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Integrin β5, a noninvasive diagnostic biomarker, is associated with unfavorable prognosis and immunotherapy efficacy in gastric cancer

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Abstract

Background Integrin β5 (ITGB5) is a pivotal player in the pathogenesis of gastric cancer (GC). We aimed to explore the potential value of ITGB5 as a predictor of diagnosis and immunotherapy in gastric cancer.

Methods The expression of ITGB5 in GC was assessed using The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases, and verified through quantitative polymerase chain reaction (qPCR) and immunohistochemistry. Kaplan-Meier curves were conducted to evaluate the prognostic significance. The immune cells infiltration, tumor mutational burden (TMB), and immunophenoscore (IPS) were examined using CIBERSORT, TIMER, and TISIDB. In addition, colony formation, scratch assays, and transwell assays were employed to determine the impact on tumor progression and metastasis. CD276 expression was detected by western blotting following the knockdown of ITGB5. ELISA was utilized to measure serum ITGB5 levels.

Results The expression of ITGB5 in GC tissue surpassed that in normal tissue, it might contribute to GC pathogenesis through pathways including PI3K-AKT, ECM-receptor interaction, and TGF-beta. The elevated ITGB5 expression is associated with poor prognosis in GC patients. In addition, a strong positive association between ITGB5 overexpression and the infiltration levels of macrophages and monocytes, and it significantly influenced immune response. Moreover, lower expression of ITGB5 was associated with better immunotherapy efficacy. Subsequent investigation demonstrated that silencing of ITGB5 suppressed the proliferation and migration of GC cell lines in vitro. ITGB5 expression was positively correlated with CD276 expression and the knockdown of ITGB5 resulted a notable decrease CD276 expression. Futhermore, a significantly high level of serum ITGB5 was observed in GC patients. The combined assessment of ITGB5, CEA, and CA19-9 improved the diagnostic accuracy.

Conclusions ITGB5 potentially serve as both a diagnostic biomarker and therapeutic target in managing GC.

Keywords Gastric cancer, ITGB5, Biomarker, Tumor immune, Bioinformatics analysis, Experiment verification

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Background

Global statistics indicate that gastric cancer (GC) ranks the fifth most prevalent malignancy and is a significant contributor to cancer-related mortality worldwide [1, 2]. Remarkable progress has been achieved in the diagnosis and treatment of GC in recent decades, with over

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30% of post-radical gastrectomy patients surviving for more than five-years [3]. However, due to unsatisfactory early diagnosis and treatment, recurrence and metastasis are still a threat to health of patients [4]. It is often the case that the bulk of GC diagnosis occur in the late stages of disease during their initial visit, inevitably leading to a 5-year survival rate of under 10% [5]. The poor survival outcomes associated with GC can be largely attributed to a lack of effective biomarkers for diagnosis and targeted therapeutic interventions. Hence, there is an urgent need to identify potential therapeutic targets and new biomarkers to enhance gastric cancer detection and prognosis.

Integrins, a superfamily of cell adhesion receptors widely expressed on the cell membrane, are heterodimers consisting of non-covalently α and β subunits [6]. Apart from binding to extracellular matrix proteins like collagen and laminin, integrins also play a significant role in tumor development and progression [6–8], and has been demonstrated in previous studies to enhance tumor cell adhesion to the extracellular matrix, regulate the endothelial cell barrier, and promote migration, invasion, vascular formation and epithelial-mesenchymal transition (EMT) by inducing TFG-β [6, 7, 9]. ITGB5 has been linked to a range of cancers, including breast cancer [10], hepatocellular cancer [11] and glioblastoma cancer [12]. Additionally, its involvement in the progression of GC has been substantiated. Hirano et al. identified the integrin αvβ5-FARP1-CDC42 pathways markedly stimulates the migratory and invasive abilities of GC cells [13]. Moreover, Li et al. have detailed how HER2 can modulate ITGB5 via the PI3K-AKT signaling pathway [14]. Concurrently, patients with positive ITGB5 protein expression in stromal cells exhibited shorter survival times than patients with negative ITGB5 expression [15]. These findings indicate that ITGB5 has various functions in multiple tumors. Nevertheless, the potential biological functions, clinical significance, and immunotherapy values of ITGB5 in GC remain unexplored.

Thus far, carcinoembryonic antigens (CEA) and carbohydrate antigen 19 − 9 (CA19-9) are commonly used markers for GC detection in clinical practice. However, previous studies showed that these biomarkers have a sensitivity of about 25.5% and 38.7%, respectively [4]. It is worth noting that some integrins have been detected in peripheral blood and have proven their effectiveness as diagnostic and prognostic tools in cancers. Notably, Li et al. reported that serum ITGB6 has emerged as a promising novel indicator for both the diagnosis and prognosis of GC [16]. Therefore, serum integrins may emerge as promising biomarkers to provide diagnostic information of GC. An earlier study found significant upregulation of exosomal ITGB5, and correlation with liver metastasis in GC [14]. However, the serum ITGB5 expression level and its diagnostic value in GC have not been investigated yet. Therefore, we hypothesized that ITGB5 levels are associated with prognosis and early diagnosis in GC.

To test this hypothesis, our study investigated the impact of ITGB5 on tumorigenesis and its clinical significance of GC. We also examined the correlation between ITGB5 and immune infiltration in GC. Additionally, ELISA was employed to confirm the diagnostic value of serum ITGB5 in GC patients for the first time. These results underscore the significant role played by ITGB5 in the emergence and progression of GC, unveiling novel targets for prognosis and immunotherapy. A flow chart detailing the analysis is shown in graphical abstract.

Methods

Data collection

We gathered data on ITGB5 expression and corresponding clinicopathological features from 375 cases of gastric cancer and 32 healthy controls through The Cancer Genome Atlas (https://portal.gdc.cancer.gov/) database. Additionally, we examined ITGB5 expression levels in both GC and normal tissues by sourcing information from three datasets (GSE66229, GSE51575, GSE65801) within The Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/).

Immunohistochemistry

Immunohistochemistry (IHC) staining was employed to evaluate the level of ITGB5 protein expression. Twenty-one pairs of frozen GC tissues and precancerous tissues from the Harbin Medical University Cancer Hospital. In summary, tumor tissues and precancerous tissues underwent fixation in 10% formalin, paraffin embedding, and slicing into 4 ~ 6 μm thick sections. After deparaffination, rehydration, and microwave antigen retrieval, the slides were incubated with ITGB5 antibody (Invitrogen, USA, PA5-118499) at a dilution of 1:200, overnight at 4 °C. Subsequently, secondary antibody incubation for 30 min at room temperature and DAB substrate staining were conducted. Finally, haematoxylin counterstaining was performed. The H-score, evaluating staining intensity (3, strong; 2, moderate; 1, weak; 0, negative) was employed ti determine the ITGB5 protein expression level. A final score ≥ 90 indicated positive ITGB5 expression. All participants provided informed consent, as approved by the Human Research Ethics Committee of the Harbin Medical University in this study.

Functional enrichment and Gene set Enrichment analyses

To identification of differentially expressed genes (DEGs) between high and low TCGA subgroups based on ITGB5 expression was carried out using the“limma” R package

[17], with the filter |log2FC|>1 and P-value < 0.05. To determine the biological processes and pathways most significantly linked with ITGB5, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses [18]. Furthermore, we utilized Gene Set Enrichment Analysis (GSEA) [19] to access statistical significance or concordant differences between the two different biological states.

Survival analysis

We evaluated the impact of ITGB5 expression on the survival rates of gastric cancer patients via the Kaplan-Meier plotter website (http://kmplot.com/analysis/index.php?p=service). additionally, the effect of ITGB5 on survival was confirmed using clinical data from the GEO database (GSE15149).

Tumor immune infiltration analysis

To evaluate the presence of tumor-infiltrating immune cells (TIICs), we employed CIBERSORT [20] to compute the relative infiltration ratios of 22 distinct types of TIICs within the TCGA samples. Upon dividing the samples into two groups based on ITGB5 median expression values, we conducted a comparative analysis of the TIICs between the groups. Additionally, we employed the TIMER (https://cistrome.shinyapps.io/timer/) and GEPIA (http://gepia2.cancer-pku.cn/#analysis) databases to establish gene expression correlations between ITGB5 and immune cell biomarkers in GC.

Relationship between ITGB5 and immune checkpoints, TMB, IPS

Our study investigated the relationship between ITGB5 expression and immune checkpoint levels. The TISIDB (http://cis.hku.hk/TISIDB) database [21] provided us with additional information concerning the correlation of ITGB5 expression with lymphocytes, major histocompatibility complexes (MHC), immunoinhibitory genes and immunostimulatory genes. Then, the correlation of ITGB5 expression with tumor mutational burden (TMB) or immunophenoscore (IPS) was conducted by Spearman's analysis.

Cell lines

We acquired GC cell lines MKN45, AGS, MKN28, HGC27, and normal gastric epithelial cells (GES) from Procell (Wuhan, China). GES, MKN45, MKN28 and HGC27 were cultured in RPMI-1640 medium (Gibco, Shanghai, China), supplemented with 10% fetal bovine serum (Excell, Jiangsu, China) and 1% penicillin-streptomycin. AGS cells were cultured in F12K (Procell, Wuhan, China) under identical culture conditions. All cells were maintained at 37℃ in a humidified incubator with 5% CO_2. Subsequent experiments involving western blotting, cell transfection, and other assays were conducted following subculturing of the cells.

Cell transfection

To target ITGB5 (Table S1), we used a negative control (NC) oligonucleotide and short interfering RNA (siRNA). According to the manufacturer's insturctions, a 3×10^5 MKN45 or HGC27 cells were seeded in 6-well plates. Liposome 2000 delivered siRNA or negative controls to the cell plates at a final concentration of 50nM.

Quantitative real-time polymerase reaction (qRT-PCR) analysis

Total RNA extraction from cells was performed using the Total RNA Kit I (Omega Biotek). The NovoStart SYBR qPCR SuperMix Plus kit (Novoprotein) was used for qRT-PCR to measure the relative expression levels of ITGB5. The primers utilized were as follows: ITGB5 (forward: 5'-GTCTGCTAATCCACCCAAAATG-3'; reverse:5'-TCTCTATCTCACCTCCACAGC-3'); GAPDH (forward: 5'-GACATGCCGCCTGGAGAAAC-3'; reverse: 5' AGCCCAGGATGCCCTTTAGT-3'). Thermal cycling conditions included initial denaturation at 95 °C for 35 s, followed by denaturation at 95 °C for 5 s, annealing and extension at 60 °C for 30 s, for 40 cycles. The calculation of relative ITGB5 mRNA expression was normalized to the mRNA expression level using the $2^{-\Delta\Delta Ct}$ method.

Colony formation assay

1×10^3 MKN45 or HGC27 cells were seeded per well in a 6-well plate for the colony formation assay, and incubated at 37℃ in 5% CO_2 for two weeks. The cells were then fixed with 4% paraformaldehyde for an additional 30 min and stained with 0.1% crystal violet for 30 min. Subsequently, the colonies were counted and analyzed.

Scratch assay

GC cells were grown in 24-well plates. When the cells reached full confluence, the cell monolayer was disrupted by gently scratching it with the tip of a 200 μl pipette. Subsequently, the culture containing 10% fetal bovine serum was substituted with a serum-free medium. Microscopic images (Olympus CKX53, Japan) of the cells were captured at both 0 and 24 h, respectively.

Transwell assay

GC cells were added to transwell chambers using a medium without serum. These chambers were then inserted into a 24-well plate filled with a medium consisting of 20% FBS. Following incubation at 37℃ with 5% CO_2 for a duration of 24 h, the transwell chamber

was removed carefully. The medium in the smaller chamber was discarded, and the cells were washed with PBS. Next, the cells were fixed with 4% paraformaldehyde for 30 min, followed by staining with 0.1% crystal violet solution for another 30 min. Finally, five random areas of the lower membrane were observed under a microscope (Olympus CKX53, Japan), and the cells were counted in order to obtain accurate measurements.

Western blotting

The expression of CD276 protein in MKN45 cell line was detected using western blotting. Total intracellular proteins were extracted through the use of a radioimmunoprecipitation analysis lysis buffer. A bicinchoninic acid protein assay kit was employed to quantify protein concentrations. Subsequently, the proteins were separated by loading onto an SDS-PAGE gel, which was then transferred to a PVDF membrane. The next step involved incubating the PVDF membrane with anti-CD276 (1:1000, Proteintech, 14453-1-AP, China) and anti-β-actin (1:1000, TA-09, ZSGB-BIO, China) overnight. After rinsing, the membrane was treated with secondary antibodies (1:5000) for one hour, and ultimately identified using the enhanced chemiluminescence.

Serum ITGB5 levels were detected by ELISA

A total of 100 serum samples from GC patients admitted to the Harbin Medical University Cancer Hospital were selected, along with 50 serum samples from healthy individuals serving as a control group. The clinical tumor stage was determined using the American Joint Committee on Cancer (AJCC) 8th edition criteria. All participants of the study approved their informed consent to the Human Research Ethics Committee of the Harbin Medical University Cancer Hospital. The enzyme-linked immunosorbent assay (ELISA, commercial kits from Meimian, China) was used to detect serum ITGB5 levels according to the manufacturer's instructions. Serum CEA and CA199 levels were assessed by electrical chemiluminescent immunoassay (ECLIA, Roche Diagnostic GmbH, Germany). The normal reference ranges for CEA and CA19-9 in our institution were 0–5 ng/mL and 0–37 U/mL, respectively, as per manufacturer guidelines.

Statistical analysis

All statistical analyses and visualizations were conducted using the R programming language (version 4.1.2) and IBM SPSS Statistics version 25 (IBM SPSS Inc., Chicago, USA). The expression levels of ITGB5 were compared between groups using the *Wilcoxon rank-sum* test, with serum data presented as median with interquartile range. The *Mann-Whitney U* test was applied to compare ITGB5 expression between groups. The diagnostic accuracy of serum ITGB5 was evaluated using ROC curves, with statistically significant set at $P < 0.05$.

Results

ITGB5 is significantly elevated in gastric cancer

ITGB5 mRNA expression levels were systematically analyzed across multiple cancer types, the result indicated that the ITGB5 transcription levels were notably higher in 10 types of tumors when compared to normal tissues (Fig. 1A, $P < 0.05$). The GC tissues had higher ITGB5 mRNA expression than normal stomach tissues was noted (Fig. 1B, $P < 0.05$). We then detected ITGB5 expression using GSE66229, GSE65801, and GSE51575 datasets and observed that normal tissues had lower ITGB5 expression than GC tissues (Fig. 1 C-E, $P < 0.001$). At the protein level, we used IHC staining on clinical specimens to compare ITGB5 expression between normal and GC tissues. In comparing GC tissues with normal tissues, we found a significant increase in ITGB5 levels (Fig. 1F), as evidenced by the IHC staining. These findings indicate the tightly regulated expression of ITGB5 in GC.

ITGB5 promotes gastric cancer tumorigenesis

A heatmap was generated to visualize the potential functions and identify critical genes associated with ITGB5. The results indicated that the ITGB5 interactive genes were predominantly elevated in GC among groups with low and high expression levels (Figure. S1A). Furthermore, the GO analysis demonstrated that the pathways linked to DEGs included extracellular matrix organization, extracellular structure organization, external encapsulating structure organization, collagen-containing extracellular matrix and extracellular matrix structural constituent (Figure. S1B). Based on KEGG analysis, the top five pathways identified were the PI3K-AKT signaling pathway, ECM-receptor interaction, proteoglycans in cancer, TGF-beta signaling pathway, and focal adhesion (Figure. S1C). GESA analysis indicated that the high-expression phenotype of ITGB5 exhibited differential associations with cell adhesion molecules, ECM receptor interaction, focal adhesion, and lysosomes found in the KEGG (Figure. S1D). These findings suggest involvement of ITGB5 in various biological processes, including cell adhesion and signalling pathways, with implications for tumour occurrence and development.

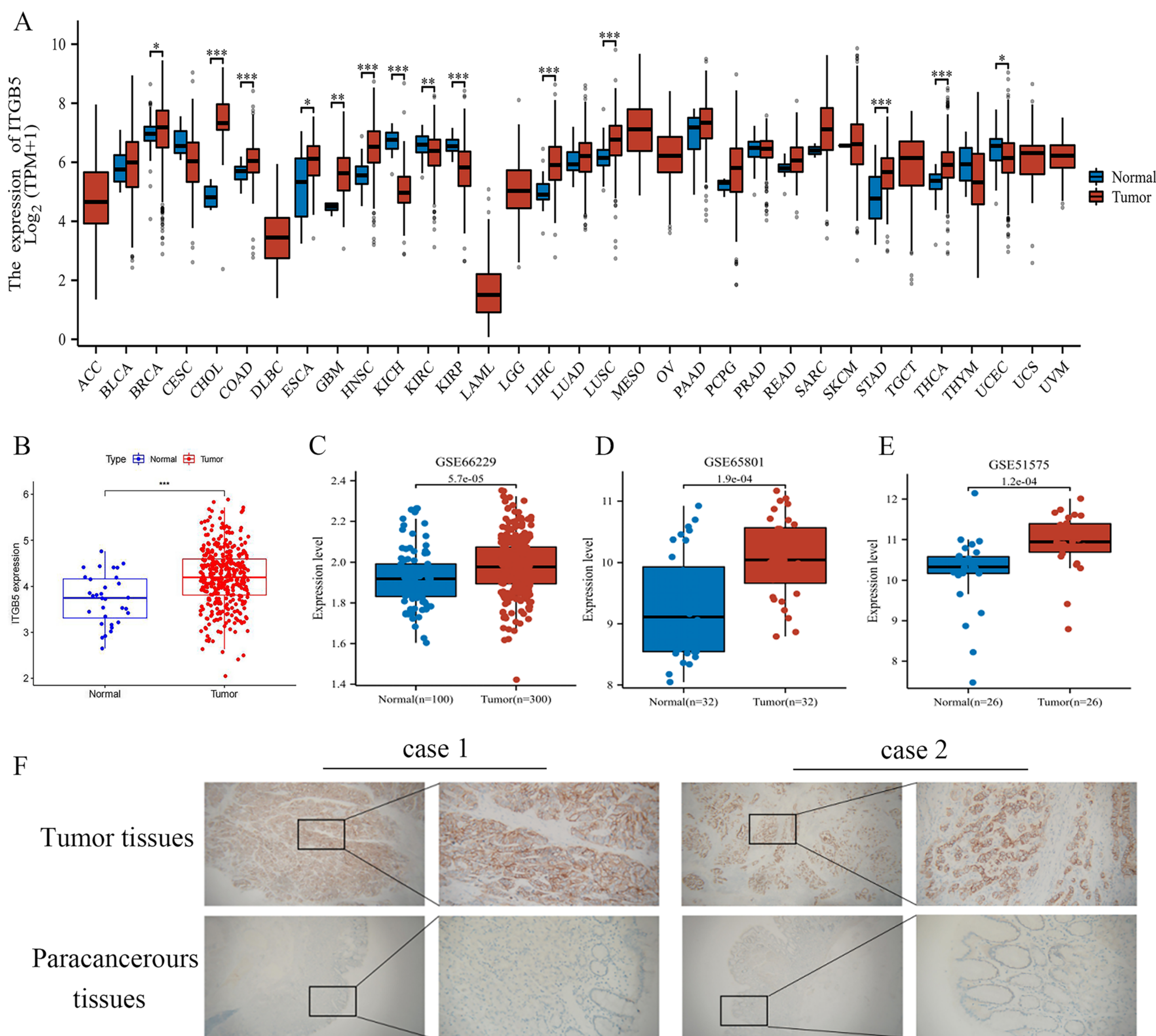

Fig. 1 ITGB5 expression level is upregulated in GC. (**A**) Expression levels of ITGB5 were determined in different tumor types from TCGA database. (**B**) A boxplot of ITGB5 expression in GC compared with normal tissues from the TCGA database. (**C-E**) The ITGB5 expression level in tumor tissues campared with normal tissues. from GSE66229, GSE65801 and GSE15715 datasets. (**F**) Representative images of ITGB5 expression in gastric cancer tissues and their matched paracancerous tissues. Original magnifications 40× and 200×

High ITGB5 expression positively correlates with clinical stage, T stage and poor prognosis in gastric cancer patients

Subsequent investigations were conducted to investigate the correlation between ITGB5 expression levels and the clinicopathological characteristics of GC patients. Our findings revealed a positive relationship between high ITGB5 expression and both clinical stage ($P<0.05$, Fig. 2 C) and T-stage ($P<0.05$, Fig. 2D). However, no significant differences in ITGB5 expression were observed with respect to age, gender, lymph node metastasis, and metastasis (Fig. 2A-B, E-F). Meanwhile, based on Kaplan-Meier curves analyses, ITGB5 overexpression often correlated with a poor prognosis to GC patients, demonstrating a strong connection to inferior overall survival (OS) and post-progression survival (PPS) rates (Fig. 2G-H). Clinical data from the GEO database (GSE15149) further supported the correlation between increased ITGB5 expression and poorer OS rates (Fig. 2I). These findings suggest that ITGB5 expression could serve as an early-stage diagnostic indicator for GC. High levels of ITGB5 expression are associated with poor outcomes in gastric cancer patients and plays a significant role in GC progression.

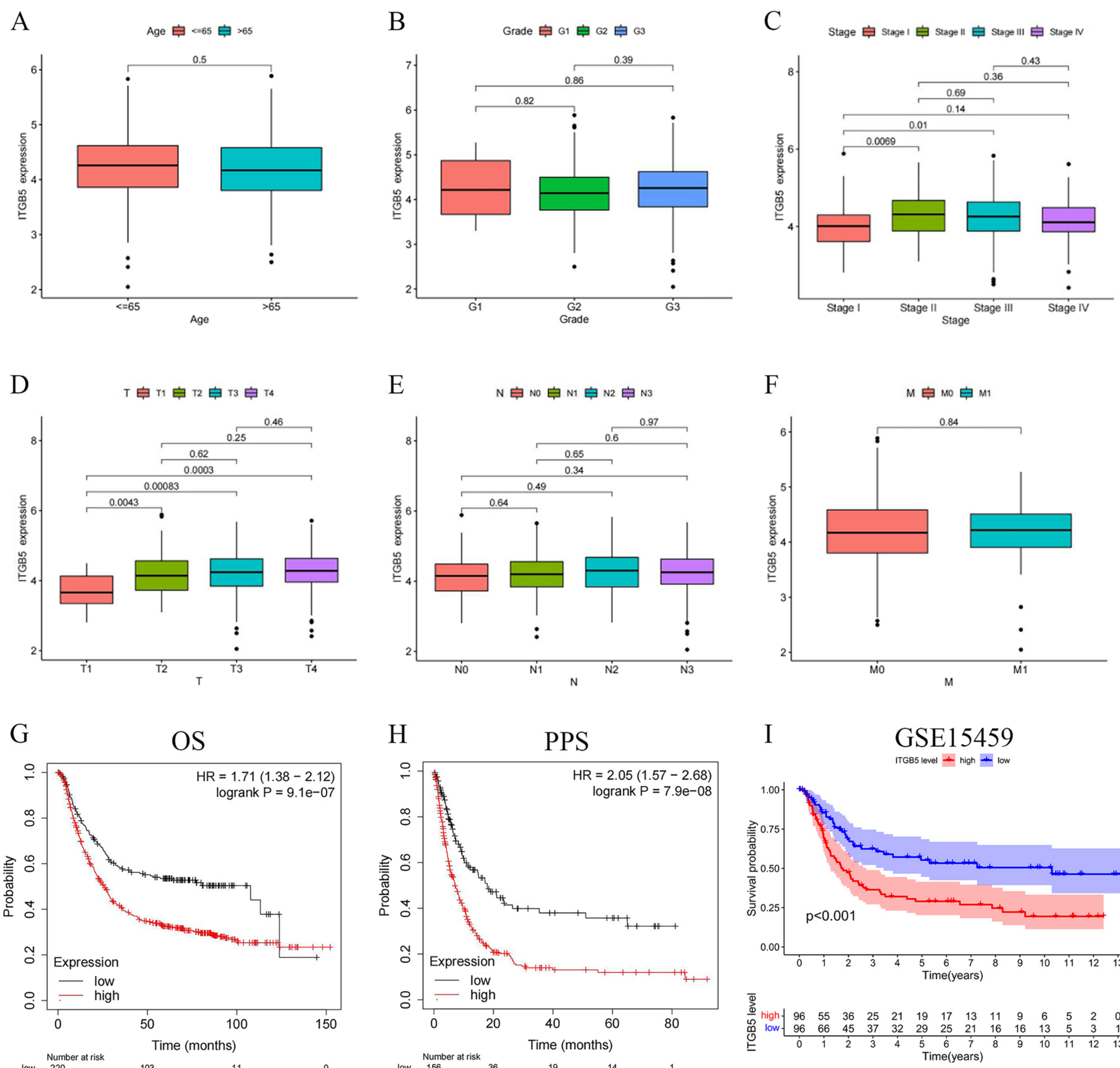

Fig. 2 Correlations between ITGB5 expression and clinical characteristics and prognosis for GC patients. (**A**) Age (≤ 65, > 65). (**B**) Gender (female, male). (**C**) Clinical stage (I, II, III, IV). (**D**) T-stage (T1, T2, T3, T4). (**E**) Lymph node metastasis (N0, N1, N2, N3). (**F**) Metastasis (M0, M1). (**G-H**) Analysis of OS and PPS of ITGB5 in GC. (**I**) Overall survival curves of GC patients from GSE15149

ITGB5 plays a role in the infiltration of M2 macrophages for gastric tumor microenvironment

We used CIBERSORT to examine the distribution of immune cell types in GC samples. The results indicated a positive correlation between CD4 + activated memory T-cells and CD8 + T cells. Conversely, a negative correlation was observed between M0 macrophages and CD8 + T cells (Fig. 3A). Figure 3B summarizes the relative proportion of 22 immune cells using a correlation heatmap. Comparatively, the high ITGB5 expression group exhibited a significant increase in both stromal score and estimate score in contrast to the low ITGB5 expression group (Fig. 3 C). Additionally, we investigated the immune cell fractions in the high and low expression groups of ITGB5. The results indicated that M2 macrophages showed statistically higher infiltration. In comparison, B-cells and plasma cells demonstrated lower infiltration in the ITGB5 high expression group compared to the low expression group (Fig. 3D). Notably, ITGB5 overexpression was positively associated with infiltration

of M2 macrophages and monocytes ($P < 0.05$), but negatively correlated with infiltration of memory B-cells, plasma cells, follicular helper T cells and activated dendritic cells ($P < 0.05$) (Fig. 3E-G; Figure S2. These findings suggest that ITGB5 is important for immune infiltration, especially of macrophages in GC.

To shed light on the potential relationship between ITGB5 expression and various immune infiltrating cells, an investigation into the correlation between ITGB5 expression and immune cell markers was conducted using the TIMER database (Table 1). M1 macrophage marker IRF5, M2 macrophage marker VSIG4, and monocyte marker CSF1R positively correlated significantly with ITGB5 expression. Meanwhile, a further investigation of the association between ITGB5 expression and immune infiltrating cell markers was performed using the GEPIA database. This analysis revealed a positive correlation between ITGB5 expression and IRF5 of M1 macrophages (Cor = 0.37, $P = 1.8e\text{-}15$), as well as COX2 (Cor = 0.19, $P = 5.6e\text{-}05$) and CSF1R of monocytes (Cor = 0.3, $P = 2.20e\text{-}10$). Altogether, several immune-related genes strongly correlate with ITGB5, demonstrating its importance in the immune microenvironment of GC (Figure. S3; Table 1).

GC patients with low ITGB5 expression experienced a better immunotherapy outcome. Our results revealed that the expression of ITGB5 shows the greatest positive correlation with immune checkpoint-related mRNA, including CD276, TNFSF4 and NRP1 (Fig. 4A). Additionally, we employed the TISIDB database to predict the relationship between ITGB5 expression and and markers of lymphocytes and immunomodulators (Fig. 4 C-F). The results reveal that ITGB5 expression had greatest correlation with CD276 ($r = 0.377$, $P = 1.75e\text{-}17$) and TNFSF4 ($r = 0.273$, $P = 0.17e\text{-}08$). In addition, a negative association was observed between TMB and ITGB5 expression ($r = -0.15$, $P = 0.0029$, Fig. 4B). We then explored the relationship between IPS and ITGB5 expression (Fig. 4G-J). The IPS values for patients in the low ITGB5 expression group were notably higher than those in the high ITGB5 expression group, except IPS_CLAT4_pos_PD1_neg. These results suggest that ITGB5 expression correlates with immunotherapy efficacy, and that GC patients with low ITGB5 expression may have a better opportunity for immune checkpoint inhibitors (ICIs) application.

Knockdown of ITGB5 inhibits the proliferation, invasion and migration of gastric cancer cells

The expression of ITGB5 was assessed in GES and four GC cell lines using qRT-PCR. Results revealed higher ITGB5 expression in the four GC cells lines compared to GES cells (Fig. 5A). Then, ITGB5 was knocked down in MKN45 and HGC27 cell lines using siRNA fragments, qRT-PCR analysis showed a significant down-regulated of ITGB5 expression in MKN45 and HGC27 cells treated with siRNA, with si-ITGB5-2 demonstrating the most effective silence in ability (Fig. 5B). Colony formation analysis demonstrated that ITGB5 downregulation significantly inhibited the cell cloning ability of MKN45 and HGC27 (Fig. 5 C-D). Scratch assays and transwell assays showed that ITGB5 gene downregulation inhibited the invasion and migration abilities of MKN45 and HGC27 cells (Fig. 5E-H). These experiments provide evidence that upregulated ITGB5 plays a role in promoting proliferation and migration in GC cells.

Expression of CD276 is correlated with ITGB5 in gastric cancer cells

CD276 belongs to the B7 family similar as PD-L1. Many solid tumors have shown an overexpression of CD276, which has inversely correlated with patient prognosis. Our analysis results indicated a strongly positive association between CD276 expression and ITGB5 expression. Following the knockdown of ITGB5, we evaluated CD276 expression in MKN45 cells through western blotting. The results revealed that CD276 expression decreased due to the silencing of ITGB5, suggesting that ITGB5 may influence immunotherapy by enhancing CD276 expression ($P < 0.05$, Fig. 6A-B).

ITGB5 was confirmed as a potential diagnostic biomarker for gastric cancer by serum validation

We further explored the diagnostic characteristics and efficacy of ITGB5 as a potential biomarker for GC. The results indicate that serum ITGB5 levels were higher in patients with GC (16.47, 13.78 ~ 18.26 ng/ml) than in healthy controls (14.56, 10.66 ~ 17.09 ng/ml) ($P = 0.01$, Fig. 7A;

(See figure on next page.)

Fig. 3 Immune infiltration results in ITGB5 high and low expression groups based on CIBERSORT analysis. (**A**) Correlation matrix of immune cell infiltration in GC samples. Red indicates positive trend and blue indicates negative trend. (**B**) An illustration of the distribution of 22 immune cells in the 212 filtered gene matrix. Red indicates higher immune infiltration levels, while green indicates lower levels. (**C**) The relationship between ITGB5 expression and immune scores in GC. (**D**) A boxplot diagram depicting the proportion of immune cells in two groups, Blue fraction represents ITGB5 low expression group and red fraction represents ITGB5 high expression group. (**E**) The correlation between ITGB5 expression and immune infiltration in GC. (**F-G**) Correlation analysis of ITGB5 expression and infiltration levels of M2 macrophages or memory B-cells. *P*-value Significant Codes: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$

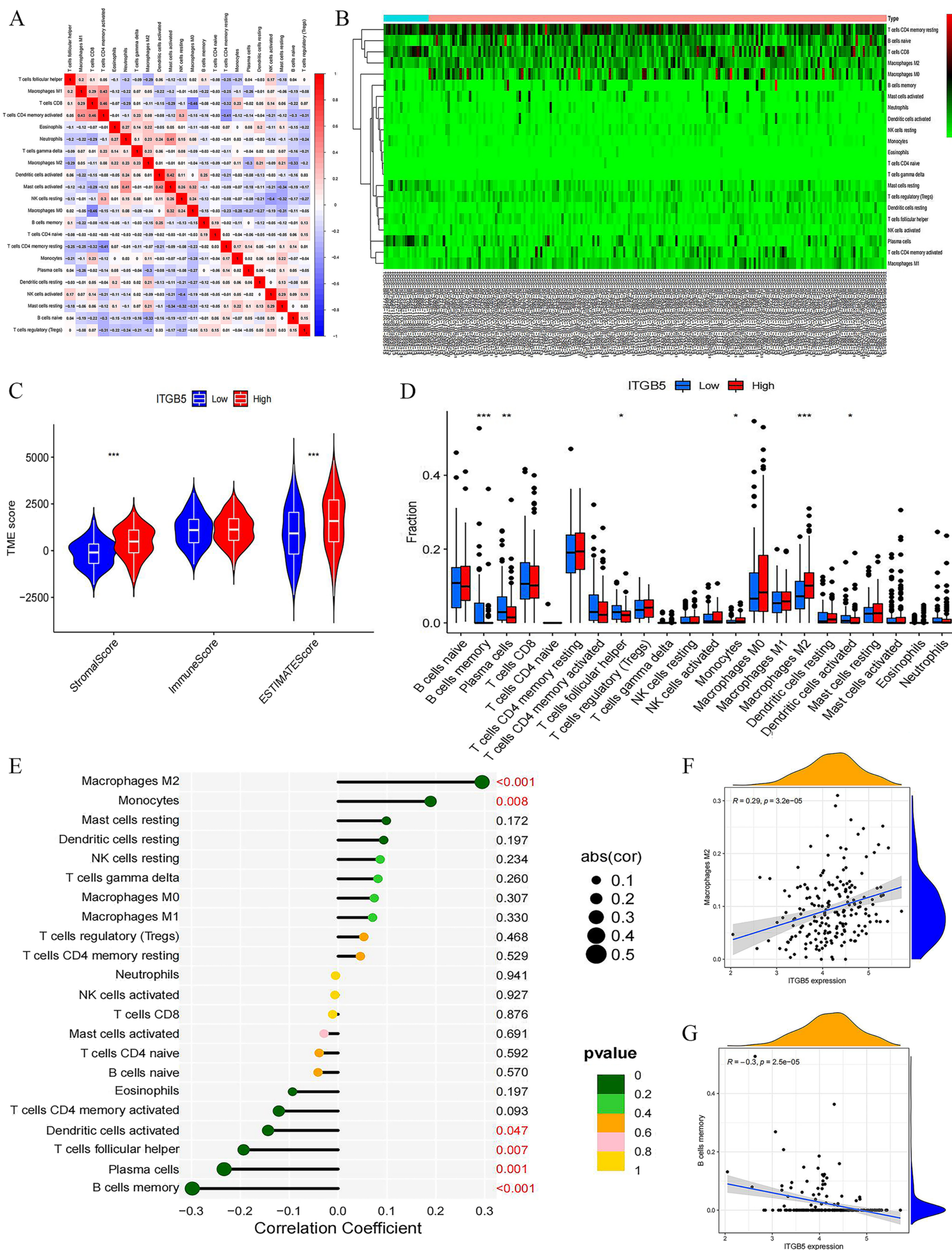

Fig. 3 (See legend on previous page.)

Table 1 Correlation analysis between ITGB5 and gene markers of immune cells using TIMER and GEPIA2 database

Description	Gene Marker	TIMER				GEPIA	
		None		Purity			
		Cor	*p*	Cor	*p*	Cor	*P*
M1-Macrophage	INOS(NOS2)	-0.165	7.34e-04 *	-0.151	3.15e-03*	-0.094	0.048 *
	IRF5	0.285	3.32e-09 *	0.3	2.70e-09 *	0.37	1.8e-15 *
	COX2(PTGS2)	0.15	2.14e -03 *	0.142	5.61e-03 *	0.19	5.6e-05 *
M2-Macrophage	CD163	0.285	3.24e-09 *	0.285	1.58e-08 *	0.19	5.9e-05 *
	VSIG4	0.281	5.97e-09 *	0.298	3.25e-09 *	0.21	1.2e-05 *
	MS4A4A	0.229	2.5e-06 *	0.238	2.78e-06 *	0.16	0.00096 *
TAM	CCL2	0.16	1.08e-03 *	0.16	1.90e-03 *	0.079	0.098
	CD68	0.252	1.95e-07 *	0.258	3.51e-07 *	0.23	5.9e-07 *
	IL10	0.194	6.66e-05 *	0.215	2.41e-05 *	-0.022	0.65
Monocyte	CD86	0.171	4.76e-04 *	0.181	4.03e-04 *	0.15	0.0016 *
	CD115(CSF1R)	0.301	3.99e-10 *	0.312	5.50e-10 *	0.3	2.2e-10 *
B cell	CD19	-0.012	8.15e-01	0.007	8.89e-01	-0.12	0.0084 *
	CD79A	-0.022	6.56e-01	-0.018	7.33e-01	-0.18	0.00014 *

Table S2). Our subsequent analysis examined the association of serum ITGB5 expression with clinical parameters in GC patients. The findings indicated a positive correlation ITGB5 expression and age, T-stage, lymph node metastasis, distant metastasis and clinical stage ($P<0.05$). In contrast, no significant differences were observed in terms of gender, smoking, drinking, tumor size, histological differentiation, pathological classification, and primary site($P>0.05$). Moreover, in patients with CA19-9 positive GC, the ITGB5 serum levels were higher compared to those with CA19-9 negative GC ($P<0.05$, Table S3).

A ROC curve analysis was performed to evaluate the diagnostic value of ITGB5. The AUC for serum ITGB5 was 0.628 (Fig. 7B). CEA and CA19-9 are commonly used as markers for GC. The AUC for CEA was 0.705 and for CA19-9 was 0.630 (Fig. 7 C-D). Thus, detecting ITGB5 alone can not compensate for the diagnostic shortcomings of common tumor markers. To improve the diagnostic efficacy of GC, we combined ITGB5 with CEA (AUC=0.740, 95% CI: 0.663~0.08, $P<0.0001$; sensitivity=46.0%; specificity=96.0%) (Fig. 7E), ITGB5 with CA19-9 (AUC=0.670, 95% CI: 0.589~0.745, $P<0.001$; sensitivity=7.0% specificity=50.0%) (Fig. 7F), and ITGB5 with CEA and CA19-9 (AUC=0.73, 95% CI: 0.676~0.80, $P<0.0001$; sensitivity=55.0%; specificity=92.0%) (Fig. 7G). Compared with a single index, combined detection of ITGB5, CEA, or CA19-9 improved the diagnostic efficacy of GC to some extent (Table S4).

Discussion

ITGB5 regulates various pathways and cellular processes, with previous studies indicating its role in promoting migration and invasion of GC cell lines [13]. However, there are limited studies on to how ITGB5 might modulate the development, diagnosis and prognosis of GC. To gain more detailed insights into the potential functions of ITGB5 in GC, we focus on ITGB5 expression, functional networks, diagnostic and prognostic value, and immunotherapy efficacy. High ITGB5 expression may be associated with poor prognosis and immune infiltration in GC. Serum ITGB5 levels were elevated in GC patients compared to healthy controls, indicating the potential for ITGB5 as a marker and therapeutic target in GC and paving the way for future functional investigations.

Multiple databases were analyzed in our study, and the findings reveal that ITGB5 was significantly elevated in multiple cancers, including GC. GC patients with unfavorable clinical and T stages often have elevated ITGB5 expression, as confirmed by subsequent serum studies. In addition, we proved that ITGB5 was significantly upregulated in GC cells and tissues. Previous research results found a similar phenomenon. Hung et al. reported that high ITGB5 protein expression was positively related to clinically aggressive behavior of GC tissues [22]. Li et al. found that increased levels of ITGB5 were consistent with those measured by western blot in GC tissues, and that exosome ITGB5 upregulation was positively correlated with liver metastasis [14]. Simultaneously, Kaplan-Meier survival curves for including OS and PPS indicated shorter survival times in GC patients with high ITGB5 expression. This finding coincides with ITGB5's effect on prognosis in liver cancer caused by HBV [23] and glioblastoma [24]. It reveals that high ITGB5 expression is linked to poor outcomes, suggesting an oncogenic role for ITGB5 in GC.

Fig. 4 Relationship between ITGB5 expression and immune checkpoints, TMB and IPS. (**A**) Analysis of correlation between ITGB5 and immune checkpoints. (**B**) Correlation of ITGB5 gene expression with TMB. (**C-F**) The correlation between TILs, immunoinhibitors, immunostimulators, MHC molecules and ITGB5 expression, top 2 displaying the highest correlation. (**G-J**) The violin plots of IPS differences between ITGB5 high and low expression groups

Functional enrichment analyses and GSEA were conducted to examine DEGs in GC, with a focus on ITGB5 expression level. GO enrichment analysis results indicated that DEGs are linked to an extracellular matrix organization, extracellular structure organization, external encapsulating structure organization, collagen-containing extracellular matrix, and the extracellular matrix structural constituent. As per KEGG pathway analysis, the enrichment of DEGs showed significant correlation with pathways including PI3K-AKT signaling, ECM-receptor interaction, proteoglycans in cancer, TGF-beta signaling pathway, and focal adhesion. Likewise, GSEA highlighted variations in enrichment within the ITGB5 high-expression phenotype, particularly in relation to the regulation of cell adhesion molecules, ECM receptor interaction, focal adhesion, and lysosomes as identified in KEGG. On the one hand, integrins could activate the intracellular and extracellular bidirectional signaling pathways while participating in the binding of cells with ECM and promoting the expression of related mRNA and proteins. On the other hand, integrins could control ECM degradation, break connective cells and enable tumor cells to separate from the local tissue structure [25]. In this study, a significant link between ITGB5 and focal adhesion and ECM signaling in GC was found. In breast [26]and cervical cancers [27], prior studies demonstrated that focal adhesion signaling via ITGB5 is important for EMT. Interestingly, we observed an upregulation of the PI3K-AKT and TGF-beta pathway. Numerous tumors have abnormal activation of the PI3K-AKT signaling and TGF-beta signaling pathways. which regulate tumor cell proliferation, migration, differentiation, apoptosis and invasion [28, 29]. Recent studies found that HER-2

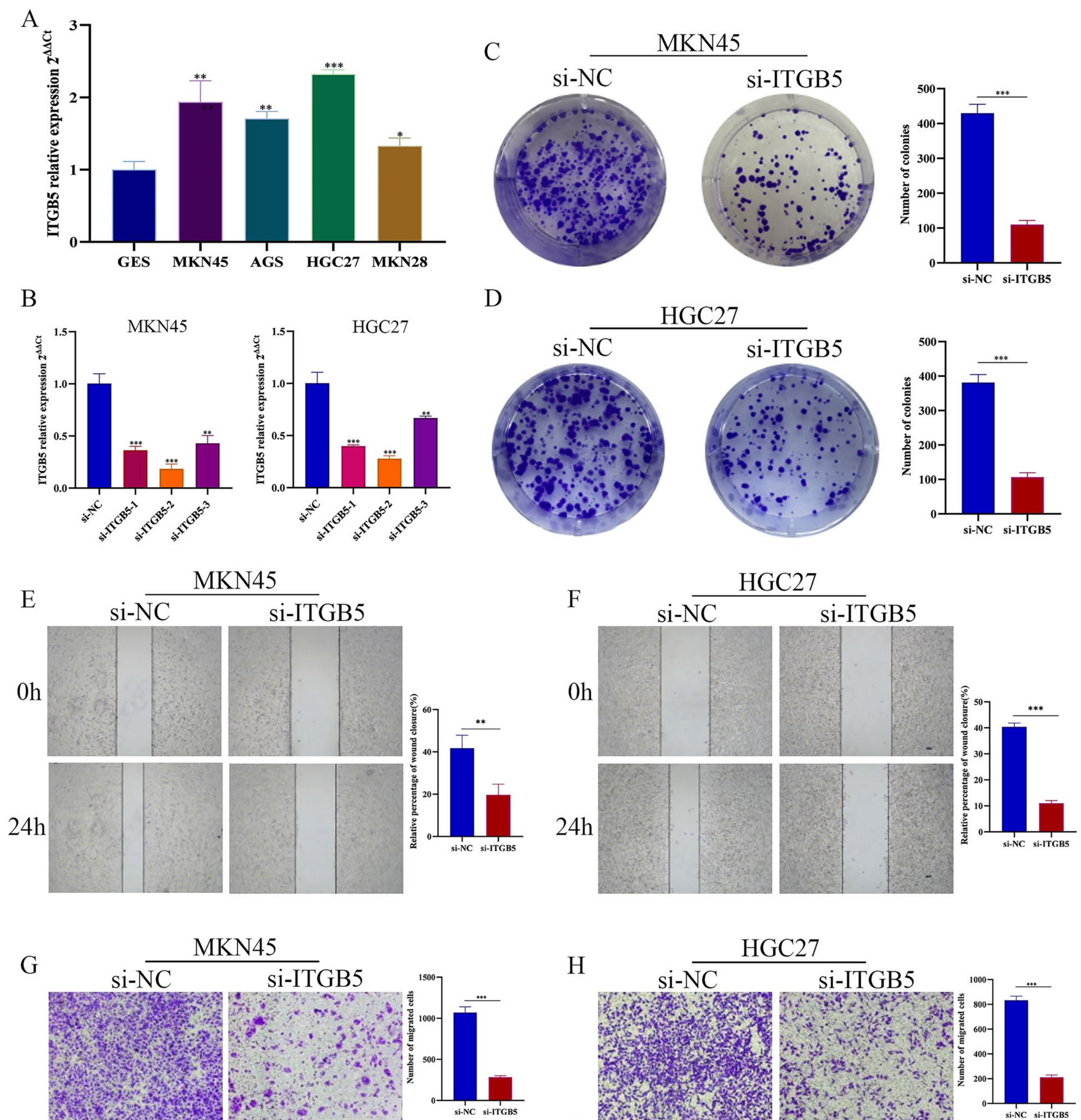

Fig. 5 Validation of ITGB5 functionality in vitro. (**A**) The expression of ITGB5 in the cells was detected using qRT-PCR. (**B**) qRT-PCR analysis was performed to assess ITGB5 expression in MKN45 and HGC27 cells treated with siRNA. (**C-D**) The proliferation ability of MKN45 and HGC27 cells transfected with NC or si-ITGB5 was examined using a plate cloning assay. (**E-H**) The migration and invasion ability of the cells was detected using wound healing and transwell assays. Data are presented as means ± SD, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001

increased ITGB5 via the PI3K-AKT pathway, resulting in HER-2 positive GC cells migrating and invading in vitro when ITGB5 was overexpressed [14]. A hypothesis by Cho et al. suggests that the unfolded type III domain of the fibronectin matrix might be responsible for TRAIL resistance through integrin αvβ5 activation via the PI3K-AKT pathway [30]. It is also noteworthy that TGF-beta signaling via ITGB5 is essential for EMT in breast cancer [9]. Despite this, research on TGF-beta signaling pathway mechanisms through ITGB5 in GC is insufficient. These results suggest that ITGB5 may influence tumor occurrence and development of

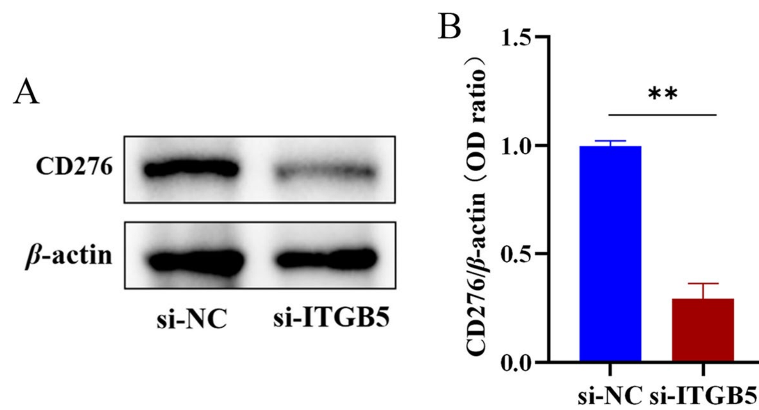

Fig. 6 After knockdown of ITGB5, the expresssion of CD276 was assessed by western blotting in MKN45 cells

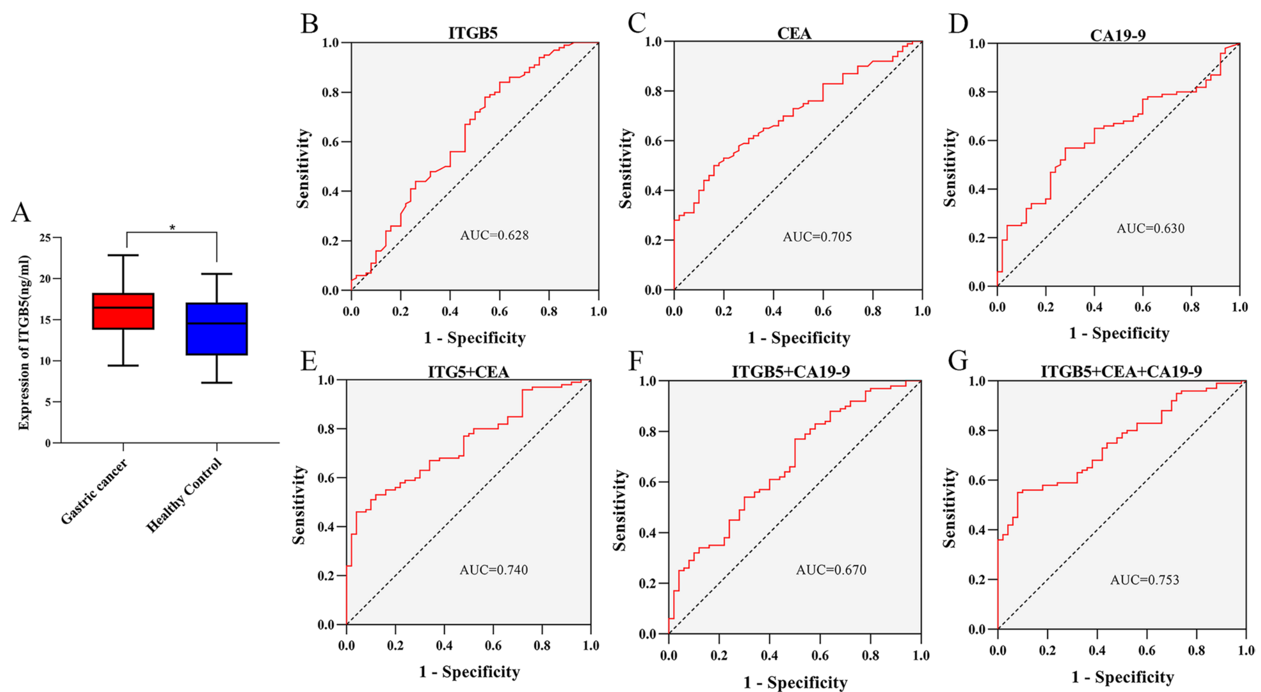

Fig. 7 Identification of ITGB5 as a diagnostic biomarker for GC by serum validation. (**A**) Expression of serum ITGB5 in GC patients and healthy controls. (**B-D**) ROC-AUC was used to compare the diagnostic performance of ITGB5, CEA and CA19-9. (**E-G**) ROC-AUC was used to compare the combined diagnostic value of ITGB5, CEA and CA19-9. P-value Significant Codes: *$P<0.05$, **$P<0.01$, and ***$P<0.001$

tumors by regulating PI3K-AKT and TGF-beta signaling. Indeed, our enrichment analysis detects novel molecular mechanisms between ITGB5 and related-mediated pathways in GC, and the functions are worthy of further investigation.

Accumulating evidence suggests that the tumor microenvironment (TME) plays a crucial role in tumor initiation, development, and metastasis [31]. TME is mainly composed of immune cells, stromal cells, and fibroblasts, which play a role in regulating immunotherapy and affect the prognosis and treatment of cancer patients [32]. Surprisingly, our study discovered a connection between the expression of ITGB5 in GC and the level of immune infiltration. By analyzing the CIBERSORT data, we observed a higher presence of M2 macrophages and monocytes in the high ITGB5 expression group, while the low ITGB5expression group had higher levels of memory B-cells, plasma cells, T follicular helper cells, and dendritic cells. Additionally, ITGB5 significantly correlated with most immune marker sets present on diverse immune cells in GC, such as M1 macrophage marker IRF5, M2 macrophage marker VSIG4, and monocyte

marker CSF1R. These findings establish a relationship between ITGB5 and tumor immunity, and indicate that high ITGB5 might have a potential impact on the immune response of GC.

ICIs are a potent class of anti-cancer therapies that trigger the immune system to eliminate cancer cells. Several ICIs target receptors and pathways associated with immune evasion, including CTLA4, PD-1, and programmed cell death protein ligand-1 (PD-L1) [33–35]. However, there is no relevant study on whether ITGB5 affect on immunotherapy in GC. In this study, we found that CD276, TNFSF4, and NRP1 were positively associated with ITGB5 expression. It is noteworthy that CD276 is a member of the B7 family of immune checkpoint proteins, and it demonstrates immunosuppressive effects by inhibiting NK cell activity and T-cell proliferation [36]. And we found that the distinct expression patterns of CD276 across a diverse range of cancer types have garnered significant attention, positioning it as a promising target for immunotherapeutic strategies [37]. In gastric cancer, research indicates that CD276 expression levels are notably elevated. Furthermore, mesenchymal stem cells derived from gastric cancer have been shown to activate the AKT/c-Myc/mTOR signaling pathway within gastric cancer cells, consequently leading to an upregulation of CD276 expression [38]. Our findings have revealed that ITGB5 was positively relugated CD276 in this research. In addition, ITGB5 expression negatively correlated with TMB. IPS was greater in the low ITGB5 expression group than in the high ITGB5 group, except for IPS_CLAT4_pos_PD1_neg. It is the first time found that ITGB5 may provide noval indictor for immunotherapy for GC. Cilengitide, an ITGB5 selective inhibitor is currently undergoing phase stage II clinical trials for advanced non-small cell lung cancer [39] and stage III clinical trials for malignant glioblastoma [40]. However, future studies are needed to determine whether cilengitide can be used for GC treatment.

To verify the clinical diagnostic efficacy of serum ITGB5 for GC, we explored the elevated expression levels of ITGB5 in serum samples from GC patients. Besides, statistically significant positive correlations were observed between ITGB5 between expression and age, tumor stage, lymphatic metastasis, and clinical stage. Patients with CA19-9 positive GC had higher serum levels of ITGB5 than patients with negative disease. Based on CEA and CA19-9, we conducted sample determination and comparison of ITGB5 as a serum diagnostic biomarker. We found that the diagnostic index combination is more reliable, thus proving that ITGB5 is a potential diagnostic marker.

This study, for the first time, investigated ITGB5 as a diagnostic biomarker for GC, which could be associated with unfavorable prognosis and immunotherapy. However, this study has some limitations. First, investigations are required through in vivo and in vitro experiments to further investigate the exact mechanism and function of ITGB5 in GC. Second, multiplex IHC and immune cell antigens should verify the link between ITGB5 and immune cell infiltration. Moreover, this study was based on a limited number of cases, warranting larger studies to validate the results and draw more definitive conclusions.

Conclusions

In conclusion, our study is the first to provide convincing evidence that ITGB5 can serve as a diagnostic biomarker and affect immunotherapy. Moreover, ITGB5 may be an ideal potential biomarker for predicting the prognosis of patients with GC, which is clinically relevant.

In a nutshell, the findings of this research provide scholars that ITGB5 may serve as a crucial factor in immunotherapy, representing a potential effective target to enhance the survival outcomes of GC patients. This study provides insights for future research, concentrating on clinical verification and molecular pathways turnover and the potential impact of ITGB5 in GC progression.

Abbreviations

ITGB5 Integrin β5
GC Gastric cancer
qPCR Quantitative polymerase chain reaction
GEO Gene Expression Omnibus
TCGA The Cancer Genome Atlas
TMB Tumor mutational burden
IPS Immunophenoscore
EMT Epithelial-mesenchymal transition
CEA Carcinoembryonic antigens
CA19-9 Carbohydrate antigen 19-9
IHC Immunohistochemistry
DEGs Differentially expressed genes
KEGG Kyoto Encyclopedia of Genes and Genomes
GSEA Gene Set Enrichment Analysis
TIICs Tumor-infiltrating immune cells
MHC Major histocompatibility complexes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-024-03447-w.

Supplementary Material 1.

Supplementary Material 2.

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Author contributions

Yihua Sun designed the study. Yangyang Cheng and Xin Lin reviewed the raw data. Huimin Xu, Mingcheng Xu and Jing Zhao confirm the authenticity of all raw data. Yangyang Cheng and Zexu Cheng performed analysis. Wanlin Xie and Baohong Cui collected data. Yangyang Cheng and Xin Lin drafted and completed the manuscript. Yihua Sun and Jingya Zeng revised the manuscript. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The study was carried out after the protocol was approved by the Human Research Ethics Committee of the Harbin Medical University Cancer Hospital. I confirm that all methods were performed in accordance with the relevant guidelines. All procedures were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, and informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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