Original Article

Inhibition of ITGB1-DT expression delays the growth and migration of stomach adenocarcinoma and improves the prognosis of cancer patients using the bioinformatics and cell model analysis

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Background: The long non-coding RNA, integrin subunit beta 1 (ITGB1) divergent transcript (ITGB1-DT), is known to be involved in cancer progression and associated with the poor prognosis of cancer patients. At present, the role of ITGB1-DT in stomach adenocarcinoma (STAD) has not been reported.

Methods: The expression level of ITGB1-DT was detected in normal gastric and STAD tissues from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. A receiver operating characteristic (ROC) analysis was used to evaluate the role of ITGB1-DT in diagnosing STAD. The relationship between ITGB1-DT overexpression and clinicopathological features, prognosis, and immune-infiltrated cells in STAD were explored using correlation, survival, and Cox regression analyses. A cell model of ITGB1-DT interference was constructed to explore the roles of ITGB1-DT on STAD cell proliferation and migration, and the signaling mechanism was investigated using Gene Set Enrichment Analysis (GSEA).

Results: ITGB1-DT was expressed up-regulated in STAD tissues. ITGB1-DT overexpression was associated with the T stage, therapeutic effect, overall survival, progression-free interval status, and poor prognosis in STAD patients. ITGB1-DT overexpression was valuable in diagnosing STAD and a negative factor affecting the prognosis of STAD patients. Interference with ITGB1-DT expression inhibited STAD cell proliferation, invasion, and migration. GSEA results showed that ITGB1-DT may be involved in STAD progression through the insulin, p53, mechanistic target of rapamycin kinase (MTOR), and other signaling pathways. Overexpression of ITGB1-DT was significantly correlated with the levels of STAD B cells, T cells, T helper cells, CD8 T cells, cytotoxic cells, and other immune cells.

Conclusions: ITGB1-DT was overexpressed and associated with poor prognosis in STAD. Interference with ITGB1-DT expression may delay the progression of STAD to improve the prognosis of STAD patients.

Keywords: ITGB1-DT; stomach adenocarcinoma; overall survival (OS); prognosis; The Cancer Genome Atlas (TCGA)

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Introduction

Stomach adenocarcinoma (STAD) is one of the most common gastrointestinal tumors, and most patients diagnosed with STAD are in a locally advanced stage (1,2). The incidence of postoperative local recurrence or distant metastasis in STAD patients remains high (1). Multiple studies have shown that long non-coding RNAs (lncRNAs) affect the prognosis of STAD patients and can also delay its progression (3-7). For example, LINC01503 expression was significantly elevated in gastric cardia adenocarcinoma (GCA) tissues and cells. LINC01503 overexpression was associated with lymph node metastasis, pathological stage, and poor prognosis in GCA patients. However, inhibition of LINC01503 expression reduced the proliferation, migration, and invasion of GCA cells. LINC01503 was shown to promote GCA progression by influencing the miR-133A-5p/Vimentin (VIM) signaling axis and epithelial-mesenchymal transformation (EMT) process (3). Compared with normal tissues, lncRNA HOX transcript antisense RNA (HOTAIR) and collagen type V alpha 1 chain (COL5A1) were overexpressed in STAD tissues and associated with poor prognosis in STAD patients. HOTAIR or COL5A1 promoted the growth of STAD cells, while miR-1277-5P played the opposite biological role. HOTAIR promoted the increased expression of COL5A1 using sponge miR-1277-5p, thus regulating STAD progression (6). Another study reported that LINC02407 was significantly upregulated in STAD tissues and cells, and could promote cell proliferation, migration, and invasion but inhibit apoptosis. LINC02407 was also shown to play a role in STAD by regulating the miR-6845-5p/miR-4455-ADGRD1 signaling mechanisms (7).

The transcription direction of integrin subunit beta 1 (ITGB1) divergent transcript (ITGB1-DT) on chromosome 10P11.22 is opposite to that of ITGB1. Previous research reported that ITGB1-DT was overexpressed in lung adenocarcinoma tissues, and a high expression of ITGB1-DT was associated with advanced clinical stage, short overall survival (OS), and disease-free survival (DFS) (8). High expression of ITGB1-DT was shown to promote the proliferation, migration, and invasion of lung adenocarcinoma cells, as well as lung metastasis in vivo. In contrast, knockdown of ITGB1-DT inhibited the proliferation, migration, and invasion of lung adenocarcinoma cells. The ITGB1-DT/ITGB1/Wnt/β-catenin/MYC positive feedback loop was involved in lung adenocarcinoma progression (8). ITGB1-DT/ARNTL2 promoted the growth and migration of lung adenocarcinoma cells, whereas miR-30b-3p reversed ITGB1/ARNTL2-mediated carcinogenesis (9). Currently, the mechanism of ITGB1-DT in STAD has not been reported. Therefore, we aimed to explore the expression levels and potential clinical values of ITGB1-DT in STAD using The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. The role of ITGB1-DT in the growth and migration of STAD cells was explored in a cell model of ITGB1-DT interference. The relationship between ITGB1-DT expression and tumor immune cells in STAD tissues was analyzed to provide new candidate molecules for STAD treatment. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-233/rc).

Methods

Data sources of STAD patients

In May 2021, the gene expression data in STAD and normal gastric tissues were downloaded from the TCGA and GTEx official websites. Data from STAD patients included the transcripts per million (TPM), and fragments per kilobase per million (FPKM) gene expression subtypes. There were 32 normal gastric tissue samples and 375 STAD tissue samples in the TCGA database. The GTEx database contained 174 normal gastric tissue samples. The clinicopathological features and survival data of 375 STAD patients were downloaded from the TCGA database. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of ITGB1-DT expression in STAD

The expression data of ITGB1-DT in normal gastric tissues and STAD tissues were obtained. The expression levels of ITGB1-DT in the unpaired STAD patient tissues of the TCGA + GTEx TPM, and FPKM types were determined, and the paired STAD patient tissues of the TCGA TPM and FPKM types were determined after one-to-one pairing.

Evaluation of the clinical values of ITGB1-DT in STAD

STAD patients were grouped according to clinicopathological features to explore differences in the expression levels of ITGB1-DT. The roles of ITGB1-DT expression in STAD diagnosis was evaluated using a receiver operating characteristic (ROC) analysis, and the area under the curve

(AUC) was used to assess the diagnostic value of ITGB1-DT in STAD. Survival analysis was conducted to explore the relationship between ITGB1-DT expression and OS, disease-specific survival (DSS), and the progression-free interval (PFI) in STAD patients after removing patients with missing prognostic information. Univariate and multivariate COX regression analysis were used to analyze the relationship between clinicopathological features and ITGB1-DT expression level and the prognosis of STAD patients. A prognostic nomogram was constructed based on the results of COX regression analysis (10).

Cell culture

The human gastric adenocarcinoma cell line AGS was used for the present study and was purchased from the cell resource center of ICELL Bioscience Inc. (Shanghai, China). The AGS cells were fed with 10% fetal bovine serum (Gibco, USA) and 1% streptomycin and penicillin (Solebo, China) and cultured in 5% CO_2 at 37 ℃.

Construction of the cell model

The sense sequence of the ITGB1-DT specific sequence was 5'-GGUCUAGCUGAGUUGACAATT-3', and the antisense sequence of the ITGB1-DT specific sequence was 5'-UUGUCAACUCAGCUAGACCTT-3' (GenePharma, China). AGS cells were placed in 6-well plates and incubated overnight at 5% CO_2 and 37 ℃. The GP-transfect-mate transfection reagent (GenePharma, China) was used to promote cell transfection at >60% cell confluency, and the AGS cells of our cell model were collected after 24 h for qRT-PCR detection of ITGB1-DT expression.

qRT-PCR

Total RNA of the AGS cells was extracted with Trizol (Takara, China) reagent. After quantification, cDNAs of ITGB1-DT were synthesized by reverse transcription using a PrimeScript RT Reagent Kit (Takara, China). A TB Green Premix Ex TaqII (Takara, China) kit was used for the PCR amplification, and the relative expression of ITGB1-DT was calculated using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as an internal reference. The primer sequences were as follows: forward of β-actin: 5'-GTGGCCGAGGACTTTGATTG-3'; reverse of β-actin: 5'-CCTGTAACAACGCATCTCATATT-3'; forward of ITGB1-DT: 5'-TTCCCTGGATGTAGCCTC TCA-3'; reverse of ITGB1-DT: 5'-TCCGAAATCCATCC ACATCT-3'.

CCK-8

A CCK8 kit (Invigentech, China) was used to measure cancer cell proliferation. AGS cells were taken in the logarithmic growth phase. 2,000/well cells were inoculated on 96-well plates and incubated at 5% CO_2 and 37 ℃. The cell activity of the two groups was detected at 0, 24, 48, 72, and 96 h. A 10 μL/well CCK-8 solution was incubated at 5% CO_2 and 37 ℃ for 1 h. The absorbance value at 450 nm was measured with a microplate reader. The experiment was repeated three times.

Wound healing

The surface of the cell layer was scratched with a 100 μL sterile spear tip when the AGS cells were fused to 90–100% in 6-well plates. After suspension, the cells were cleaned with PBS, and a serum-free medium was added to the 6-well plates. After routine incubation, the wound distance was photographed at 0, 12, and 24 h after scratching to observe the cell migration under the 10× microscope. The migration distance was calculated by Image J (Version: 1.8.0) software. The experiment was repeated three times.

Migration and invasion experiments

Diluted matrigel was slowly added to the inner compartment surface of the basement membrane of the transwell chamber and air-dried at 37 ℃. A single cell of AGS suspension was prepared. 200 μL cell suspension was added to the transwell chamber of the experimental and control groups, and 500 μL 15% fetal bovine serum medium was added to the outer chamber. After conventional culture for 24 h, the remaining adherent cells in the basement transwell membrane were removed with cotton swabs, fixed with 4% paraformaldehyde at room temperature for 20 min, and stained with 0.5% crystal violet for 20 min. At high magnification, five fields were counted randomly. The experiment was repeated three times.

The ITGB1-DT underlying signaling mechanism

The tumor tissues of the TCGA STAD patients were sequenced and divided into two groups using the median ITGB1-DT value. Gene Set Enrichment Analysis (GSEA) was used to investigate the effect of ITGB1-DT on the

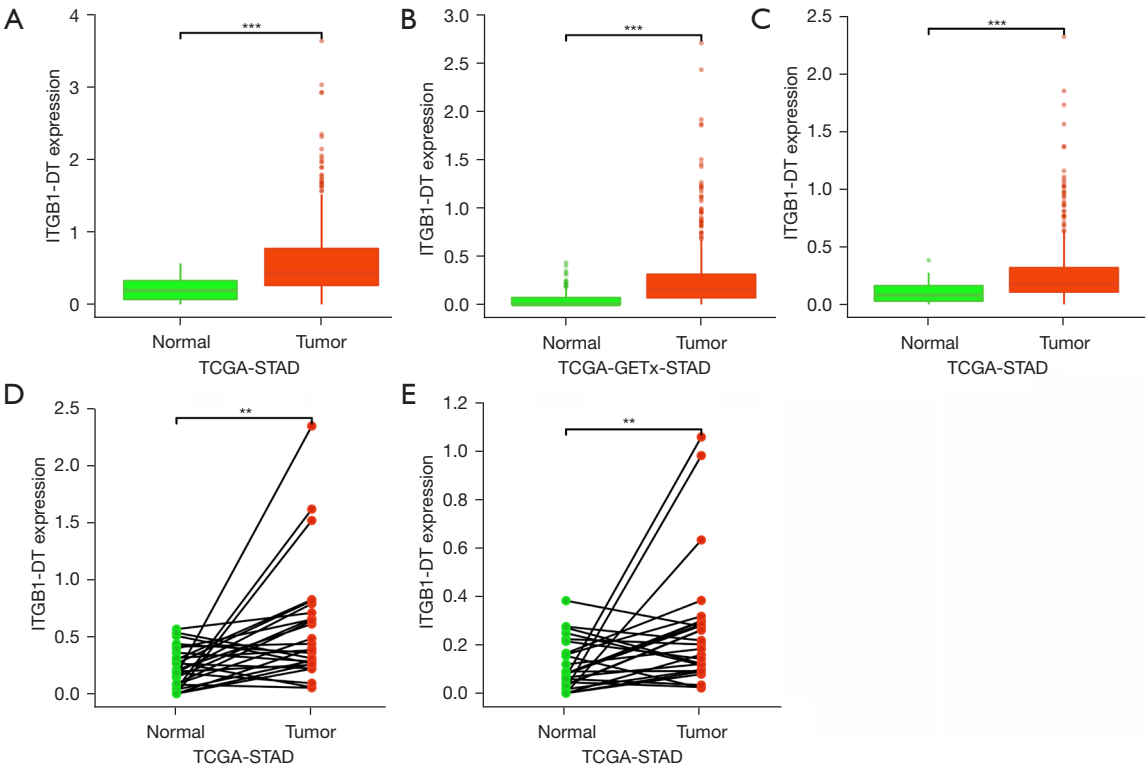

Figure 1 ITGB1-DT overexpression in STAD tissues. (A) Unpaired tissues in the TCGA TPM types; (B) unpaired tissues in the TCGA + GTEx TPM type; (C) unpaired tissues in the TCGA FPKM type; (D,E) paired tissues in the TCGA TPM and FPKM types. TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; STAD, stomach adenocarcinoma; TPM, the transcripts per million; FPKM, fragments per kilobase per million; **, P<0.01; ***, P<0.001.

TCGA gene set to understand the potential mechanisms of ITGB1-DT involving STAD progression (10,11).

Identification of the relationship between ITGB1-DT expression and immune infiltration in STAD

STAD tissues from the TCGA database were evaluated using immune scoring by a ssGSEA algorithm. The relationship between the ITGB1-DT expression level and STAD immune infiltration cells was explored using the Spearman method. On this basis, the relationship between ITGB1-DT expression and STAD immune infiltrating cell marker levels were explored.

Statistical analysis

The expression of ITGB1-DT in STAD tissues and cells was investigated using the *t*-test. The ROC analysis assessed the value of ITGB1-DT in diagnosing STAD, and the AUC was the index of diagnostic significance. A survival analysis was conducted to investigate the relationship between ITGB1-DT expression and poor prognosis in STAD patients. The association between ITGB1-DT expression levels and STAD immune infiltrating cells was analyzed using correlation analysis. A P value <0.05 was considered to be statistically significant.

Results

ITGB1-DT was overexpressed in STAD

The results based on the TCGA and GTEx data showed that ITGB1-DT was overexpressed in unpaired STAD tissues compared with normal stomach tissues and showed a significant statistical difference (*Figure 1A-1C*). After one-to-one matching, analysis showed that ITGB1-DT was overexpressed in the paired STAD tissues compared with normal stomach tissues, and this difference was statistically significant (*Figure 1D,1E*).

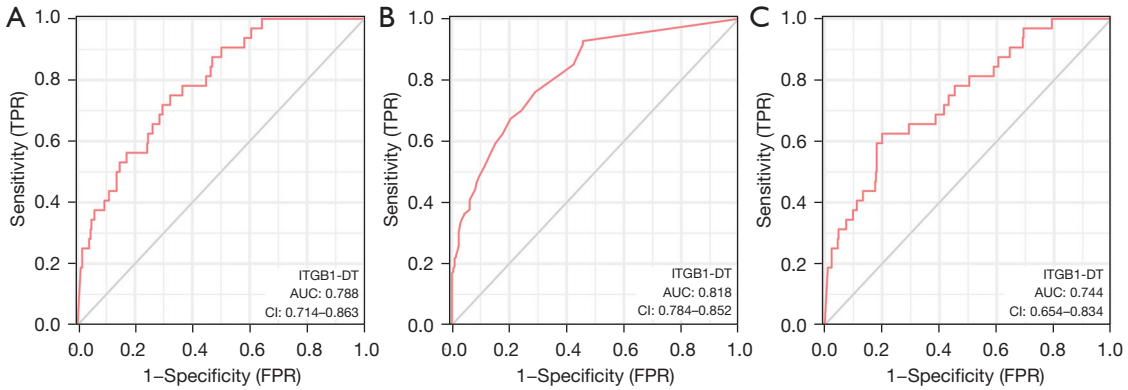

Figure 2 ROC analysis indicates the diagnostic value of ITGB1-DT in STAD. AUC, the area under the curve; ROC, receiver operating characteristic; STAD, stomach adenocarcinoma; CI, confidence interval; TPR, true positive rate; FPR; false positive rate.

Table 1 ITGB1-DT expression is correlated with the clinicopathological features in STAD

Characteristic	Low expression of ITGB1-DT	High expression of ITGB1-DT	P
T stage, n (%)			0.007
T1	16 (4.4)	3 (0.8)	
T2	42 (11.4)	38 (10.4)	
T3	73 (19.9)	95 (25.9)	
T4	52 (14.2)	48 (13.1)	
N stage, n (%)			0.507
N0	62 (17.4)	49 (13.7)	
N1	46 (12.9)	51 (14.3)	
N2	35 (9.8)	40 (11.2)	
N3	35 (9.8)	39 (10.9)	
M stage, n (%)			0.398
M0	168 (47.3)	162 (45.6)	
M1	10 (2.8)	15 (4.2)	

STAD, stomach adenocarcinoma.

ITGB1-DT demonstrated diagnostic value for STAD

The ROC analysis showed that the ITGB1-DT expression level has significant value in diagnosing STAD. In detail, the results based on the TPM data of the TCGA database showed an ITGB1-DT AUC of 0.788 (*Figure 2A*). The results based on the TPM data of the TCGA + GTEx database showed an ITGB1-DT AUC of 0.818 (*Figure 2B*). The results based on the FPKM data of the TCGA database showed an ITGB1-DT AUC of 0.744 (*Figure 2C*), indicating that ITGB1-DT has significant value in diagnosing STAD.

ITGB1-DT expression was correlated with the clinicopathological features of STAD patients

Grouping by the ITGB1-DT expression median value, we found that the expression level of ITGB1-DT was correlated with the T stage of STAD patients (*Table 1*). Based on the clinicopathological characteristics in the TCGA database, ITGB1-DT expression levels showed significant differences in T stage, therapeutic effect, DSS, and PFI of STAD patients (*Figure 3*). Specifically, the expression level of ITGB1-DT was significantly increased in T3 and T4 compared with T1 (*Figure 3A*). Compared with patients who had a progressive disease (PD) response to treatment, the ITGB1-DT expression level was significantly decreased in the tissues of patients with a complete response (CR) to treatment (*Figure 3B*). ITGB1-DT expression was significantly increased in tissues of deceased versus living STAD patients in the OS and PFI conditions (*Figure 3C,3D*).

ITGB1-DT overexpression level was associated with poor prognosis in STAD patients

The prognostic data analysis based on the TPM and FPKM types from the TCGA database showed that ITGB1-DT overexpression was correlated with a poor prognosis for STAD patients (*Figure 4*). Specifically, ITGB1-DT overexpression was correlated with a shorter OS for STAD patients and was statistically significant (*Figure 4A*). ITGB1-DT overexpression was also associated with a shorter DSS in

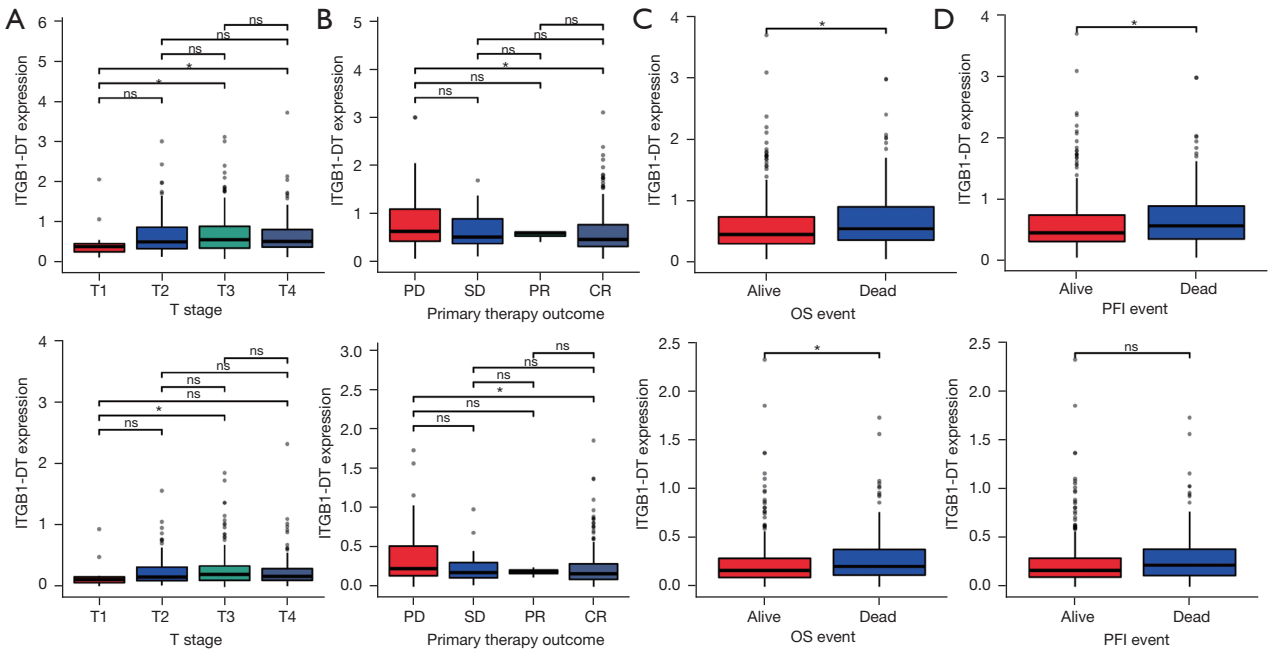

Figure 3 ITGB1-DT expression changes in the clinicopathological features of STAD patients. (A) Clinical stage; (B) therapeutic effect; (C) OS; (D) PFI. STAD, stomach adenocarcinoma; OS, overall survival; PFI, progression-free interval; ns, not statistically significant; PD, progression disease; SD, stable disease; PR, partial remission; CR, complete remission; *, P<0.05.

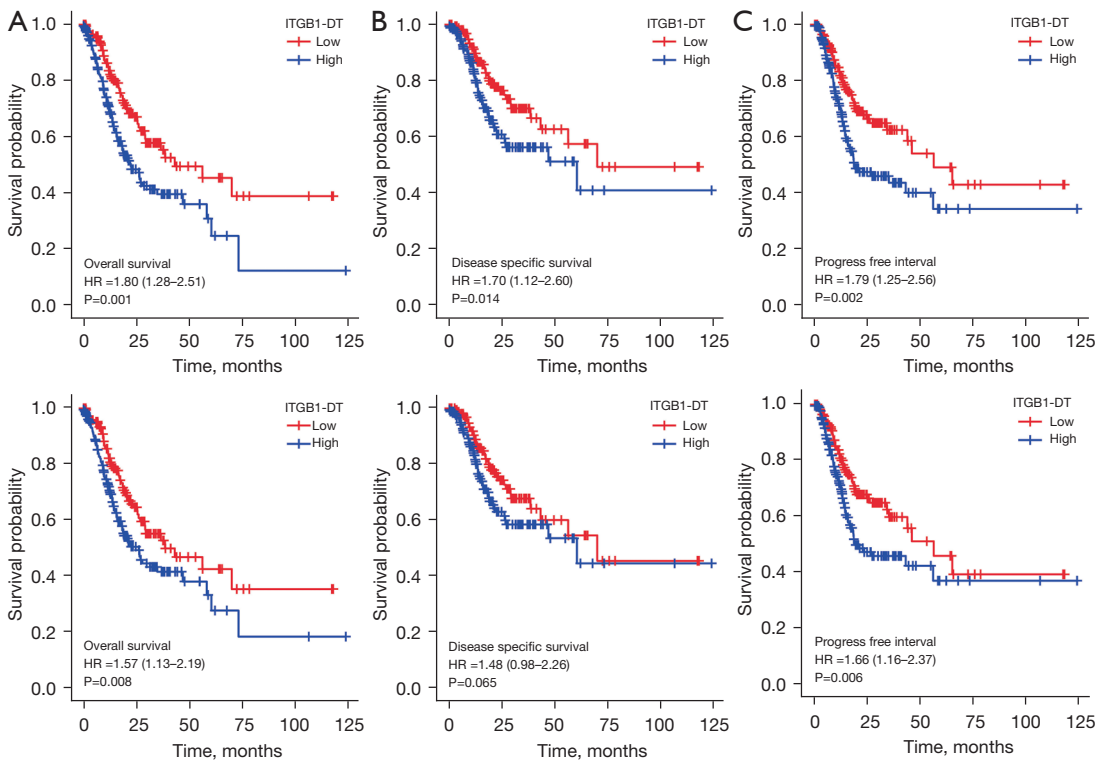

Figure 4 ITGB1-DT overexpression level is associated with poor prognosis in STAD patients. (A) Overall survival; (B) disease-specific survival; (C) progression-free interval. STAD, stomach adenocarcinoma; HR, hazard ratio.

Table 2 Cox regression analysis shows the independent risk factors for poor prognosis in STAD patients

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
T stage	362		0.06		
T1	18	Reference			
T2	78	6.725 (0.913–49.524)	0.061		
T3	167	9.548 (1.326–68.748)	0.025		
T4	99	9.634 (1.323–70.151)	0.025		
N stage	352		<0.001		
N0	107	Reference			
N1	97	1.629 (1.001–2.649)	0.049	1.073 (0.561–2.051)	0.832
N2	74	1.655 (0.979–2.797)	0.060	1.158 (0.542–2.473)	0.705
N3	74	2.709 (1.669–4.396)	<0.001	1.819 (0.842–3.932)	0.128
M stage	352		0.004		
M0	327	Reference			
M1	25	2.254 (1.295–3.924)	0.004	1.276 (0.544–2.993)	0.575
Pathologic stage	347		<0.001		
Stage I	50	Reference			
Stage II	110	1.551 (0.782–3.078)	0.209	1.657 (0.741–3.706)	0.219
Stage III	149	2.381 (1.256–4.515)	0.008	1.860 (0.717–4.824)	0.202
Stage IV	38	3.991 (1.944–8.192)	<0.001	3.375 (1.092–10.432)	0.035
Gender	370		0.188		
Female	133	Reference			
Male	237	1.267 (0.891–1.804)	0.188		
Race	309		0.153		
Asian	73	Reference			
White	236	1.448 (0.872–2.404)	0.153		
Age	367		0.005		
≤65 years	163	Reference			
>65 years	204	1.620 (1.154–2.276)	0.005	1.804 (1.249–2.605)	0.002
ITGB1-DT	370		0.012		
Low	185	Reference			
High	185	1.531 (1.100–2.132)	0.012	1.552 (1.087–2.216)	0.016

STAD, stomach adenocarcinoma; HR, hazard ratio; CI, confidence interval.

STAD patients. However, the association between ITGB1-DT and DSS in STAD patients was not significant based on the TCGA FPKM data (*Figure 4B*). Overexpression of ITGB1-DT was correlated with a shorter PFI for STAD patients and was statistically significant (*Figure 4C*). Univariate Cox regression analysis showed that clinical stage, T stage, lymph node metastasis, distant metastasis, age, and ITGB1-DT expression were the factors influencing the poor prognosis of STAD patients (*Table 2*). Multivariate Cox regression analysis showed that clinical stage, age, and ITGB1-DT overexpression were independent risk factors for the poor prognosis of STAD patients (*Table 2*). As the

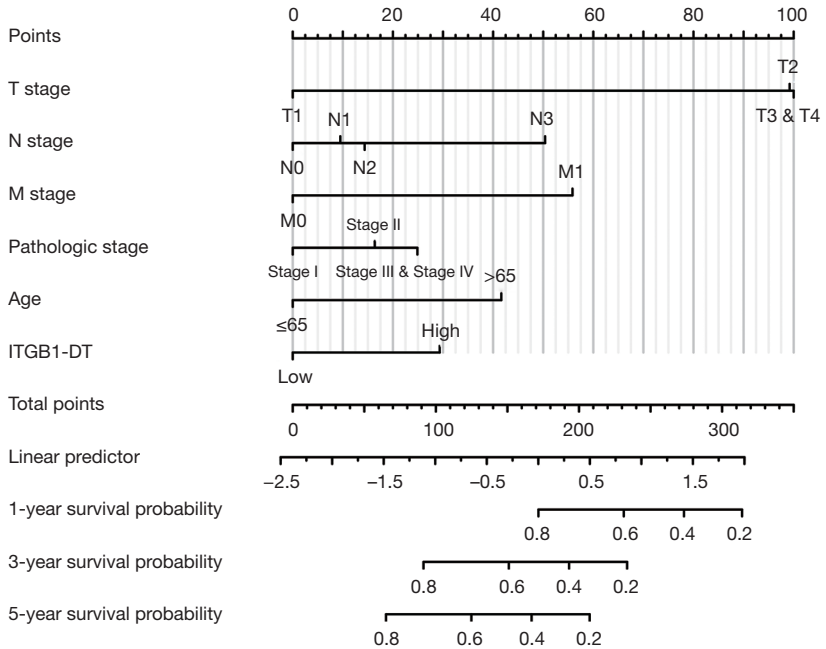

Figure 5 Construction of the ITGB1-DT related prognostic nomogram of STAD patients. STAD, stomach adenocarcinoma.

TNM stage is a commonly used the TNM stage system, we constructed a related nomogram of ITGB1-DT combined with the T, N, and M stages (*Figure 5*).

ITGB1-DT expression promoted the growth and migration of STAD cells

Using the GO analysis in GSEA software, we identified that the low expression of ITGB1-DT involved RNA interference, DNA modification, regulation of cell cycle arrest, positive regulation of cell cycle phase transition, regulatory t cell differentiation, cell cycle g1-s phase transition, positive regulation of mitotic cell cycle, cell cycle arrest, and other functions (*Figure 6* and Table S1). In AGS cells, interference with ITGB1-DT expression inhibited the growth of AGS cells and blocked the migration and invasion of AGS cells (*Figure 7*).

The signaling mechanisms of ITGB1-DT may be involved in STAD growth and migration

The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that interference in ITGB1-DT expression involved base excision repair, cell cycle, progesterone mediated oocyte maturation, insulin signaling pathway, p53 signaling pathway, MTOR signaling pathway, peroxisome, oocyte meiosis, and other mechanisms (Figure S1 and *Table 3*).

ITGB1-DT expression was correlated with immune infiltrated cells in STAD

The expression level of ITGB1-DT was correlated with T helper cells, TFH, Th17 cells, Th2 cells, B cells, Tem, TReg, CD8 T cells, T cells, Cytotoxic cells, and Tcm levels (*Figure 8* and Figure S2). In this case, we explored the relationship between ITGB1-DT expression and STAD immune cell marker levels (*Figure 9* and *Table 4*). The results showed that the expression level of ITGB1-DT was significantly correlated with the levels of CD8B, CD3D, CD3E, CD2, CD19, CD79A, IL17A, FOXP3, PD-1, CTLA4, LAG3, and GZMB.

Discussion

STAD is one of the most common digestive tract malignancies and has high morbidity and mortality. Several recent studies have shown that lncRNAs are involved in the progression of various cancers (12-14). For example, lncRNA GAS5 was shown to be downregulated in laryngeal carcinoma tissues. Low GAS5 expression was closely related

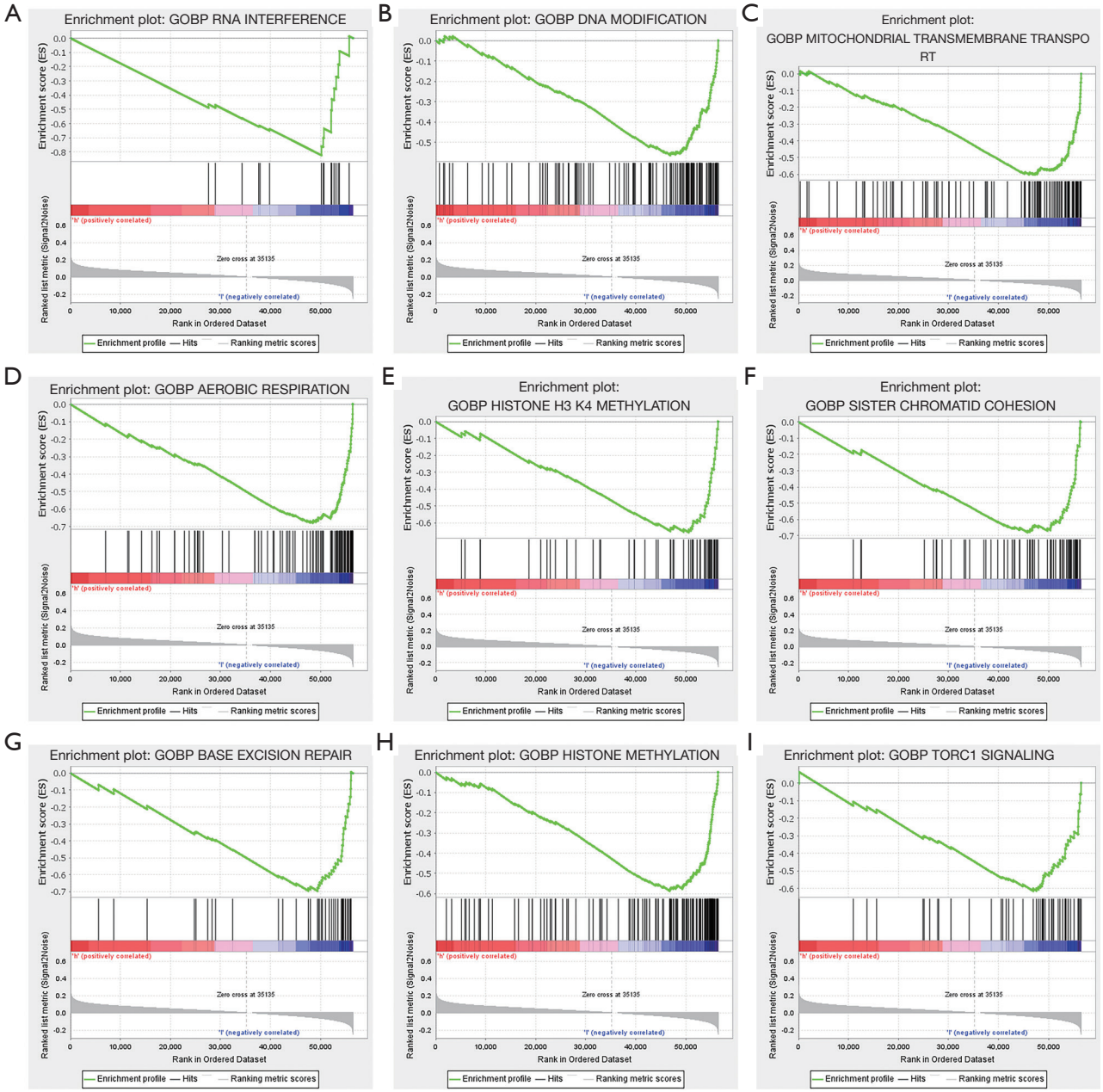

Figure 6 The biological functions of ITGB1-DT involved in STAD. STAD, stomach adenocarcinoma.

to low tumor differentiation, late TNM stage, lymph node metastasis, and short OS. GAS5 overexpression inhibited laryngeal cancer cell proliferation, G2/M phase arrest, migration, and invasion, accompanied by cell apoptosis rate. GAS5 overexpression regulated the PI3K/AKT/mTOR signaling pathway to participate in cancer progression (12). ASB16-AS1 is an under-studied novel lncRNA highly expressed in colorectal cancer (CRC) cells. ASB16-AS1 silencing has been shown to inhibit the proliferation, migration, and invasion of CRC cells and accelerate cell apoptosis. ASB16-AS1 drives CRC progression by modulating the miR-185-5p/TEAD1 signaling axis (13). LncRNAs have also been shown to participate in gastric cancer progression (15-17). The expression of lncRNA CYTOR was significantly increased in metastatic gastric cancer. The expression level of CYTOR was positively

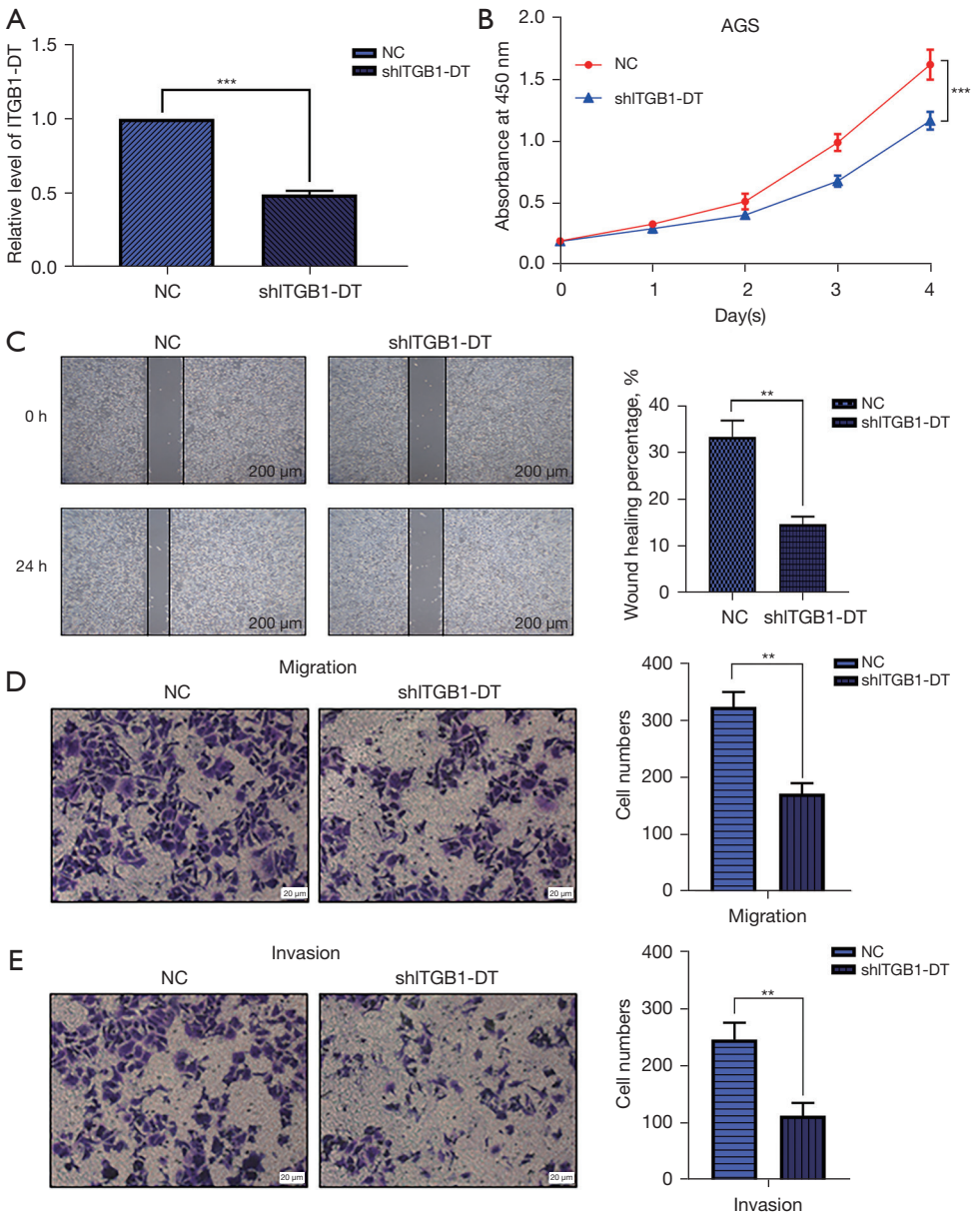

Figure 7 Interfering with ITGB1-DT expression inhibits STAD growth and migration. STAD, stomach adenocarcinoma. (A) The cell model of interfering with ITGB1-DT expression; (B) cell proliferation using CCK-8; (C,D) cell migration using the scratch test and transwell test (the wound distance was photographed at 0 and 24 h after scratching to observe the cell migration under the ×10 microscope); (E) cell invasion using the transwell test (0.5% crystal violet staining). STAD, stomach adenocarcinoma; **, P<0.01; ***, P<0.001.

correlated with the aggressiveness, lymph node metastasis, and late clinical stage of gastric cancer. Downregulated CYTOR expression inhibited cell proliferation and migration, induced apoptosis, and inhibited tumor growth in BGC823 mice (15). TGB1-DT is a newly discovered lncRNA, opposite to the ITGB1 gene transcription direction. ITGB1-DT was overexpressed in lung adenocarcinoma tissues and was related to the prognosis of lung adenocarcinoma patients. ITGB1-DT as an oncogenic RNA promoted the growth and migration of cancer cells (8). Currently, the relationship between TGB1-DT and STAD progression has not been reported. In this study, ITGB1-

Table 3 GSEA analysis indicates the signaling mechanisms of ITGB1-DT may be involved in STAD

Name	Size	NES	NOM P
Valine leucine and isoleucine degradation	44	−2.2412837	0
Butanoate metabolism	34	−2.2301126	0
Lysine degradation	44	−2.081703	0.003795066
Fatty acid metabolism	42	−2.0419292	0
Beta alanine metabolism	22	−2.0368598	0
Glyoxylate and dicarboxylate metabolism	16	−1.9944289	0.001941748
Propanoate metabolism	33	−1.9319856	0.004040404
Citrate cycle TCA cycle	31	−1.9138249	0.005802708
Base excision repair	35	−1.9109693	0.005736138
Pyruvate metabolism	40	−1.8659407	0.005836576
Glycolysis gluconeogenesis	62	−1.8403244	0.007633588
Parkinsons disease	128	−1.8293734	0.022044089
Glycine serine and threonine metabolism	31	−1.8067198	0.003883495
One carbon pool by folate	17	−1.7854892	0.021153847
Cell cycle	124	−1.7764975	0.031746034
Cysteine and methionine metabolism	34	−1.7510746	0.013592233
Fructose and mannose metabolism	33	−1.7368857	0.017175572
Selenoamino acid metabolism	26	−1.7363652	0.009689922
Progesterone mediated oocyte maturation	85	−1.729401	0.027559055
Huntington's disease	180	−1.7265979	0.03992016
Tryptophan metabolism	40	−1.7209246	0.015296367
Alanine aspartate and glutamate metabolism	32	−1.7001189	0.023166023
Insulin signaling pathway	137	−1.6936874	0.00990099
P53 signaling pathway	68	−1.6707178	0.024253732
MTOR signaling pathway	52	−1.6706328	0.0295858
Type ii diabetes mellitus	47	−1.6642514	0.016129032
Peroxisome	78	−1.6470382	0.047904193
Purine metabolism	158	−1.6314803	0.022177419
Arginine and proline metabolism	54	−1.594828	0.037401576
Oocyte meiosis	112	−1.5657029	0.042801555

STAD, stomach adenocarcinoma; NES, Normalized Enrichment Score; NOM, Nominal; GSEA, Gene Set Enrichment Analysis; TCA, tricarboxylic acid; MTOR, mechanistic target of rapamycin kinase.

DT was found to be overexpressed in STAD and shows diagnostic value. ITGB1-DT was associated with T stage, treatment effect, DSS, and PFI and was an independent risk factor for poor prognosis, suggesting that ITGB1-DT could be a prognostic biomarker for STAD.

ITGB1-DT plays an important role in lung adenocarcinoma, and it has been reported that interference with ITGB1-DT induces the growth and migration of

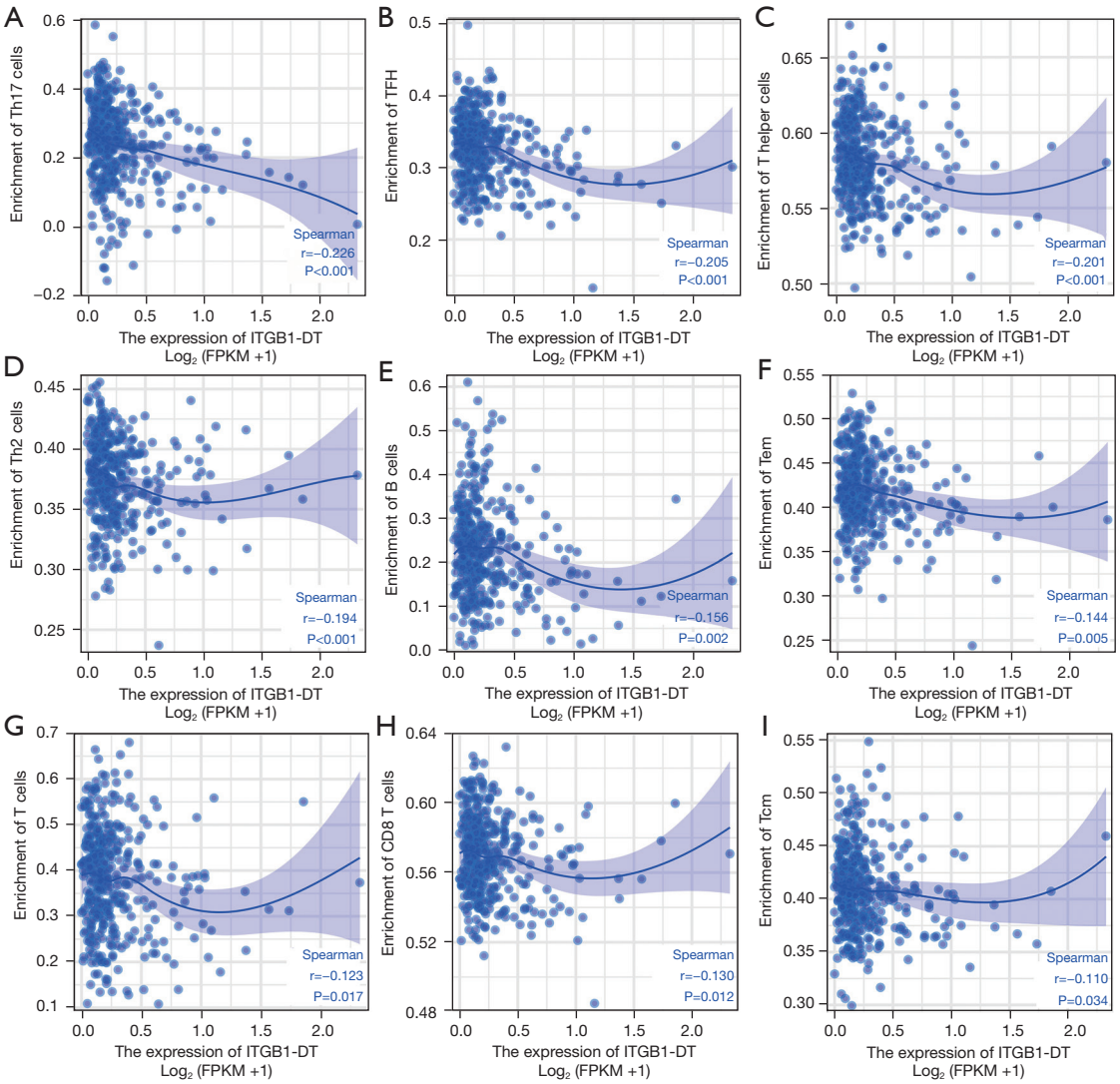

Figure 8 ITGB1-DT expression is correlated with immune infiltrated cells.

lung adenocarcinoma cells (8,9). Using the GO analysis, we found that interference with ITGB1-DT expression was related to cell growth. A KEGG analysis showed that interference of ITGB1-DT expression involved base excision repair, cell cycle, progesterone mediated oocyte maturation, insulin signaling pathway, p53 signaling pathway, MTOR signaling pathway, peroxisome, oocyte meiosis, and other mechanisms. In the STAD cell model constructed by us, interfering with the expression of ITGB1-DT inhibited the growth of AGS cells and blocked the migration and invasion of AGS cells. However, the signaling mechanisms by which ITGB1-DT is involved in STAD progression need to be further understood by western blotting in future studies.

Many studies have confirmed that immune cells and immune factors in the immune microenvironment play an important role in cancer progression, and an abnormal immune microenvironment is inseparable from cancer progression (18-24). One recent study reported that NDRG2 overexpression inhibited PD-L1 expression in human breast cancer cells through the NF-κB signaling pathway (24). NDRG2 overexpression in mouse breast cancer cells promoted the downregulation of PD-L1 expression and then blocked the inhibitory activity of cancer cells on T cell proliferation. Knockdown of NDRG2 expression enhanced pD-L1 expression, leading to tumor cells inhibiting T cell proliferation (24). Targeting members of the Lysyl oxidase (LOX) family could be used

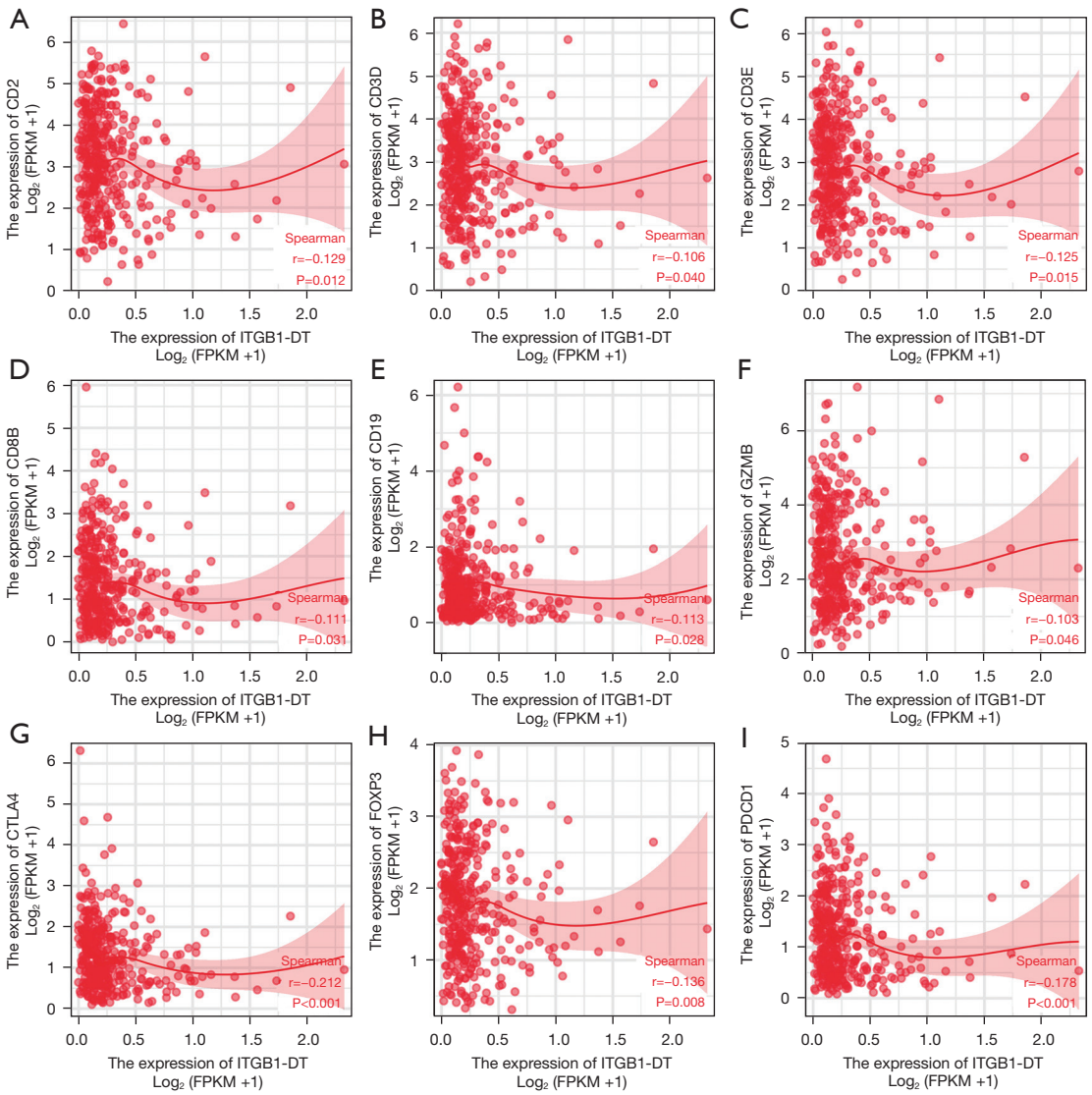

Figure 9 ITGB1-DT expression is correlated with immune infiltrated cell markers.

as an anticancer strategy. LOX is overexpressed in gastric cancer. LOX overexpression is associated with a shorter OS, progression-free survival (PFS), and post-progression survival (PPS) in STAD patients. LOX overexpression leads to a poor prognosis in STAD patients, perhaps by promoting M2 macrophage polarization and tumor immune escape, as well as enhancing tumor cell resistance to chemotherapy drugs (18). In our study, ITGB1-DT overexpression was significantly correlated with the levels of T helper cells, TFH, Th17 cells, Th2 cells, B cells, Tem, TReg, CD8 T cells, T cells, Cytotoxic cells, and Tcm. ITGB1-DT overexpression was also significantly correlated with levels of the STAD immunocyte markers CD8B, CD3D, CD3E, CD2, CD19, CD79A, IL17A, FOXP3, PD-1, CTLA4, LAG3, and GZMB, suggesting that ITGB1-DT might be involved in the progression of STAD via the immune microenvironment. However, further basic research is needed to confirm this.

In this study, basic research and bioinformatics were used to explore the roles and potential signaling mechanisms of ITGB1-DT in the progression of STAD. ITGB1-DT was found to be overexpressed in STAD. ITGB1-DT overexpression was associated with the T stage, therapeutic effect, OS, PFI status, and poor prognosis in STAD patients. ITGB1-DT overexpression was valuable in the diagnosis of STAD and was a negative factor affecting the

Table 4 ITGB1-DT expression is correlated with immune infiltrated cell markers in STAD

Description	Gene markers	Cor	P
$CD8^+$ T cell	*CD8A*	−0.091	0.079
	CD8B	−0.111	0.031
T cell	*CD3D*	−0.106	0.040
	CD3E	−0.125	0.015
	CD2	−0.129	0.012
B cell	*CD19*	−0.113	0.028
	CD79A	−0.102	0.048
Th2	*GATA3*	0.076	0.142
	STAT6	−0.021	0.690
	STAT5A	−0.099	0.057
	IL13	−0.066	0.205
Tfh	*BCL6*	0.027	0.600
	IL21	−0.091	0.077
Th17	*STAT3*	−0.046	0.378
	IL17A	−0.122	0.018
Treg	*FOXP3*	−0.136	0.008
	CCR8	−0.064	0.215
	STAT5B	−0.082	0.113
	TGFB1	0.059	0.251
T cell exhaustion	*PD-1 (PDCD1)*	−0.178	<0.001
	CTLA4	−0.212	<0.001
	LAG3	−0.119	0.021
	HAVCR2	−0.092	0.076
	GZMB	−0.103	0.046

STAD, stomach adenocarcinoma.

prognosis of STAD patients. Interference with ITGB1-DT expression inhibited STAD cell proliferation, invasion, and migration. Interference with ITGB1-DT expression may delay the progression of STAD through the insulin, p53, MTOR, and other signaling mechanisms to improve the prognosis of STAD patients. In our future studies, the STAD tissues from our hospital should be collected to confirm the expression level of ITGB1-DT, and the roles of interference ITGB1-DT expression in insulin, p53, MTOR signaling mechanisms, and the tumor microenvironment by using western blotting, cell transfection, co-culture, and other basic experiments.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-233/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-233/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-233/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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