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Original Article

TNFSF13B correlated with poor prognosis and immune infiltrates in KIRC: a study based on TCGA data

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Abstract: TNFSF13B is highly expressed in RCC tissues and is closely related to tumor immune escape strategy. The purpose of this study was to investigate the prognostic value of TNFSF13B and immune infiltrates in KIRC, based on data obtained from TCGA. Gene expression profiles and clinical information were downloaded from the TCGA database. Overall survival and disease-free analysis of hub genes was performed using GEPIA. Differential-expression and pairwise difference maps of TNFSF13B were analyzed using R software (version 3.5.1). The Molecular Signatures Database (MSigDB) was performed by the GSEA. In addition, TIMER was used to explore correlation levels between gene expression and abundance of immune infiltrates. FYB expression was high in cancer tissues of KIRC, compared with normal tissues. KIRC patients with TNFSF13B alteration showed worse overall survival and worse disease-free survival. Univariate analysis revealed that age at diagnosis, grade, stage, T, N, M, and TNFSF13B were statistically significant factors for KIRC progression. However, T and TNFSF13B were not statistically significant, according to multivariate analysis. Moreover, KIRC with high TNFSF13B expression was shown to be more prone to progression and metastasis than KIRC with low TNFSF13B expression. In addition, signaling pathways of TNFSF13B activated in KIRC were mainly enriched in cytokine-cytokine receptor interaction, natural killer cell-mediated cytotoxicity, and antigen processing. Importantly, TNFSF13B expression showed a significant positive correlation with infiltrating levels of B-cells, macrophages, neutrophils, and dendritic cells.

Keywords: Kidney cancer, TNFSF13B, prognosis, immune

Introduction

Kidney renal clear cell carcinoma (KIRC) is a distinct subtype of renal cell carcinoma. Radical resection is the primary treatment approach for KIRC, followed by radiotherapy, chemotherapy, and targeted therapy. However, even after successful treatment, 30% of patients develop tumor recurrence or distant metastasis [1-3]. KIRC can be hereditary or non-hereditary. Both types have been associated with short arm structure abnormalities in chromosome 3 [4].

With clinical applications of new immunotherapies, traditional TNM staging has been unable to meet the clinical needs of curative effects predictions [5]. Immunological features of the tumor immune microenvironment play important roles in tumor prognosis evaluation. Recently, the immune-score system, based on local immune cell distribution and density levels, has become an important indicator for prognosis evaluation. It has been verified in several tumor studies [6, 7].

Previous studies have confirmed that TNFSF13B deficiencies can lead to low immune function, especially humoral immune function. TNFSF13B overexpression has been shown to be closely involved in systemic lupus erythematosus, involving both B and T lymphocyte hyperfunction. Moreover, TNFSF13B has been associated with the development of certain tumors [8]. Recent studies have found that serum TNFSF13B levels are three times higher than in normal subjects. TNFSF13B receptors of B-cells on the surface were shown to be significantly decreased in patients with follicular non-Hodgkin's lymphoma. Results suggested that occurrence of these tumors may be related to TNFSF13B [9]. For the first time, the current study examined the association of TNFSF13B expression for KIRC.

Therefore, the purpose of the current study was to investigate the prognostic value of TNFSF13B in KIRC. GSEA was used to further understand the pathogenesis of KIRC, as well as the biologi-

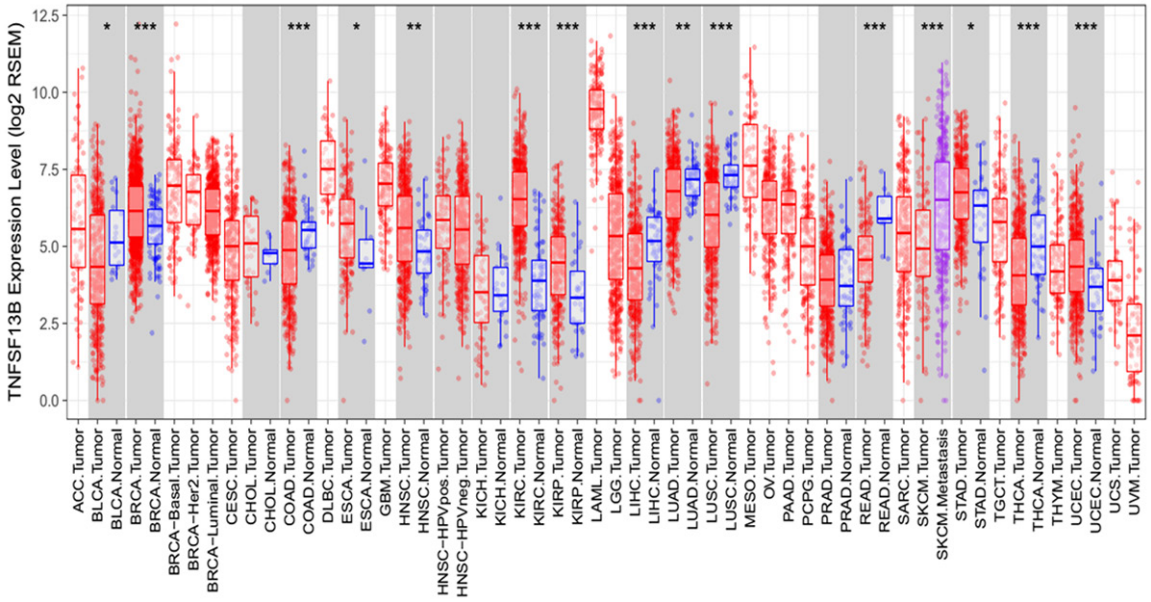


Figure 1. TNFSF13B expression levels in different types of human cancers. Human PRDM1 expression levels in different tumor types from TCGA database were determined by TIMER. (*P < 0.05, **P < 0.01, ***P < 0.001).

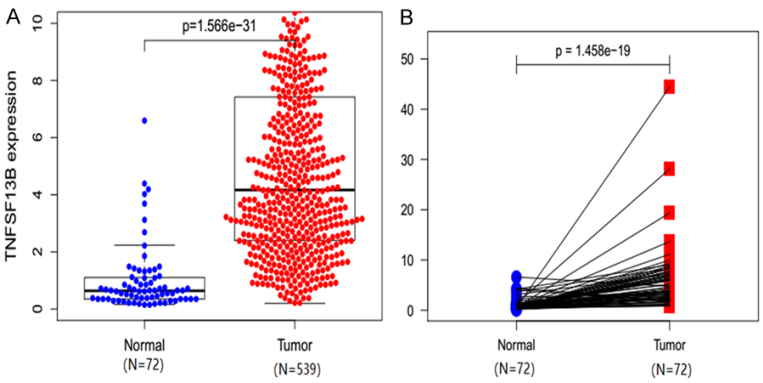


Figure 2. Differential expression and the pairwise difference map of TNFSF13B in KIRC from TCGA database were examined using R software (version 3.5.1).

cal pathways involved in the regulatory network associated with TNFSF13B. Importantly, the current study assesses the association between expression of TNFSF13B and immune infiltration levels in KIRC in TIMER.

Materials and methods

Microarray data and bioinformatics analysis

Gene expression profiles and clinical information were downloaded from the TCGA database. Overall survival and disease-free analysis of the hub genes was performed using GEPIA, an opening website. Differential-expression and pairwise difference maps of TNFSF13B were analyzed using R software (version 3.5.1).

Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) can be used to evaluate the distribution of genes in a pre-defined gene set in a list of genes sorted by phenotypic correlation, aiming to determine their contribution to phenotypes. In the current study, it was used explore the Molecular Signatures Database (MSigDB), a collection of annotated gene sets for use with GSEA software. Pathways enrichment was analyzed based on nominal *P* values and normalized enrichment scores (NES).

TIMER database analysis

TIMER is a comprehensive resource for systematic analysis of immune infiltration in different cancer types. Abundances of six immune infiltrates (B-cells, CD4+T-cells, CD8+T-cells, neutrophils, macrophages, and dendritic cells) are estimated. This web server allows users to input function-specific parameters, with resulting figures dynamically displayed to conveniently access tumor immunological, clinical, and genomic features. The Gene module was used

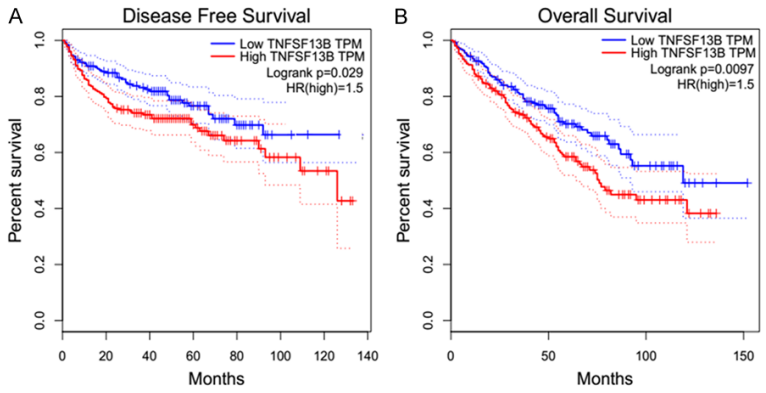


Figure 3. Kaplan-Meier survival curves comparing high and low expression of TNFSF13B in KIRC from TCGA database.

Table 1. Characteristics of patients with KIRC based on TCGA

Clinical characteristics	Number of cases	Percentages (%)
Ages		
50	106	20.2
50-60	154	29.3
60-70	140	26.7
70-80	101	19.2
80	23	4.3
Gender		
Female	185	35.3
Male	339	64.7
Grade		
G1	14	2.6
G2	227	43.3
G3	206	39.3
G4	77	14.6
Stage		
I	263	50.1
II	54	10.3
III	124	23.6
IV	83	15.8
Topography (T)		
T1	269	51.3
T2	66	12.5
T3	178	33.9
T4	11	2
Lymph node (N)		
N0	236	45
N1	15	3
NX	273	52
Metastasis (M)		
M0	418	79.7
M1	78	15
MX	27	5.3

to explore the correlation between genes expression and abundance of immune infiltrates. The Diff Exp module was used to explore differential gene expression between tumors (**Figure 1**). Gene expression levels were displayed with log2 RSEM.

Statistical analysis

Expression of TNFSF13B in patients was evaluated using box plots. The relationship between clinical features and TNFSF13B was analyzed using Wilcoxon signed rank tests and logistic regression analysis. Multivariate cox and univariate cox analysis were used to compare the effects of survival and clinical features. Survival curves were generated by GEPIA. *P* values < 0.05 indicate statistical significance.

Results

Differential expression of TNFSF13B in different tumor tissues

TNFSF13B expression in tumor and normal tissues was analyzed by TIMER. As shown in **Figure 1**, results illustrated that FYB expression was high in cancer tissues of BRCA, ESCA, NHSC, KIRC, KIRP, and UCEC, compared with normal tissues. Expression was low in BLCA, COAD, LIHC, LUSC, READ, and THCA. Next, the current study examined TNFSF13B expression from the TCGA database in 72 cancer tissues, compared with 539 normal tissues. Results showed that PRDM1 expression was significantly higher in cancer tissues of KIRC than in normal tissues (**Figure 2A**). Furthermore, expression of TNFSF13B was analyzed in 72 pairs of KIRC cancer tissues and adjacent tissues. Results showed that TNFSF13B was significantly overexpressed in KIRC ($P < 0.001$) (**Figure 2B**). Present results were handled using the R language package.

Survival and prognostic analyses of TNFSF13B

Overall survival and disease-free analysis was performed using GEPIA. KIRC patients with TNFSF13B alteration showed worse overall survival and worse disease-free survival (**Figure 3**). Clinical information was downloaded from the TCGA database. Results of multivariate and

Table 2. TNFSF13B expression associated with clinical pathological characteristics (logistic regression)

Characteristics	Univariate analysis			Multivariate analysis		
	Odds ratio		*P*-value	Odds ratio		*P*-value
Age	1.03	(1.02-1.05)	2.29E-06	1.03	(1.02-1.05)	5.20E-06
Gender	0.93	(0.68-1.28)	0.66	1.01	(0.72-1.41)	0.942
Grade	2.29	(1.85-2.84)	1.94E-14	1.57	(1.22-2.01)	0.0003
Stage	1.89	(1.65-2.16)	4.67E-20	1.6	(1.01-2.52)	0.041
T	1.94	(1.64-2.3)	1.50E-14	0.9	(0.6-1.37)	0.65
M	4.28	(3.11-5.9)	7.45E-19	1.4	(0.7-2.78)	0.03
N	0.9	(0.81-0.99)	0.04	0.88	(0.79-0.98)	0.02
TNFSF13B	1.04	(1.02-1.06)	0.001	0.97	(0.1-9.27)	0.98

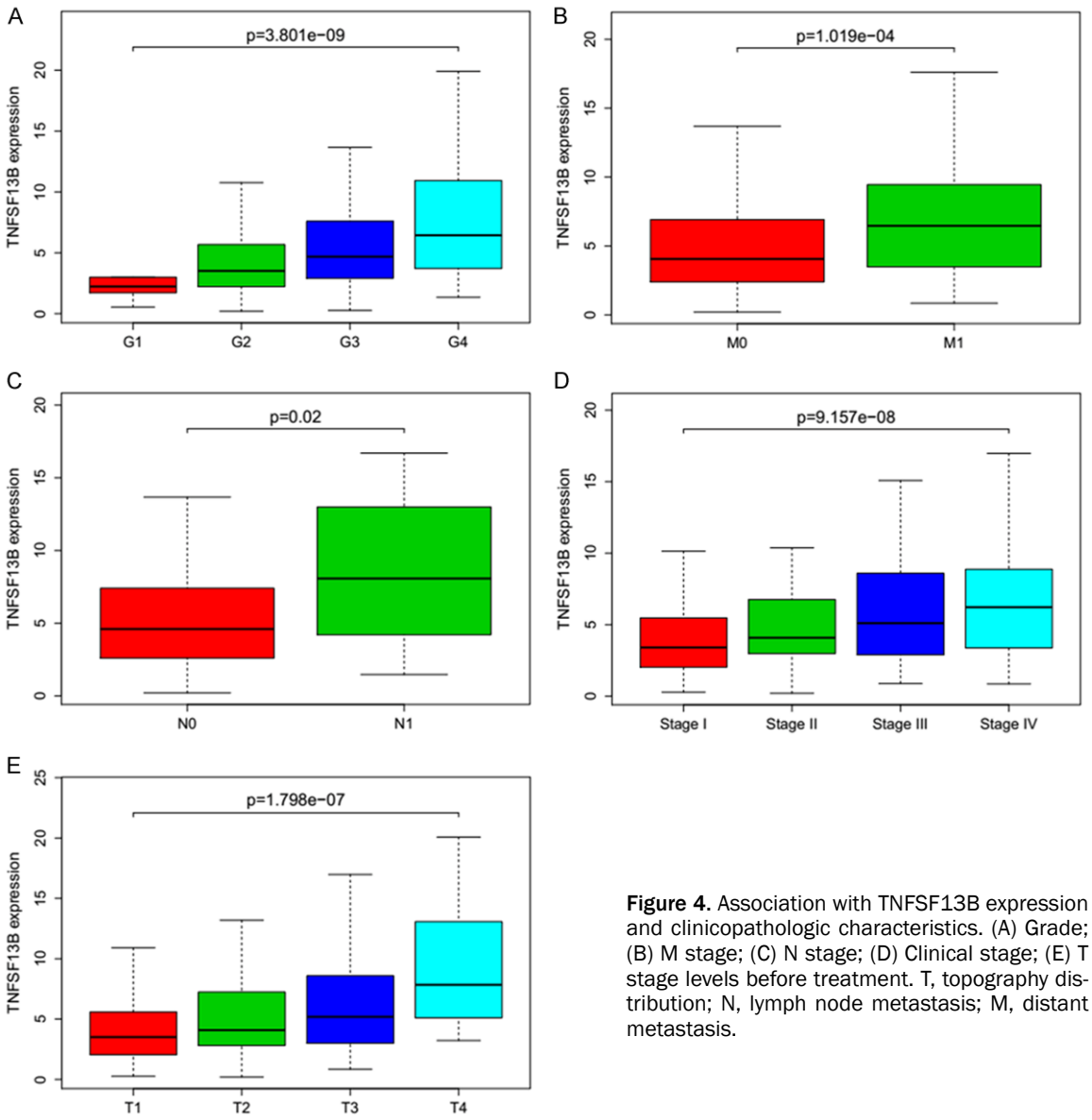


Figure 4. Association with TNFSF13B expression and clinicopathologic characteristics. (A) Grade; (B) M stage; (C) N stage; (D) Clinical stage; (E) T stage levels before treatment. T, topography distribution; N, lymph node metastasis; M, distant metastasis.

univariate analysis were examined using the R language package (**Table 2**). Univariate analysis revealed that age at diagnosis, grade, stage, T, N, M, and TNFSF13B were statistically signifi-

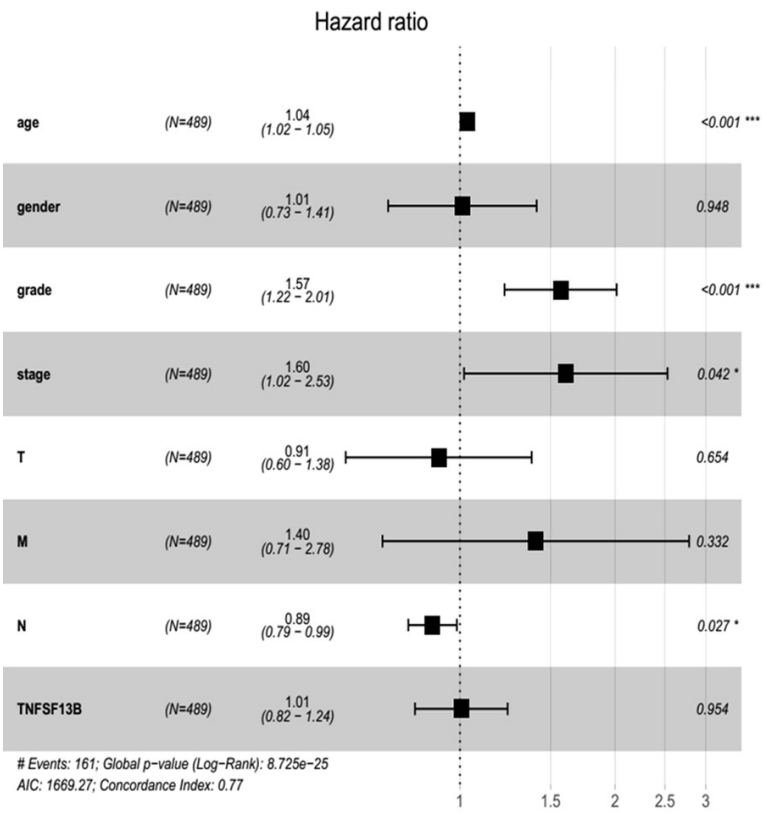


Figure 5. Multivariate analysis of the correlation of TNFSF13B expression among KIRC patients.

cant factors for KIRC progression. However, T and TNFSF13B were not statistically significant, according to multivariate analysis (**Figure 5**).

Patient characteristics

Gene expression profiles and clinical information were downloaded from the TCGA database. In the present study, a total of 611 tissue samples were downloaded from TCGA, including 539 tumor samples and 72 normal samples. Of these, stage I cases accounted for 50.1%, 10.3% for grade II, and 23.6% for grade III, with 83 (15.8%) cases of grade IV. No disease was found in 236 patients (45%). N1 was found in 15 (3%) and NX was found in 273 (52%). Clinicopathologic characteristics of the patients in TCGA are listed in **Table 1**.

Association with TNFSF13B expression and clinicopathologic variables

A total of 539 KIRC samples with TNFSF13B were downloaded from TCGA. As shown in **Figure 4A-E**, higher expression of TFAP2B correlated significantly with stage, grade, topography, lymph node, and metastasis. Results suggest that KIRC with high TNFSF13B expression is more prone to progression and metastasis than KIRC with low TNFSF13B expression.

TNFSF13B-related signaling pathways based on GSEA

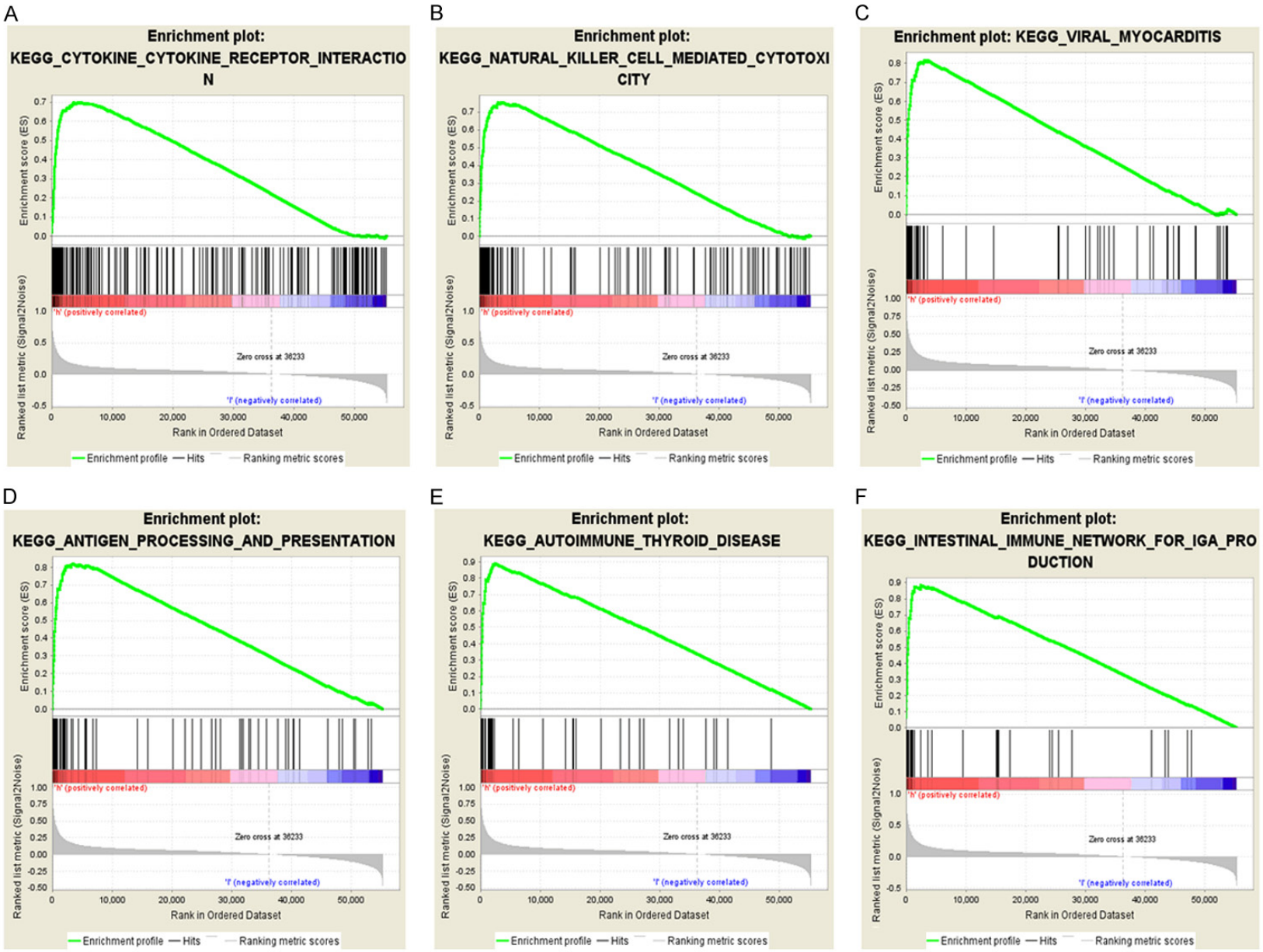
Aiming to identify signaling pathways activated in KIRC, GSEA was used to compare low and high TNFSF13B expression datasets. As shown in **Figure 6**, cytokine-cytokine receptor interactions, natural killer cell-mediated cytotoxicity, antigen processing and presentation, autoimmune thyroid disease, intestinal immune network, hematopoietic cell lineage, chemokine signaling pathway, cell adhesion molecules, and T-cell receptor signaling pathways (**Table 3**) were related to human immune infiltration.

Association with immune infiltration levels in KIRC

The current study examined the association between expression of TNFSF13B and immune infiltration levels in KIRC in TIMER. Results revealed that TNFSF13B expression has a significant positive correlation with infiltrating levels of B-cells (Cor=0.466, P=6.94e-26), macrophages (Cor=0.537, P=8.78e-35), neutrophils (Cor=0.578, P=3.73e-42), and dendritic cells (Cor=0.634, P=1.35e-52) (**Figure 7**).

Discussion

TNFSF13B can inhibit the growth of prostate cancer, colon cancer, cervical cancer, breast cancer, and embryonic kidney cells. Degradation of TNFSF13B induced by caspase-3 is a marker of tumor cell apoptosis. Interestingly, treatment of U937 cells with TNFSF13B for 2 hours can cause partial cleavage of PARP, indi-


A
Enrichment plot:
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
B
Enrichment plot:
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY
C
Enrichment plot: KEGG_VIRAL_MYOCARDITIS
D
Enrichment plot:
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
E
Enrichment plot:
KEGG_AUTOIMMUNE_THYROID_DISEASE
F
Enrichment plot:
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'h' (positively correlated)
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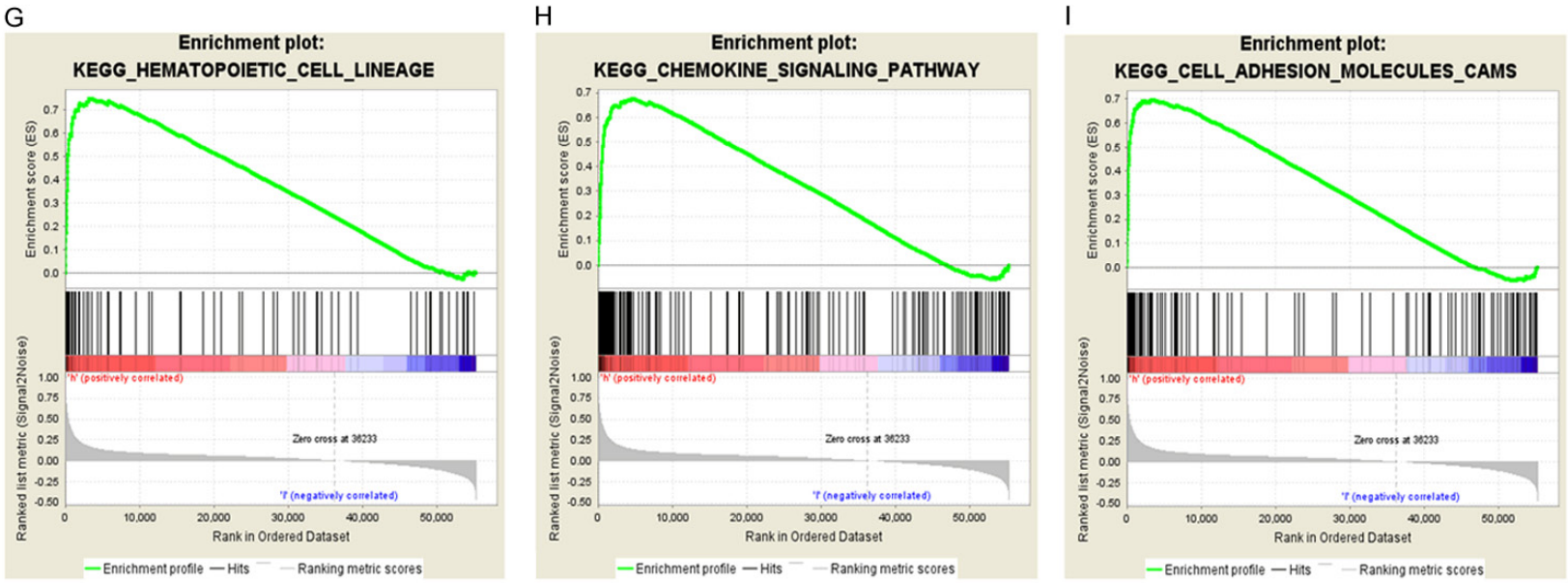

Figure 6. Enrichment plots from gene set enrichment analysis (GSEA). GSEA results showing cytokine-cytokine receptor interaction (A); Natural killer cell-mediated cytotoxicity (B); Antigen processing and presentation (C); Autoimmune thyroid disease (D); Intestinal immune network (E); Hematopoietic cell lineage (F); Chemokine signaling pathway (G); Cell adhesion molecules (H); and T-cell receptor signaling pathway (I); Differentially enriched in TNFSF13B-related KIRC. NES, normalized ES; FDR, false discovery rate.

Table 3. Gene sets enriched in phenotype high

GS follow link to MSigDB	NES	NOM *p*-val	FDR q-val	FWER *p*-val
CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	2.9500372	0	0	0
NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	2.8480852	0	0	0
ANTIGEN_PROCESSING_AND_PRESENTATION	2.7046082	0	0	0
AUTOIMMUNE_THYROID_DISEASE	2.6608996	0	0	0
INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	2.6450207	0	0	0
HEMATOPOIETIC_CELL_LINEAGE	2.6392586	0	0	0
CHEMOKINE_SIGNALING_PATHWAY	2.633466	0	0	0
CELL_ADHESION_MOLECULES_CAMS	2.6324408	0	0	0
T_CELL_RECEPTOR_SIGNALING_PATHWAY	2.6232328	0	0	0

NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate. Gene sets with NOM *p*-val b0.05 and FDR q-val b0.25 are considered as significant.

cating that its inhibition of tumor growth is induced by apoptosis [10, 11]. However, TNFSF13B has also been found in normal and pathological tissues [12], including various malignancies [13, 14]. In kidney tissues, TNFSF13B is highly expressed in RCC tissues and is closely related to tumor immune escape strategy [15].

The present study found that FYB expression was high in cancer tissues of BRCA, ESCA, NHSC, KIRC, KIRP, and UCEC, compared with normal tissues. Next, expression of TNFSF13B in was analyzed in 72 pairs of KIRC cancer tissues and adjacent tissues. Results showed that TNFSF13B was significantly overexpressed in KIRC. Moreover, KIRC patients with TNFSF13B alteration showed worse OS and DFS (**Figure 3**). Univariate analysis revealed that age at diagnosis, grade, stage, T, N, M, and TNFSF13B were statistically significant factors for KIRC progression. However, TNFSF13B was not statistically significant, according to multivariate analysis (**Figure 5**). Present results suggest that the gene is associated with poor prognosis in patients with KIRC tumors. Interestingly, higher expression of TFAP2B correlated significantly with stage, grade, topography, lymph node, and metastasis, indicating that KIRC with high TNFSF13B expression is more prone to progression and metastasis than KIRC with low TNFSF13B expression.

B-cell-activating factors are encoded on TNFSF13B genes [16]. TNFSF13B and TNFSF13 were originally thought to be unique elements of the immune system, regulating differentiation and the fate of lymphocytes [17, 18]. TNFSF13B stimulates B-cells and induces overexpression of the gene Bcl2, a key anti-apoptotic gene, originally described for its carcinogenic effects in B-cell transformation. This suggests that TNFSF13B acts as a survival factor rather than a true growth factor [19]. TNFSF13B and TNFSF13 are involved in the development and differentiation of B lymphocytes in solid tumors [20]. Furthermore, TNFSF13B gene polymorphisms have been associated with autoimmune diseases, including immune thrombocytopenic purpura and rheumatoid arthritis [21, 22]. Present results suggest that the signaling pathways of TNFSF13B activated in KIRC were mainly enriched in cytokine-cytokine receptor interaction, natural killer cell-mediated cytotoxicity, antigen processing and presentation, autoimmune thyroid disease, intestinal immune network, hematopoietic cell lineage, chemokine signaling pathways, cell adhesion molecules, and T-cell receptor signaling pathways. Some studies have shown that TNFSF13B signaling may contribute to cancer progression and cancer cachexia via its proinflammatory role [23]. Activation of NF-γB produces more cytokines by positive feedback [24, 25]. In combination with its receptor, TNFSF13B promotes inflammatory cytokine production by enhancing NF-γB signaling. Thus, it promotes inflammation during malignant tumors [26-28].

In addition, the current study assessed the association between expression of TNFSF13B and immune infiltration levels in KIRC in TIMER. Results revealed that TNFSF13B expression has a significant positive correlation with infiltrating levels of B-cells, macrophages, neutrophils, and dendritic cells. Present findings strongly suggest that TNFSF13B plays specific roles in immune infiltration in KIRC.

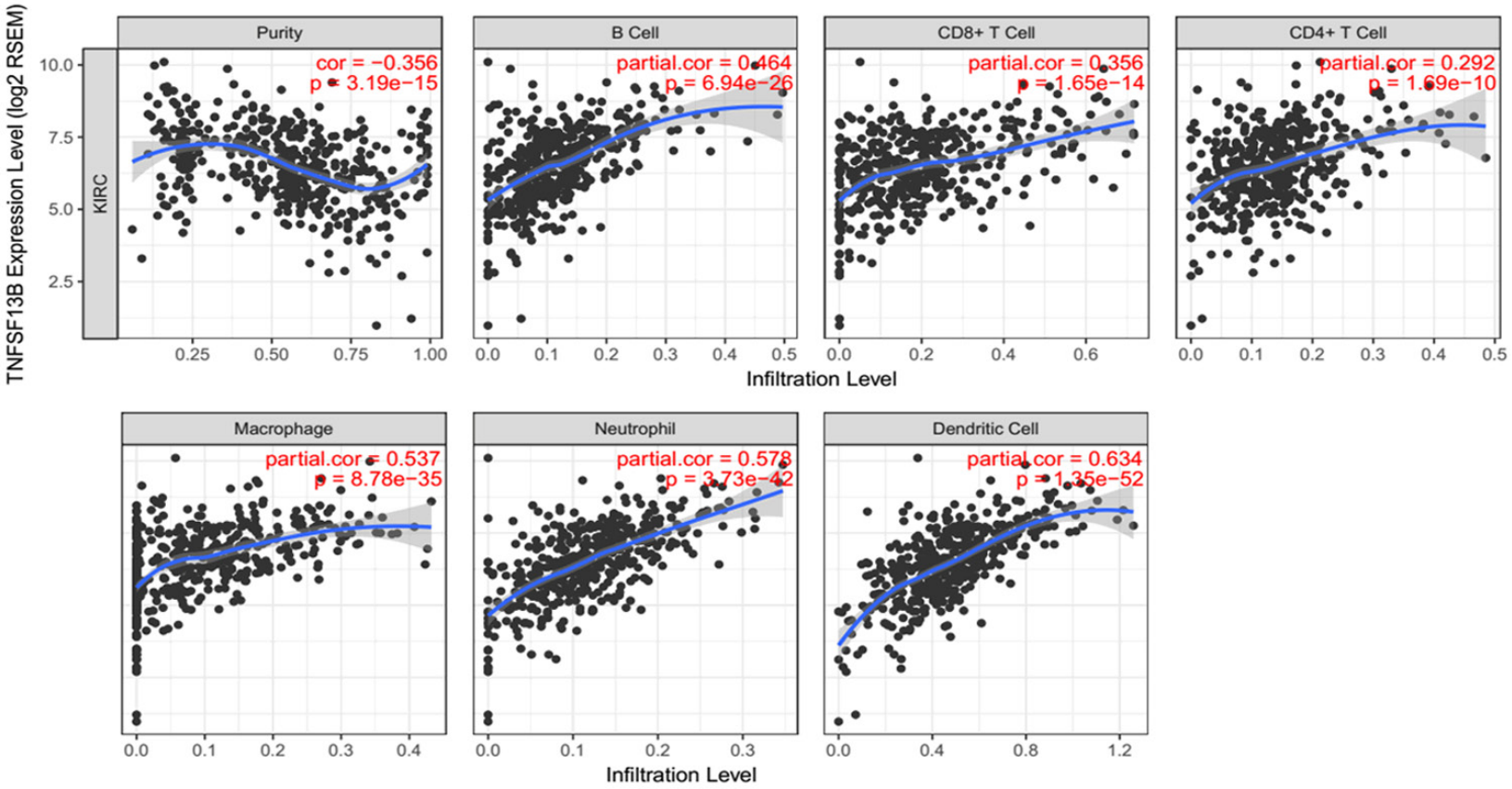

Figure 7. Correlation of TNFSF13B expression with immune infiltration levels in KIRC. TNFSF13B expression was significantly positively related to tumor purity and has a significant positive correlation with infiltrating levels of B-cells, macrophages, neutrophils, and dendritic cells.

Conclusion

TNFSF13B expression may be a potential prognostic molecular marker of poor survival in KIRC. Moreover, cytokine-cytokine receptor interaction, natural killer cell-mediated cytotoxicity, antigen processing, and presentation signaling pathways may be key pathways regulated by TNFSF13B in KIRC.

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Disclosure of conflict of interest

None.

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