ITGA5 Is a Prognostic Biomarker and Correlated with Immune Infiltration In Gastrointestinal Cancers

Hai Zhu
First Affiliated Hospital of Anhui Medical University
Gang Wang
First Affiliated Hospital of Anhui Medical University
Haixing Zhu
First Affiliated Hospital of University of Science and Technology of China
Aman Xu (amanxuahmu@126.com)
First Affiliated Hospital of Anhui Medical University

Research article

Keywords: ITGA5, gastrointestinal tumor, immune, prognosis, tumor-associated macrophages

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58757/v1

Version of Record: A version of this preprint was published on March 12th, 2021. See the published version at https://doi.org/10.1186/s12885-021-07996-1.

Abstract

Background: Integrin Subunit Alpha 5 (ITGA5) belongs to the integrin alpha chain family, is vital for promoting cancer cell invasion, metastasis. However, the correlation between ITGA5 expression and immune infiltration in gastrointestinal (GI) tumors remain unclear.

Methods: The expression levels of ITGA5 were detected by Oncomine and Tumor Immune Estimation Resource (TIMER). The association between ITGA5 and prognosis of patients was identified by Kaplan–Meier plotter, Gene Expression Profiling Interactive Analysis 2(GEPIA2) and PrognoScan. We evaluated the correlation between ITGA5 expression and immune infiltrating level via TIMER. Besides, TIMER and immunohistochemistry (IHC) staining were used to explore correlations between ITGA5 expression and markers of immune infiltrates cells. Furthermore, we constructed protein-protein interaction (PPI) network and performed functional enrichment by GeneMANIA and Metascape.

Results: ITGA5 was generally overexpressed and correlated with worse prognosis in multiple types of GI tumors. In addition, ITGA5 expression levels was significantly associated with tumor purity and immune infiltration levels of different immune cells in GI tumors. Interestingly, Immune markers for monocytes, tumor - associated macrophages (TAMs), macrophages 2 (M2) cells and T-helper 2 (Th2) cells were found to be significantly and positively correlated with ITGA5 expression levels in colon and gastric cancer. Results from IHC staining further proved that markers of Th2 and M2 cell were significantly increased in gastric cancer patients with high ITGA5 expression levels. Lastly, interaction network and function enrichment analysis revealed ITGA5 were mainly involved in “integrin mediated signaling pathway”, “leukocyte migration”, “cell-substrate adhesion”.

Conclutions: our study demonstrated that ITGA5 may act as an essential regulator of tumor immune cell infiltration and a valuable prognostic biomarker in GI tumors. Additional work is needed to fully elucidate the underlying mechanisms behind these observations.

Introduction

Gastrointestinal (GI) tumors are a common malignant tumor affecting individuals worldwide (1). The high mortality rate associated with GI tumors is a result of the tumor invading and metastasizing to other tissues. The increasing resistance observed in GI tumors receiving chemotherapy and targeted therapy has led to an increased challenge when treating this disease (2-4). There is extensive evidence that tumor-infiltrating lymphocytes, such as tumor - associated macrophages (TAMs), influence both the prognosis and efficacy of chemotherapy and immunotherapy(5, 6), as well as tumor angiogenesis, progression and metastasis(7). Therefore, it is crucial to identify novel immune-related therapeutic targets and elucidate the potential mechanisms behind the immune interactions linked to GI tumors.

Integrins are a large family of heterodimeric integral membrane proteins that function in cell surface adhesion and signaling pathways. Integrin Subunit Alpha 5 (ITGA5), primarily binds to Integrin Subunit Beta 1 (ITGB1) to form a α5β1 heterodimer (8, 9). Recently, it has become clear that ITGA5 is essential for cancer proliferation, migration, invasion and metastasis (10-13). In addition, ITGA5 was shown to maintain cancer

cell stemness and chemotherapy resistance (14, 15). Interestingly, ITGA5 was identified as a critical factor for promoting the differentiation of human mesenchymal stromal cells *in vivo* (16). Recent studies revealed that ITGA5 plays a role in different tumor cell subgroups and cell-cell interactions. Importantly, there is evidence revealing that immune components existing in the tumor microenvironment (TME) comprehensively regulate the biological behavior of the tumor through mutual interactions (17). Furthermore, immunotherapy, especially the application of immune checkpoint modulators, is an efficient treatment strategy for solid tumors (18, 19). Here, we aim to identify the role of ITGA5 in gastrointestinal tumors and its relationship with tumor immunity.

Detailed exploration investigating the expression level and prognostic value of ITGA5 in various cancers was performed in this study. The correlation between ITGA5 and tumor-infiltrating immune cells and markers in different tumor microenvironments was analyzed. Moreover, immunohistochemistry was used to detect the correlation between ITGA5 protein levels and relative markers of Th2 and M2 cells in gastric cancer. Lastly, a protein-protein interaction (PPI) network of genes interacting with ITGA5 was constructed and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed. The data obtained in this study suggest that ITGA5 is vital for the occurrence and development of GI tumors, and the overexpression of ITGA5 may have a potential and important relationship with tumor-immune infiltration.

Materials And Methods

Oncomine Analysis

Expression of ITGA5 mRNA levels were analyzed to compare normal and tumor tissues in various cancer types using the Oncomine database (https://www.oncomine.org/) (20, 21). The filter condition was set to a p value < 0.001, fold change > 1.5, gene rank: 10%, data type: mRNA.

PrognoScan Analysis

PrognoScan database (http://www.abren.net/PrognoScan)was used to identify the correlation between ITGA5 expression and prognosis in various cancers across a large collection of publicly available microarray datasets (22). The threshold was adjusted to a Cox *P*-value < 0.05.

Kaplan-Meier Plotter Analysis

Kaplan-Meier plotter (https://kmplot.com/) possesses gene expression data and survival information associated with 1,065 gastric cancer patients (23). To evaluate differential expression of ITGA5 mRNA levels and the effects on patient progression, Kaplan–Meier survival curves for overall survival (OS) and disease or progression free survival (DFS or PFS) were generated for patients containing low and high ITGA5 mRNA expression levels. Results contained a hazard ratio (HR) with a 95 percent confidence interval (CI) and log rank p value.

TIMER Analysis

Tumor Immune Estimation Resource (TIMER) database (https://cistrome. shinyapps.io/timer) is a publicly available comprehensive resource for systematic analysis of tumor immune-infiltrates (24). It includes 10,897 samples across 32 different cancer types from The Cancer Genome Atlas (TCGA). The correlation between ITGA5 expression levels and immune infiltrates including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells, were explored using the gene module in various cancer types. The correlation between genes markers of tumor-infiltrating immune cells and ITGA5 expression was analyzed using the correlation module. Gene markers included CD8+ T cells, general T cells, B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs and exhausted T cells. ITGA5 expression was plotted on the X-axis and the expression of related marker genes were represented as gene symbols on the Y-axis. Correlation coefficients were estimated using Spearman's correlation method. Gene expression levels were shown as log2 RSEM.

GEPIA2 Analysis

Gene expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/#index) is an updated and enhanced version of GEPIA that supports 198,619 isoforms and 84 cancer subtypes (25, 26). In our study, this tool was used to determine the correlation between ITGA5 expression and prognosis in pancreatic and esophageal cancers.

Immunohistochemistry staining Analysis

Collected tissue specimens were formalin fixed and embedded with paraffin. Tissue sections (4μm thickness) were used in immunohistochemistry (IHC) staining analyses as previously described (27). The intensity of immunostaining and the percentage of positive cells were used to determine the immunoreactivity to proteins. The density grading of intensity was analyzed as follows: 0 represented no staining, 1 represented weak staining; 2 represented medium staining, and 3 represented strong staining. The proportion of positive cells was further classified as 0: <1%; 1: 1-30%, 2: 30-70%, and 3:> 70 %. Semi-quantitative assessment and final immunostaining scores were determined. Scores 0, 1, 2 and 3 indicated low expression levels, while scores 4, 6 and 9 indicated high expression levels. Immunohistochemistry results were evaluated by two pathologists using a blind test and differences were resolved through consensus. Antibody information is listed in Supplementary Table 1.

GeneMANIA Analysis

GeneMANIA (http://genemania.org/) is used to construct PPI networks and analyze the function of interactive genes (28). This online tool uses bioinformatic methods to display a list of interacting genes, including gene co-expression, physical interactions, gene co-localization, gene enrichment analysis and website prediction. GeneMANIA was used to construct the PPI network for ITGA5.

Functional Enrichment Analysis

Metascape (https://metascape.org/) is a gene function annotation website with obvious advantages of wide coverage and fast updating. It integrates multiple authoritative data resources such as GO, KEGG, UniProt

and DrugBank to complete thorough pathway enrichment and biological process annotation. Genes interacting with ITGA5 were put into Metascape to perform GO and KEGG annotation.

Statistical Analysis

All statistical analyses were performed using SPSS 21.0 software (IBM Corp; Armonk, NY). Survival curves and relative results generated from PrognoScan, Kaplan-Meier Plotter and GEPIA2 databases were shown with HR and *P* or Cox *P* values from a log-rank test. Correlation analyses in TIMER were evaluated using Spearman's correlation. Associations between ITGA5 expression and IHC results in patients were evaluated using Pearson's $\chi 2$ test. A *P*-value of <0.05 was considered as statistically significant.

Results

Pan-cancer analysis of ITGA5 expression levels

To determine ITGA5 mRNA expression levels in both normal and tumor tissues, Oncomine was interrogated to analyze ITGA5 expression in various cancer types. The expression levels of ITAG5 were greater in colorectal, esophageal, gastric and pancreatic cancers relative to normal tissues (Figure 1A). In addition, the transcriptional levels for ITGA5 were analyzed using RNA-seq data for multiple malignancies in the TCGA with the use of TIMER. Results revealed significant differences in ITGA5 expression levels when comparing normal and tumor tissues. This was significantly higher in, of which was liver hepatocellular carcinoma (LIHC) and stomach adenocarcinoma (STAD) compared to normal tissue levels (Figure 1B).

Correlation between ITGA5 expression levels and patient prognosis

The PrognoScan database was used to investigates the relationship between ITGA5 mRNA levels and the survival of cancer patients using high-throughput analysis and detailed clinical prognosis data. ITGA5 expression was found to significantly impact prognosis in colorectal and ovarian cancers. Specifically, the cohorts (GSE17536) (29) included 177 samples at different stages of colorectal cancer and showed a remarkable association between high ITGA5 expression levels and poor prognosis (OS HR = 1.66, 95% CI = 1.16 to 2.37, Cox *P* = 0.005; DFS HR = 2.97, 95% CI = 1.70 to 5.21, Cox *P* = 0.0004) (Figures 2A–B). Similarly, the cohorts (GSE26712) (30) showed that high ITGA5 expression levels were correlated with poor prognosis (OS HR = 1.58, 95% CI = 1.06 to 2.36, Cox *P* = 0.025; DFS HR = 1.54, 95% CI = 1.06 to 2.24, Cox *P* = 0.025) in ovarian cancers (Figures 2C–D). Subsequently, clinical data from TCGA were used to explore the prognostic value of ITGA5 expression using GEPIA2. High expression of ITGA5 was marginally correlated with poor prognosis in pancreatic cancer (OS HR=1.6, *P* = 0.12; DFS HR = 2.1, *P* = 0.015) and esophageal cancer (OS HR=1.4, *P* = 0.37; DFS HR = 1.2, *P* = 0.52) (Figure 2E-H).

To further identify the prognostic potential of ITGA5 in various cancers, the Kaplan-Meier plotter database was used to determine the prognostic value of ITGA5. Significant differences were observed between ITGA5 expression and prognosis in gastric cancer (OS HR=2.4, 95% CI = 1.96 to 2.97, *P* < 1e-16; DFS HR = 2.69, 95% CI = 2.04 to 3.54, *P* = 2.2e-13) patients. Interestingly, high expression of ITGA5 was also associated with poor prognosis in breast cancer (OS HR=1.21, 95% CI = 0.96 to 1.52, *P* = 0.11; DFS HR = 1.28, 95% CI = 1.14 to

1.44, *P* = 4.1e-05) and lung cancer (OS HR=1.6, 95% CI = 1.4 to 1.81, *P* = 5.9e-13; DFS HR = 1.75, 95% CI = 1.44 to 2.12, *P* = 1.2e-08) patients (Figure 2I-P).

Relationship between ITGA5 expression and immune infiltration levels in gastrointestinal cancers

Recent work has shown that gastrointestinal tumors exhibit extensive immune infiltration characteristics (31-33). Therefore, to better understand the role of ITGA5 in GI tumors, the relationship between ITGA5 expression and immune infiltration in GI tumors was investigated using the TIMER database. ITGA5 expression was found to be significantly correlated with tumor purity in colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), STAD and rectum adenocarcinoma (READ) (*P* < 0.05), CD8+ cell in COAD, LIHC and pancreatic adenocarcinoma (PAAD), CD4+T cells in COAD, PAAD, LIHC, READ and STAD (*P* < 0.05) (Figure 3A-F). Furthermore, ITGA5 expression was significantly associated with macrophages and dendritic cells in COAD, ESCA, READ, LIHC, PAAD and STAD (*P* < 0.05). ITGA5 expression was also significantly associated with neutrophils in COAD, LIHC, READ, PAAD and STAD (*P* < 0.05) (Figure 3A-F).

Specifically, ITGA5 expression levels showed significantly positive correlations with infiltrating levels of CD8+T cells (r =0.248, *P* = 4.18e-07), CD4+ T cells (r = 0.423, *P* = 6.67e-19), macrophages (r = 0.545, *P* = 1.20e-32), neutrophils (r = 0.590, *P* = 4.92e-39) and dendritic cells (r = 0.556, *P* = 5.48e-34) in COAD (Figure 3A). Similarly, positive correlations were identified with infiltrating levels of CD4+ T cells (r = 0.426, *P* =1.52e-17), macrophages (r = 0.447, *P* = 1.42e-19), neutrophils (r = 0.189, *P* = 2.47e-04) and dendritic cells (r = 0.28, *P* = 4.19e-08) in STAD (Figure 3F). These results revealed that ITGA5 expression is closely associated with immune infiltration in GI tumors.

Correlation between ITGA5 expression levels and immune markers

Gastric cancer and colorectal cancer are the most common GI-related malignancies. Therefore, COAD and STAD were chosen to further investigate the relationship between ITGA5 expression and immune marker genes of diverse immune cells using TIMER databases. The immune cells analyzed included CD8+ T cells, general T cells, different functional T cells, B cells, monocytes, TAMs, M1 macrophages and M2 macrophages, neutrophils, NK cells and dendritic cells. After adjusting correlation by purity, ITGA5 expression was found to be significantly correlated with immune markers of most immune cells in STAD and COAD (Table 1 and Figure 4).

There is a great deal of evidence suggesting that an increased number of M2 macrophages is associated tumor growth, angiogenesis, invasion and metastasis (34, 35). M2 cells promote tumor and local immunosuppression (36). Th2 cells release IL4 and IL13 and induce M2 cell polarization (37, 38). Immune markers for monocytes, TAMs, M2 and Th2 phenotypes were found to be significantly and positively correlated with ITGA5 expression levels in COAD and STAD (*P* < 0.05; Figures 4A–H). These findings support the hypothesis that ITGA5 may be a critical factor for macrophage polarization and immune escape in COAD and STAD.

High ITGA5 expression affects the prognosis of gastric cancer patients exhibiting lymph node metastasis

To better understand the relationship between ITGA5 expression levels and clinicopathological features, further research was focused on gastric cancer. According to Kaplan-Meier plotter database analyses, overexpression of ITGA5 is strongly correlated with the deterioration of OS and progression-free survival (PFS) based on gender, differentiation and Lauren classification ($P < 0.05$). In addition, stage 1 through 4 cancer patients with high expression levels of ITGA5 showed worse OS ($P < 0.05$). Similarly, high expression levels of ITGA5 were associated with worse PFS in stage 2 through 4 gastric cancer patients ($P < 0.05$) but not in stage 1 gastric cancer patients (PFS). However, there was no strong correlation observed between ITGA5 expression levels and patient prognosis in stage N0 (OS HR = 1.92, $P = 0.13$; PFS HR = 1.95, $P = 0.11$), mixed Lauren classification (OS HR = 1.88, $P = 0.22$; PFS HR = 0.48, $P = 0.16$) or poor differentiation (OS HR = 0.75, $P = 0.16$; PFS HR = 1.39, $P = 0.25$) (Table 2). N category refers to lymph node involvement [31]. Moreover, high ITGA5 levels express the highest HR value of N1 with OS and PFS among four N categories (Table 2). These results indicate that the expression levels of ITGA5 are related to lymph node metastasis in gastric cancer patients.

ITGA5 protein expression and M2 and Th2 immune marker genes in STAD

To confirm the results obtained from publicly available databases, IHC was performed on adjacent normal and STAD tumor tissues obtained from 40 gastric patients. Compared with adjacent normal tissues, ITGA5, CD163, STAT6 and GATA3 levels were greater in most tumor tissues (Figure 5). In addition, these results revealed that the expression levels of CD163 ($\chi^2 = 8.750$, $P = 0.003$), STAT6 ($\chi^2 = 8.174$, $P = 0.004$) and GATA3 ($\chi^2 = 5.079$, $P = 0.024$) in the ITGA5 high-expression group were significantly higher than what was observed in the ITGA5 low-expression group (Table 3 and Figure 5).

ITGA5 PPI network and functional enrichment

PPI network analysis revealed interactions between ITGA5 and specific genes. As shown by GeneMANIA, genes interacting with ITGA5 included SPP1, ITGB1, ITGB3, COL18A1, HOXD3, ANGPTL3, CD9, FBN1, VEGFD, ITGA2B, ITGA4, FN1, FLT4, TLN1, ACVRL1, FUBP1, THBS4, ACTN1, ANGPT2 and ITGA8 (Figure 6A).

To further predict enriched functional information of ITGA5 interacting genes, GO and KEGG pathway analyses were performed using Metascape. According to GO term analysis, genes interacting with ITGA5 in the PPI network are mainly related with cell-substrate adhesion, regulation of leukocyte migration, response to wounding and myeloid cell differentiation. KEGG pathway analysis showed these genes are enriched in focal adhesion, PID avb3 integrin pathway and PID integrin5 pathway. Based on these results, ITGA5 and its interacting proteins were shown to play an important role in the integrin mediated signaling pathway, leukocyte migration, cell-substrate adhesion and other essential biological processes (Figure 6B). In addition, Cytoscape was used to determine the relationship for the enriched terms and to build a network diagram (Figure 6C and 6D)

Discussion

ITGA5 belongs to the integrin alpha chain family and interacts with ITGB1 to form a heterodimeric integral membrane protein - integrin α5β1 complex that performs diverse biological functions. Despite not knowing

all of the functions performed by ITGA5, multiple studies have demonstrated that increased expression of ITGA5 is associated with poor prognosis in multiple tumors types, such as triple negative breast cancer (14), lung cancer (39), hepatocellular carcinoma (40) and ovarian cancer (41). Here, public databases were used to determine ITGA5 expression levels in different cancer types. Compared to normal tissues, ITGA5 was generally overexpressed in different cancers including multiple types of GI tumors. We explored whether the expression of ITGA5 was correlated with patient prognosis in these different tumors. Results revealed that high expression levels of ITGA5 were significantly correlated with worse OS and DFS in four types of GI tumors, including colorectal, pancreatic, gastric and liver cancers. Although the results did not show statistical significance, esophageal cancer patients with high ITGA5 expression levels still showed a trend of high survival risk. To understand the clinical significance of ITGA5 in gastric cancer, the prognostic values for ITGA5 expression levels with various clinicopathological factors were determined. High expression of ITGA5 was correlated with poor prognosis in gastric cancer stages 2–4, T2 - T4, N1 - N3 and M0 – M1. These results demonstrated that ITGA5 has potential as a prognostic biomarker in GI tumors.

Both innate and adaptive immune cells regulate the biological behavior of tumors and the response to treatment through direct contact or signal transduction mechanisms (17). We therefore explored the relationship between ITGA5 expression levels and immune infiltration levels. Our results indicated that ITGA5 expression was significantly and negatively correlated with immune purity in four types of GI tumors, including COAD, ECAD, STAD and READ. In STAD and COAD, significant and positive relationships were observed between ITGA5 expression and infiltration levels for CD4 + T cells, macrophages, neutrophils and dendritic cells. In addition, the relationship between ITGA5 expression levels and immune cell markers implicates underlying regulation in STAD and COAD. First, gene markers for Th2 cells (GATA3, STAT6, STAT5A and IL13) showed a positive correlation with ITGA5 expression. The M2 macrophage markers (CD163, VSIG4 and MS4A4A) showed a moderate or strong correlation. In addition, we also observed a significant correlation between ITGA expression and monocytes and TAMs in COAD and STAD. These results revealed a potential regulatory role for ITGA5 in the polarization of macrophages. TAMs, derived from mononuclear cells, are induced to differentiate into M2 cells by cytokines such as IL-4, IL-10 and IL-13, which are secreted by Th2 cells (42). Moreover, M2 cells exhibit pro-tumoral activity by promoting genetic instability, angiogenesis, stem cell nurturing and local immunosuppression (36, 43). Subsequently, IHC was performed on adjacent normal and tumor tissues extracted from gastric cancer patients to find that Th2 cell markers such as STAT6, GATA3 and the M2 cell polarization marker CD163 were significantly increased in patients with high ITGA5 expression levels. This suggested that ITGA5 may play an important role in promoting the recruitment and activation of monocytes, TAMs, Th2 cells and M2 cells in the tumor microenvironment.

Emerging evidence provides potential mechanisms to explain the correlation between ITGA5 expression levels and immune infiltration. Integrin α5β1 usually acts as a receptor for fibronectin to perform biological functions. Previous studies mainly focused on the role of ITGA5 in tumor cells, but recent studies found that ITGA5 is also expressed in cancer-associated fibroblasts (CAFs)(44), TAMs (45) and chimeric antigen receptor expressing T cells (CAR-T) (46). Based on previous research, CAFs, which are regulators of immune cell recruitment and function, affect both innate and adaptive immune responses (47, 48). Similarly, ITGA5 is expressed on the surface of TAMs and CAR-T cells and can directly regulate the recruitment and alternative activation of immune cells. Interestingly, further work has shown that decreased expression of ITGA5 down-

regulates pancreatic stellate cell (PSC) differentiation into CAFs in pancreatic ductal cancer (15). Importantly, PSCs are seen as the main source of CAFs (49). The study presented here revealed an important role for ITGA5 in the differentiation and maturation of cells. These results partly explain the effects of ITGA5 expression on immune cell infiltration in malignant tumors and provides insight for further work. ITGA5 and its interacting proteins play an important role in integrin-mediated signaling pathways, leukocyte migration and cell-matrix adhesion as shown by the PPI network that was constructed as well as gene functional enrichment analysis. ITGA5 may act as an "anchor" for cell positioning, promoting the aggregation, adhesion and migration of partial immune cells and changing components of the tumor microenvironment. This may result in immune escape and suppression, ultimately accelerating tumor development and metastasis. In the future, the possible role and mechanisms behind ITAG5 in tumor immunity should be explored.

Conclusions

In summary, our study demonstrated that ITGA5 may be an essential regulator of tumor immune cell infiltration and a valuable prognostic biomarker in GI tumors. Additional work is needed to fully elucidate the underlying mechanisms behind these observations.

Abbreviations

ITGA5: Integrin subunit alpha 5; Gastrointestinal: GI; TAMs: Tumor - associated macrophages:; ITGB1: Integrin subunit beta 1; TME: tumor microenvironment; PPI: Protein-protein interaction; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; OS: Overall survival; DFS: Disease free survival; PFS: Progression free survival; HR: Hazard ratio; CI: confidence interval; TCGA: The cancer genome atlas; NK: Natural killer; DCs: Dendritic cells; Th1: T-helper 1; Th2: T-helper 2; Tfh: follicular helper T; Th17: T-helper 17; GEPIA2: Gene expression profiling interactive analysis 2; IHC: Immunohistochemistry; LIHC: liver hepatocellular carcinoma; STAD: Stomach adenocarcinoma; COAD: Colon adenocarcinoma; ESCA: Esophageal carcinoma; READ: Rectum adenocarcinoma; PAAD: Pancreatic adenocarcinoma; CAFs: Cancer-associated fibroblasts; CAR-T: Chimeric antigen receptor expressing T cells; PSC: Pancreatic stellate cell.

Declarations

Ethics Statement

This work was approved by the Academic Committee of The First Affiliated Hospital of Anhui Medical University and was conducted following the Declaration of Helsinki. Each data set was retrieved from published literature to confirm that written informed consent was obtained for all patients included in this study.

Acknowledgements

Not applicable.

Funding

This research was supported by the National Natural Science Foundation of China (No. 81572350 to A. M. X.).

Author Contributions

H.Z. and G.W conceived and designed the experiments. H.Z., G.W and A.M.X. extracted the data and performed the analysis, H.Z., H.X.Z. and A.M.X. wrote the manuscript. H.Z. and A.M.X. revised the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing Interests

The authors declared no conflicts of interest in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
2. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. Lancet (London, England). 2016;388(10060):2654-64.
3. Becht E, de Reyniès A, Giraldo NA, Pilati C, Buttard B, Lacroix L, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2016;22(16):4057-66.
4. Myint ZW, Goel G. Role of modern immunotherapy in gastrointestinal malignancies: a review of current clinical progress. Journal of hematology & oncology. 2017;10(1):86.
5. Waniczek D, Lorenc Z, Śnietura M, Wesecki M, Kopec A, Muc-Wierzgoń M. Tumor-Associated Macrophages and Regulatory T Cells Infiltration and the Clinical Outcome in Colorectal Cancer. Archivum immunologiae et therapiae experimentalis. 2017;65(5):445-54.
6. Zhang H, Liu H, Shen Z, Lin C, Wang X, Qin J, et al. Tumor-infiltrating Neutrophils is Prognostic and Predictive for Postoperative Adjuvant Chemotherapy Benefit in Patients With Gastric Cancer. Annals of surgery. 2018;267(2):311-8.
7. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231-5.

8. Morgan MR, Byron A, Humphries MJ, Bass MD. Giving off mixed signals–distinct functions of alpha5beta1 and alphavbeta3 integrins in regulating cell behaviour. IUBMB life. 2009;61(7):731-8.
9. Marelli UK, Rechenmacher F, Sobahi TR, Mas-Moruno C, Kessler H. Tumor Targeting via Integrin Ligands. Frontiers in oncology. 2013;3:222.
10. Tani N, Higashiyama S, Kawaguchi N, Madarame J, Ota I, Ito Y, et al. Expression level of integrin alpha 5 on tumour cells affects the rate of metastasis to the kidney. British journal of cancer. 2003;88(2):327-33.
11. Sawada K, Mitra AK, Radjabi AR, Bhaskar V, Kistner EO, Tretiakova M, et al. Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. Cancer research. 2008;68(7):2329-39.
12. Qian F, Zhang ZC, Wu XF, Li YP, Xu Q. Interaction between integrin alpha(5) and fibronectin is required for metastasis of B16F10 melanoma cells. Biochemical and biophysical research communications. 2005;333(4):1269-75.
13. Deng Y, Wan Q, Yan W. Integrin α5/ITGA5 Promotes The Proliferation, Migration, Invasion And Progression Of Oral Squamous Carcinoma By Epithelial-Mesenchymal Transition. Cancer management and research. 2019;11:9609-20.
14. Xiao Y, Li Y, Tao H, Humphries B, Li A, Jiang Y, et al. Integrin α5 down-regulation by miR-205 suppresses triple negative breast cancer stemness and metastasis by inhibiting the Src/Vav2/Rac1 pathway. Cancer letters. 2018;433:199-209.
15. Kuninty PR, Bansal R, De Geus SWL, Mardhian DF, Schnittert J, van Baarlen J, et al. ITGA5 inhibition in pancreatic stellate cells attenuates desmoplasia and potentiates efficacy of chemotherapy in pancreatic cancer. Science advances. 2019;5(9):eaax2770.
16. Hamidouche Z, Fromigué O, Ringe J, Häupl T, Vaudin P, Pagès JC, et al. Priming integrin alpha5 promotes human mesenchymal stromal cell osteoblast differentiation and osteogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(44):18587-91.
17. Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer letters. 2017;387:61-8.
18. Valsecchi ME. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England journal of medicine. 2015;373(13):1270.
19. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. The New England journal of medicine. 2015;373(17):1627-39.
20. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia (New York, NY). 2007;9(2):166-80.
21. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia (New York, NY). 2004;6(1):1-6.
22. Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC medical genomics. 2009;2:18.
23. Lánczky A, Nagy Á, Bottai G, Munkácsy G, Szabó A, Santarpia L, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast cancer

research and treatment. 2016;160(3):439-46.
24. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer research. 2017;77(21):e108-e10.
25. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic acids research. 2017;45(W1):W98-w102.
26. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic acids research. 2019;47(W1):W556-w60.
27. Wang G, Zhong WC, Bi YH, Tao SY, Zhu H, Zhu HX, et al. The Prognosis Of Peroxiredoxin Family In Breast Cancer. Cancer management and research. 2019;11:9685-99.
28. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic acids research. 2010;38(Web Server issue):W214-20.
29. Smith JJ, Deane NG, Wu F, Merchant NB, Zhang B, Jiang A, et al. Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology. 2010;138(3):958-68.
30. Bonome T, Levine DA, Shih J, Randonovich M, Pise-Masison CA, Bogomolniy F, et al. A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. Cancer research. 2008;68(13):5478-86.
31. Bijlsma MF, Sadanandam A, Tan P, Vermeulen L. Molecular subtypes in cancers of the gastrointestinal tract. Nature reviews Gastroenterology & hepatology. 2017;14(6):333-42.
32. Leone RD, Powell JD. Metabolism of immune cells in cancer. Nature reviews Cancer. 2020.
33. Solinas C, Pusole G, Demurtas L, Puzzoni M, Mascia R, Morgan G, et al. Tumor infiltrating lymphocytes in gastrointestinal tumors: Controversies and future clinical implications. Critical reviews in oncology/hematology. 2017;110:106-16.
34. Tan B, Shi X, Zhang J, Qin J, Zhang N, Ren H, et al. Inhibition of Rspo-Lgr4 Facilitates Checkpoint Blockade Therapy by Switching Macrophage Polarization. Cancer research. 2018;78(17):4929-42.
35. Donzelli S, Milano E, Pruszko M, Sacconi A, Masciarelli S, Iosue I, et al. Expression of ID4 protein in breast cancer cells induces reprogramming of tumour-associated macrophages. Breast cancer research : BCR. 2018;20(1):59.
36. Genard G, Wera AC, Huart C, Le Calve B, Penninckx S, Fattaccioli A, et al. Proton irradiation orchestrates macrophage reprogramming through NFκB signaling. Cell death & disease. 2018;9(7):728.
37. Chen D, Xie J, Fiskesund R, Dong W, Liang X, Lv J, et al. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. Nature communications. 2018;9(1):873.
38. Xiang W, Shi R, Kang X, Zhang X, Chen P, Zhang L, et al. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nature communications. 2018;9(1):2574.
39. Zheng W, Jiang C, Li R. Integrin and gene network analysis reveals that ITGA5 and ITGB1 are prognostic in non-small-cell lung cancer. OncoTargets and therapy. 2016;9:2317-27.

40. Zhao X, Wu Y, Lv Z. miR-128 modulates hepatocellular carcinoma by inhibition of ITGA2 and ITGA5 expression. American journal of translational research. 2015;7(9):1564-73.
41. Gong C, Yang Z, Wu F, Han L, Liu Y, Gong W. miR-17 inhibits ovarian cancer cell peritoneal metastasis by targeting ITGA5 and ITGB1. Oncology reports. 2016;36(4):2177-83.
42. Chen Y, Song Y, Du W, Gong L, Chang H, Zou Z. Tumor-associated macrophages: an accomplice in solid tumor progression. Journal of biomedical science. 2019;26(1):78.
43. Najafi M, Hashemi Goradel N, Farhood B, Salehi E, Nashtaei MS, Khanlarkhani N, et al. Macrophage polarity in cancer: A review. Journal of cellular biochemistry. 2019;120(3):2756-65.
44. Lu L, Xie R, Wei R, Cai C, Bi D, Yin D, et al. Integrin α5 subunit is required for the tumor supportive role of fibroblasts in colorectal adenocarcinoma and serves as a potential stroma prognostic marker. Molecular oncology. 2019;13(12):2697-714.
45. Xu M, Zhang S, Jia L, Wang S, Liu J, Ma X, et al. E-M, an Engineered Endostatin with High ATPase Activity, Inhibits the Recruitment and Alternative Activation of Macrophages in Non-small Cell Lung Cancer. Frontiers in pharmacology. 2017;8:532.
46. Guha P, Cunetta M, Somasundar P, Espat NJ, Junghans RP, Katz SC. Frontline Science: Functionally impaired geriatric CAR-T cells rescued by increased α5β1 integrin expression. Journal of leukocyte biology. 2017;102(2):201-8.
47. Harper J, Sainson RC. Regulation of the anti-tumour immune response by cancer-associated fibroblasts. Seminars in cancer biology. 2014;25:69-77.
48. Jiang H, Hegde S, DeNardo DG. Tumor-associated fibrosis as a regulator of tumor immunity and response to immunotherapy. Cancer immunology, immunotherapy : CII. 2017;66(8):1037-48.
49. Schnittert J, Bansal R, Prakash J. Targeting Pancreatic Stellate Cells in Cancer. Trends in cancer. 2019;5(2):128-42.

Tables

Table 1
Correlation analysis between ITGA5 and relate genes and markers of immune cells in TIMER.

Description	Gene markers	COAD				STAD			
		None		Purity		None		Purity	
		Cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*
CD8 + T cell	CD8A	0.34	***	0.235	***	0.152	**	0.134	**
	CD8B	0.22	***	0.168	***	0.094	0.055	0.094	0.066
T cell(general)	CD3D	0.284	***	0.127	*	0.103	*	0.07	0.175
	CD3E	0.381	***	0.241	***	0.381	***	0.102	0.046
	CD2	0.348	***	0.216	***	0.348	***	0.118	0.021
B cell	CD19	0.226	***	0.072	0.150	0.226	***	0.205	***
	CD79A	0.303	***	0.147	**	0.303	***	0.146	**
Monocyte	CD86	0.651	***	0.583	***	0.651	***	0.313	***
	CD115 (CSF1R)	0.65	***	0.584	***	0.65	***	0.423	***
TAM	CCL2	0.607	***	0.607	***	0.659	***	0.427	***
	CD68	0.556	***	0.497	***	0.556	***	0.21	***
	IL10	0.461	***	0.404	***	0.392	***	0.388	***
M1 Macrophage	INOS (NOS2)	0.064	0.172	-0.125	*	0.035	0.475	0.042	0.411
	IRF5	0.296	***	0.323	***	0.193	***	0.197	***
	COX2(PTGS2)	0.41	***	0.359	***	0.402	***	0.398	***
M2 Macrophage	CD163	0.700	***	0.648	***	0.444	***	0.437	***
	VSIG4	0.633	***	0.567	***	0.437	***	0.444	***
	MS4A4A	0.597	***	0.526	***	0.378	***	0.374	***
Neutrophils	CD66b (CEACAM8)	-0.218	***	0.207	***	0.018	0.714	0.045	0.384
	CD11b (ITGAM)	0.684	***	0.624	***	0.427	***	0.418	***
	CCR7	0.405	***	0.274	***	0.319	***	0.292	***
Natural killer cell	KIR2DL1	0.224	***	0.173	***	0.11	*	0.117	****

Description	Gene markers	COAD				STAD			
	KIR2DL3	0.168	***	0.121	0.015	0.039	0.433	0.031	0.553
	KIR2DL4	0.222	***	0.135	**	-0.03	0.549	-0.046	0.375
	KIR3DL1	0.267	***	0.201	***	0.092	0.062	0.076	0.138
	KIR3DL2	0.249	***	0.177	0.177	0.08	0.102	0.074	0.149
	KIR3DL3	0.052	0.265	0.039	0.431	-0.069	0.159	-0.033	0.525
	KIR2DS4	0.194	***	0.162	**	0.051	0.296	0.055	0.284
Dendritic cell	HLA-DPB1	0.501	***	0.389	***	0.136	**	0.101	0.051
	HLA-DQB1	0.297	***	0.183	***	0.068	0.169	0.04	0.441
	HLA-DRA	0.46	***	0.355	***	0.076	0.120	0.044	0.394
	HLA-DPA1	0.486	***	0.385	***	0.09	0.069	0.056	0.281
	BDCA-1(CD1C)	0.304	***	0.199	***	0.246	***	0.213	***
	BDCA-4(NRP1)	0.83	***	0.794	***	0.639	***	0.63	***
	CD11c (ITGAX)	0.738	***	0.675	***	0.408	***	0.392	***
Th1	T-bet (TBX21)	0.43	***	0.336	***	0.183	***	0.172	***
	STAT4	0.343	***	0.231	***	0.222	***	0.222	***
	STAT1	0.451	***	0.411	***	0.076	0.124	0.071	0.169
	IFN-γ (IFNG)	0.242	***	0.183	***	-0.043	0.380	-0.052	0.312
	TNF-α (TNF)	0.383	***	0.334	***	0.236	***	0.204	***
Th2	GATA3	0.442	***	0.36	***	0.22	***	0.204	***
	STAT6	0.132	**	0.121	*	0.199	***	0.214	***
	STAT5A	0.307	***	0.275	***	0.367	***	0.368	***
	IL13	0.3	***	0.247	***	0.167	***	0.19	***
Tfh	BCL6	0.643	***	0.583	***	0.503	***	0.471	***
	IL21	0.202	***	0.154	**	0.029	0.563	0.012	0.809
Th17	STAT3	0.417	***	0.37	***	0.462	***	0.457	***
	IL17A	0.198	***	0.224	***	-0.103	*	-0.112	*
Treg	FOXP3	0.537	***	0.454	***	-0132	0.244	-0.107	0.395

Description	Gene markers	COAD				STAD			
	CCR8	0.543	***	0.481	***	0.123	0.280	0.055	0.642
	STAT5B	0.356	***	0.385	***	0.381	***	0.418	***
	TGFβ (TGFB1)	0.69	***	0.606	***	0.6	***	0.591	***
T cell exhaustion	PD-1 (PDCD1)	0.395	***	0.28	***	0.175	***	0.17	***
	CTLA4	0.465	***	0.28	***	0.155	**	0.144	**
	LAG3	0.419	***	0.317	***	0.134	**	0.126	*
	TIM-3 (HAVCR2)	0.645	***	0.584	***	0.33	***	0.33	***
	GZMB	0.099	*	0.077	0.122	0.068	0.169	0.05	0.332

Table 2
Correlation of ITGA5 expression and prognosis in STAD with different clinicopathological factors

Clinicopathological characteristics	Overall survival (n = 875)			Progression-free survival (n = 640)		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
SEX						
Female	236	2.74(1.68–4.46)	2.6e-05	201	2.69(1.58–4.58)	0.00014
Male	544	2.75(2.12–3.57)	2.2e-15	437	2.55(2.00-3.25)	4.2e-15
SATGE						
1	67	3.62(1.00-13.12)	0.036	60	2.42(0.63–9.31)	0.19
2	140	2.18(1.09–4.33)	0.023	131	1.96(0.98–3.90)	0.051
3	305	2.31(1.61–3.30)	2.4e-06	186	1.87(1.29–2.72)	8e-04
4	148	1.78(1.2–2.64)	0.0039	141	1.60(1.07–2.38)	0.02
STAGE T						
2	241	2.00(1.29–3.11)	0.0015	239	1.98(1.26–3.11)	0..0024
3	204	1.73(1.19–2.51)	0.0036	204	1.37(0.98–1.91)	0.065
4	38	2.73(1.10–6.75)	0.025	39	3.39(1.37–8.40)	0.0054
STAGE N						
0	74	1.92(0.82–4.46)	0.13	72	1.95(0.85–4.49)	0.11
1	225	3.13(1.99–4.94)	2.3e-07	222	2.84(1.83–4.42)	1.2e-06
2	121	2.56(1.62–4.05)	3.1e-05	125	2.08(1.33–3.25)	0.00098
3	76	2.1(1.22–3.61)	0.0061	76	1.84(1.02–3.33)	0.04

Please put Table 2 **at the end of the paragraph which begin with the subtitle " High ITGA5 expression affects the prognosis of gastric cancer patients exhibiting lymph node metastasis".**

Clinicopathological characteristics	Overall survival (n = 875)			Progression-free survival (n = 640)		
1 + 2 + 3	422	2.14(1.64–2.80)	9.8e-09	423	2.30(1.66–3.20)	2.8e-07
STAGE M						
0	444	2.31(1.66–3.21)	2.6e-07	443	2.12(1.55–2.91)	1.6e-06
1	56	2.66(1.45–4.88)	0.0011	56	1.69(0.92–3.12)	0.088
Lauren classification						
Intestinal	320	2.84(2.07–3.90)	1.6e-11	263	2.70(1.74–4.20)	4.2e-06
Diffuse	241	2.00(1.33–2.99)	0.00061	231	2.10(1.33–3.32)	0.0012
Mixed	32	1.88(0.68–5.20)	0.22	28	0.48(0.17–1.35)	0.16
Differentiation						
Poor	165	0.75(0.50–1.12)	0.16	121	1.39(0.79–2.45)	0.25
Moderate	67	2.16(1.08–4.30)	0.025	67	2.23(1.15–4.32)	0.015

Please put Table 2 **at the end of the paragraph which begin with the subtitle " High ITGA5 expression affects the prognosis of gastric cancer patients exhibiting lymph node metastasis".**

Table 3
The protein expression level of ITGA5 and immune marker genes of M2 and Th2 cells in STAD

		ITGA5 high(n = 28)	ITGA5 low (n = 12)	χ2	*P* value
CD163	High(n = 24)	21	3	8.750	0.003
	Low (n = 16)	7	9		
STAT6	High(n = 29)	24	5	8.174	0.004
	Low (n = 11)	4	7		
GATA3	High(n = 24)	20	4	5.079	0.024
	Low(n = 16)	8	8		

Please put Table 3 **at the end of the paragraph which begin with the subtitle " ITGA5 protein expression and M2 and Th2 immune marker genes in STAD".**

Figures

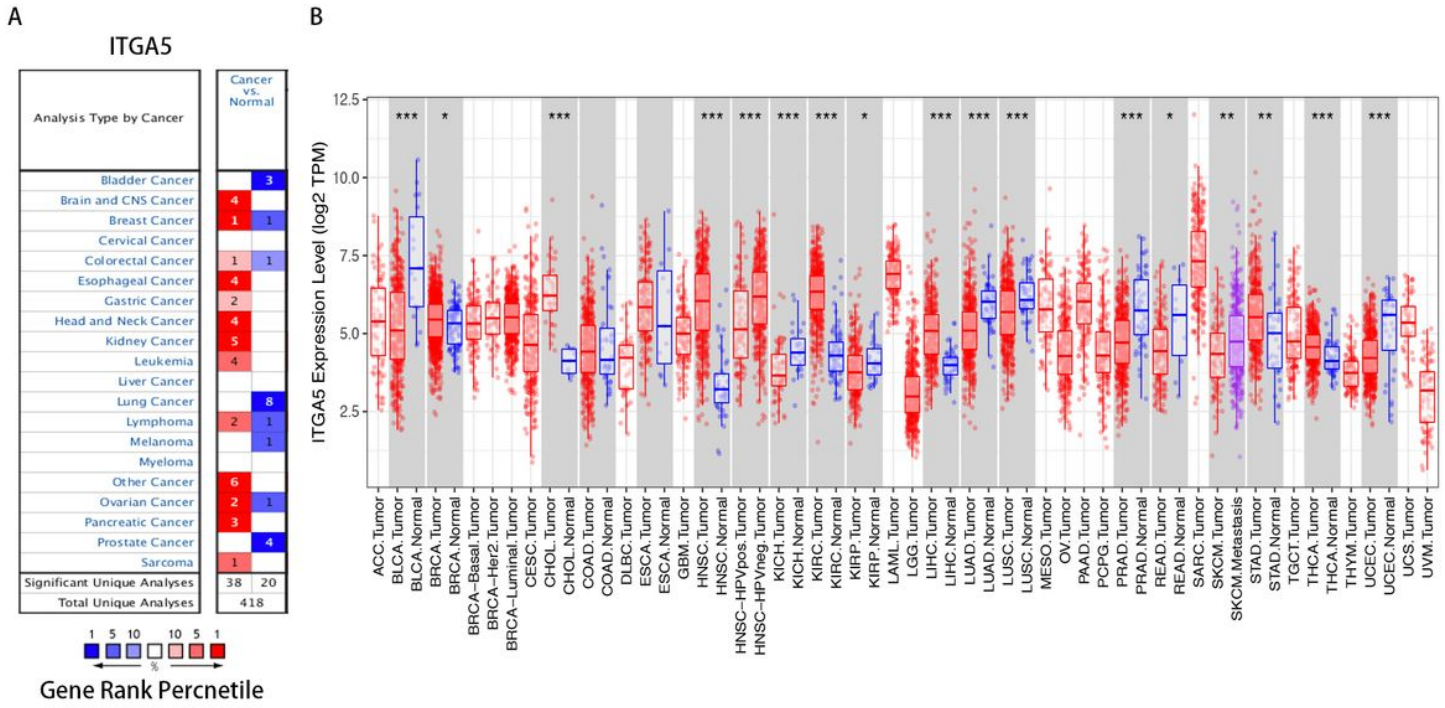

Figure 1

ITGA5 expression in different types of human cancers. (A) Different expression of ITGA5 between tumors and normal tissues in Oncomine database. The filter condition was set as: p value < 0.0001, fold change > 2, gene rank: 10%, data type: mRNA. (B) Different expression of ITGA5 between tumors and normal tissues in TIMER database (*P < 0.05, **P < 0.01, ***P < 0.001).

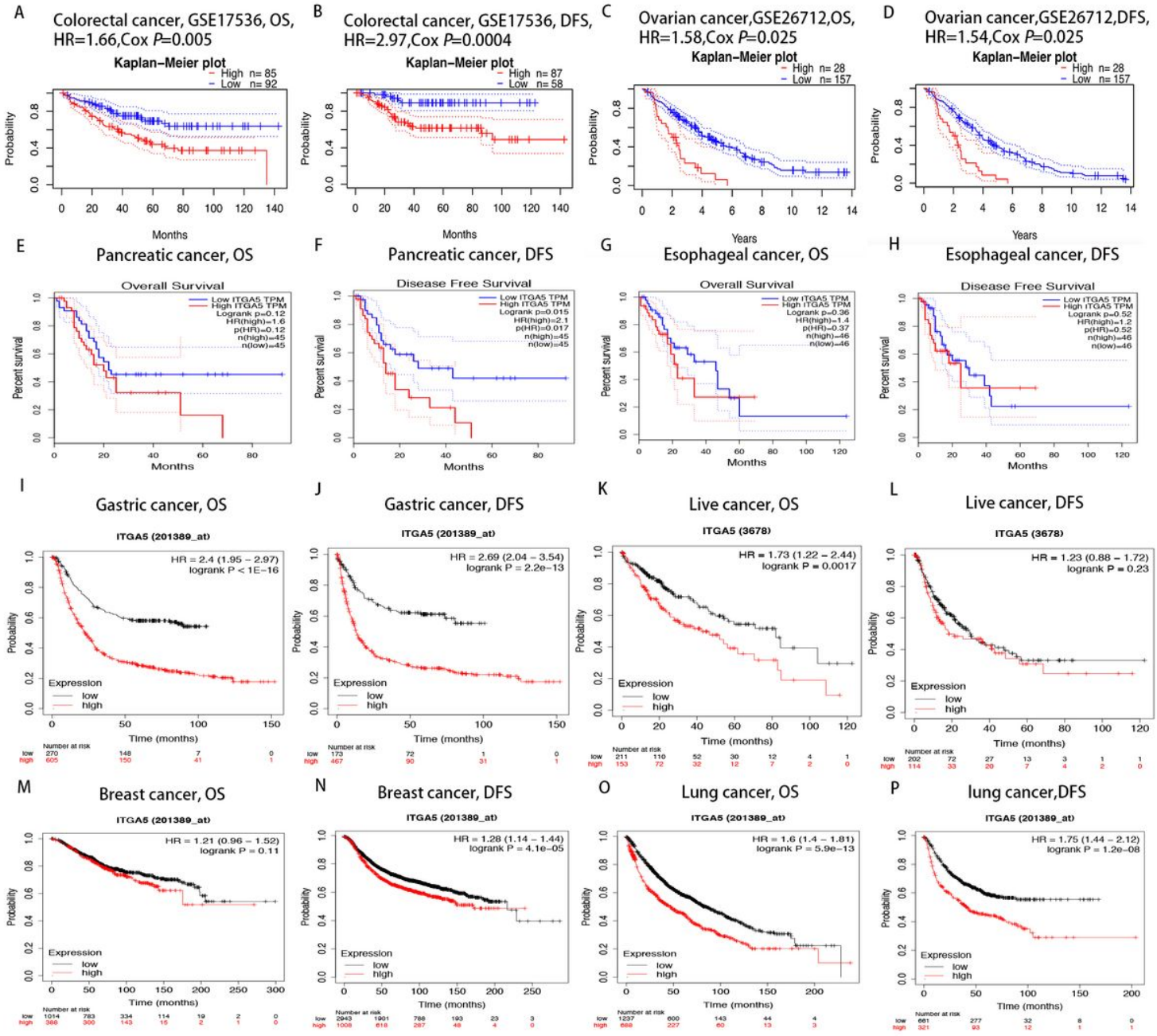

Figure 2

The prognostic value of ITGA5 expression in different types of human cancers. (A-D) Correlation between ITGA5 expression and prognosis of colorectal cancer and ovarian cancer in PrognoScan; (E-H) Correlation between ITGA5 expression and prognosis of pancreatic cancer and esophageal cancer in GEPIA2; (I-P) Correlation between ITGA5 expression and prognosis of gastric cancer, liver cancer, breast cancer and lung cancer in Kaplan-Meier plotter. OS, overall survival; DFS, disease free survival.

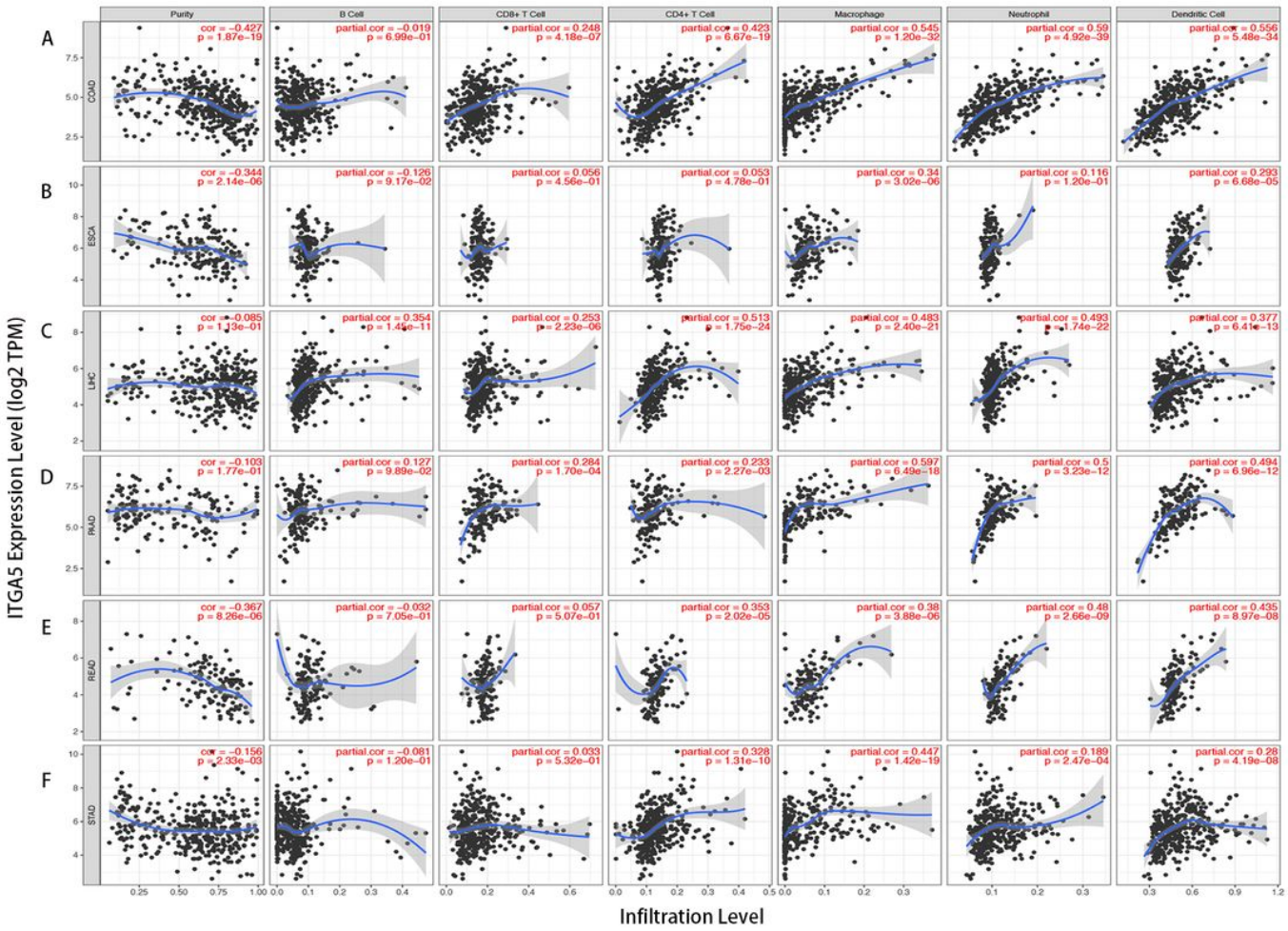

Figure 3

Correlation of ITGA5 expression with immune infiltration level in GI tumors. (A) Colon adenocarcinoma (COAD); (B) Esophageal carcinoma (ESCA); (C) Liver hepatocellular carcinoma (LIHC); (D) Pancreatic adenocarcinoma (PAAD); (E) Rectum adenocarcinoma (READ); (F) Stomach adenocarcinoma (STAD).

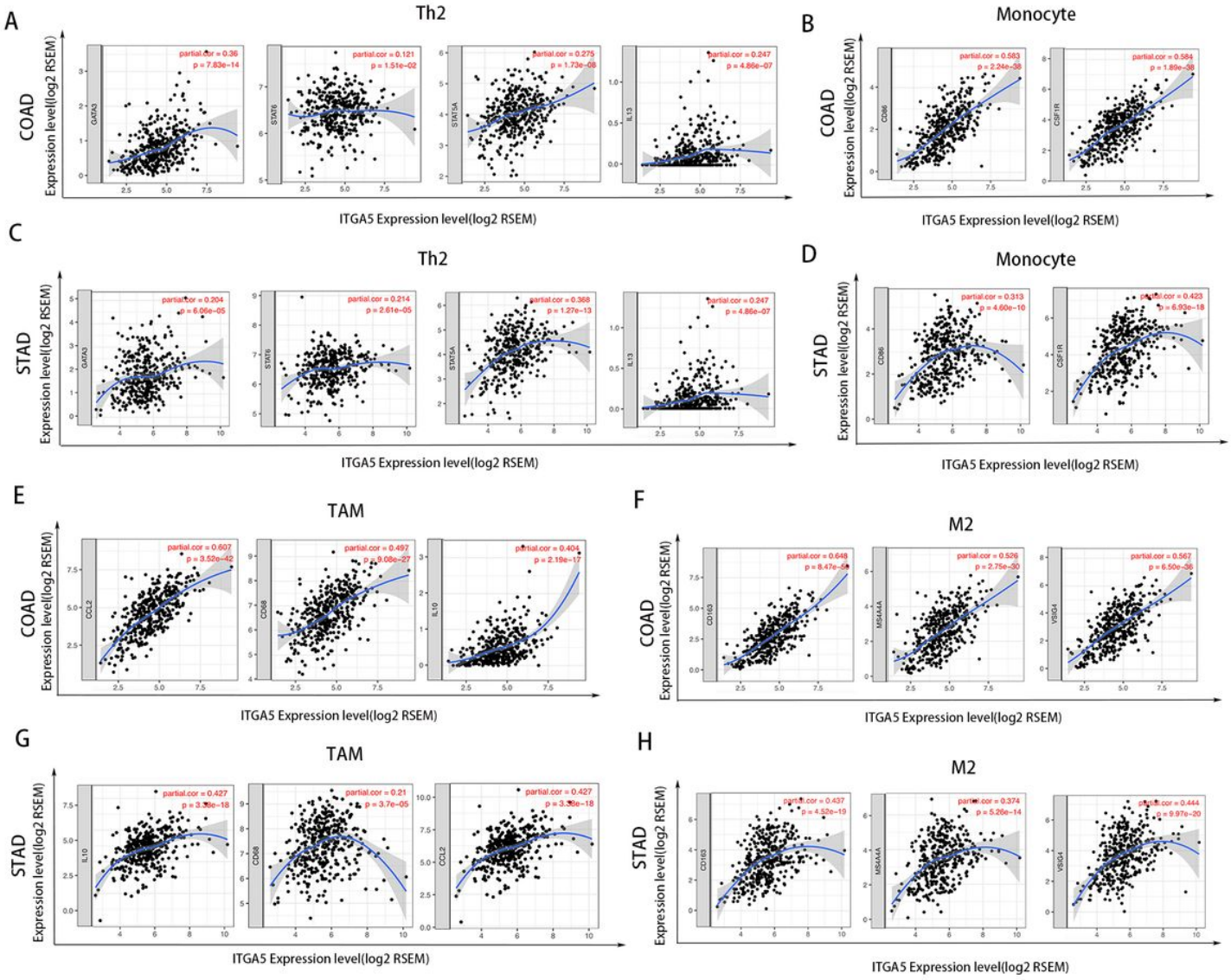

Figure 4

Correlation analysis between ITGA5 expression and immune marker set in STAD and COAD. (A) Scatterplots of correlations between ITGA5 expression and gene markers of Th2 cells in COAD; (B) Scatterplots of correlations between ITGA5 expression and gene markers of Monocyte in COAD; (C) Scatterplots of correlations between ITGA5 expression and gene markers of Th2 cells in STAD; (D) Scatterplots of correlations between ITGA5 expression and gene markers of Monocyte in STAD; (E) Scatterplots of correlations between ITGA5 expression and gene markers of TAM in COAD; (F) Scatterplots of correlations between ITGA5 expression and gene markers of M2 in COAD; (G) Scatterplots of correlations between ITGA5 expression and gene markers of TAM in STAD; (H) Scatterplots of correlations between ITGA5 expression and gene markers of M2 cells in STAD.

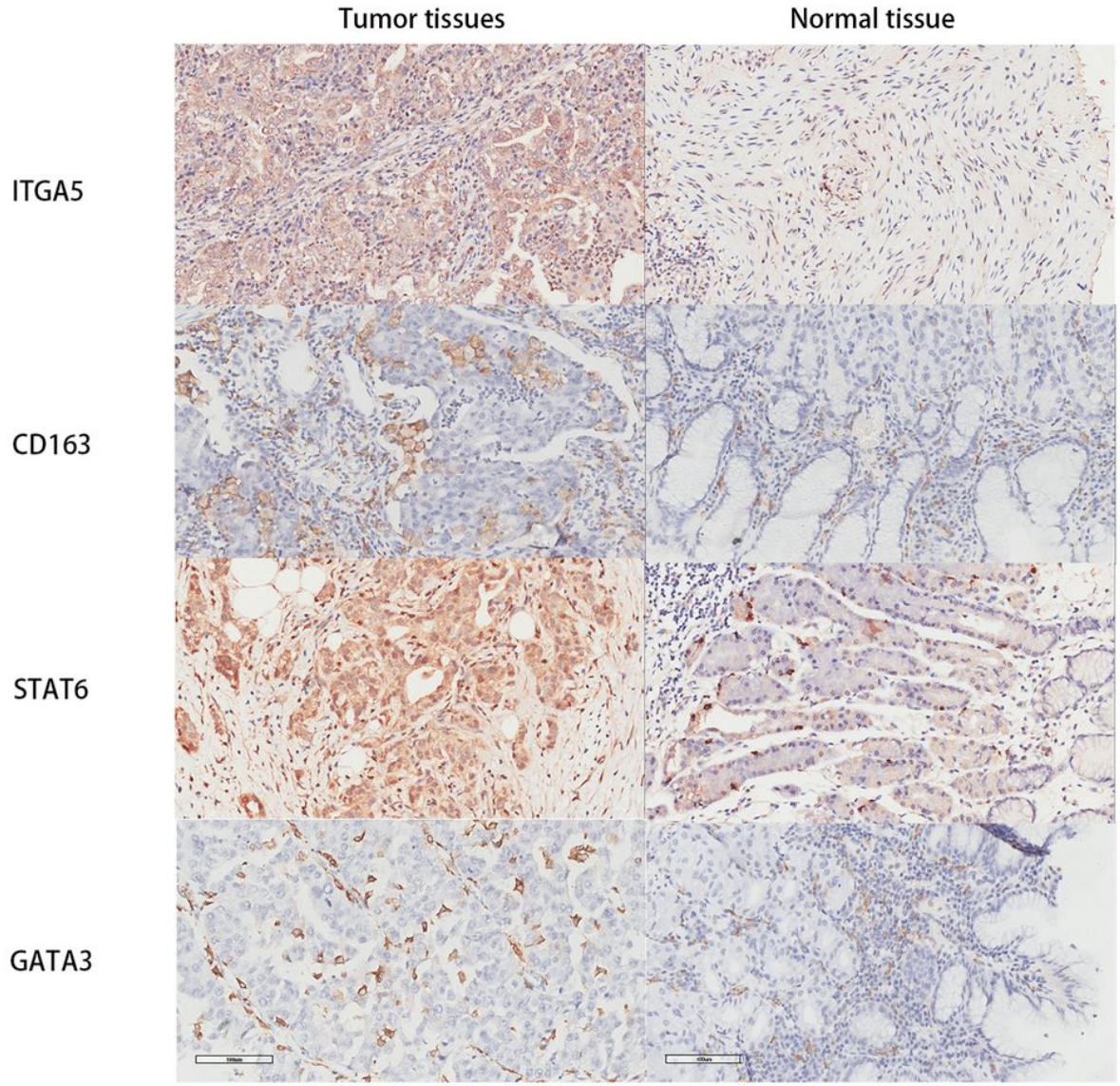

Figure 5

Representative IHC images between ITGA5 and different markers of Th2 and M2 cells in gastric cancer tissues and adjacent normal tissues (A) Representative IHC images of ITGA5 in gastric cancer tissues and adjacent normal tissues; (B) Representative IHC images of CD163 in gastric cancer tissues and adjacent normal tissues; (C) Representative IHC images of STAT6 in gastric cancer tissues and adjacent normal tissues; (D) Representative IHC images of GATA3 in gastric cancer tissues and adjacent normal tissues.

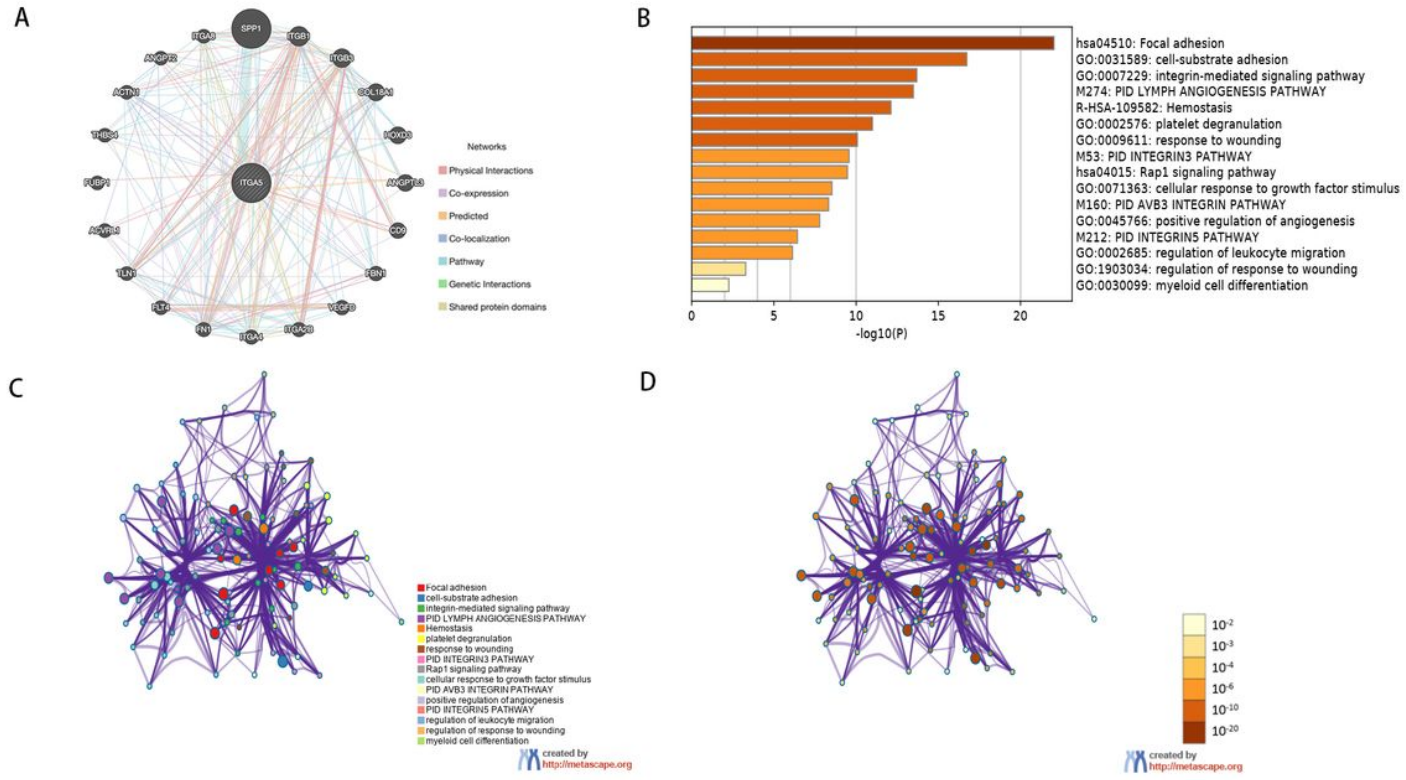

Figure 6

PPI network of ITGA5 and functional enrichment analysis; (A) PPI network of ITGA5 in GeneMANIA, different colors of the network edge indicate the bioinformatics methods applied: physical interactions, co-expression, predicted, co-localization, pathway, genetic interactions, and shared protein domains; (B) A heat map of GO and KEGG analysis of ITGA5 and it's 20 most interactive genes, orange represents the enrichment terms colored by p values; (C,D) Interactive network of the top enrichment terms colored by cluster ID, different colors represent various enrichment pathways of ITGA5 correlated genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx