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A Pan-cancer Analysis Revealing the Role of TIGIT in Tumor Microenvironment

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ABSTRACT

T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), an immune checkpoint, plays a pivotal role in immune suppression. However its role in tumor immunity and correlation with the genetic and epigenetic alterations remains unknown. Here, we comprehensively analyzed the expression patterns of the TIGIT and its value of prognostic prediction among 33 types of cancers based on the data collected from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression projects (GTEx). Furthermore, the correlations of TIGIT with tumor-infiltrating immune cells (TIICs), immune checkpoint genes, the degree of Estimation of STromal and Immune cells in MAlignant Tumor tissues using the Expression data (ESTIMATE), tumor mutation burden (TMB), microsatellite instability (MSI), mismatch repair (MMR) genes, and DNA methyltransferases (DNMTs) were also explored. Gene functional enrichment was conducted by Gene Set Enrichment Analysis (GSEA). Our results showed that the expression of TIGIT was upregulated in most of the cancer types. Cox regression model showed that high expression of TIGIT in tumor samples correlates with poor prognosis in KIRC, KIRP, LGG, UVM, and with favorable prognosis in BRCA, CECS , HNSC, SKCM. TIGIT expression significantly correlated with TIICs and the degree of ESTIMATE in KIRC, KIRP and UVM. TIGIT expression also correlated with CTLA4, PDCD1 (PD-1), CD274 (PD-L1), ICOS in most of the cancer types. Furthermore, the expression of TIGIT was correlated with TMB, MSI, MMR genes and DNMTs in different types of cancers. GSEA analysis showed that the expression of TIGIT was related to cytokine-cytokine receptor interaction, allograft rejection, oxidative phosphorylation.These findings suggested that TIGIT could serve as a potential biomarker for prognosis and a novel target for immunotherapies in cancers.

Introduction

T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT, also called WUCAM, Vstm3, VSIG9), an immune inhibitory receptor (IR) and immune checkpoint upregulated by immune cells including activated T cells, natural killer (NK) cells, and regulatory T cells (Tregs), plays critical roles in limiting adaptive and innate immunity against tumors[1-3]. Upregulation of TIGIT was observed in a variety of cancers, such as lung cancers[4], kidney cancers[5], liver cancers[6].Several mechanisms of the TIGIT inhibition of T cells in the tumor microenvironment (TME) have been revealed[7]. Currently, TIGIT has been viewed as a promising biomarker to predict the prognosis and a potential target to develop novel immunotherapies[8] . However, a specific function of TIGIT in pan-cancers remains largely unknown.

The initiation and development of the cancers are largely dependent on immune dysfunction[9].TME consists of a variety of cells including immune cells, stromal cells, etc. The tumor and immune cells interact with each other dynamically in TME, which determines the characteristics and heterogenicity of the cancers[10,11]. Under chronic exposure to tumor antigens, T cells become dysfunctional/exhausted and upregulate several IRs including programmed cell death receptor 1 (PD-1) and TIGIT[12,13]. Immunotherapies such as Immune checkpoint blockade (ICB) have achieved great progress and show tremendous potentiality, especially for those patients with resistance to chemoradiotherapy[14,15]. However, the clinical options of the immunotherapies are still lacking[16]. Thus it is of great significance and urgency to explore and validate more effective immune-related targets.

In this research, taking advantages of TCGA and GTEx datasets, we conducted a comprehensive analysis at pan-cancer level to illustrate the TIGIT expression profiles, prognostic values and its correlation with immune infiltration level, tumor mutation burden (TMB), microsatellite instability (MSI), mismatch repair (MMR) genes, and DNA methyltransferases (DNMTs).

Methods

Data source and processing

The TIGIT expression data of 33 types of cancers and corresponding clinical information were acquired from The Cancer Genome Atlas through the UCSC cancer genome browser (https://tcga.xenahubs.net, accessed April 2020)[17]. To compare with the TIGIT expression level in normal tissues, we extracted RNA sequences in normal tissues from Genotype-tissue expression (GTEx; http://commonfund.nih.gov/GTEx/).

Data of 33 types of cancer (N =17,398) were introduced into the final analysis, including Bladder Urothelial Carcinoma (BLCA, n =408), Breast invasive carcinoma (BRCA, n =1098), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, n=306), Cholangiocarcinoma (CHOL, n=36), Colon adenocarcinoma (COAD, n=458),Esophageal carcinoma (ESCA, n=162), Glioblastoma multiforme (GBM, n=167), Head and Neck squamous cell carcinoma (HNSC, n=502), Kidney Chromophobe (KICH, n=65), Kidney renal clear cell carcinoma (KIRC, n=531), Kidney renal papillary cell carcinoma (KIRP, n=289), Acute Myeloid Leukemia (LAML, n = 151), Brain Lower Grade Glioma (LGG, n = 525), Liver hepatocellular carcinoma (LIHC, n = 373), Lung adenocarcinoma (LUAD, n = 515), Lung squamous cell carcinoma (LUSC, n = 501),Ovarian serous cystadenocarcinoma (OV, n = 379), Pancreatic adenocarcinoma (PAAD, n= 178), Prostate

adenocarcinoma (PRAD, n = 496), Rectum adenocarcinoma (READ, n = 167), Skin Cutaneous Melanoma (SKCM, n = 471), Stomach adenocarcinoma (STAD, n = 375), Testicular Germ Cell Tumors (TGCT, n = 156), Thyroid carcinoma (THCA, n = 510), Uterine Corpus Endometrial Carcinoma (UCEC, n = 544), Uterine Carcinosarcoma (UCS, n = 56).

Gene expression and Survival analysis

The TIGIT expression data of 33 cancer types from TCGA and normal samples from GTEx were extracted and formed an expression matrix. Using univariate cox model to evaluate the correlation between TIGIT expression and patient survival for the 33 cancer types. Based on the median TIGIT expression levels, we stratified patients into the high and low group. The Kaplan-Meier (KM) analysis by log rank test was applied to compared patient prognosis from these 2 groups. A $p<0.05$ was considered as statistical significance.

Relationship Between TIGIT Expression and Immunity

We explored the abundance of tumor-infiltrated immune cells (TIICs) among 33 types of cancers through Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/)[18] and Cell-type identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT)[19] respectively. The correlation between the TIGIT expression level and the abundance of TIICs including CD4+ T cells, CD8+ T cells, B cells, neutrophils, macrophages and dendritic cells. we chose log2 (TPM+1) transformed expression data as parameter selection for plotting.

We also utilized the Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) to generate 3 scores including stromal score, immune score, and ESTIMATE score, which represented the immunocyte infiltration level, stromal cells, tumor purity respectively in tumor tissues[20]. We further analysed the correlation between TIGIT expression and these 3 scores.

In addition, to explore the potential mechanism of immune inhibition of TIGIT signaling, the correlations of TIGIT expression with other checkpoint markers were compared across diverse cancer types with preference to previous researches[21-23], with the generation of estimated statistical significance and Spearman's correlation coefficient.

Through the extraction of somatic mutation profiles of all patients from TCGA, we calculated the TMB scores, MSI scores and analysed their correlation with TIGIT expression. We also conducted correlation analysis between TIGIT expression and MMR genes, DNMTs, respectively.

Gene Set Enrichment Analysis

To explore the biological signaling pathway of TIGIT, gene set enrichment analysis (GSEA) was performed by KEGG and HALLMARK analyses. Significant enrichment results were demonstrated using normalized enrichment scores (NES), gene ratio and P value[24]. A $p< 0.05$ and FDR ≤ 0.25 were considered as statistical significance.

Statistical Analysis

Gene expression profiles acquired from TCGA and GTEx were analysed by Students' t-test. Spearman's correlation analysis was applied to evaluated the correlation between TIGIT expression and the abundance of TIICs and scores of immune cells. All analyses were performed with the R package (ggplot2, circlize, clusterProfifiler, DOSE and enrichplot) to visualize the results. A $p<0.05$

indicated statistical significance.

Results

Pan-cancer expression landscape of TIGIT

Comparison of expression of TIGIT between normal and tumor samples across TCGA cancer types and the combined datasets based on integrated database of GTEx and TCGA datasets were conducted and showed in Figure 1. Consistent upregulated expression of TIGIT were seen in BRCA, CHOL, ESCA, GBM, HNSC, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, STAD, USEC compared with normal tissues based on both comparisons. The TIGIT expression was downregulated in THCA based on TCGA datasets. On the contrary, the integrated database showed that TIGIT expression was significantly higher in THCA than in normal tissues. Besides THCA, patients with ACC, CESC, COAD, LAML, OV, PAAD, PRAD, TGCT, THCA also exhibited significantly higher expression of TIGIT in integrated database.

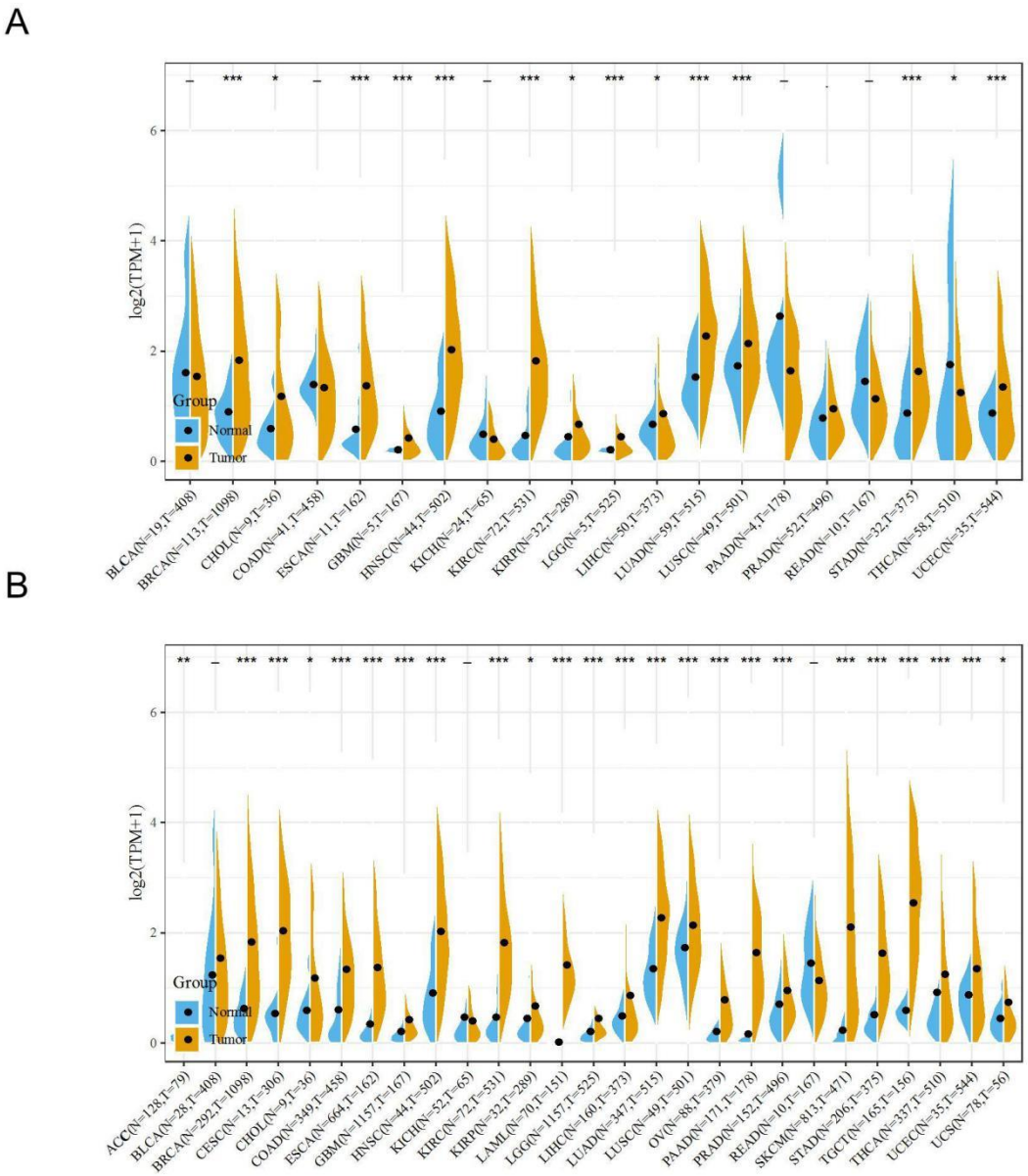

Fig. 1 | TIGIT expression levels in different types of human cancers. The expression level of TIGIT

between tumor and normal tissues were compared in twenty cancer types based on the TCGA database (A) and twenty-seven cancer types based on the integrated database from TCGA and GTEx datasets (B). The expression level of TIGIT in tumor samples of BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRH, KIRC, LGG, LIHC, LUAD,LUSC,STAD,THCA were significantly higher than in normal tissues based on both comparisons, and the expression level of TIGIT were also significantly increased in ACC, BLCA, CESC, CHOL, COAD,ESCA, GBM, HNSC, KIRC, LAML, LGG, LUSC, OV, PAAD, PRAD, SKCM, STAD, TGCT,THCA, UCS compared with normal tissues based on the integrated database.

Prognostic value of TIGIT in cancers

Figure 2 summarized the results of overall survival (OS) analyses of TIGIT expression across the 33 cancer types. Cox regression model showed that high expression of TIGIT in tumor samples correlates with poor prognosis in KIRC(HR, 1.05, 95% CI: 1.02-1.08), KIRP(HR, 1.14, 95% CI: 1.06-1.22), LGG(HR, 1.18, 95% CI: 1.01-1.36), UVM(HR, 1.2, 95% CI: 1.04-1.38), and with favorable prognosis in BRCA(HR, 0.96, 95% CI: 0.93-1), CECS(HR, 0.9, 95% CI: 0.84-0.97) , HNSC(HR, 0.95, 95% CI: 0.91-0.98), SKCM(HR, 0.96, 95% CI: 0.94-0.98)(Figure 2A). Univariate analysis confirmed the prognostic impact of TIGIT in KIRC(p=0.0057), KIRP(p<0.0001) and UVM(p<0.0001) with the same trend (Figure 2B).

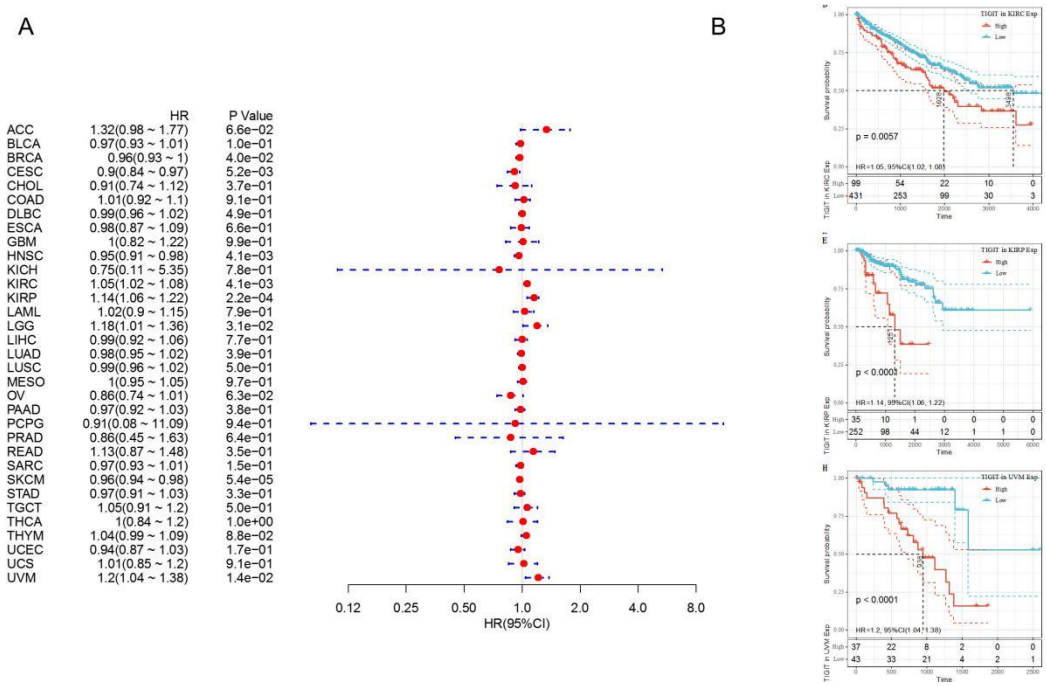

Fig. 2 | Selected Kaplan-Meier plots and forest plot comparing the high and low expression of TIGIT on overall survival (OS) across different cancers (A) Forest plot exhibiting the influence of high expression of TIGIT on OS across thirty three cancer types using Cox regression model. (B) Kaplan-Meier Method showed high expression of TIGIT correlated with unfavorable prognosis in KIRC, KIRP and UVM.

Correlation between TIGIT and immune infiltration level

Considering several studies have revealed the regulatory function of TIGIT in TME, we analyzed its effect on the abundance of immune infiltration levels in tumors that harbor prognostic value. TIMER showed that TIGIT positively correlated with the abundance of CD8+ T cell in KIRC, KIRP, UVM and also positively correlated with the abundance of B cell, CD4+ T cell, Neutrophil, Macrophage and Dendritic cell in KIRC, KIRP, while TIGIT negatively correlated with the abundance of B cell in UVM (Figure 3).

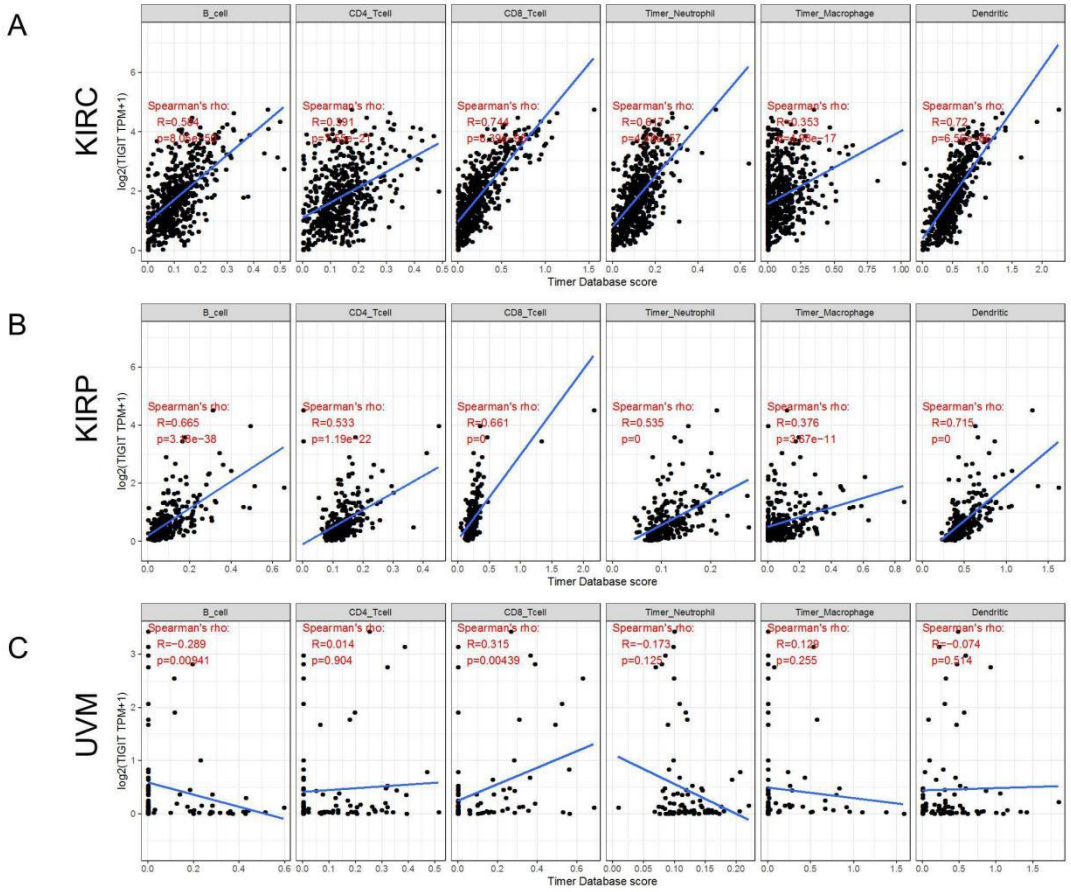

Fig. 3 | Correlation of TIGIT expression with immune infiltration level in KIRC, KIRP and UVM (A-C).

We calculated the immune, stromal and estimate scores respectively through ESTIMATE method. Later we evaluated the correlation between TIGIT expression and immune/stromal/estimate scores in three cancer types. As shown in Figure 4, TIGIT expression was significant correlated with the stromal, immune and estimate scores in all these cancers (all value of $p<0.05$).

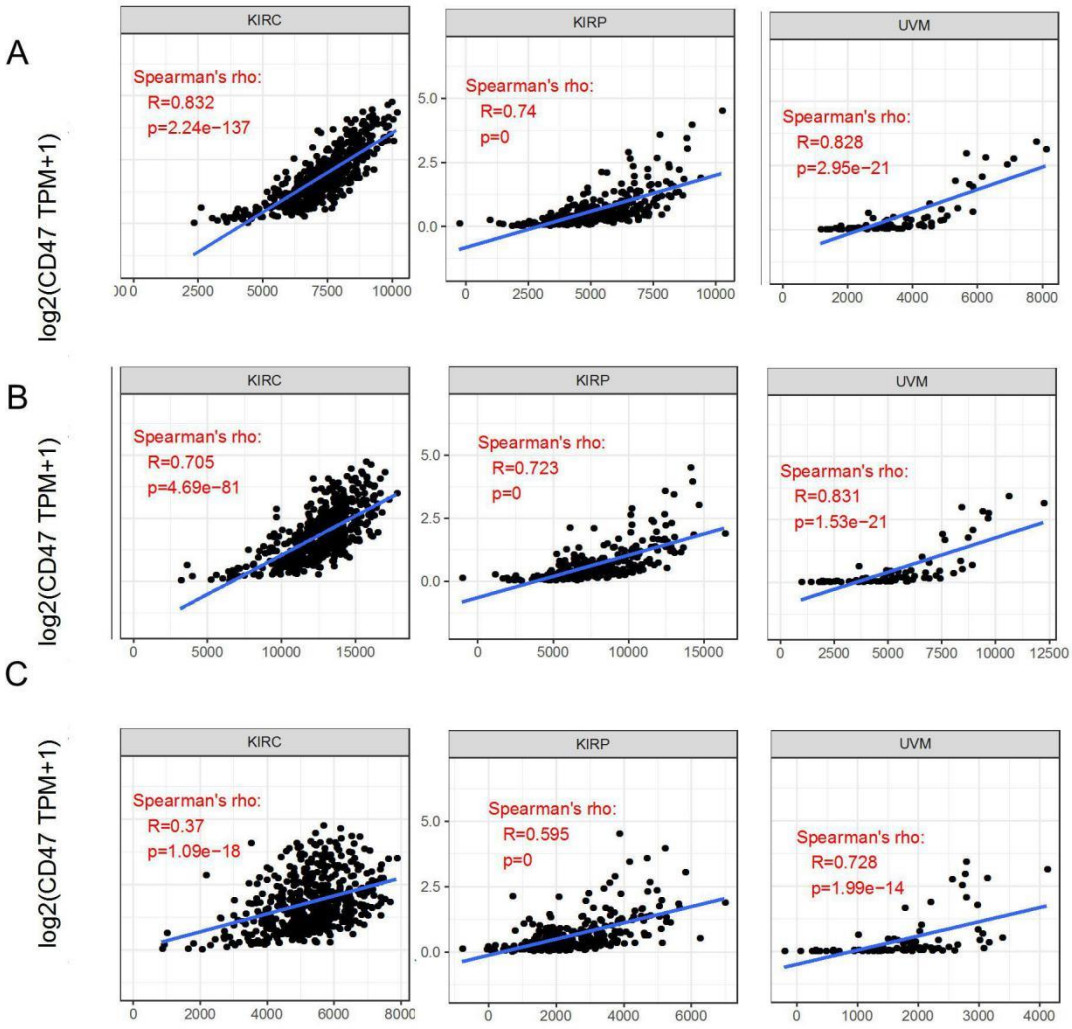

Fig. 4 | Correlation of TIGIT expression with Immune Score, Estimate Score and Stromal Score in KIRC, KIRP and UVM (A-C).

To further investigate the underlying mechanism of immune inhibition of TIGIT signaling, we analyzed the relationship of TIGIT expression with multiple immune checkpoint markers across 33 cancer types (Figure 5). Generally, our result suggests that TIGIT expression was significantly correlated with many immune checkpoints in diverse immunocytes and distinct T cells, such as the positive correlation of TIGIT with CTLA4, PDCD1 (PD-1), CD274 (PD-L1), ICOS in most of the cancer types.

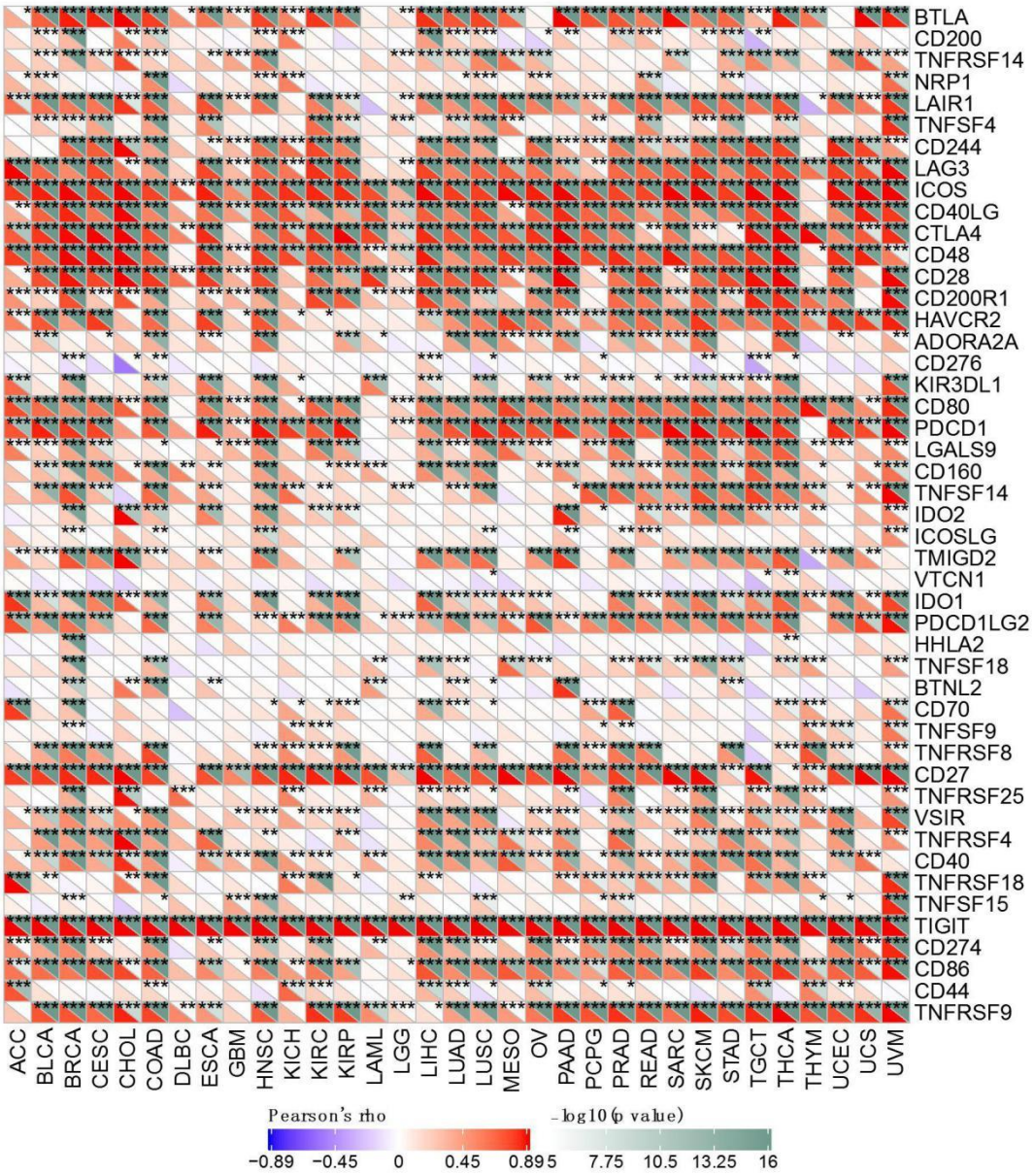

Fig. 5 | Correlation of TIGIT expression with expression of immune checkpoint genes across 33 cancer types.

Correlation analysis on TMB, MSI, MMR and DNMT

Moreover, we evaluated the association of TMB/MSI with TIGIT expression (Figure 6). We found that TIGIT expression was positively correlated with the TMB in BRCA ($p<0.0001$), CESC ($p=0.0058$), COAD ($p<0.0001$), LAML ($p=0.021$), SARC ($p=0.0034$), SKCM ($p=0.042$), THYM ($p=0.0016$), UCEC ($p<0.0001$), USC ($p=0.071$) while negatively correlated with the TMB in BLCA ($p=0.00013$), PAAD ($p=0.0062$), THCA ($p=0.0063$), as shown in Figure 6A. Moreover, TIGIT expression was found to be positively correlated to the MSI in COAD ($p<0.0001$), READ ($p=0.046$), UCEC ($p<0.0001$) while negatively correlated to the MSI in ESCA ($p=0.0063$), HNSC ($p=0.00024$), KIRP ($p=0.015$), LUSC ($p=0.028$), OV ($p<0.0001$), SKCM ($p=0.0029$), TGCT ($p<0.0001$), as

presented in Figure 6B.

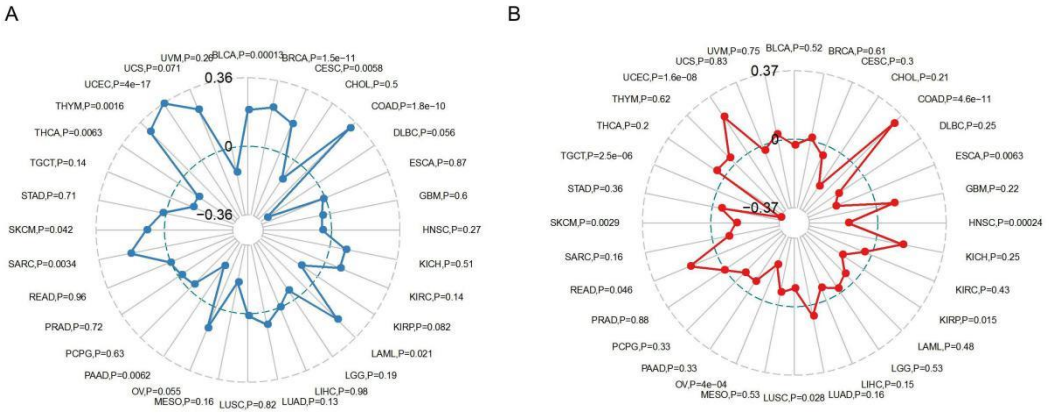

Fig. 6 | Radar map displaying the correlations between TIGIT expression and Tumor mutation burden (A), as well as the correlations between TIGIT expression and microsatellite instability (B) across 33 cancer types.

Furthermore, we analyzed the correlation between MMR genes (MLH1, MSH2, MSH6, PMS2, EPCAM). 21 of the 33 cancer types were correlated with at least one MMR genes, PAAD was even correlated with all 5 MMR genes (Figure 7A). Besides, we also performed a correlation analysis between DNMT (DNMT1, DNMT2, DNMT3A, and DNMT3B) and TIGIT expression. As shown in Figure 7B, 20 of the 33 cancer types were correlated with at least one DNMT. Notably, BRCA and HNSC were correlated with all 4 DNMTs.

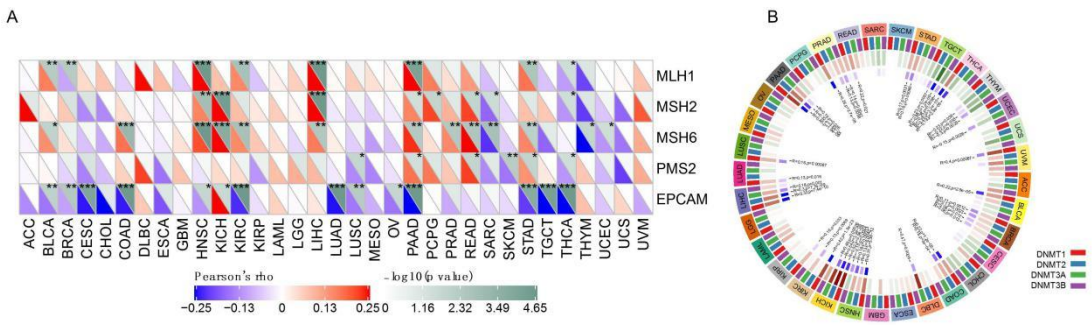

Fig. 7 | The correlations between TIGIT expression and five mismatch repair genes (A), as well as the correlations between TIGIT expression and DNA methyltransferase (B) across 33 cancer types.

Functional analysis by GSEA

GSEA was performed to explore the biological role of the TIGIT. Generally, the top three negatively enriched KEGG terms in high TIGIT subgroup were cytokine-cytokine receptor interaction, chemokine signaling pathway and natural killer cell mediated cytotoxicity (Figure 8A) and the top three negatively enriched HALLMARK terms included allograft rejection, interferon-gamma

response and IL6-JAK-STAT3 signaling (Figure 8C). The top positively enriched terms were oxidative phosphorylation and propanoate metabolism (Figure 8B, D).

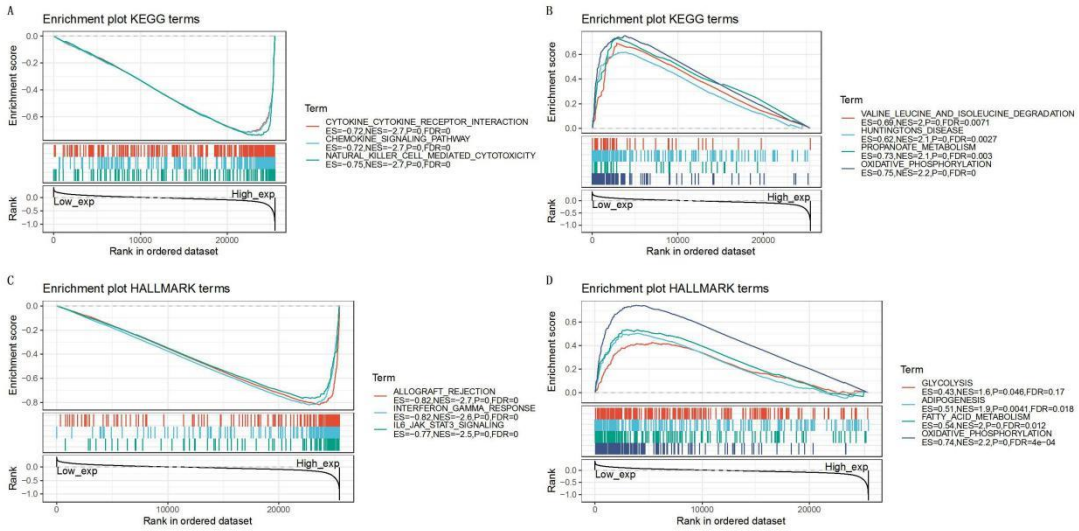

Fig 8 | Functional Enrichment of KEGG and HALLMARK terms on TIGIT through GSEA. The top three negative and positive enriched KEGG terms were displayed in (A) and (B) respectively. The top three negative and positive enriched HALLMARK terms were displayed in (C) and (D) respectively.

Discussion

The present work illustrated a comprehensive workflow for pan-cancer analysis and thoroughly investigated the role of TIGIT in cancers. The results showed the prognostic impact of TIGIT across the different cancer types. TIGIT expression mediated infiltrated immune cells and positively correlated with the expression of LAG3, CTLA4, PDCD1 (PD-1), CD274 (PD-L1), PDCD1LG2 (PD-L2) in most of the cancer types. TIGIT expression was also correlated with TMB/MSI/DNMTs/MMR genes in multiple cancers. GSEA results demonstrated the high TIGIT patient group negatively enriched terms including cytokine-cytokine receptor interaction, chemokine signaling pathway, natural killer cell mediated cytotoxicity, allograft rejection, interferon gamma response and IL6-JAK-STAT3 signaling.

Our study showed the great prognostic values of TIGIT across different cancer types. Upregulated TIGIT expression has been reported in KIRC[25], LGG[26] and correlated to poor prognosis, which was consistent with our results, probably due to its inhibitory effect on T cells and NK cells. Generally, the mechanism of the TIGIT inhibition contains 1) binding CD155 and triggering direct inhibitory signals on T/NK cells[3,27] , 2) binding CD155 on APC to produce more anti-inflammatory cytokines[3], 3) binding CD155 competing with CD226[2] and disrupting CD226 homodimerization to impede CD226-mediated T cell activation[28], 4) stabilizing and enhancing the immunosuppressive functions of Tregs[29,30], 5) binding Fap2 from the gut bacteria Fusobacterium nucleatum and triggering inhibitory signals[31]. The tumor tissues with the upregulated expression of TIGIT also exhibited aberrant immune characteristics. For example, in colorectal tumor tissues, TIGIT+ CD8+ T cells exhibited significantly higher infiltration and an exhausted phenotype with lower expression of proinflammtory cytokines such as IFN-γ, IL-2, TNF-α and higher expression of inhibitory receptors such as PD-1, LAG-3, and

TIM-3 on the surface[32]. In gastric tumor tissues, TIGIT and CD155 were also upregulated in CD8+ T cells and could suppress the function of infiltrated CD8+ T cell[33]. Not only these two researches but also some others reported that TIGIT could serve as a potential prognostic biomarker[4,6,34]. Our ESTIMATE revealed the positive correlation between TIGIT expression and the stromal cells, immunocyte infiltration level and tumor purity through analysis of immune/stromal/estimate scores, confirming the great impact of TIGIT on TME. Given all the information above, it is likely that the immunosuppressive effect of TIGIT leads to the tumor cells escape and survival, influencing the initiation and development of the cancers and the patients' prognosis.

To further investigate the underlying mechanism of the relationship between TIGIT and tumors, we conducted an analysis on the correlation between TIGIT and TMB, MSI, MMR genes, DNMTs. MSI is characterized as the expansion or deletion of microsatellites and leads to tumorigenesis, as the consequence of mutations of MMR genes[35]. Emerging evidence revealed that most of the tumors with MSI-H/dMMR status exhibited high TMB[36,37]. These features are related to the increased neoantigen, affecting tumor-infiltrating lymphocytes and response to ICB, thus could predict the response to immunotherapies[38-40]. TIGIT was also found to be correlated with MSI/dMMR in prostate[41] and colorectal cancer[42]. Our results not only show similar results with previous studies but also revealed more correlations between TIGIT expression and TBM/MSI/MMR genes in multiple other cancer types at pan-cancer level. Besides genetic mutations, epigenetic alterations also impact the growth, proliferation, metastasis and immunosuppression of the tumors profoundly. DNA methylation is one of the most important epigenetic regulation. Aberrant levels of DNA methylation were associated with tumorigenesis and immune evasion in cancers[43]. Our results found certain positive and negative correlations between DNMTs and TIGIT expression in different cancer types, suggesting DNA methylation may also participate in the modulation of TIGIT, as previous studies reported[44,45]. Its mechanism is related to the reduced expression of the genes concerned with tumor suppression and anti-tumor immunity by DNA hypermethylation and overexpression of the genes responsible for tumorigenesis and immune suppression by DNA hypomethylation[46,47]. Altogether, different kinds of tumors and its immune microenvironment are driven by different methylation patterns, which is complicated and needs deeper investigation in the future. The relationship between DNMTs and TIGIT also indicates the possible strategy to target these checkpoint by methylation modulators or combine methylation modulators with ICBs to elevate the response rates[48,49].

As one of the most commonly targeted immune checkpoint[50] and the core of a complex regulatory network included CD96, CD112R, CD226, CD155 and CD226[7], TIGIT has been considered as a potential ICB to develop novel immunotherapy strategies. Several preclinical studies have shown that TIGIT blockade alone could impede the growth and proliferation of the tumors[51-53], even in anti-PD-1 resistant tumor model[2]. Moreover, combining the TIGIT blockade with PD-1 blockade[54,55], IL-15 stimulation[56] or optimized fractionated radiotherapy[57], could promote the response to immunotherapy and increase the survival in animal models. Multiple clinical trials are also ongoing to test whether TIGIT blockade could translate into an actual benefit for patients with cancers (NCT04354246, NCT04150965, NCT04570839)

In this study, we showed the pan-cancer landscape of aberrant TIGIT expression across different tumors for the first time. Our finding will allow us to take the next step into a further functional

investigation of TIGIT and clinical application of TIGIT blockade in specific cancers, providing new insights and options for the patients with cancers. Our study has several limitations. First, there's no experimental validation of the predicted results. The relationship between the TIGIT expression and the nature of the tumors are needed to be validated in future experiments. Second, more data from other public datasets are needed to validate our results.

Conflict of Interest

None.

Author Contributions

Jie Wen and Fangkun Liu performed data extraction and prepared the manuscript. All authors contributed to writing and revising the manuscript.

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Data Availability Statement

The datasets for this study can be found in the TCGA Research Network (https://www.cancer.gov/tcga), GTEx (http://commonfund.nih.gov/GTEx/), and GEO (https://www.ncbi.nlm.nih.gov/geo/).

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