Comprehensive Characterization of Transforming Growth Factor Beta Receptor 1 in Stomach Adenocarcinoma Identifies a Prognostic Signature for Predicting Clinical Outcomes and Immune Infiltrates

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Research

Keywords: TGFBR1, STAD, prognosis, immune infiltration, Bioinformatics

Posted Date: December 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1119461/v1>

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Version of Record: A version of this preprint was published at International Journal of General Medicine on March 1st, 2022. See the published version at <https://doi.org/10.2147/IJGM.S353879>.

Abstract

Background: Stomach adenocarcinoma (STAD) is the common cancer and ranks third leading cause of cancer death worldwide. TGF- β receptor 1 (TGFB1), serving important roles in the TGF- β family, the mechanisms whereby TGF β 2 governs tumor progression, immune cell infiltration and its correlation with tumor microenvironment (TME) in STAD remains unintelligible.

Methods: First, we used the data in the TCGA, GEPIA, and HPA databases to explore the expression level of TGFB1 in STAD, the correlation between TGFB1 expression and the clinical features of STAD, its impact on the survival of STAD. Subsequently,

a receiver operating characteristic (ROC) curve and nomogram were constructed and LASSO (the Least Absolute Shrinkage and Selection Operator)-selected features were used to build the TGFB1 prognostic signature. Moreover, GSEA enrichment analysis is used to find the potential molecular mechanism of TGFB1 to promote the malignant process of STAD. Finally, we further explored the influence of theTGFB1 expression on the immune microenvironment of STAD patients through the TIMER2.0 and GEPIA database.

Results: In our study, TGFB1 expression was significantly elevated in patients with STAD and positively co-expression with pathologic stage, lymph node metastases (LNM) stage and histopathological grade of STAD. LASSO-selected features were used to build the TGFB1 prognostic signature. 9 factors with non-zero coefficients were identified. The corresponding risk scores were computed, according to the following formula: Risk score = $(-0.2914) * DIXDC1 + (0.1113) * STON1-GTF2A1L + (0.3092) * FERMT2 + (-0.0146) * BHMT2 + (0.1798) * ABCC9 + (0.068) * MSRB3 + (-0.1007) * SYNC + (-0.0891) * SORBS1 + (0.0828) * TGFB1$. Survival analysis revealed that patients with high TGFB1 had shorter OS, FP, and PPS. Multivariate Cox analysis revealed TGFB1 was an independent prognostic factor for OS in STAD. The receiver operating characteristic (ROC) analysis suggested high diagnostic value with the area under curve (AUC) of TGFB1 was 0.739, and a prognostic nomogram involving age, T, N, M classification, pathologic stage, primary therapy outcome, histologic grade and TGFB1 to predict the 1, 3, 5-year OS was constructed. GSEA revealed that high TGFB1 expression was correlated with pathway in cancer, MAPK signaling pathway, NOTCH signaling pathway, focal adhesion and VEGF-C production. ssGSEA showed that TGFB1 is correlated with NK cells, Tem and Th17 cells. Furthermore, elevated TGFB1 expression was found to be significantly correlated with several immune checkpoint and immune markers associated with immune cell subsets.

Conclusion: In summary, TGFB1 could be a prognostic biomarker and an important regulator of immune cell infiltration in STAD. The present study revealed the probable underlying molecular mechanisms of TGFB1 in STAD and provided a potential target for improving the prognosis.

Introduction

Stomach adenocarcinoma (STAD) is a disease of the common cancer worldwide and ranks third leading cause of cancer death[1, 2]. Despite improvements in STAD treatment, the prognosis of patients with advanced STAD remains poor[3]. Importantly, there are neither effective prognostic biomarkers nor validated predictive for this lethal tumor[4]. Tumorigenesis is a complicated process involving multiple factor sand stages[5], among which the linked gene and immune cells play an essential regulatory role[6, 7]. Thus, it is imperative to investigate the molecular mechanism underlying the development and progression of STAD and explore the targeted signaling pathways for cancer treatment. Besides, immune-related mechanisms and markers are involved in the occurrence and progression of STAD[7]. And immune-related targeted therapy provides a promising option for the treatment of STAD[7, 8]. However, the developments of the novel molecular and high effective markers are in its infancy. Thus, it's necessary to identify novel immune-related markers to realize early diagnosis and treatment in STAD.

TGF- β receptor 1 (TGFB1), serving important roles in the TGF- β family, and have vital effects on many biological processes, including cell growth, reproductive capacity and immunological reactions[9, 10]. Transforming growth factor (TGF)- β signaling plays an important role in both the regulation of the growth and differentiation of tumor cells and the functional regulation of tumor interstitial[11, 12]. TGF- β binds to TGFB2, forming a complex with TGFB1, which then phosphorylates R-SMADs, enabling the formation of a complex with SMAD4 that could enter the nucleus to regulate gene expression [8, 13, 14]. It has been reported that TGFB1 is an irreplaceable downstream molecule of TGF- β 1 that participates in the life cycle of cells, such as cell differentiation, adsorption, fission and death[15, 16]. It's observed that TGFB1 expression taking part in the Linc00462/miR-665/TGFB1-TGFB2/SMAD2/3 pathway influences pancreatic cancer invasiveness[17]. TGFB1 participate in TGFB1-epidermal growth factor receptor (EGFR)-CTNNB1-CDH1 axis in regulating the invasion and migration in lung cancer[18]. Additionally, the activation or overexpression of TGFB1 is observed in different types of tumors and can serve an important role in tumor cell proliferation and migration to other tissues [8, 9]. Cells that lack TGFB1 do not respond to TGF- β 1, which further affects the transduction of the TGF- β signaling pathway[19, 20]. It's reported that some inhibitors of TGFB1 had been join in phase 1 study in patients with advanced cancer, such as galunisertib[21, 22].

Increasing evidence indicates that the tumor microenvironment (TME) supports tumor growth and survival and regulates immune responses[22, 23]. TGFB1 is a potential regulator of the tumor microenvironment, playing an important role in tumor immune evasion, leading to tumor progression and metastasis[22, 24]. TGF- β signaling pathway operates as a major suppressor of the adaptive and innate immune responses during tumor progression[6, 15]. Mutations in TGFB1 is frequently observed, the loss of function mutations provide evidence for the tumor suppressor role of the TGF- β signaling pathway in tumor[6]. TGFB1 mediated phosphorylation of ILK and its subsequent binding to CD103, thus initiating inside-out signaling leading to activation of the integrin and strengthening of CD103-E-cadherin adhesion[25]. The existing body of research have shown a vital role for TGF- β signals in regulation of NK cells[19, 26], T-Lymphocyte Accumulation and Antitumor Activity[25] and safe-guarding specific Treg cell functions[27]. However, the mechanisms whereby TGF β 2 governs tumor progression, immune cell

infiltration and the prognosis value of TGFBR1 expression and its correlation with tumor microenvironment (TME) in STAD remains unintelligible.

Here we investigated the TGFBR1 expression in STAD and its diagnostic and prognostic value in patients with STAD. Then, gene set enrichment analysis (GSEA) of the STAD data set from TCGA was applied to evaluate the underlying mechanisms of TGFBR1, and the correlation between TGFBR1 expression and tumor infiltrating immune cells (TIILs), immune checkpoint in the TME were assessed.

Methods

Sample Data Sets

All raw data of STAD, with transcriptome RNA-seq data and the corresponding clinical information, were downloaded from TCGA database (<https://portal.gdc.cancer.gov/repository>)[28]. Performing the differential expression analysis in TCGA-STAD samples according to TGFBR1 expression, we determined the DEmRNAs with thresholds of $|\log FC| > 1.5$ and $\text{adj } p < 0.05$. Next, we used the “Similar Gene Detection” module of GEPIA2 to obtain the top 100 TGFBR1-correlated targeting genes based on the datasets of all STAD-TCGA tumor tissues. Then, we conduct an intersection analysis to compare the TGFBR1-binding and interacted genes through a website (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

GEPIA database analysis

GEPIA is an online database which facilitates the standardized analysis of RNA-seq data in the TCGA and GTEx data sets (<http://gepia.cancer-pku.cn/index.html>)[29, 30]. We therefore employed this database to assess the link between TGFBR1 expression and patient prognosis in STAD, and we further assessed the link between TGFBR1 expression and the expression of particular markers associated with immune cell infiltration of tumors.

Kaplan-Meier plotter analysis

The Kaplan-Meier plotter offers a means of readily exploring the impact of a wide array of genes on patient survival in 21 different types of cancer [31]. We explore the association between TGFBR1 expression and outcome therefore through this database in STAD (<http://kmplot.com/analysis/>).

TIMER database analysis

TIMER2.0 (<http://timer.comp-genomics.org/>) is a database designed for analyzing immune cell infiltrates in multiple cancers. This database employs pathological examination-validated statistical methodology in order to estimate tumor immune infiltration by monocyte, neutrophils, M1/M2macrophages, TMA, dendritic cells, B cells and CD4/CD8 T cells so on[32, 33]. We initially employed this database to assess differences in TGFBR1 expression levels in particular tumor types using the TIMER2.0 database, and we then explored the relationship between this TGFBR1 expression and the degree of infiltration by particular immune cell subsets. We further conducted Kaplan-Meier curve analyses to explore the differences of

survival as a function of gene expression or immune cell infiltration. Lastly, we assessed how TGFBR1 expression correlated with the expression of particular immune infiltrating cell subset markers.

Gene Set Enrichment Analysis (GSEA)

GSEA was conducted to identify potential biological processes and pathways in high-risk groups in the “cluster Profiler” R package[34, 35]. The c2.cp.v7.2.symbols.gmt (KEGG) and c5.all.v7.2.symbols.gmt (Gene ontology) were downloaded from the Molecular Signatures Database (MSigDB). Gene sets with $|NES| > 1$, $NOM\ p < 0.05$ and $FDR < 0.05$ were considered as statistical significance.

Immune Cells Infiltration of ssGSEA

immune infiltration analysis of STAD was performed by single-sample gene set enrichment analysis (ssGSEA) in the “GSVA” R package[36], and the infiltration levels of 24 immune cell types[37] were quantified from gene expression profiles. In addition, a Spearman correlation was performed to analyze the relationship of immune cells infiltration with TGFBR1 expression and Wilcoxon rank sum test was conducted to analyze the correlation between infiltration level of immune cells and TGFBR1.

Statistical analysis

The least absolute shrinkage and selection operator (LASSO) regression algorithm for feature selection, using 10-fold cross-validation, the above analysis uses the R software package glmnet[38–40]. For Kaplan–Meier curves, p-values and hazard ratio (HR) with 95% confidence interval (CI) were generated by log-rank tests and univariate Cox proportional hazards regression. All analytical methods above and R packages were performed using R software version 4.0.3 (The R Foundation for Statistical Computing, 2020). $p < 0.05$ was considered as statistically significant.

All figure construction in this study was conducted by using R package software (version 4.1.1). Univariate and multivariate Cox regression model were performed by using “survival”, “survminer” packages. Wilcox test was used to determine statistical differences of categorical variables. Genes included in the multivariate analysis were selected through stepwise Cox regression analysis with both directions. Bootstrap method was used to perform internal validation in the TCGA dataset.

Results

TGFBR1 levels are increased in STAD

We used Timer database to analyze the TGFBR1 expression in tumor tissues, and our results showed that the expression level of TGFBR1 was increased in a variety of tumors, including STAD compared with those corresponding NT tissues (Fig. 1A). At the same time, we investigated the expression of TGFBR1 in TCGA database by GEPIA. The results suggested that TGFBR1 was elevated in STAD compared with the corresponding normal tissues (Fig. 1B and C). Furthermore, IHC results of normal tissues and tumor tissues were obtained from the HPA database (Fig. 1D). The results suggested that the level of TGFBR1 protein increased in STAD compared with the corresponding normal stomach tissue. Collectively, these

results revealed that TGFBR1 is consistently upregulated in STAD tissue compared with corresponding normal cells and tissue.

High TGFBR1 Expression is Correlated with Clinicopathologic Features in Patients with STAD

To clarify the correlation between the overexpression of TGFBR1 and the clinical, pathological, and molecular characteristics. Using the GEPIA2 dataset, we compared the mRNA expression of TGFBR1 between STAD with different pathologic stage (Fig. 2A). The results indicated that increased expression of TGFBR1 associated significantly with different pathologic stage ($P < 0.05$). The characteristics of 375 STAD patients including clinical and gene expression data were achieved from TCGA database (Table 1). Based on the mean value of TGFBR1 expression, the patients were divided into high- and low-TGFBR1 expression groups, then we conducted Wilcoxon signed-rank test and logistic regression to analyze the correlation between TGFBR1 expression and clinical characteristics. As shown in Table 1, Fig S1B,1C and 1D, the expression level of TGFBR1 was correlated with the pathologic stage, T stage, N stage, LNN, and degree of Histologic grade in STAD. Therefore, we further explore that a high level of TGFBR1 may be associated with a poor prognosis of STAD patients.

Table 1

Correlation Between TGFBR1 Expression and Clinicopathologic Characteristics of Patients with STAD

Characteristic	Low expression of TGFBR1	High expression of TGFBR1	p
n	187	188	
T stage, n (%)			0.001
T1	15 (4.1%)	4 (1.1%)	
T2	49 (13.4%)	31 (8.4%)	
T3	84 (22.9%)	84 (22.9%)	
T4	38 (10.4%)	62 (16.9%)	
N stage, n (%)			0.004
N0	69 (19.3%)	42 (11.8%)	
N1	53 (14.8%)	44 (12.3%)	
N2	32 (9%)	43 (12%)	
N3	28 (7.8%)	46 (12.9%)	
M stage, n (%)			0.965
M0	167 (47%)	163 (45.9%)	
M1	12 (3.4%)	13 (3.7%)	
Gender, n (%)			0.617
Female	64 (17.1%)	70 (18.7%)	
Male	123 (32.8%)	118 (31.5%)	
Age, n (%)			0.880
<=65	81 (21.8%)	83 (22.4%)	
>65	105 (28.3%)	102 (27.5%)	
Histological type, n (%)			0.249
Diffuse Type	27 (7.2%)	36 (9.6%)	
Mucinous Type	6 (1.6%)	13 (3.5%)	
Not Otherwise Specified	113 (30.2%)	94 (25.1%)	
Papillary Type	3 (0.8%)	2 (0.5%)	

Characteristic	Low expression of TGFBR1	High expression of TGFBR1	p
Signet Ring Type	4 (1.1%)	7 (1.9%)	
Tubular Type	34 (9.1%)	35 (9.4%)	
Histologic grade, n (%)			0.291
G1	6 (1.6%)	4 (1.1%)	
G2	75 (20.5%)	62 (16.9%)	
G3	103 (28.1%)	116 (31.7%)	
Pathologic stage, n (%)			< 0.001
Stage I	37 (10.5%)	16 (4.5%)	
Stage II	64 (18.2%)	47 (13.4%)	
Stage III	60 (17%)	90 (25.6%)	
Stage IV	17 (4.8%)	21 (6%)	
Primary therapy outcome, n (%)			0.187
PD	35 (11%)	30 (9.5%)	
SD	7 (2.2%)	10 (3.2%)	
PR	0 (0%)	4 (1.3%)	
CR	117 (36.9%)	114 (36%)	
Barretts esophagus, n (%)			1.000
No	107 (51.4%)	86 (41.3%)	
Yes	8 (3.8%)	7 (3.4%)	
Age, mean $\pm$ SD	66.2 $\pm$ 10.57	65.46 $\pm$ 10.74	0.504

Identification of the TGFBR1 Related Prognostic Signature

Performing the differential expression analysis in TCGA-STAD samples according to TGFBR1 expression, we determined the 470 DEGs with thresholds of $|\log FC| > 1.5$ and $\text{adj } p < 0.05$. Volcano plots visually displaying the distribution of DE mRNAs were generated (Figure 2A). Next, we obtain the top 100 TGFBR1-correlated targeting genes based on the GEPIA2 datasets (Table S1). Then, we conduct an intersection analysis to compare the TGFBR1-binding and interacted genes through a website

(<http://bioinformatics.psb.ugent.be/webtools/Venn/>) (Figure 2B, Table S2). To stratify the clinical outcomes of patients with the TGFBR1 readily and efficiently, we applied the LASSO Cox regression algorithm to the 20 factors and TGFBR1 in the TCGA dataset. A total of 9 factors with non-zero coefficients were identified. These LASSO-selected features were used to build the TGFBR1 prognostic signature (Figure 2 and Figure S2). The corresponding risk scores were computed, according to the following formula:

Risk score = $(-0.2914) \cdot \text{DIXDC1} + (0.1113) \cdot \text{STON1-GTF2A1L} + (0.3092) \cdot \text{FERMT2} + (-0.0146) \cdot \text{BHMT2} + (0.1798) \cdot \text{ABCC9} + (0.068) \cdot \text{MSRB3} + (-0.1007) \cdot \text{SYNC} + (-0.0891) \cdot \text{SORBS1} + (0.0828) \cdot \text{TGFBR1}$. We divided patients in TCGA datasets into high-risk and low-risk groups using their respective median risk score as the cutoff. Kaplan-Meier survival analysis determined that patients with high-risk scores had poorer OS than those with low-risk scores ($p < 0.001$, HR = 1.579, 95% CI: 1.134, 2.198; Figures 2C-D). The area under curve (AUC) for 1-year, 3-year and 5-year time-dependent ROC were 0.645 and 0.639, 0.669 respectively (Figures 2E).

High Expression of the TGFBR1 Related Prognostic Signature

The survival analysis of the TCGA-STAD dataset demonstrated that high TGFBR1 expression was correlated with poor OS ($P = 0.039$) (Figure 3A). Next, we conduct Kaplan–Meier survival analysis by KM Plotter. The result of survival analysis represented that high TGFBR1 expression was associated with poor OS (206943_at, $P = 5.7 \times 10^{-7}$) and (224793_s_at, $P = 7.3 \times 10^{-7}$) (Figure 3B and C), poor FP (206943_at, $P = 4 \times 10^{-4}$) and (224793_s_at, $P = 5.5 \times 10^{-12}$) (Figure 3D and E), and poor PPS (206943_at, $P = 6 \times 10^{-9}$) and (224793_s_at, $P = 0.00011$) (Figure 3F and G). Patients with TGFBR1 overexpression have a shorter overall survival time, and the results are consistent with the TCGA databases. Then, the receiver operating characteristic (ROC) curve was carried out to analyze the effectiveness of TGFBR1 expression in normal samples of GTEx combined adjacent STAD tissues and STAD samples. AUC of TGFBR1 was 0.739, CI (0.629, 0.848), which suggested high diagnostic value (Figure 4A). Then, the nomogram used age, T, N, M classification, pathologic stage, primary therapy outcome, histologic grade and TGFBR1 to predict the 1, 3, 5-year OS in the TCGA-STAD (Figure 4B). Univariate and multivariate analyses were performed to explore whether TGFBR1 is an independent prognostic factor affecting the prognosis of STAD. Univariate Cox analysis showed that high TGFBR1 expression was significantly correlated with poor OS (hazard ratio [HR] = 1.645, 95% CI = 1.179–2.295, $P = 0.003$ (Table 2 and Figure 5A). Moreover, multivariate regression analysis further verified that TGFBR1 expression was an independent prognostic factor for OS in patients with STAD (HR = 1.733, 95% CI = 1.165–2.578, $P = 0.007$) (Table 2 and Figure 5B). The univariate and multivariate analyses results revealed that the upregulation of TGFBR1 could be used as a prognostic factor for STAD. Hence, the results suggested that TGFBR1, as a prognostic factor, its upregulation can significantly affect the prognosis of patients with STAD.

Table 2: Associations with Clinicopathologic Characteristics in STAD Patients Using Cox Regression

Characteristics	HR (95% CI)	P value
Univariate analysis		
T3&T4 vs (T1&T2)	1.719 (1.131-2.612)	0.011
N stage (N1&N2&N3 vs N0)	1.925 (1.264-2.931)	0.002
M stage (M1 vs M0)	2.254 (1.295-3.924)	0.004
TGFBR1(High vs Low)	1.645 (1.179-2.295)	0.003
Age (>65 vs <=65)	1.620 (1.154-2.276)	0.005
Gender (Male vs Female)	1.267 (0.891-1.804)	0.188
Histologic grade G3 vs(G1&G2)	1.353 (0.957-1.914)	0.087
Primary therapy outcome (SD&PR&CR vs PD)	0.241 (0.165-0.352)	<0.001
multivariate analysis		
T3&T4 vs (T1&T2)	1.492 (0.853-2.607)	0.161
N stage (N1&N2&N3 vs N0)	1.287 (0.774-2.137)	0.331
M stage (M1 vs M0)	1.765 (0.830-3.755)	0.140
TGFBR1(High vs Low)	1.733 (1.165-2.578)	0.007
Age (>65 vs <=65)	1.923 (1.281-2.886)	0.002
Histologic grade G3 vs(G1&G2)	1.717 (1.124-2.623)	0.012
Primary therapy outcome (SD&PR&CR vs PD)	0.227 (0.151-0.341)	<0.001

TGFBR1-Related Signaling Pathways Based on GSEA

From the above results, we can know that TGFBR1 plays an important role in the tumorigenesis of STAD, but the specific molecular mechanism that leads to the poor prognosis of STAD is still unclear. To reveal the mechanism of TGFBR1, GSEA be used to evaluate the potential signaling pathway of TGFBR1 in STAD. We found some signaling pathways and biological processes that may be related to the effect of TGFBR1 on STAD progression, including pathway in cancer, MAPK signaling pathway, NOTCH signaling pathway, focal adhesion and VEGF-C production (Fig. 6A-F). The result further illustrates the complexity of the mechanism of TGFBR1 in STAD and it also clarifies the potential biological pathway regulation mechanism that TGFBR1 may participate in the regulation of STAD.

Relationship between TGFBR1 expression and tumor-infiltrating immune cells

Tumor immune infiltration is an important factor in neoplasm progress and significantly influences the survival probability of tumor patients. Using the TIMER database, we analyze the relationship between expression of TGFBR1 and immune cell infiltration (Fig. 7A). The results showed that the expression level of TGFBR1 was positively correlated with the infiltration levels of CD4+ T cells, CD8+ T cells, Macrophages, Neutrophils and Myeloid dendritic cells. This suggests that TGFBR1 may be a potential factor affecting the immune microenvironment of STAD. We also analyze the OS between expression of TGFBR1 and immune cell infiltration level through Timer database (Fig. 7B). The results showed that the patient with higher TGFBR1 expression and lower CD8+ T cells infiltration level could be had poor prognosis ($p<0.05$). In high TGFBR1 expression group, the patient with higher Macrophage infiltration level could be have poor prognosis ($p<0.05$). We further explored the relationship between TGFBR1 expression and infiltration level of 24 immune cells quantified by ssGSEA in STAD through Spearman correlation (Fig. 8A and FigS3). The results showed that high TGFBR1 expression was significantly positively correlated with infiltration levels of natural killer (NK) cells and Tem cells and negatively correlated with infiltration levels of Th17 cells (Fig. 8B-D). Collectively, the above results demonstrated that the expression level of TGFBR1 is positively correlated with the level of tumor immune cell infiltration.

Table3 Correlation analysis between TGFBR1 and markers of immune cells in TIMER2

Description	Gene markers	STAD			
		None		Purity	
		cor	p	cor	p
CD8+T cell	CD8A	0.193176	7.47E-05	0.191376	***
	CD8B	0.116758	*	0.122913	*
B cell	CD19	0.145225	**	0.143698	*
	CD79A	0.080533	0.101359	0.069968	0.174053
T cell (general)	CD2	0.166071	**	0.177884	**
	CD3D	0.082814	0.092015	0.089776	0.080897
	CD3E	0.103589	*	0.11805	*
Dendritic cell	CD1C	0.248421	***	0.266934	***
	HLA-DPA1	0.086889	0.07705	0.087213	0.089986
	HLA-DPB1	0.089836	0.067509	0.087484	0.088985
	HLA-DQB1	0.002143	0.965286	0.003934	0.939146
	HLA-DRA	0.058686	0.232889	0.06168	0.230935
	ITGAX(CD11C)	0.349478	***	0.351671	***
	NRP1	0.522294	***	0.52699	***
Monocyte	CD86	0.271163	***	0.284061	***
	CSF1R(CD115)	0.422028	***	0.417374	***
M1	IRF5	0.191952	8.31E-05	0.202119	7.41E-05
Macrophage	NOS2	-0.00307	0.95033	-0.00253	0.960839
	PTGS2	0.25425	***	0.262486	***
M2	CD163	0.43154	***	0.437968	***
Macrophage	MS4A4A	0.362993	***	0.370423	***
	VSIG4	0.361185	***	0.373065	***
TAM	CCL2	0.299029	***	0.305362	***
	CD68	0.151294	***	0.152384	**
	IL10	0.287491	***	0.301947	***
NK cell	KIR2DL1	0.180803	***	0.183648	***

	KIR2DL3	0.108487	*	0.087666	0.088323
	KIR2DL4	-0.02344	0.634017	-0.03423	0.506391
	KIR2DS4	0.112557	*	0.102084	*
	KIR3DL1	0.151271	**	0.128413	*
	KIR3DL2	0.123974	*	0.11811	*
	KIR3DL3	0.0133	0.787051	0.008714	0.865734
Neutrophils	CCR7	0.232123	***	0.248176	***
	CEACAM8	0.091303	0.063132	0.098906	0.054373
	ITGAM	0.351498	***	0.363363	***
Th2	GATA3	0.198144	*	0.205701	5.47E-05
	IL13	0.060757	0.216783	0.076006	0.139697
	STAT5A	0.381184	***	0.389848	***
	STAT6	0.261588	***	0.265655	***
Th1	IFNG	0.001592	0.974204	0.004794	0.925889
	STAT1	0.173163	***	0.1644	**
	STAT4	0.329852	***	0.341129	***
	TBX21	0.149525	**	0.159203	**
	TNF	-0.0072	0.883787	-0.00297	0.954111
Tfh	BCL6	0.486884	***	0.482278	***
	IL21	0.043422	0.377607	0.045623	0.375771
Th17	IL17A	-0.1392	***	-0.12542	*
	STAT3	0.552904	***	0.546375	***
Treg	CCR8	0.300346	***	0.298788	***
	FOXP3	0.175633	***	0.172984	**
	STAT5B	0.587878	***	0.584431	***
	TGFB1	0.37103	***	0.357758	***
T cell exhaustion	CTLA4	0.15921	**	0.168492	**
	GZMB	-0.01039	0.832891	-0.03254	0.527684
	HAVCR2	0.302736	***	0.312788	***

LAG3	0.059521	0.226302	0.051196	0.320204
PDCD1	0.122171	*	0.123658	*

Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. *P < 0.05; **P < 0.005; ***P < 0.0005.

Assessment of the correlation between TGFBR1 and immune marker expression

We next further explored the link between TGFBR1 expression and levels of immune cell infiltration based on sets of immunological markers in STAD using the TIMER and GEPIA database. Especially, we assessed the correlation between TGFBR1 expression and levels of parkers for particular cell subsets including CD8+ T cells, total T cells, B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK

cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs and exhausted T cells. We adjusted these results based on tumor purity, revealing a significant correlation between TGFBR1 expression and CD8+T cell markers (CD8A,CD8B), B cell marker (CD19), T cell (general) markers (CD2,CD3E), monocyte markers (CD86, CSF1R), TAM markers (CCL2, IL10,CD68), M1 macrophage markers (PTGS2), M2 macrophage markers (CD163, VSIG4, MS4A4A), neutrophils markers (CCR7, ITGAM), NK cell markers (KIR2DL1, KIR2DS4, KIR3DL1, KIR3DL2), Dendritic cell markers (CD1C, ITGAX, NRP1), Th1 markers (STAT1, STAT4, TBX21), Th2 markers (STAT5A, STAT6), Tfh markers (BCL6), Th17 markers (IL17A, STAT3), T cell exhaustion markers (HAVCR2, PDCD1) and Treg markers (CCR8, FOXP3, STAT5B, TGFB1) in STAD (Table 3). TGFBR1 expression was related to that of the majority of monocyte, TAM, M1 and M2 macrophage markers in STAD (Table 4). In particular, it was significantly correlated with monocyte markers (CD86, CSF1R), TAM markers (CCL2, IL10), M1 macrophage markers (PTGS2) and M2 macrophage markers (CD163, VSIG4, MS4A4A) in STAD (P<0.05; Figure 9A–D). Thus, we further evaluate the relationship between TGFBR1 expression and these markers in STAD through the GEPIA2 database revealing similar correlations in TIMER2 (Table 4). The result suggests that TGFBR1 may be capable of regulating the polarization of macrophages in STAD. Elevated TGFBR1 expression is also associated with increased NK cell infiltration in STAD, and consistent with this, the NK cell markers KIR2DL1, KIR2DS4, KIR3DL1 and KIR3DL2 were correlated with the expression of TGFBR1 expression. We further observed that there was a significant correlation between TGFBR1 and markers of Tregs and exhausted T cells including CCR8, FOXP3, STAT5B, TGFB, HAVCR2, PDCD1 (Table 4), indicating that TGFBR1 may play a role in immune escape in STAD, although further work will be necessary to confirm the underlying mechanisms. In addition, we also explored the relationship between TGFBR1 and the 15 Immune checkpoint (10 proteins from B7 family and 5 proteins from CD28 family) through GEPIA2. As shown in Fig. 10A-F and FigS4, the TGFBR1 expression level is positively correlated with the expression levels of CD86, CD276, PDCD1LG2, C10orf54, CTLA4 and NCR3LG1 in STAD. Collectively, the above results demonstrated that the expression level of TGFBR1 is positively correlated with the level of tumor immune cell infiltration, indicating that

TGFBR1 may play a vital role in immune escape in STAD, while further work will be needed to confirm the mechanisms.

Table4 Correlation analysis between TGFBR1 and relate genes and markers of monocyte, TAM and macrophages in GEPIA

Description	Gene markers	TCGA-STAD			
		Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.12	*	-0.3	0.077
	CSF1R	0.29	***	0.043	0.8
TAM	CCL2	0.15	**	0.74	***
	CD68	0.052	0.29	-0.44	**
	IL10	0.13	*	-0.18	0.3
M1 Macrophage	NOS2	-0.075	0.13	-0.19	0.26
	IRF5	0.033	0.5	-0.37	*
	PTGS2	0.19	***	0.83	***
M2 Macrophage	CD163	0.16	9e-04	0.29	0.082
	VSIG4	0.17	**	0.11	0.51
	MS4A4A	0.18	***	0.23	0.18

Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. *P < 0.05; **P < 0.005; ***P < 0.0005.

Discussion

STAD is common cancer and remains the leading cause of cancer-related deaths in the worldwide[2]. In recent years, the OS of STAD patients has been improved to a certain extent with development of therapy and medicine, but there is still space for improvement. As well known, it is of great significance for improving the clinical prognosis of patients that well understanding the mechanism of the occurrence and development of cancer. Immunological mechanisms regulate the progression of STAD, and many different immunotherapies have been proposed as a means of effectively treating methods[7]. TGFBR1 act as important roles in the TGF- β family, and have important effects on cell reproductive capacity, growth and immunological reactions[19].

In this report, we assessed the expression of TGFBR1 as it related to the prognosis of 33 different types of cancers using the TIMER2.0 databases, revealing obvious differences between tumor and normal tissue expression of TGFBR1 in many cancers. TCGA data set analysis indicated that there was elevated TGFBR1 expression in CHOL, COAD, GBM, HNSC, LIHC, PCPG, STAD and THCA, whereas expression was decreased in BLCA, BRCA, KIRP, KIRC, LUAD, LUSC, PRAD and UCEC relative to adjacent controls. Altered TGFBR1 expression in a range of different cancers may relate to differences in the underlying biological mechanisms. Particularly, elevated expression of TGFBR1 also was observed in STAD through GEPIA. We confirmed the prognostic value of a signature built with 20 TGFBR1-related genes (Figure 3 and Table S2). The risk score of the TGFBR1-related signature was a stable, independent prognosis factor in TCGA datasets. Furthermore, elevated TGFBR1 correlated with poorer patient prognosis, as well as, stage, T stage, N stage, and differentiation. Elevated TGFBR1 expression in STAD correlated with a higher stage, N stage, T stage, and poor differentiation. This elevated TGFBR1 expression was also a reliable predictor of the presence of lymph node metastasis in STAD patients, indicating that TGFBR1 may be a valuable prognostic indicator of metastatic progression in STAD. These results together thus suggest that TGFBR1 may have value as a STAD prognostic biomarker and associated with progression of STAD.

In this study, we found that TGFBR1 expression correlated with patient prognosis in STAD, with a particularly strong correlation between high TGFBR1 expression and a poor STAD prognosis. Across these databases, we consistently observed a correlation between elevated TGFBR1 expression and a poor STAD prognosis. In the TCGA database, elevated TGFBR1 levels were correlated with a poorer outcome for patients with STAD. Similarly, the Kaplan-Meier plotter database found elevated TGFBR1 to correlate with poor GC outcome. What's more important, ROC analysis also confirmed the diagnostic value. In recent years, there was no predictive nomogram for STAD combining the TGFBR1 expression reported. Therefore, a prognostic nomogram involving T, M, N classification, age, pathologic stage, primary therapy outcome, histologic grade and TGFBR1 was constructed, which can be used by the physician to improve the accuracy of identifying high risk STAD patients. Multivariate Cox analysis further confirmed that high TGFBR1 expression was an independent risk factor for OS in STAD patient. Moreover, high TGFBR1 expression was correlated with clinicopathologic features in STAD, including primary therapy outcome, histologic grade and age. The result showed that high TGFBR1 expression was correlated with advanced gastric cancer, which indicated that it may be a marker to identify early STAD and advanced STAD. We conclude that the poor survival in STAD has a probable prognostic molecular marker known as the TGFBR1 expression. Also, possible key pathways in STAD that is regulated by TGFBR1 are the focal adhesion, pathway in cancer, NOTCH signaling pathway, MAPK signaling pathway, chemokine binding, and positive regulation of VEGF-C production. Finally, the results further suggest that for improved clinical outcomes among STAD patients, we could use an independent prognostic factor known as the high TGFBR1 mRNA expression. We recommend further research on the subject matter to progressively improve the evidence on the biological impact of TGFBR1.

A key finding in this study is that the TGFBR1 expression correlated with the degree of immune infiltration level in STAD. We found that TGFBR1 expression was positively correlated with the degree of CD4+ T cells, CD8+ T cells, Macrophages, Neutrophils and Myeloid dendritic cells infiltration in STAD. The results

showed that the patient with higher TGFBR1 expression and lower CD8+ T cells infiltration level could be had poor prognosis. We further observed that macrophage infiltration to be significantly associated with prognosis in STAD (Figure 7B). In high TGFBR1 expression group, the patient with higher Macrophage infiltration level could be have poor prognosis. We observed a correlation between TGFBR1 and M1/M2 macrophage markers including PTGS2, CD163, VSIG4 and MS4A4A (Table 4). This suggests that TGFBR1 play an important role in regulating TAM polarization. Also, we found TGFBR1 levels in STAD was related to markers of Treg cells and T cell exhaustion (CCR8, FOXP3, STAT5B, TGFB, HAVCR2, PDCD1 (Table 4). This suggests that TGFBR1 could participated in regulating Treg responses to suppress T cell-mediated immunity. Besides, we observed a correlation between TGFBR1 and NK cell markers including KIR2DL1, KIR2DS4, KIR3DL1, KIR3DL2 (Table 4). NK cells respond rapidly to tumor cells and virus-infected cells through the unique capacity to recognize stressed cells in the absence of an adaptive response, allowing a rapid immune reaction[6]. The result may reveal that TGFBR1 may participate in regulating NK cell-mediated rapid immune reaction or innate immunity. What's more, we observed that TGFBR1 expression correlated with multiple T cell markers (Th1, Th2, Tfh and Th17). This may correspond to the ability of TGFBR1 to regulate T cell responses in STAD. Together, the correlation observed between TGFBR1 and certain immunological marker genes demonstrated that TGFBR1 could regulate immune cell infiltration and interactions within the tumor microenvironment in STAD.

In addition, the TGFBR1 expression level is positively correlated with the expression levels of CD86, CD276, PDCD1LG2, C10orf54, CTLA4, CD28 and NCR3LG1 in STAD. The correlation observed between TGFBR1 and the expression of 15 Immune checkpoint (B7 and CD28 family) suggests that TGFBR1 participated in regulating immune cell infiltration and interactions within the tumor microenvironment in STAD tumors. Together, these results highlight the ability of TGFBR1 to take part in potentially regulating immune cell recruitment and activation in STAD. The TGFBR1 expression correlated with the expression of several immune checkpoint, highlighting a possible role for TGFBR1 in the immunological interaction in STAD, making it a valuable biomarker worthy of further research in this type of cancer.

Conclusion

In summary, TGFBR1 could be a prognostic biomarker and an important regulator of immune cell infiltration in STAD. However, some limitations still exist in our study. There are some biases caused by confounding factors because of data from public databases. In addition, cell function assay and validation in vivo which are solid evidence were lacking. Some mechanisms of TGFBR1 in STAD also require further investigation.

Abbreviations

A list of abbreviations used in the manuscript and their meanings as show in Table S3.

Declarations

Availability of data and materials

All datasets generated for this study are included in the article/Supplementary Material.

Acknowledgements

Thank all members of TCGA, Timer, and GEPIA database for providing a good platform for researchers.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 82072664, 81772629, 81974374, 82173125, 81802363, 81702431), Tianjin Science Foundation (Nos. 18JCQNJC81900, 18JCYBJC92000, 18JCYBJC25400, 18JCYBJC92900) and the Science & Technology Development Fund of the Tianjin Education Commission for Higher Education (2018KJ046, 2017KJ227). The funders had no role in the study design, the data collection and analysis, the interpretation of the data, the writing of the report, and the decision to submit this article for publication.

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Competing interests

The authors declare that they have no competing interests.

Ethics declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Tianjin Medical University Cancer Institute and Hospital.

Consent for publication

Yes.

Competing interests

None.

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Figures

Figure 1

TGFBR1 levels are increased in STAD. A. TGFBR1 expression levels in different types of cancers from TCGA datasets in TIMER. B. Expression of TGFBR1 based on GEPIA2 database in STAD. C. Differential expression of TGFBR1 in different disease state (Tumor or Normal). D. IHC of normal stomach tissue and STAD tissue obtained from the HPA database. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2

Identification of the TGFBR1-related prognostic signature. A. The volcano plots describe DEGs ($|\log_2\text{fold change}| > 1.5$ and adjusted p value < 0.05) between the expression of TGFBR1-high and TGFBR1-low in STAD samples. B. An intersection analysis of the DEGs and TGFBR1-correlated genes was conducted. C. STAD patients' survival status and risk score distribution in the TCGA database. D. Kaplan-Meier curves

of OS between high-risk and low-risk groups in the TCGA database. E. ROC curves predict the 1-, 3- and 5-year OS in the TCGA database according to LASSO regression in TCGA database.

Figure 3

High TGFBR1 Expression indicated a poor prognosis for STAD. A. The survival analysis of overall survival (OS) of TCGA-STAD database. B, C. Kaplan–Meier survival analysis of OS. D, E. Kaplan–Meier survival analysis of free progression (FP). F, G. Kaplan–Meier survival analysis of post progression survival (PPS).

Figure 4

A. ROC curve for TGFBR1 in normal samples of GTEx combined adjacent STAD tissues and STAD samples. B. A nomogram for predicting probability of patients with 1-, 3- and 5-year OS.

A

B

Figure 5

A. Forest plot of the univariate Cox regression analysis in TCGA-STAD. B. Forest plot of the multivariate Cox regression analysis in TCGA-STAD.

Figure 6

The GSEA analysis results of TCGA RNA-seq data. A–C. TGFBR1-related signaling pathways in c2.cp.v7.2.symbols.gmt. D–F. TGFBR1-related signaling pathways in c5.all.v7.2.symbols.gmt (Gene ontology).

Figure 7

A. TGFBR1 expression is correlated with the level of immune infiltration based on TIMER2 database in STAD. B. Kaplan-Meier plots of immune infiltration and TGFBR1 expression levels based on TIMER2 database in STAD.

Figure 8

Immune cell infiltration analysis of TGFBR1 in the TCGA-STAD. A. The forest plot shows the correlation between TGFBR1 expression level and 24 immune cells. B. the correlation between TGFBR1 expression and NK cells. C. The correlation between TGFBR1 expression and Tem. B. the correlation between TGFBR1 expression and Th17 cells. Tcm, T central memory; Tem, T effector memory; Tgd, T gamma delta; Tfh, T follicular helper; NK, natural killer; pDC, plasmacytoid dendritic cells.

Figure 9

Correlation analysis between TGFBR1 expression and immunological marker set in STAD. A. Scatterplots of correlations between TGFBR1 expression and gene markers of M1 macrophages in STAD. B. Scatterplots of correlations between TGFBR1 expression and gene markers of M2 macrophages in STAD. C. Scatterplots of correlations between TGFBR1 expression and gene markers of TAMs in STAD. D. Scatterplots of correlations between TGFBR1 expression and gene markers of monocytes in STAD.

Figure 10

A-F. Correlation analysis between TGFBR1 expression and immune checkpoint based on GEPIA2 in STAD.

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