0.01, ***P < 0.001). (B-C) Differences in *TRIB3* expression between cancers from the TCGA database and normal samples from the GTEx database (*P < 0.05). Red indicates high expression, and blue indicates low expression.

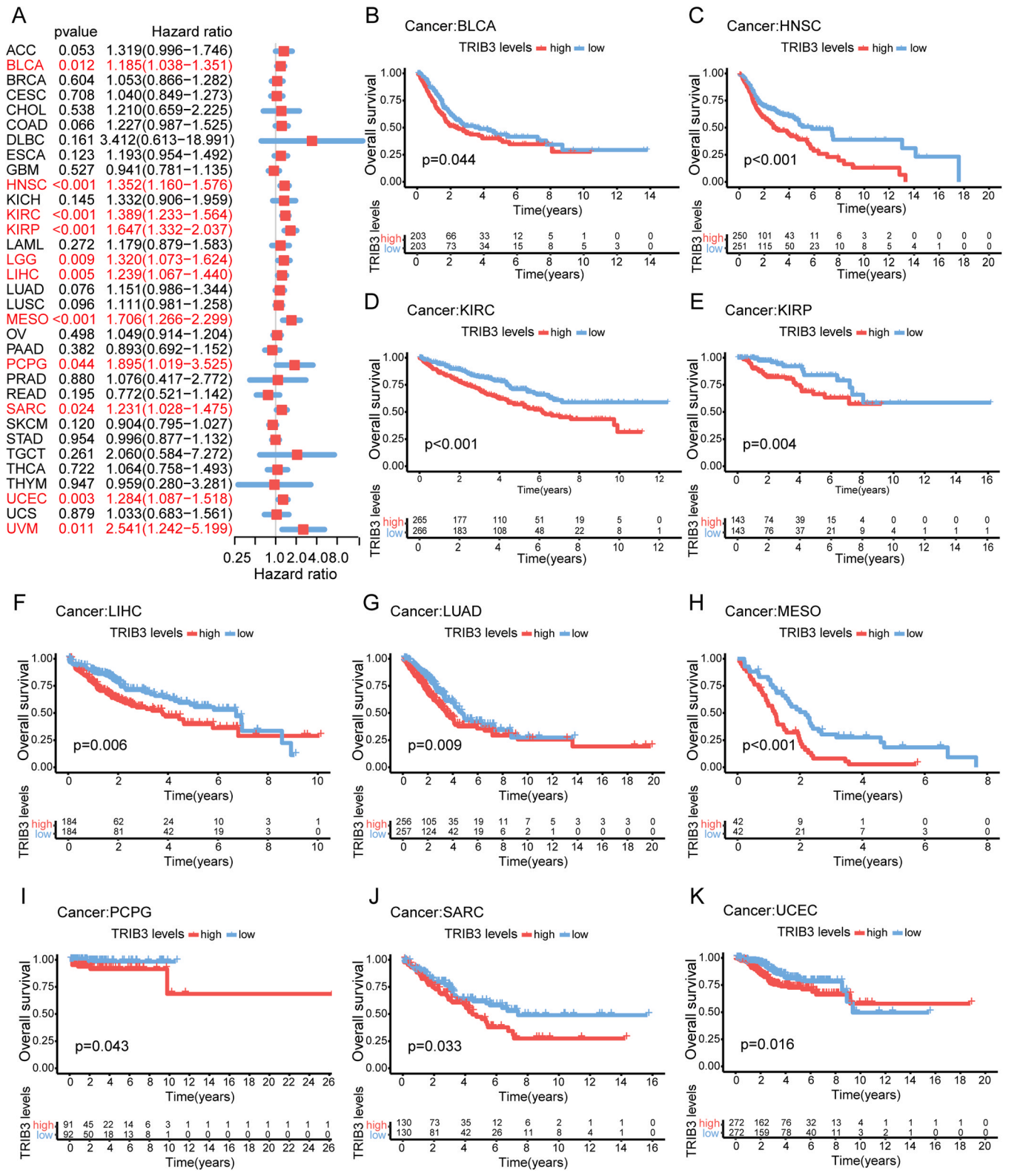

Fig. 2. *TRIB3* expression is correlated with an unfavorable prognosis of OS in 10 types of cancer patients. (A) Forest plot depicting the results of survival analysis of *TRIB3* expression on OS in pan-cancer; cancers marked in red are significant ($P < 0.05$). (B-K) Results of Kaplan—Meier analysis of significance between *TRIB3* expression and OS ($P < 0.05$).

UCEC, and UVM patients determined via a single-factor Cox regression analysis (Fig. S4A). Kaplan—Meier survival curve analysis also showed a significant correlation between high *TRIB3* expression and poor prognosis in ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, MESO, PCPG, UCEC, and UVM patients (Fig. S4B-K). The results showed that high *TRIB3* expression is closely related to poor prognosis of various cancers, revealing the potential of *TRIB3* as a pan-cancer prognostic biomarker.

3.4. *TRIB3* exhibits mutations in a wide spectrum of 22 distinct cancer types, with the predominant mutation types being missense mutations and gene amplifications

Due to the significant role of gene mutations in tumor development, we utilized the CBioPortal platform to conduct a comprehensive analysis of *TRIB3* mutations in pan-cancer using data from 10,953 patients in the TCGA database. The findings revealed that missense mutations and amplifications were the primary types of *TRIB3* mutations, with OV (4.97%), UCEC (4.35%), and LUSC (4.31%) being the top three mutated cancer types (Fig. 3A). Additionally, we identified 87 mutation points within amino acids 0–358, with 72 missense mutations, 12 truncating mutations, 1 splice site mutation, and 2 SV/fusion mutations mainly located in TRIB3 protein structural domains. The C46Afs* 5 mutation is the most frequent mutation site and has been found in LUSC and COADREAD cases (Fig. 3B).

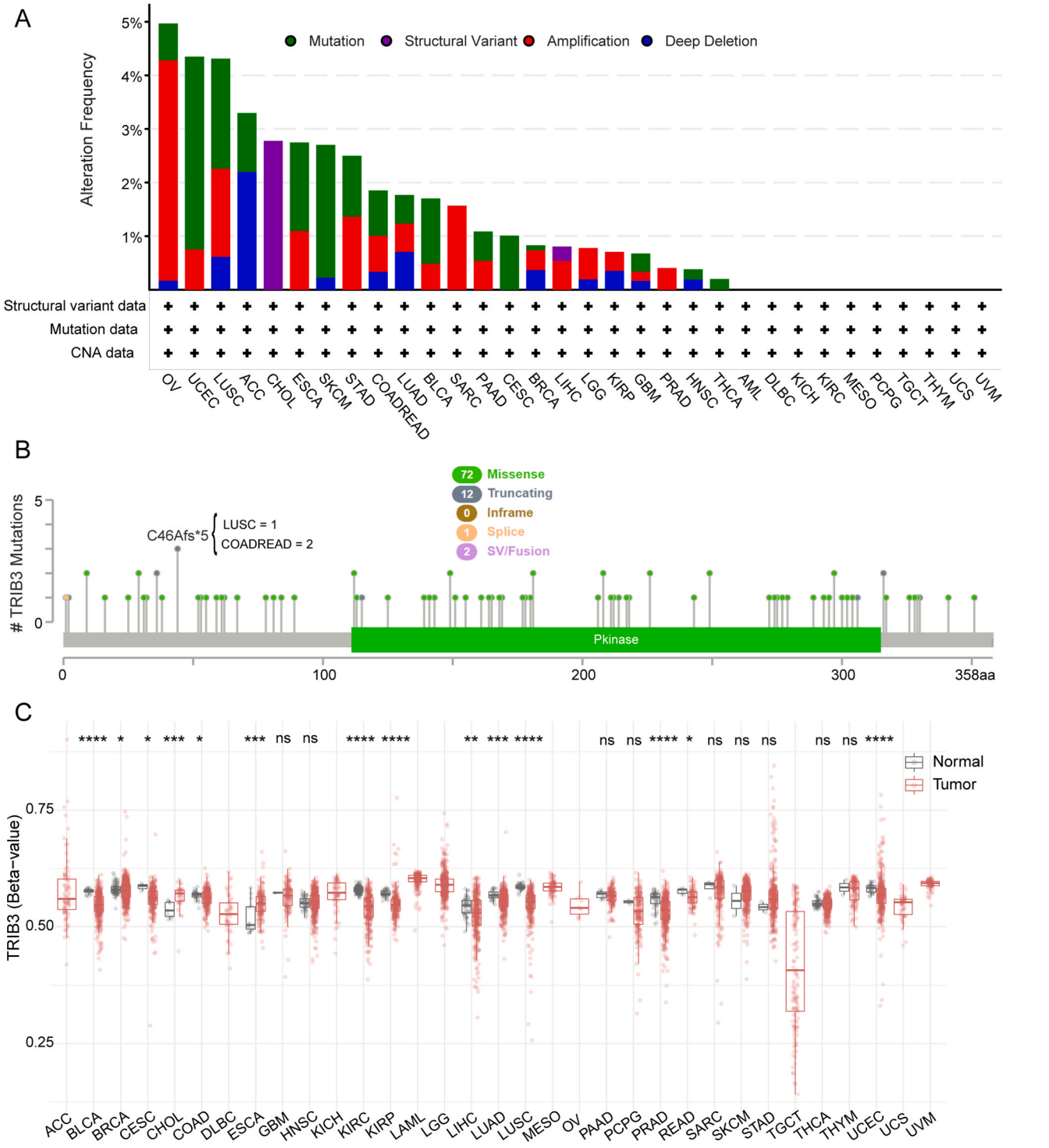

Fig. 3. Genetic alterations of *TRIB3* in pan-cancer. (A) Frequency and mutation types of *TRIB3* gene alterations in pan-cancer. COADREAD refers to the COAD and READ. (B) *TRIB3* mutations across protein structural domains. (C) DNA methylation levels of *TRIB3* in pan-cancer; *P < 0.05, * *P < 0.01, * **P < 0.001, * ** *P < 0.0001.

3.5. TRIB3 shows significant changes in DNA methylation levels in 14 types of cancer

There is mounting evidence that DNA methylation could be a promising diagnostic, prognostic, and predictive biomarker in cancer [43]. Therefore, we employed the SMART platform to examine the DNA methylation levels of *TRIB3* in tumor tissues and their corresponding normal tissues in the TCGA database (Fig. 3C). The results showed significant variations in *TRIB3* DNA methylation across 14 types of cancer. Notably, *TRIB3* methylation levels were significantly decreased in BLCA, BRCA, CESC, COAD, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, and UCEC patient tissues, while the opposite was true in CHOL and ESCA. Furthermore, the findings indicated that DNA methylation levels of *TRIB3* were negatively correlated with its mRNA expression in most cancers.

3.6. The expression of TRIB3 is significantly associated with immune-related pathways in 11 types of cancers

Following above results, we conducted a deeper investigation into the biological functions of *TRIB3* across different types of cancers. To this end, we utilized the LinkedOmics database to conduct gene set enrichment analysis (GSEA) on *TRIB3*. The results of the analysis revealed the top 10 ranked KEGG pathways in GSEA. Remarkably, we found that immune-related pathways were enriched in 11 types of cancers, including BRCA, CHOL, COADREAD, DLBC, ESCA, HNSC, LUAD, LUSC, PAAD, PRAD, and STAD (Fig. 4). Furthermore, we observed that the high expression of *TRIB3* was negatively associated with immune-related pathways, such as cytokine—cytokine receptor interaction, chemokine signaling pathway, T cell receptor signaling pathway, and Th17 cell differentiation pathway. Conversely, we found that the high expression of *TRIB3* was the positively enriched KEGG pathways primarily included ribosome, proteasome, cell cycle, RNA transport, and carbon metabolism. Taken together, these findings suggested potential correlation between high *TRIB3* expression and immune suppression in the TIME.

3.7. High TRIB3 expression indicates lower abundance of stromal and immune cells in the TME across 14 cancer types

The TME plays a critical role in promoting tumor occurrence, development, metastasis, recurrence, drug resistance, and immune suppression through complex cell-to-cell signaling [44]. Our functional enrichment analysis of *TRIB3* revealed its association with immune-related signaling pathways. Therefore, it is of great significance to further investigate the pan-cancer relationship between *TRIB3* expression and the TME. To do so, we used the ESTIMATE algorithm to calculate StromalScore, ImmuneScore, ESTIMATEScore, and tumor purity of *TRIB3* in 33 types of cancers. According to our research findings, we observed a significant negative correlation between the expression of TRIB3 and the StromalScore, ImmuneScore, and ESTIMATEScore in 14 different types of cancers, namely, BRCA, CHOL, COAD, DLBC, ESCA, HNSC, LAML, LUAD, LUSC, PAAD, PRAD, SKCM, STAD, and THCA. However, the opposite result was observed in BLCA. Interestingly, among 15 types of cancers, including BRCA, CHOL, COAD, DLBC, ESCA, HNSC, LAML, LUAD, LUSC, PAAD, PRAD, SKCM, STAD, THCA, and UCEC, the expression of *TRIB3* was significantly positively correlated with tumor purity, while the opposite result was observed in BLCA, KIRC, and TGCT (Fig. 5A). Moreover, the scatterplot provides a more detailed illustration of the correlation between *TRIB3* expression and StromalScore, ImmuneScore, and tumor purity in BRCA, DLBC, and LUAD (Fig. 5B-J). Specifically, our results suggested that the proportions of immune cells and stromal cells are lower in most tumors with high *TRIB3* expression.

3.8. TRIB3 expression is positively associated with the infiltration of immunosuppressive cells, such as $CD4^+$ Th2 cells and myeloid-derived suppressor cells (MDSCs), in the majority of cancer types

The TIME refers to all the immune components within the TME that have been shown to play crucial roles in tumor immune suppression, metastasis, recurrence, and resistance [45]. Previous research has indicated that the expression of *TRIB3* is closely related to the TME. To investigate this further, we used the TIMER 2.0 database, which employs multiple immune prediction methods, to evaluate the correlation between *TRIB3* expression and immune infiltrating cells. Our results demonstrate that *TRIB3* expression was positively correlated with most MDSCs, $CD4^+$ Th2 cells, M0 macrophages, resting mast cells, and common lymphoid progenitors in most tumors, whereas *TRIB3* expression was negatively correlated with $CD8^+$ T cells, B cells, and hematopoietic stem cells in most cancers (Fig. 6). The above results provided further evidence that high expression of TRIB3 promotes the formation of an immunosuppressive TME.

3.9. The expression of TRIB3 is significantly correlated with immune-related genes in multiple types of cancer

We conducted gene co-expression analysis to explore the relationship between *TRIB3* expression and the expression of genes encoding MHC, immunostimulator, immunoinhibitor, chemokines, and chemokine receptor proteins. Our findings indicate that *TRIB3* is significantly correlated with most immunostimulator genes in various cancers. Specifically, *TRIB3* expression was negatively correlated in BRCA, CHOL, COAD, ESCA, HNSC, LUAD, LUSC, PAAD, PRAD, SKCM, STAD, and THCA, while it was significantly positively correlated in BLCA, KIRC, and KIRP. Interestingly, *TRIB3* expression was significantly positively correlated with the expression of the ULBP1, PVR, and CD276 genes in almost all cancers (Fig. S5A). Additionally, *TRIB3* expression was negatively correlated with immunoinhibitor genes, particularly in BRCA, COAD, ESCA, HNSC, LUSC, PAAD, PRAD, SKCM, STAD, and THCA. In contrast, *TRIB3* expression was positively correlated with immunoinhibitor in BLCA, KIRC, KIRP, and TGCT (Fig. S5B). With respect to chemokine receptors, *TRIB3* expression was negatively correlated in BRCA, CHOL, COAD, HNSC, LUAD, LUSC, PAAD, PRAD, SKCM, STAD, and THCA (Fig. S5C) and positively correlated with most chemokines in most cancers, except for CHOL, PAAD, and PRAD (Fig. S5D). Finally, *TRIB3* expression was positively correlated with almost all MHC genes in BLCA and TGCT, while it was negatively correlated with most MHC genes in BRCA, COAD, ESCA, LUAD, LUSC, PAAD, PRAD, SKCM and STAD (Fig. S5E). Taken together, these findings suggested that TRIB3 might be involved in regulating the biological functions of immune cell infiltration and various immune-related genes.

3.10. The expression of TRIB3 is significantly correlated with cancer immune-related TMB, MSI, and MMR in multiple types of tumors

Many studies have shown that patients with MSI-H and TMB-H tumors have a higher response rate to immunotherapy [41,42]. Thus, we investigated the relationship between TMB, MSI, and *TRIB3* expression across cancers. *TRIB3* expression was significantly correlated with TMB in 18 types of cancer, with positive correlations observed in BLCA, BRCA, HNSC, KICH, KIRC, LGG, LUAD, LUSC, OV, PAAD, PCPG, PRAD, SARC, SKCM, STAD, and UCEC and negative correlations in COAD and KIRP (Fig. 7A). Additionally, we found that *TRIB3* expression was significantly positively correlated with MSI in 6 types of cancer, including BLCA, HNSC, KICH, PRAD, STAD, and UCEC, but significantly negatively correlated with MSI in COAD, LAML, and TGCT (Fig. 7B).

Moreover, MMR can repair base mismatches during DNA replication to maintain genomic stability, and MMR deficiency can generate new antigens, increasing the sensitivity of tumor patients to immunotherapy [42]. Therefore, we evaluated the correlation between *TRIB3* and MMR

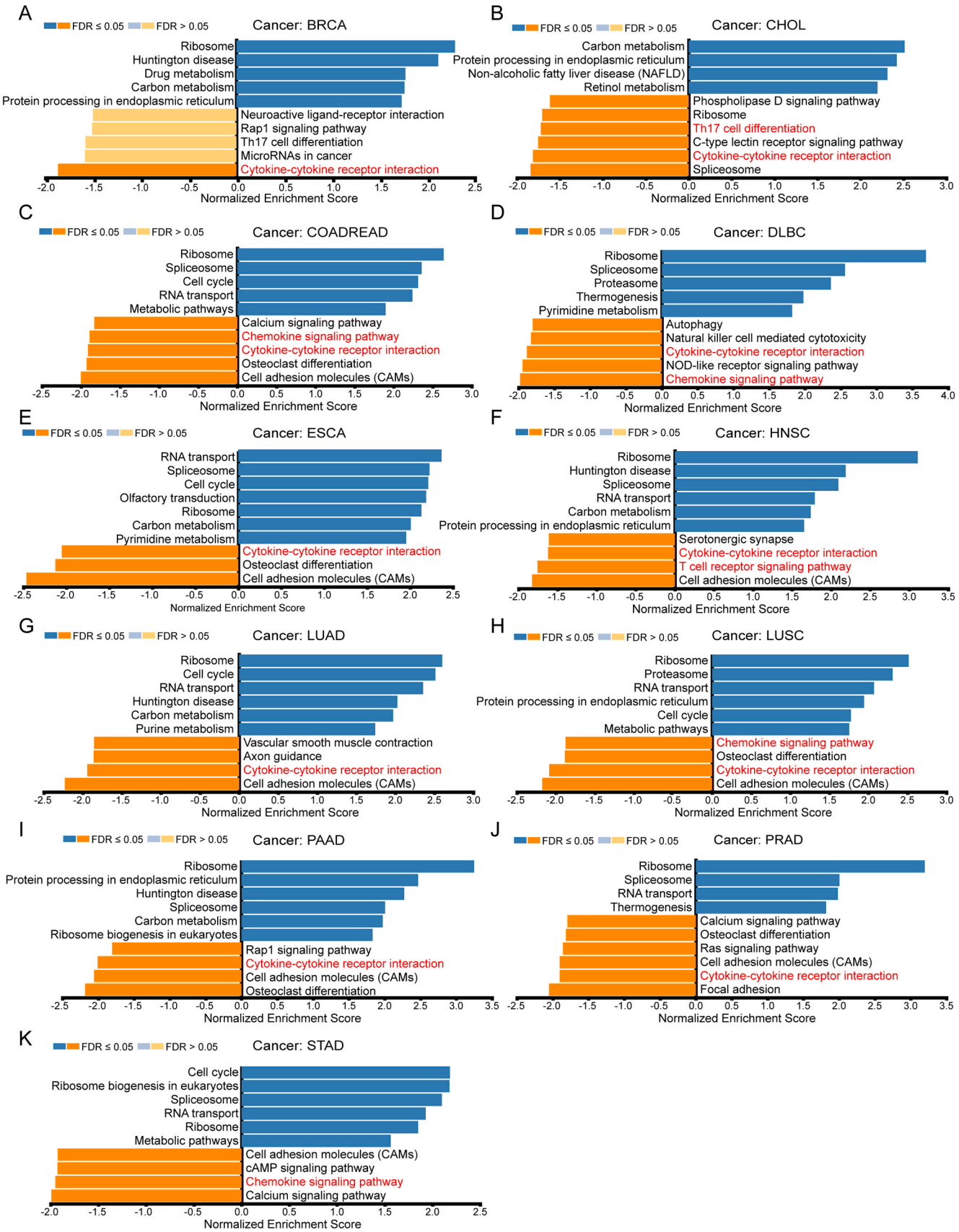

Fig. 4. *TRIB3* expression in 11 cancers with GSEA-KEGG functional enrichment results significantly correlated with immune-related pathways. Top 10 GSEA functionally enriched pathways of *TRIB3* in (A) BRCA, (B) CHOL, (C) COADREAD, (D) DLBC, (E) ESCA, (F) HNSC, (G) LUAD, (H) LUSC, (I) PAAD, (J) PRAD, and (K) STAD. Those marked in red are immune-related pathways (FDR $\leq$ 0.05). Blue represents positive enrichment, and yellow represents negative enrichment.

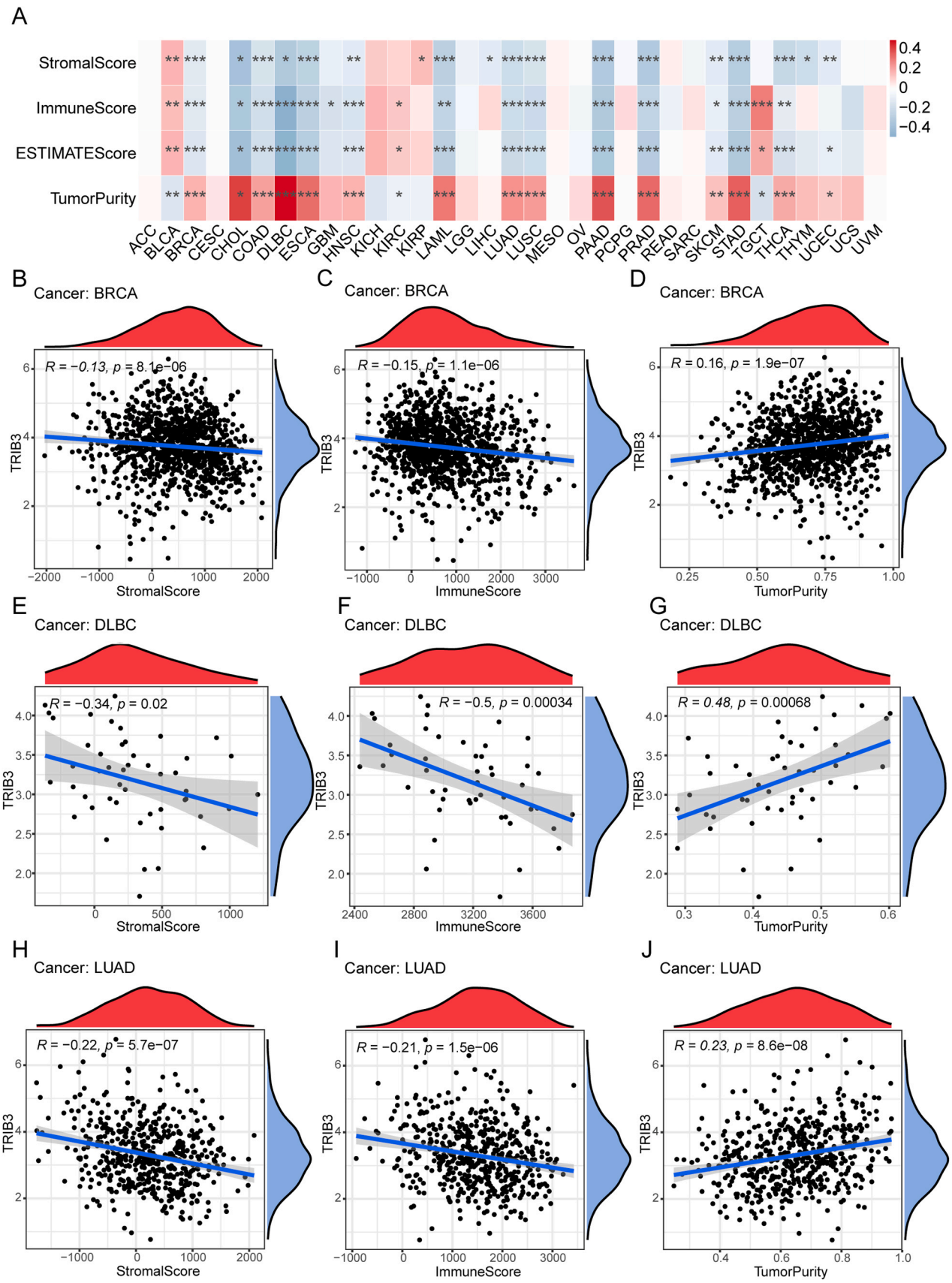

Fig. 5. Elevated *TRIB3* expression is linked to reduced levels of stromal and immune cells in the TME across 14 cancer types. (A) Heatmap showing the correlation between *TRIB3* in pan-cancer and StromalScore, ImmuneScore, ESTIMATEScore and tumor purity; red represents positive correlation, and blue represents negative correlation. (B-J) Specific correlation plots between *TRIB3* expression in BRCA, DLBC, LUAD and StromalScore, ImmuneScore, tumor purity; * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$.

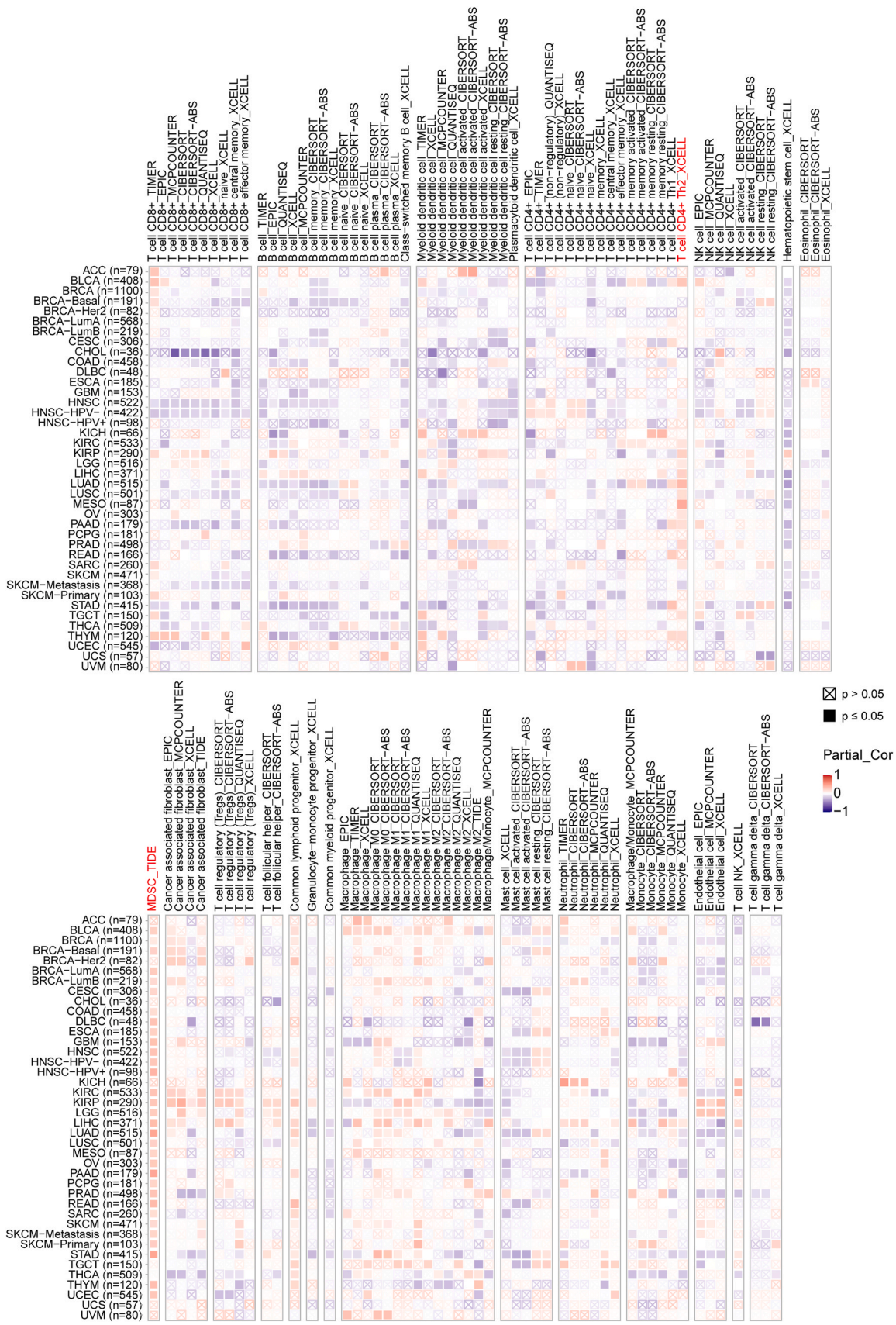

Fig. 6. *TRIB3* expression positively correlates with recruitment of immunosuppressive cells, such as $CD4^+$ Th2 cells and MDSCs, in most cancer types, while showing a negative correlation with $CD8^+$ T cell infiltration in certain cancers. Orange color indicates significant positive correlation and purple color indicates significant negative correlation.

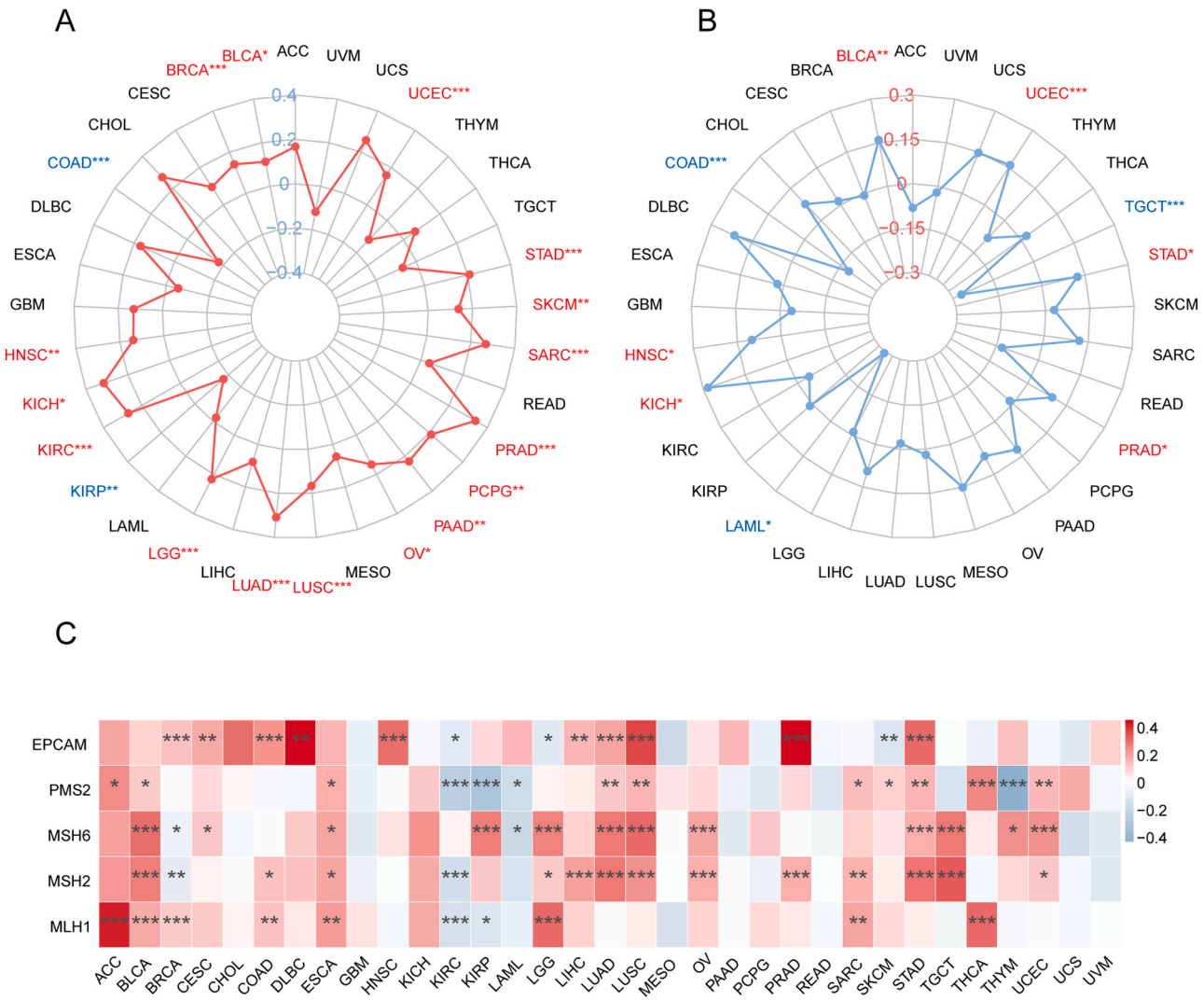

Fig. 7. The expression of *TRIB3* is significantly associated with TMB, MSI, and MMR in multiple types of cancer. (A) Significant positive correlation between *TRIB3* expression and TMB in 16 cancer types (marked in red) and significant negative correlation in 2 cancer types (marked in blue). (B) Significant positive correlation between *TRIB3* expression and MSI in 6 cancer types (marked in red) and significant negative correlation in 3 cancer types (marked in blue). (C) Heat map of the correlation between *TRIB3* expression and MMR genes; red represents positive correlation, and blue represents negative correlation; *P < 0.05, * *P < 0.01, * **P < 0.001.

genes, including MLH1, MSH2, MSH6, PMS2, and EPCAM. The results showed that *TRIB3* expression was closely related to MMR genes in 12 types of cancer, including BLCA, BRCA, COAD, ESCA, KIRC, KIRP, LGG, LUAD, LUSC, SARC, STAD, and UCEC (Fig. 7C). Overall, these findings suggested that *TRIB3* is closely related to various tumors and may be a potential immune biomarker for indicating the immune therapy response of these tumor types.

3.11. Loss-of-function TRIB3 inhibits the proliferation and promotes the apoptosis of breast cancer cells

Due to the limited research on the *TRIB3* gene in breast cancer, some studies have yielded conflicting results. For instance, while increase in *TRIB3* mRNA has been linked to a poorer prognosis [46], other studies have found that high TRIB3 protein expression is associated with a better prognosis in luminal breast cancer [47]. Although, the expression of *TRIB3* was not significantly correlated with the prognosis of BRCA in TCGA, we found that high *TRIB3* expression was significantly associated with a poor OS prognosis in breast cancer patients in METABRIC database (Fig. 8A). Furthermore, several studies have indicated that *TRIB3* contributes to the advancement of TNBC [48,49]. So, we speculated that *TRIB3* could be a potential target for TNBC. To this end, we delved deeper into the influence of *TRIB3* on the proliferation and apoptosis of TNBC cells. To accomplish this, we transfected HCC1806 and MDA-MB-231 cells with five distinct siRNAs targeting *TRIB3*. The results indicate that si*TRIB3*-2, si*TRIB3*-3, si*TRIB3*-4, and si*TRIB3*-5 achieved a higher gene knockout efficiency (Fig. 8B-E, Fig. S6A-B). Subsequently, we chose these four siRNAs for cloning formation experiments and apoptosis experiments. The findings demonstrated a significant reduction in the proliferation capacity of HCC1806 and MDA-MB-231 cells as a result of *TRIB3* function loss (Fig. 8F-I, Fig. S6C-F). Additionally, this loss-of-function of *TRIB3* induces apoptosis in HCC1806 cells (Fig. 8J-K). These observations furnished compelling evidence highlighting the oncogenic role of *TRIB3* in breast cancer.

3.12. The functional deficiency of TRIB3 in breast cancer cells might reverse the immunosuppressive TME

Differential expression analysis was performed on the mRNA expression profile derived from *TRIB3* silencing in MCF-7 cells, confirming the significant downregulation of *TRIB3* and indicating the high quality of the GSE116671 dataset (Fig. 9A). The KEGG enrichment

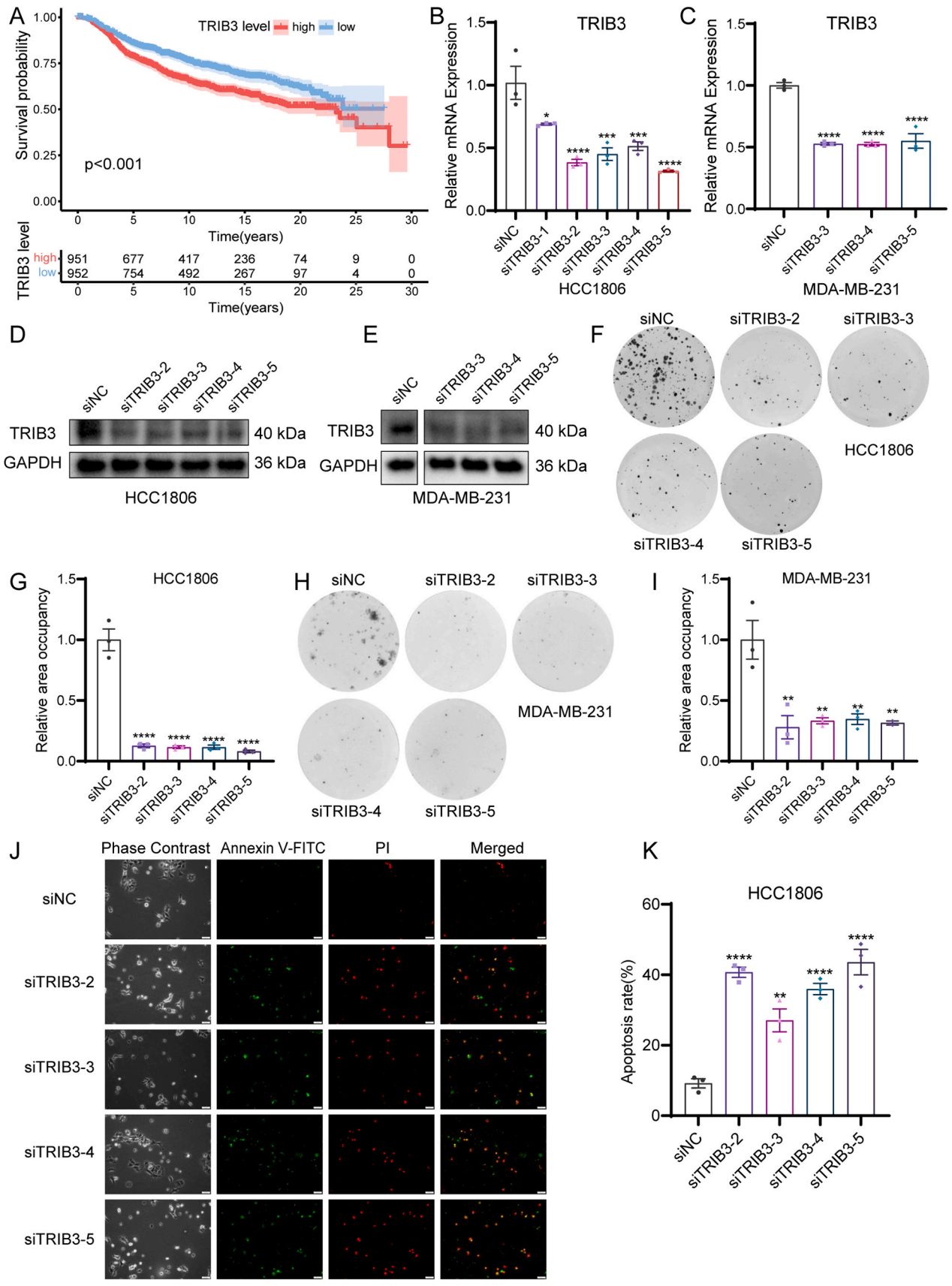

Fig. 8. Loss-of-function *TRIB3* in breast cancer cells significantly suppresses cell proliferation and induces apoptosis. (A) METABRIC data analysis showed that patients with high *TRIB3* expression had a worse prognosis for OS. (B-C) The knockdown efficiency of *TRIB3* mRNA in HCC1806 and MDA-MB-231 cells. (D-E) The knockdown efficiency of TRIB3 protein levels in HCC1806 and MDA-MB-231 cells. (F-I) Silencing of *TRIB3* significantly inhibited the proliferation of HCC1806 and MDA-MB-231 cells. (J-K) Silencing TRIB3 can significantly induce apoptosis in HCC1806 cells. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$, * *** * $P < 0.0001$.

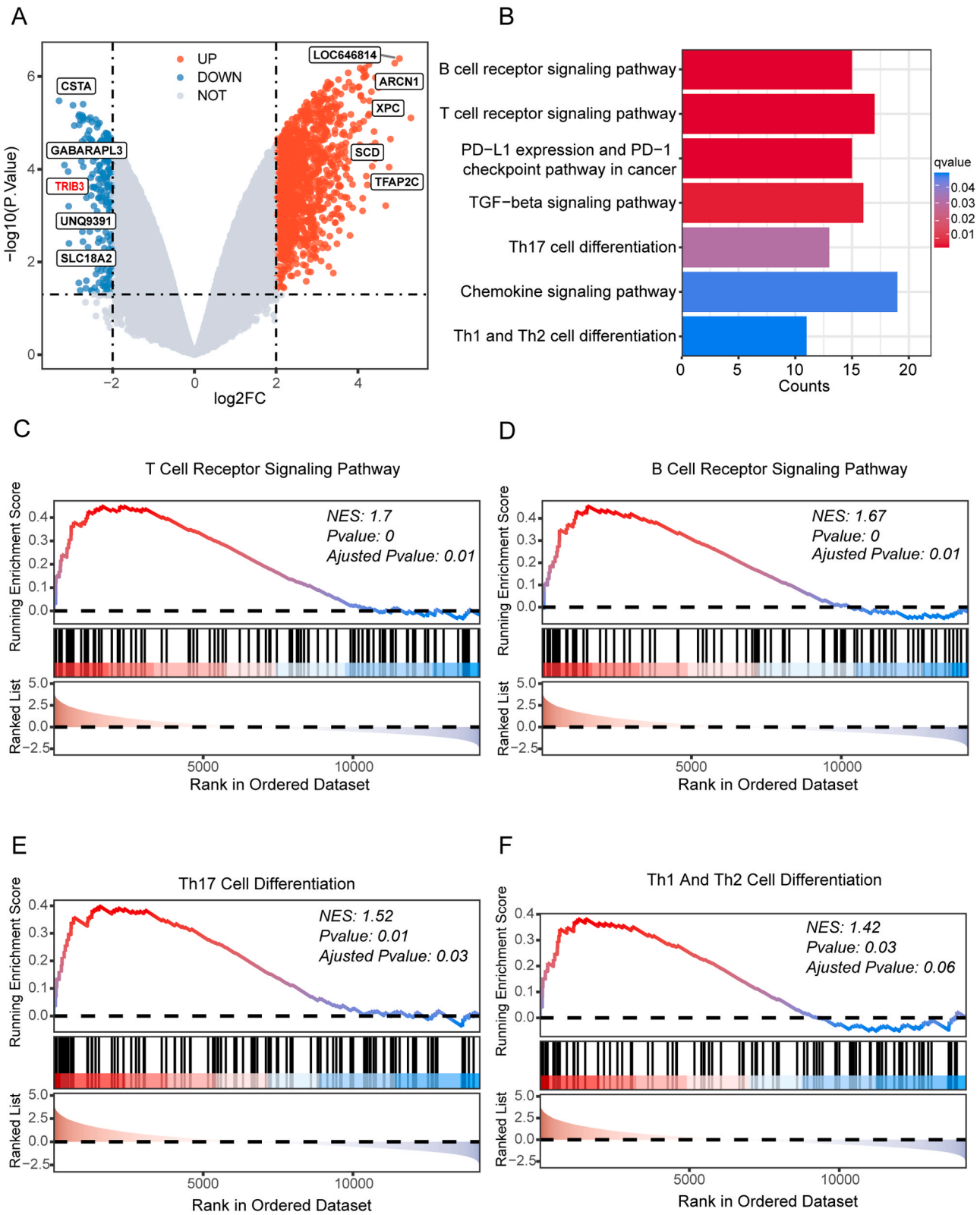

Fig. 9. Loss-of-function *TRIB3* in breast cancer cells is associated with a significant enrichment of multiple immune-related pathways. (A) The volcano plot depicts the differential expression of genes (DEGs) in *TRIB3*-silenced MCF-7 breast cancer cells, where upregulated genes are denoted in red and downregulated genes in blue. (B) The DEGs were enriched in immune-related pathways as identified by KEGG analysis. (C-F) The GSEA-KEGG enrichment analysis of *TRIB3*-silenced MCF-7 cells demonstrated a positive enrichment of immune-related pathways.

analysis demonstrated significant enrichment of "hot" tumor-related pathways, such as the chemokine signaling pathway and the T-cell receptor signaling pathway (Fig. 9B). Notably, the GSEA results also exhibited a similar trend with positive enrichments in the T cell receptor signaling pathway (Fig. 9C-F) and cytokine signaling in immune system (Fig. S6G-J). Together, these results further supported the role of *TRIB3* in fostering the formation of an immunosuppressive tumor microenvironment.

4. Discussion

Through comprehensive pan-cancer analysis, our study revealed that *TRIB3* exhibited elevated mRNA expression levels in as many as 24 different cancers. This abnormal expression pattern was influenced by gene mutations and DNA methylation and showed a significant correlation with poor prognosis across multiple cancers. Additionally, we performed functional enrichment analysis of *TRIB3* and found a significant enrichment of immune-related pathways in 11 cancers. Notably, we observed associations between the expression of *TRIB3* and the recruitment of immunosuppressive cells such as $CD4^+$ Th2 cells and MDSCs. Of particular interest, in breast cancer, our study revealed that loss-of-function of *TRIB3* inhibits cell proliferation, promotes apoptosis, and leads to the significant enrichment of signaling pathways associated with "hot" tumors. These findings suggested that high *TRIB3* expression might reshape the TIME, thereby impeding the transition of "cold" tumors to "hot" tumors. Hence, our research revealed the latent potential of TRIB3 as a prognostic biomarker, and a target for pharmaceutical development and immunotherapeutic interventions.

Genetic mutations and aberrant DNA methylation are known to contribute to dysregulated gene expression in cancer, thereby impacting the onset and progression of cancer [50,51]. The utilization of gene expression profiling data offers a powerful means to investigate the clinical prognosis of genes in cancer patients. Our study reveals consistent and significant overexpression of *TRIB3* in almost all types of cancer, corroborating previous findings. This widespread upregulation of *TRIB3* suggests its potential significance across multiple cancer types, prompting us to further investigate its clinical prognostic implications. Through rigorous analysis of clinical prognosis data, we identified *TRIB3* as an unfavorable prognostic factor in diverse cancers.

Currently, the molecular mechanisms of *TRIB3* have primarily been investigated in specific cancer types, and its role in pan-cancer remains unclear. Previous studies have suggested that TRIB3 functions as an adapter/scaffold protein for various proteins, mediating interactions with transcription factors, ubiquitin ligases, and kinase-dependent proteins to regulate diverse signaling pathways, including MAPK, PI3K/AKT, NF-κB, and TGF-β [19]. However, the precise involvement of *TRIB3* in pan-cancer contexts remains incompletely understood. Notably, pathway enrichment analyses based on GSEA-KEGG in pan-cancer have demonstrated a negative enrichment of immune-related pathways, such as cytokine—cytokine receptor interaction and chemokine signaling pathways. Consistently, functional enrichment analysis of mRNA expression profile from *TRIB3* silencing experiments in MCF-7 cells revealed enrichment of immune-related pathways, including the chemokine signaling pathway and T cell receptor signaling pathway, thereby further confirming the immunomodulatory properties of *TRIB3*. Remarkably, it has been reported that *TRIB3* inhibits the release of macrophage cytokines [52] and can also suppress *CXCL10* expression by inhibiting *STAT1* activation [53]. Consistent with previous findings, our study's results corroborate the notion that *TRIB3* exerts immunoregulatory effects by modulating the release of cytokines and chemokines.

Tumor immunotherapy has shown significant efficacy in various solid tumors and is considered a groundbreaking advancement. However, its clinical response rates remain relatively low [54]. Therefore, the need to identify more effective targets for immune-based therapies is urgent. Numerous studies have indicated that immune cells present in the TME can either promote or inhibit tumor immune responses, thus representing a dual-edged sword in tumor development [55,56]. The composition and activity of immune cells within the TME play a critical role in determining the response to immunotherapy [57]. Prior investigations have demonstrated that cytotoxic $CD8^+$ T lymphocytes (CTLs), $CD4^+$ T helper 1 cells (Th1 cells), and natural killer (NK) cells predominantly exert antitumor effects, while $CD4^+$ Th2 cells and MDSCs are often associated with immune-suppressive microenvironment formation [58,59].

Our study exploring the relationship between *TRIB3* expression and immune cell infiltration reveals a positive correlation between *TRIB3* expression and MDSCs, $CD4^+$ Th2 cells, macrophages of the M0 phenotype, and resting mast cells. Conversely, *TRIB3* expression exhibits a negative correlation with $CD8^+$ T cells and B cells in multiple cancer types. Notably, existing research has demonstrated that overexpression of *TRIB3* inhibits cytokine release from macrophages [52] and contributes to the survival and vitality of bone marrow-derived mast cells [60]. Furthermore, single-cell transcriptome analysis has indicated elevated expression of *TRIB3* in $CD4^+$ Th2 cells, highlighting its high specificity [61]. Another study confirms that *TRIB3* impedes $CD8^+$ T-cell infiltration and induces immune evasion by suppressing the release of the chemokine $CXCL10^{53}$. Consistent with previous reports, our results indicate that *TRIB3*, through its regulation of chemokine and cytokine release, not only hinders the infiltration of $CD8^+$ T cells but also potentially promotes the recruitment of MDSCs and $CD4^+$ Th2 cells into the TME, thereby contributing to the establishment of an immunosuppressive microenvironment.

Due to the limited research on *TRIB3* in breast cancer and the contradictory conclusions, the role of *TRIB3* in breast cancer remains complex and poorly understood. While some studies have associated high *TRIB3* mRNA expression with poor prognosis in breast cancer patients [46], others have found that elevated TRIB3 protein levels are linked to a favorable prognosis [30]. Additionally, the effects of *TRIB3* in breast cancer are further complicated by conflicting findings, such as enhanced cloning formation ability in breast cancer cells (BT474) following *TRIB3* knockdown [62], while *TRIB3* gene silencing suppresses the growth of MDA-MB-231 xenograft tumors [48] and inhibits proliferation in radiation-resistant MDA-MB-231 cells [49]. Consequently, we conducted cloning formation assays and apoptosis assays, which further confirmed that *TRIB3* silencing strongly inhibits cell proliferation in the TNBC cell lines, HCC1806 and MDA-MB-231. Additionally, this loss of function induces apoptosis in HCC1806 cells. Our research results align with the conclusions from previous studies on TRIB3 in TNBC, which leads us to believe that TRIB3 plays an oncogenic role in breast cancer, particularly in TNBC.

Encouragingly, recent research has demonstrated that *TRIB3* expression is suppressed by knocking down *P300* or treating 4T1 murine breast cancer cells with C646, a *P300* inhibitor. Moreover, the combined treatment of C646 and PD-L1 antibody in situ breast cancer models (4T1) showed partial inhibition of tumor progression, with significantly enhanced antitumor efficacy compared to using either treatment alone. Furthermore, treatment with C646, either alone or in combination, significantly increased the infiltration of $CD8^+$ T cells [53]. These findings suggest that targeting *TRIB3* is a crucial strategy for converting "cold tumors" into "hot tumors", particularly in the case of TNBC, where effective therapeutic targets are lacking. These findings underscore the potential of *TRIB3* as a promising immunotherapeutic target in TNBC. Notably, studies have shown that *TRIB3* gene knockout mice do not exhibit significant phenotypic differences compared to wild-type mice, indicating that the gene may not be essential [63]. However, this observation also suggests that *TRIB3* represents a highly promising target that can be selectively modulated for therapeutic purposes.

Although this study systematically revealed the relationship between *TRIB3* and various types of cancer, it still has some limitations. The research primarily relies on bioinformatics analysis, while *TRIB3* is closely associated with multiple cellular signaling pathways. Although

there are reports suggesting that *TRIB3* inhibits immune infiltration of $CD8^+$ T cells and exhibits high expression and specificity in $CD4^+$ Th2 cells, the precise biological functions of *TRIB3* in T cells and its specific relationship with $CD4^+$ Th2 cells have not been fully elucidated. Additionally, there is a lack of research linking *TRIB3* to MDSCs. Therefore, further investigations using basic experimental approaches are needed to clarify the biological functions and molecular mechanisms of *TRIB3* in tumor immunity. Moreover, due to the absence of a crystalline protein structure for TRIB3, we plan to employ gene expression profiling after drug perturbation to identify small molecule compounds that can effectively inhibit the functional activity of *TRIB3*.

5. Conclusion

Our research reveals that *TRIB3* is significantly overexpressed in 24 different cancer types and is closely associated with adverse prognosis. Additionally, *TRIB3* appears to play a critical role in orchestrating the infiltration of immune cells into the tumor microenvironment. It promotes the recruitment of MDSCs and $CD4^+$ Th2 cells by regulating chemokines and cytokines, while concurrently suppressing the infiltration of $CD8^+$ T cells. This intricate modulation might ultimately contribute to the formation of an immunologically "cold" tumor phenotype, facilitating tumor immune escape. These findings provide valuable insights into the potential of *TRIB3* as a target for drug development and immunotherapy, as well as its promising prospects as a prognostic biomarker.

Author Statement

Chao Hu, Yumei Wang, and Dong Wang contributed to the conception of the study. Data collection, analysis, and figure generation were performed by Chao Hu, Lei Xiang, Shengrong Li, Xiankuo Yu, and Guochen Zhang. Experiments were conducted by Qingzhou Li, Jun An, Yan Luo, Yuhui Chen. The manuscript was drafted by Chao Hu. Chao Hu, Yumei Wang, and Dong Wang revised the manuscript. All authors made contributions to the article and have approved the final submitted version.

Declaration of Competing Interest

The authors confirm that they have no known conflicting financial interests or personal associations that might have influenced the findings presented in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 82172723), Science and Technology Department of Sichuan Province (No. 2021ZYD0079 and No. 2023NSFSC1828), and Innovation Team and Talents Cultivation Program of National Administration of Traditional Chinese Medicine (No. ZYYCXTD-D-202209).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.11.043.

References

[1] Sung H, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J Clin 2021;71:209–49.
[2] Miller KD, et al. Cancer treatment and survivorship statistics, 2022. CA Cancer J Clin 2022;72:409–36.
[3] DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res 2008;68:8643–53.
[4] Zhu S, et al. Combination strategies to maximize the benefits of cancer immunotherapy. J Hematol Oncol 2021;14:156.
[5] Meric-Bernstam F, Larkin J, Tabernero J, Bonini C. Enhancing anti-tumour efficacy with immunotherapy combinations. Lancet 2021;397:1010–22.
[6] Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017;541:321–30.
[7] Zhang J, Huang D, Saw PE, Song E. Turning cold tumors hot: from molecular mechanisms to clinical applications. Trends Immunol 2022;43:523–45.
[8] Stefanovska B, André F, Fromigué O. Tribbles pseudokinase 3 regulation and contribution to cancer. Cancers (Basel) 2021;13:1822.
[9] Liew CW, et al. The pseudokinase tribbles homolog 3 interacts with ATF4 to negatively regulate insulin exocytosis in human and mouse beta cells. J Clin Invest 2010;120:2876–88.
[10] Ohoka N, Hattori T, Kitagawa M, Onozaki K, Hayashi H. Critical and functional regulation of CHOP (C/EBP homologous protein) through the N-terminal portion. J Biol Chem 2007;282:35687–94.
[11] Qi L, et al. TRB3 links the E3 ubiquitin ligase COP1 to lipid metabolism. Science 2006;312:1763–6.
[12] Yokoyama T, et al. Trib1 links the MEK1/ERK pathway in myeloid leukemogenesis. Blood 2010;116:2768–75.
[13] Kiss-Toth E, et al. Human Tribbles, a Protein Family Controlling Mitogen-activated Protein Kinase Cascades. J Biol Chem 2004;279:42703–8.
[14] Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science 2003;300:1574–7.
[15] Takahashi Y, Ohoka N, Hayashi H, Sato R. TRB3 suppresses adipocyte differentiation by negatively regulating PPARgamma transcriptional activity. J Lipid Res 2008;49:880–92.
[16] Zhou Y, et al. E3 ubiquitin ligase SIAH1 mediates ubiquitination and degradation of TRB3. Cell Signal 2008;20:942–8.
[17] Yu J-J, et al. TRIB3-EGFR interaction promotes lung cancer progression and defines a therapeutic target. Nat Commun 2020;11:3660.
[18] Hua F, et al. TRIB3 interacts with β-catenin and TCF4 to increase stem cell features of colorectal cancer stem cells and tumorigenesis. Gastroenterology 2019;156:708–721.e15.
[19] Arif A, et al. The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. Int Immunopharmacol 2023;114:109581.
[20] Salazar M, et al. Oncosuppressive functions of tribbles pseudokinase 3. Biochem Soc Trans 2015;43:1122–6.
[21] Yu J, et al. TRIB3 supports breast cancer stemness by suppressing FOXO1 degradation and enhancing SOX2 transcription. Nat Commun 2019;10:5720.
[22] Wang R-Q, et al. Tribbles pseudokinase 3 (TRIB3) contributes to the progression of hepatocellular carcinoma by activating the mitogen-activated protein kinase pathway. Ann Transl Med 2021;9:1253.
[23] Zhou W, Ma J, Meng L, Liu D, Chen J. Deletion of TRIB3 disrupts the tumor progression induced by integrin αvβ3 in lung cancer. BMC Cancer 2022;22:459.
[24] Tang Z, et al. TRIB3 facilitates glioblastoma progression via restraining autophagy. Aging (Albany NY) 2020;12:25020–34.
[25] Wang S, et al. Down-regulation of TRIB3 inhibits the progression of ovarian cancer via MEK/ERK signaling pathway. Cancer Cell Int 2020;20:418.
[26] Shen P, Zhang T-Y, Wang S-Y. TRIB3 promotes oral squamous cell carcinoma cell proliferation by activating the AKT signaling pathway. Exp Ther Med 2021;21:313.
[27] Dong S, et al. Overexpression of TRIB3 promotes angiogenesis in human gastric cancer. Oncol Rep 2016;36:2339–48.
[28] Miyoshi N, et al. Abnormal expression of TRIB3 in colorectal cancer: a novel marker for prognosis. Br J Cancer 2009;101:1664–70.
[29] Yang J, et al. TRIB3 promotes the malignant progression of bladder cancer: an integrated analysis of bioinformatics and in vitro experiments. Front Genet 2021;12:649208.
[30] Wennemers M, Bussink J, Grebenchtchikov N, Sweep FCGJ, Span PN. TRIB3 protein denotes a good prognosis in breast cancer patients and is associated with hypoxia sensitivity. Radio Oncol 2011;101:198–202.
[31] Li Y, et al. TRB3 reverses chemotherapy resistance and mediates crosstalk between endoplasmic reticulum stress and AKT signaling pathways in MHCC97H human hepatocellular carcinoma cells. Oncol Lett 2018;15:1343–9.
[32] Goldman MJ, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol 2020;38:675–8.
[33] Cerami E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.
[34] Barrett T, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41:D991–5.
[35] T L, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48.
[36] Z T, B K, C L, T C, Z Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;(47).
[37] Uhlen M, et al. A pathology atlas of the human cancer transcriptome. Science 2017;357:eaan2507.
[38] Li Y, Ge D, Lu C. The SMART App: an interactive web application for comprehensive DNA methylation analysis and visualization. Epigenetics Chromatin 2019;12:71.
[39] Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res 2018;46:D956–63.
[40] Yoshihara K, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun 2013;4:2612.
[41] Chan TA, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol 2019;30:44–56.
[42] Yi M, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. Mol Cancer 2018;17:129.

[43] Koch A, et al. Analysis of DNA methylation in cancer: location revisited. Nat Rev Clin Oncol 2018;15:459–66.
[44] Anderson NM, Simon MC. The tumor microenvironment. Curr Biol 2020;30: R921–5.
[45] Fu T, et al. Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response. J Hematol Oncol 2021;14:98.
[46] Wennemers M, et al. Tribbles homolog 3 denotes a poor prognosis in breast cancer and is involved in hypoxia response. Breast Cancer Res 2011;13:R82.
[47] Orea-Soufi A, et al. The Pseudokinase TRIB3 negatively regulates the HER2 receptor pathway and is a biomarker of good prognosis in luminal breast cancer. Cancers (Basel) 2021;13:5307.
[48] Izrailit J, Berman HK, Datti A, Wrana JL, Reedijk M. High throughput kinase inhibitor screens reveal TRB3 and MAPK-ERK/TGFβ pathways as fundamental Notch regulators in breast cancer. Proc Natl Acad Sci USA 2013;110:1714–9.
[49] Lee Y-C, et al. Tribbles homolog 3 involved in radiation response of triple negative breast cancer cells by regulating notch1 activation. Cancers (Basel) 2019;11:127.
[50] Endicott JL, Nolte PA, Shen H, Laird PW. Cell division drives DNA methylation loss in late-replicating domains in primary human cells. Nat Commun 2022;13:6659.
[51] Nishiyama A, Nakanishi M. Navigating the DNA methylation landscape of cancer. Trends Genet 2021;37:1012–27.
[52] Steverson D, et al. Tribbles homolog 3 promotes foam cell formation associated with decreased proinflammatory cytokine production in macrophages: evidence for reciprocal regulation of cholesterol uptake and inflammation. Metab Syndr Relat Disord 2016;14:7–15.
[53] Shang S, et al. TRIB3 reduces CD8+ T cell infiltration and induces immune evasion by repressing the STAT1-CXCL10 axis in colorectal cancer. Sci Transl Med 2022;14: eabf0992.
[54] Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. Nat Rev Cancer 2021;21:345–59.
[55] Locy H, et al. Immunomodulation of the tumor microenvironment: turn foe into friend. Front Immunol 2018;9:2909.
[56] LV B, et al. Immunotherapy: reshape the tumor immune microenvironment. Front Immunol 2022;13.
[57] Petitprez F, Meylan M, de Reyniès A, Sautès-Fridman C, Fridman WH. The tumor microenvironment in the response to immune checkpoint blockade therapies. Front Immunol 2020;11:784.
[58] Basu A, et al. Differentiation and regulation of TH cells: a balancing act for cancer immunotherapy. Front Immunol 2021;12.
[59] Zhang Q, et al. CCL5-mediated Th2 immune polarization promotes metastasis in luminal breast cancer. Cancer Res 2015;75:4312–21.
[60] Ord T, Ord D, Kuuse S, Plaas M, Ord T. Trib3 is regulated by IL-3 and affects bone marrow-derived mast cell survival and function. Cell Immunol 2012;280:68–75.
[61] Jaroušek R, et al. Single-cell RNA sequencing analysis of T helper cell differentiation and heterogeneity. Biochim Biophys Acta Mol Cell Res 2022;1869: 119321.
[62] Salazar M, et al. Loss of tribbles pseudokinase-3 promotes Akt-driven tumorigenesis via FOXO inactivation. Cell Death Differ 2015;22:131–44.
[63] Okamoto H, et al. Genetic deletion of Trb3, the mammalian Drosophila tribbles homolog, displays normal hepatic insulin signaling and glucose homeostasis. Diabetes 2007;56:1350–6.