High expression ITGA2 affects the expression of MET, PD-L1, CD4 and CD8 with the immune microenvironment in pancreatic cancer patients

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Abstract

Purpose:
Pancreatic cancer has a poor prognosis and is considered one of the most lethal tumors. ITGA2, a gene highly expressed in various tumor tissues, is a promising candidate for cancer therapy. The objective of this study is to assess the presence of ITGA2, MET and PD-L1 in pancreatic cancer, while also identifying ITGA2, CD4 and CD8 as potential survival indicators for patients suffering from this disease.

Experimental Design:
We examined the expression of ITGA2, MET, E-cad, PD-L1, CD4, and CD8 proteins in 62 pancreatic cancer tissue samples using multi-tissue immunofluorescence and immunohistochemistry techniques. This study examined how the protein expression of ITGA2, E-cad, and PD-L1 relate to clinicopathological features in patients diagnosed with pancreatic cancer. Additionally, the study examined the correlation between protein expression of ITGA2, CD4, and CD8 in pancreatic cancer and their relationship with clinicopathological features and prognosis.

Results
In our study, we observed the expression of ITGA2, E-cad, and PD-L1 in both tumor and stroma tissues of pancreatic cancer. Interestingly, the expression of E-cad and PD-L1 was higher in the stroma (average = 25.827% and average = 34.346%, respectively) compared to the tumor (average = 19.973% and average = 20.042%, respectively). Additionally, we found a positive correlation between ITGA2 and E-cad, as well as PD-L1 in the tumor region (r = 0.55, P < 0.001 and r = 0.51, P < 0.001, respectively), and PD-L1 in the stroma region (r = 0.51, P < 0.001). In this study, the correlation between ITGA2, E-cad, and PD-L1 with tumor marker CA-199 and lymph node metastasis was observed (P < 0.05). Additionally, the expression levels of ITGA2, CD4, and CD8 were found to be significantly higher in pancreatic cancer tissues compared to adjacent tissues (P < 0.05) as determined by immunohistochemical analysis. The protein expressions were found to be correlated with the degree of differentiation, TNM stage, lymph node metastasis, and local invasion in pancreatic cancer patients (all P < 0.05), while no significant correlation was observed with age, gender, tumor location, and tumor size (all P > 0.05). The study found that the protein expression of ITGA2 was negatively correlated with CD4 and CD8 (r = -0.344, P < 0.005 and r = -0.398, P < 0.005). The follow-up was successful in 95.0% of the 62 patients with pancreatic cancer, with a follow-up time ranging from 3 to 64 months. Furthermore, the study found that the expression of ITGA2, CD4, and CD8 was correlated with the survival time of patients after surgery (all P < 0.05). The study analyzed the clinicopathological data of 62 patients diagnosed with pancreatic cancer through univariate Cox regression analysis. The findings indicated that the prognosis of patients was associated with various factors such as histological grade, TNM stage, local invasion, lymph node metastasis, and the expression intensity of ITGA2, CD4, and CD8 (all P < 0.05). However, multivariate regression analysis highlighted that only local invasion was an independent prognostic factor for overall survival (P < 0.05).

Conclusions
ITGA2 has been identified as a potential target for the treatment and prevention of pancreatic cancer. Studies have shown that increased expression of ITGA2 is associated with poor prognosis in pancreatic cancer patients, and may impact the immune microenvironment by affecting the expression of PD-L1, CD4, and CD8. This suggests that ITGA2 could serve as a valuable entry point for developing new therapies for pancreatic cancer.

Background
Pancreatic cancer (PC) is a highly lethal tumor with a poor prognosis, ranking as the fourth or fifth most common cause of cancer death in developed countries [1]. Unfortunately, the incidence of pancreatic cancer is on the rise, with projections suggesting it will become the second leading cause of cancer deaths by 2020 [2]. Despite significant advances in cancer research, pancreatic cancer remains a formidable and often fatal disease. The latest epidemiological data shows that in the United States, 55,440 individuals were newly diagnosed with pancreatic cancer, with 44,330 of those unfortunately passing away from the disease. However, in China, there has yet to be a reported epidemiological study on the national mortality rate of pancreatic cancer. When examining regional distribution, it is noted that the mortality rate of pancreatic cancer is higher in northeast and North China compared to North China, central China, South China, Northwest, and southwest China. The incidence of pancreatic cancer is higher in urban areas, with rates 2 to 3 times higher than in rural areas. Unfortunately, the survival time for pancreatic cancer patients has not improved as much as other cancer types. In fact, the survival time for pancreatic cancer has shown a slight decrease over time. This is likely due to the fact that most pancreatic cancer cases are diagnosed at an advanced stage, resulting in a low 5-year survival rate of only 8%[3]. Pancreatic cancer is characterized by KRAS mutations in 90% of patients, which are believed to be the driving
force behind the cancer's progression. Additionally, 50 to 80% of patients have inactivating mutations in TP53, CDKN2A, and SMAD4. The most prevalent form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), as confirmed by pathological results. Pancreatic cancer has a high mortality rate and poor prognosis primarily due to the fact that it is often diagnosed at an advanced stage with metastatic cells contributing to the formation of the pancreatic cancer microenvironment.

Pancreatic cancer research has increasingly focused on the tumor microenvironment. This microenvironment consists of cancer cells, other cells, and extracellular components. Stromal cells, such as pancreatic stellate cells (PSCs), regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), play a crucial role in pancreatic cancer progression. Cells, including cancer cells, are capable of secreting various extracellular components such as extracellular matrix (ECM), matrix metalloproteinase (MMP), and growth factors like transforming growth factor (TGF) in order to maintain their microenvironment. Recent research has highlighted the crucial role played by the pancreatic tumor microenvironment in the progression of PDAC, with a clear link established between the microenvironment and metastasis. The microenvironment of pancreatic cancer is characterized by two main features: dense fibrous tissue proliferation and extensive immunosuppression [6]. These properties can promote the proliferation of pancreatic cancer cells and evade immune surveillance by inhibiting anti-tumor immunity directly or inducing the proliferation and metastasis of immunosuppressive cells. The epithelial-to-mesenchymal transition (EMT) plays a crucial role in the tumor microenvironment of pancreatic cancer. Specifically, EMT is characterized by the loss of adhesion ability between cells during the process of metastasis.

EMT plays a crucial role in several pathological processes such as wound healing, tissue fibrosis, and cancer progression [7–8]. It is closely linked to the proliferation of dense fibrous tissue in the microenvironment of pancreatic cancer. Specimens from pancreatic cancer patients have shown that the EMT status is connected to portal vein invasion and lymph node metastasis [9]. Pancreatic epithelial cells have been observed to invade and enter circulating epithelial cells (CECs) in precancerous lesions and pancreatic cancer mouse models. Studies have shown that maintenance of the mesenchymal phenotype, as well as inflammation, can enhance EMT, invasiveness, and dissemination of pancreatic epithelial cells, even at the PanIN stage [10]. Intratumoral papillary mucinous neoplasm (IPMN) is another precancerous lesion that falls under the category of EMT. It is classified as low grade dysplasia (adenoma), moderate dysplasia (borderline dysplasia), and high grade dysplasia (carcinoma in situ) [11]. Early detection of IPMN is possible and it can be detected at an early stage. CECs can be detected in 88% of patients with IPMNs. RNA-seq analysis has shown that the MUC gene may drive EMT of pancreatic epithelial cells in IPMNs [12]. Tumor-associated macrophages (TAMs) and pancreatic stellate cells (PSCs) are cellular elements in the tumor microenvironment that can promote epithelial-mesenchymal transition (EMT) and cancer development. Research has found that the co-activation of ZEB1 and YAP1 can activate ITGA3 transcriptional regulation via YAP1/TEAD binding sites in pancreatic cancer cells and tissues, leading to EMT plasticity and metastasis [13]. There is increasing evidence to suggest that activated integrin signaling plays a crucial role in cell-cell adhesion, cell proliferation, and EMT. Specifically, activated α5β1 integrin has been shown to increase cell adhesion to fibronectin during EMT [14]. In recent clinical trials, Li et al. have highlighted the effectiveness of integrin antagonists, such as αvβ3 and αvβ5 integrins, in treating lung, liver, and prostate cancer. The overexpression of ITGA2 has been found to enhance cell proliferation and promote cancer cell invasion through the activation of the PD-L1/STAT3 axis [16]. In gastric cancer cells, upregulation of miR-135b-5p has been shown to reverse ITGA2-induced chemotherapy resistance and inhibit the MAPK/ERK and EMT pathways [17]. Numerous studies have suggested that integrins play a critical role in regulating tumor progression and metastasis [18]. ITGA2 plays a crucial role in regulating cell adhesion and invasion by activating various pathways such as FAK, src, paxillin, Rac, JNK, and increasing the activity of matrix metalloproteinase-2 [19]. Additionally, ITGA2 has been found to regulate the expression of PD-L1 and the tumor immune microenvironment in various tumors, making it a promising candidate gene for tumor therapy. Little is known so far about the prognostic impact of TIGA2_EMT and PD-L1 in PDAC, and there are no studies concerning expression and a possible interrelationship between ITGA2 and MET PD-L1. Targeting ITGA2 could be a novel cancer treatment strategy.

In this study, we conducted multiplex immunofluorescence and immunohistochemical analysis of ITGA2, PD-L1, E-cadherin (an epithelial-mesenchymal transition marker protein), and T cell markers CD4 and CD8 in postoperative specimens obtained from 62 patients diagnosed with pancreatic cancer. The study's findings indicate that ITGA2 expression in pancreatic cancer tissues is greater compared to stromal tissues. Additionally, ITGA2 expression is positively correlated with PD-L1 and E-cad, while being negatively correlated with CD4 and CD8 in the immune microenvironment of pancreatic cancer. High expression of ITGA2 in pancreatic cancer patients affects the status of MET and the expression of PD-L1, CD4, and CD8 in the immune microenvironment. This ultimately leads to poor patient prognosis, indicating the significant role of ITGA2 in tumor development and immune tolerance in pancreatic cancer.

Methods

samples

This study included 62 patients with pancreatic cancer who underwent resection surgeries with curative intent at the Unit of General Surgery, University - The First Affiliated Hospital of Dali. Of these patients, 6 had high differentiation, 45 had moderate differentiation, and 11 had low
Multiplexed Immunofluorescence Staining Protocol

Freshly cut pancreatic tumor tissue was subjected to deparaffinization and rehydration by incubating sections in Biodewax and Clear Solution for 10 minutes each, with three changes. The tissue was then dehydrated in pure ethanol for 5 minutes with three changes and washed in distilled water. To wash the sample, use a Rocker device and PBS (pH 7.4) three times for 5 minutes each. Choose the appropriate antigen retrieval buffer and heat extent based on the tissue characteristics. Circle the sample and block endogenous peroxidase. Then, wash the sample three times with PBS (pH 7.4) in a Rocker device for 5 minutes each and eliminate any excess liquid. Use a liquid blocker pen to mark the objective tissue. Immerse the sample in 3% H2O2 and incubate at room temperature for 15 minutes in a dark place. After washing the tissue samples three times with PBS (pH 7.4) in a Rocker device for 5 minutes each, block with serum by eliminating any excess liquid and marking the objective tissue with a liquid blocker pen. Cover the objective tissues with 10% donkey serum (if the primary antibody originated from goat) or 3% BSA (if the primary antibody originated from other sources) at room temperature for 30 minutes. For the first primary antibody, discard the blocking solution and incubate the slides overnight at 4°C with E-cad (1:1000, Servicebio Technology Co., Ltd., Wuhan, China, No: GB14076). Place the slides in a wet box with a small amount of water and use the corresponding secondary antibody marked with HRP. Wash the slides three times with PBS (pH in a Rocker device) for 5 minutes each, and then discard the liquid. To cover the objective tissue with a secondary antibody, respond appropriately to the first primary antibody in the species and incubate at room temperature for 45 minutes in dark conditions. For the CY3-TSA solution, wash slides three times with PBS (pH 7.4) in a Rocker device, 5 minutes each. Dilute the CY3-TSA (1:1000, servicebio technology co, LTD, Wuhan, China, No: G1223) solution appropriately with TBST and incubate slides with it for 10 minutes in dark conditions. Following the washing of the slides three times with TBST using a Rocker device for 5 minutes each, immerse them in EDTA antigen retrieval buffer (pH 8.0, Servicebio Technology Co., Ltd., Wuhan, China, No: G1206) for microwave treatment. Maintain the slides at a sub-boiling temperature for 8 minutes, followed by standing for 8 minutes and another sub-boiling temperature for 7 minutes. This process removes the primary antibodies and secondary antibodies combined with tissue. To prevent buffer solution from evaporating, incubate slides with the second primary antibody PD-L1 (1:3000, Servicebio Technology Co., Ltd., Wuhan, China, No: GB14196) diluted with PBS appropriately overnight at 4°C in a wet box containing a small amount of water. After incubation, wash the slides three times with PBS (pH 7.4) in a Rocker device for 5 minutes each. Then, remove the liquid slightly. To cover objective tissue with secondary antibody PD-L1, the second primary antibody in the species must be appropriately responded to. The incubation should be done at room temperature for 50 minutes in dark conditions. For the FITC-TSA solution, the slides should be washed three times with PBS (pH 7.4) in a Rocker device for 5 minutes each. The slides should then be incubated with FITC-TSA solution (1:3000, servicebio technology co, LTD in Wuhan, China, No: G1222) for 10 minutes in dark conditions. After washing the slides three times with TBST in a Rocker device for 5 minutes each, immerse them in EDTA antigen retrieval buffer (pH 8.0, Servicebio Technology Co., Ltd., Wuhan, China, No: G1206) and maintain at a sub-boiling temperature for 8 minutes. Stand for 8 minutes and then repeat the sub-boiling temperature for 7 minutes to remove the primary and secondary antibodies combined with tissue. To prevent buffer solution from evaporating, incubate the third primary antibody ITGA2 (1:200, biosis, Beijing, China, No: BSM-52613R) overnight at 4°C in a wet box containing a little water. Afterwards, wash the slides three times with PBS (pH 7.4) in a Rocker device for 5 minutes each and then discard the liquid. Finally, incubate the third corresponding secondary antibody marked with CY5 (1:500, servicebio technology co, LTD in Wuhan, China, No: G1232). Cover objective tissue with secondary antibody (appropriately respond to second primary antibody in species), incubate at room temperature for 50 min in dark condition. DAPI (servicebio technology co, LTD, Wuhan, China, No: G1012) counterstain in nucleus: incubate with DAPI solution at room temperature for 10 min, kept in dark place. Spontaneous fluorescence quenching: wash slides three times with PBS (pH 7.4) in a Rocker device, 5 min each. Eliminate obvious liquid, incubate slides with spontaneous fluorescence quenching reagent for 5 min, then wash slides under flowing water for 10 min. Mount the sample by washing it three times with PBS (pH 7.4) in a rocker device for 5 minutes each time. After washing, remove excess liquid and then coverslip the sample with an anti-fade mounting medium. Images were detected and collected using a slice scanner. DAPI was excited by a UV wavelength of 330–380 nm and emitted a blue glow at 420 nm. FITC was excited by a wavelength of 465–495 nm and emitted a green glow at 515–555 nm. CY3 was excited by a wavelength of 510–560 nm and emitted a red glow at 590 nm. CY5, which was originally red, was set to emit a pink glow at an excitation wavelength of 608–648 nm and an emission wavelength of 672–712 nm in order to distinguish it from CY3.

Immunofluorescence analysis

The tissue slices were placed on the PANNORAMIC panoramic slice scanner (3DHISTECH, Hungary) and moved gradually under the scanner’s lens. During this process, imaging was performed, and all the tissue information on the slices was scanned and imaged to create a folder containing all the tissue information. After opening the CaseViewer2.4 software from 3DHISTECH (Hungary), observations can be made at a
magnification of 1-400 times. The Indica Labs-HighPlex FL v3.1.0 module in the Halo v3.0.311.314 analysis software was utilized for quantifying the number of positive and total cells in the target area, with the positive rate (%) being subsequently calculated.

**Immunohistochemical**

Postoperative specimens were collected from pancreatic cancer patients and embedded in paraffin for routine analysis. Immunohistochemical methods were used, with serial sections made at a thickness of 4µm. After dehydration and hydration, the sections were repaired with sodium citrate antigen, blocked with goat serum, blocked with peroxide, added with the first antibody ITGA2 monoclonal antibody (1:400, bioss, Beijing, China, No: BS-M-05259R), CD4 monoclonal antibody (1:50, bioss, Beijing, China, No: bs-02659) under high temperature and high pressure environment, and finally stained with DAB. In this study, hematoxylin was used for blue reverse staining, followed by dehydration and fixation. Cells expressing ITGA2 were identified by brownish yellow staining in either the cell membrane or cytoplasm. For each section, we randomly selected five fields and averaged the results. We then divided the area of positive cells into three grades: weak positive (+) if it was ≤ 5%-25%, positive (++) if it was ≥ 25%-50%, and strong positive (+++) if it was > 50% [20]. Positive cells were identified by brown staining in the membrane or cytoplasm and were counted as CD4+ T cells and CD8+ T cells. The study measured the presence of CD4+ and CD8+ T cells in relation to tumor cells, with the results categorized as weakly positive (+) for ≤ 10/100 tumor cells, positive (++) for > 10–20/100 tumor cells, and strong positive (+++) for > 20/100 tumor cells, with three grades in total [21]. The staining results were interpreted by three pathologists in a double-blind manner, and the section numbers were randomly determined, without any correspondence to the clinicopathological data.

**Statistical analysis**

In this study, the \( x^2 \) test was utilized to examine the differences in categorical variables among groups of patients. Additionally, the Wilcoxon rank-sum test was employed to detect differences in continuous variables between groups of patients, due to the non-normal distribution of the data (as determined by the Kolmogorov-Smirnov test). To analyze correlations between continuous variables, we utilized Spearman’s rank correlations. The Kaplan-Meier method was used to estimate the overall survival (OS). OS was defined as the time interval from the start of treatment or surgery until the patient's death from any cause or the last known date of being alive. Censoring of OS data was done at 5 years. To determine the difference in survival between groups, a log-rank test was performed. The OS data was analyzed using multivariate models of Cox regression. P value of 0.05 was considered significant for all analyses. Statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA) and JMP Pro 15 software (SAS Institute, Cary, NC, USA). Hypothesis testing was conducted at a two-sided significance level with a set to 0.05.

**Result**

**The expression of ITGA2, PD-L1, and E-cad in pancreatic cancer samples was analyzed by multiplex immunofluorescence**

In the study analyzing 62 postoperative specimens of pancreatic cancer, it was found that on average, ITGA2+ cells density were expressed in 79.156% of pancreatic cancer tissues, while E-cad+ and PD-L1+ cells density were expressed in 19.973% and 20.042% respectively (Fig. 1A, C). In the stroma, the average expression of ITGA2+, E-cad+, and PD-L1+ cells density was 70.752%, 25.827%, and 34.346% respectively (Figs. 1B, D). Interestingly, the density of ITGA2+ cells density was not found to be correlated with age, sex, tumor marker CA125, or tumor size. In this study, ITGA2+ cells density were found to be significantly correlated with CA-199 (P = 0.004), CEA (P = 0.03), TNM stage (P = 0.037), lymph node metastasis (P = 0.001) and local invasion (P = 0.02), indicating its importance in the diagnosis and growth of pancreatic cancer. On the other hand, E-cad+ cells density was not found to be correlated with age, sex, CEA, CA-125, tumor size, and local invasion. However, it was significantly correlated with CA-199 (P = 0.013), TNM stage (0.023), and lymph node metastasis (P = 0.003). The density of PD-L1+ cells density that exhibit regulatory T cell immunity showed significant associations only with CA-199 (P = 0.014), CEA (P = 0.032), and lymph node metastasis (P = 0.009) in cases of pancreatic cancer, as shown in Table 1. To better understand the difference in PD-L1 cells density expression between the tumor and stroma areas in pancreatic cancer, we compared the density of positive cells in both regions. Our findings revealed that the expression of PD-L1 cells density in the stroma area was significantly higher than that in the tumor area (P = 0.012, Fig. 2F). This comparison is crucial for the treatment of pancreatic cancer. Our findings suggest that there is a significant correlation between ITGA2+ cells in the tumor area and PD-L1+(\( r= \rho <0.001 \)) and E-cad+(\( r= \rho <0.001 \)) (Fig. 2A,B,C). Additionally, we observed a positive correlation between ITGA2+ cells (\( r= \rho <0.001 \)) and E-cad+ (\( r= \rho <0.001 \)) with PD-L1+ cells in the stromal region (Fig. 2D,E), indicating a regulatory relationship among them.

**Table 1. Relationship between ITGA2+ cells, E-cad+ cells, PD-L1+ cells and clinicopathological features**
Expression of ITGA2, CD4 and CD8 in pancreatic cancer tissues and adjacent tissues

To investigate whether ITGA2 regulates immune function in pancreatic cancer, we conducted ITGA2, CD4, and CD8 assays on postoperative specimens from 62 patients with pancreatic cancer. The ITGA2 immunohistochemical positive reaction products were predominantly brown-yellow or tan, and were mainly distributed in the cell membrane and cytoplasm (Fig. 3A). CD4 and CD8 were mainly distributed in the cytoplasm and cell membrane (Fig. 3B, C).

In cancer tissues, the weak expression rate of ITGA2 was 25.8% (16/62), moderate intensity expression rate was 22.6% (14/62), and high expression rate was 51.6% (32/62). On the other hand, in adjacent tissues, the weak expression rate of ITGA2 was 54.8% (34/62), moderate expression rate was only 1.6% (1/62), and high intensity expression rate was 0%. The study found that in cancer tissues, the weak expression rate of CD4 was 46.8% (29 out of 60), while the moderate expression rate was 32.3% (20 out of 62), and the high expression rate was 21% (13 out of 62). In paracancerous tissues, the weak expression rate of CD4 was 38.75% (24 out of 62), while the moderate and high intensity expression rates were 0.

In cancer tissues, CD8 expression was weak in 48.4% (30/62) of cases, moderate in 38.7% (24/62) of cases, and high in 12.9% (8/62) of cases. In adjacent tissues, the weak expression rate was 29% (18/62) and there were no cases of moderate or high intensity expression.

The expression rates of ITGA2, CD4, and CD8 were found to be significantly higher in cancer tissues compared to adjacent tissues. Statistical analysis showed that the difference was significant (P < 0.05). Moreover, the protein expression level of ITGA2 in pancreatic cancer tissues was negatively correlated with the protein expression levels of CD4 and CD8 (r = -0.344, P < 0.05 and r = -0.398, P < 0.05, respectively). The study found that as the expression level of ITGA2 increased, the expression level of CD4 and CD8 protein decreased. This correlation suggests that ITGA2 may play a significant role in the formation of a tumor’s immunosuppressive microenvironment, as shown in Fig. 4A and 4B.

Correlation between the expression levels of ITGA2, CD4, CD8 and clinicopathological parameters in pancreatic cancer tissues

Pancreatic cancer exhibits high expression of ITGA2, however, the expression of CD4 and CD8 gradually decreases with tumor progression, rendering immunotherapy for advanced pancreatic cancer ineffective. In addition, we conducted further analysis on the expression of ITGA2, CD4, and CD8 in pancreatic cancer tissues, as well as their correlation with various clinicopathological parameters such as gender, age, tumor size, tumor location, histological grade, TNM stage, lymphatic metastasis, and local invasion. Our findings shed light on the potential significance of these markers in predicting clinical outcomes. The study findings indicate that the levels of ITGA2, CD4, and CD8 in tumor tissues are significantly associated with histological grade, TNM stage, lymph node metastasis, and local invasion (P < 0.05).

However, no
significant correlation was found with other clinicopathological parameters as shown in Table 2. In this study, univariate analysis of Cox model was conducted to determine the factors affecting the prognosis of patients with pancreatic cancer. The results revealed that histological grade, TNM stage, lymph node metastasis, local invasion, and the expression of ITGA2, CD4, and CD8 in tumor tissues were all associated with patient prognosis. However, upon conducting multivariate analysis, only local invasion was found to be an independent prognostic factor for overall survival time in patients with pancreatic cancer. These findings are presented in Table 3.

Table 2

The relationship between the expression of ITGA2, CD4 and CD8 in pancreatic cancer tissues and the clinicopathological features

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Table 3
Univariate and multivariate analysis of Cox model for clinical prognostic factors of pancreatic cancer (n = 62)

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<th>Factor</th>
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<th>Multivariate</th>
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<td>HR(95%CI)</td>
<td>P</td>
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<tr>
<td>Gender(Male vs Female)</td>
<td>1.138(0.598–2.166)</td>
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<tr>
<td>Age(years) (≤ 60 vs 60)</td>
<td>0.652 (0.352–1.208)</td>
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<td>Tumor size(cm)(≤ 2, ≥ 2)</td>
<td>1.126(0.614–2.064)</td>
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<td>Tumor site(head vs body and tail)</td>
<td>0.766(0.398–1.487)</td>
<td>0.426</td>
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<td>Differentiation(well,moderate,Poor)</td>
<td>0.557(0.323–0.963)</td>
<td>0.036</td>
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<td>TNM staging(I-II vs III-IV)</td>
<td>2.249(1.166–4.337)</td>
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<td>Lymph metastasis(Yes vs No)</td>
<td>0.524(0.276–0.966)</td>
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<td>ITGA2 expression(+ vs ++ vs +++ )</td>
<td>1.521(1.003–2.306)</td>
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<td>Partial invasion(Yes vs No)</td>
<td>0.092(0.022–0.383)</td>
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<td>CD4 expression(+ vs ++ vs +++ )</td>
<td>0.472(0.303–0.735)</td>
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<tr>
<td>CD8 expression(+ vs ++ vs +++ )</td>
<td>0.403(0.233–0.696)</td>
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**Prognostic Significance of ITGA2, CD4 and CD8 in Pancreatic Cancer**

This study evaluated the overall survival (OS) of pancreatic cancer patients by analyzing the expression of ITGA2, CD4, and CD8. The study had a success rate of 95.0% in following up with 62 patients with pancreatic cancer. The follow-up period ranged from 3–64 months, with an average survival time of (12.6 ± 10.1) months. The study found that patients with weak ITGA2 expression had a median survival of 9.5 months (range 4–15). Patients with moderate ITGA2 expression had a median survival of 9 months (range 9–20), while those with strong ITGA2 expression had a median survival of 9 months (range 5–13). Patients with strong positive ITGA2 expression had a slightly longer median survival of 10 months (range 5–15). The study found that patients with strong ITGA2 expression had a significantly lower 5-year survival rate compared to those with weak or moderate ITGA2 expression (P = 0.033, Fig. 4C). Furthermore, patients with weak CD4 expression had a median survival time of 9 months (range: 5–12 months), while those with moderate CD4 expression had a median survival time of 11 months (range: 4–14 months), and patients with strong CD4 expression had a median survival time of 16 months (range: 8–22 months). According to Fig. 4D, patients with strong CD4 expression had a significantly higher 5-year survival rate compared to those with weak and moderate CD4 expression (P = 0.001). The median survival time for patients with weak CD8 expression was 9 (5,14) months, while those with moderate CD4 expression had a median survival time of 13 (5,22) months. Patients with strong CD8 expression had a median survival time of 10 (8,17) months. The study found that patients with strong CD8 expression had a significantly higher 5-year survival rate compared to those with weak or moderate CD8 expression (P = 0.002, Fig. 4E).

**Discussion**

The development of tumors is influenced by various factors, including the tumor microenvironment. This environment promotes the continuous proliferation of tumor cells, the generation of new blood vessels, invasion and metastasis, and immune escape. Therefore, the generation of tumor cells is not solely attributed to a single factor, but is a result of multiple factors working together [22]. The relationship between ITGA2 and cancer has been a popular research topic in recent years. Currently, it has been confirmed that the FAK/ATK signaling pathway is associated with the epithelial-mesenchymal transition (EMT) of various malignant tumors, including esophageal cancer, primary liver cancer, and pancreatic cancer [23]. In esophageal malignant tumors, ITGA2 overexpression promotes tumor growth, invasion, and migration. However, by silencing or knocking out the ITGA2 gene, the FAK/AKT pathway can be inhibited, and the EMT phenotype can be downregulated [24]. Pleckstrin homology like domain family A member 1 (PHLDA1) plays a crucial role in promoting the migration and proliferation of colon cancer cells by regulating the expression level of ITGA2 in colonic malignant tumors. Furthermore, ITGA2 has been found to be associated with the progression of acral melanoma and the clinical prognosis of patients suffering from the disease [26]. ITGA2, a downstream effector molecule of the HMGA2-FOXL2 pathway, is implicated in early distant metastasis of tumor cells in gastric malignant tumors [27]. While ITGA2 has been shown to promote cancer in a variety of tumors, its role in immune regulation within the tumor microenvironment of pancreatic cancer has yet to be confirmed.
The study enrolled 62 patients with newly diagnosed resectable pancreatic cancer. Multiplexed Immunofluorescence was used to detect the expression of ITGA2, E-cad, and PD-L1. Given the association between aging, invasion, and metastasis of pancreatic cancer, ITGA2 mutation should be considered as important as CDKN2A, BRCA1/2, and PALB2 mutations in the diagnosis and treatment of pancreatic cancer. In our study, we investigated the expression of EMT markers E-cad and PD-L1 in the same sample. Surprisingly, we found that both markers were expressed mainly in the stroma. Specifically, PD-L1 expression was not significantly high, with 20% in the tumor area and 34% in the stroma. This expression level is lower than the 50% observed in lung cancer (28), which could explain the limited efficacy of current anti-PD-1 immunotherapy in pancreatic cancer. Our analysis revealed a positive correlation between ITGA2, E-cad, and PD-L1 in both the tumor and stroma. This reinforces the crucial role played by the integrin family in the regulation of metastasis and EMT plasticity in pancreatic cancer (13). Additionally, the overexpression of ITGA2 activates the STAT3 signaling pathway, leading to the up-regulation of PD-L1 expression and promoting malignant tumor invasion (29). ITGA2, E-cad, and PD-L1 have been found to be associated with pancreatic cancer tumor markers CA199 and lymph node metastasis. CA199 is a specific marker used in the diagnosis of pancreatic cancer and is widely used in clinical practice. Additionally, if CEA + CA125+/CA19-9 > 1000 U/mL is present, it indicates a poor outcome for pancreatectomy in pancreatic cancer patients (30). The regulation of EMT by ITGA2 plays a crucial role in the growth of pancreatic cancer. Additionally, ITGA2 also regulates the expression of PD-L1 in pancreatic cancer, which leads to changes in the immune microenvironment and further promotes the progression and metastasis of the cancer. Therefore, understanding the role of ITGA2 in these processes is of great significance in the development of effective treatments for pancreatic cancer.

To investigate the effect of ITGA2 on the immune microenvironment of pancreatic cancer, we conducted immunohistochemistry to detect the expression of ITGA2, CD4 + T cells, and CD8 + T cells in tumor tissues. We also analyzed the relationship between these markers and the clinicopathological characteristics and prognosis of the patients. Our study revealed a significant correlation between ITGA2 and histological grade, TNM stage, lymph node metastasis, and local invasion (P < 0.05). These findings suggest that ITGA2 may have a pro-tumor effect in the development and occurrence of pancreatic tumors. Regulatory T cells (Treg cells) are a subset of CD4 + T cells that possess negative regulatory functions. They are capable of secreting various immunosuppressive factors, such as TGF-β, IL-10, and CCL5, within tumor cells, thereby contributing to the formation of the tumor immune microenvironment (31). Research has indicated that Treg cells have the ability to hinder the function of CD4 + T and CD8 + T cells. A significant quantity of Treg cells can be found in the tumor tissues and peripheral blood of pancreatic cancer patients, which correlates with a poor prognosis (32). Moreover, CD4 + T cells and CD8 + T cells play an essential role in the anti-tumor response of various cancers, including colorectal cancer and breast cancer (33). In the tumor immune microenvironment, the number of CD4 + T and CD8 + T cells decreases gradually with tumor development, leading to an imbalance in immune function and eventual immune escape (34). Furthermore, recent research has indicated that interleukin-35 (IL-35) is highly expressed in pancreatic cancer tissues and is closely associated with the prognosis of pancreatic cancer patients (35). Treg cells secrete IL-35 which can activate GP130-STAT1 signaling pathway, leading to overexpression of ICAM1 and secretion of CCL5. This further mediates the recruitment of monocytes and macrophages into tumor tissues, promoting the generation and metastasis of tumor neovas (36). Our study found that the expression of CD4 + and CD8 + T cells in pancreatic cancer tissues was significantly reduced with increased expression of ITGA2. This suggests that ITGA2 not only promotes the occurrence and development of pancreatic cancer, but also has an immunosuppressive function in the tumor immune microenvironment that promotes the progression of pancreatic cancer. Further studies are required to determine the correlation between ITGA2 and Treg cells in promoting the formation of an immunosuppressive microenvironment, as well as their role in the development and occurrence of tumors. Additionally, the underlying mechanisms of this relationship should also be investigated. The study included clinicopathological data of pancreatic cancer and the expression of ITGA2, CD4 + T and CD8 + T cells in tumor tissues for Cox model analysis. Results from the univariate analysis revealed that the expression of ITGA2 + cells was significantly associated with overall survival time, as well as the expression of CD4 + and CD8 + T cells, degree of tissue differentiation, TNM stage, lymphatic metastasis, and local invasion in pancreatic cancer. The multivariate analysis results indicated that local invasion was the only independent prognostic factor for overall survival in pancreatic cancer. Although the expression level of ITGA2 + cells in pancreatic tumor tissues was not found to be an independent prognostic factor for patients, it is still considered a potential prognostic indicator for patients.

In our study, we observed the expression of ITGA2, E-cad, and PD-L1 in both tumor tissues and stroma of patients with pancreatic cancer. We found a positive correlation between ITGA2 and E-cad as well as PD-L1. Additionally, we observed significant correlations between these markers and CA-199 levels and lymph node metastasis. In our study, we examined the expression profile of ITGA2 in pancreatic cancer and found that it was present in the majority of patients, particularly in those with moderately and poorly differentiated tumors. Additionally, we observed that the significance of ITGA2 in pancreatic cancer was influenced by factors such as lymph node status, degree of differentiation, and local invasion status. Further experimental verification is needed to confirm the immunosuppressive role of ITGA2 in the immune regulation of pancreatic cancer microenvironment, as there is a negative correlation between ITGA2 and CD4 and CD8.

**Conclusion**
Taken together, ITGA2 can serve as a novel target for both the development and treatment of pancreatic cancer. Its high expression has been found to impact the expression of MET, PD-L1, CD4, and CD8 in the immune microenvironment of pancreatic cancer patients, leading to a poor prognosis.

**Abbreviations**

KRAS: Kirsten rat sarcoma viral oncogene homologue; PD-L1: Programmed cell death 1 ligand 1; E-cad: E-cadherin.

**Declarations**

**Ethics approval and consent to participate**

The Biomedical Research Ethics Committee of the School of Medicine at Dali University approved the present study [approval number: SYXK(yunnan)2018-0002]. All methods was conducted in accordance with the principles outlined in the Helsinki Declaration. Informed consent was obtained from all the participants.

**Consent for publication**

Not applicable.

**Availability of data and materials**

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

**Competing interests**

The authors declare that they have no competing interests.

**Founding**

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**Authors’ contributions**

LJ initiated the research and drafted the manuscript; YT and YC designed the project and performed the statistical analysis. YD drafted the manuscript and completed all experiments; LJ, YT and YC confirm the authenticity of all the raw data. YT, HS, YC, ZS completed surgical procedures and specimen collection. ZL, XL, ZL, and BD analyzed the data and assisted in the experiments; YT provided practical technical guidance and analyzed the data; and JH checked the language issues and assisted in the experiments. All authors read and approved the final manuscript.

**Acknowledgements**

Not applicable.

**References**


Figures
Multiple immunofluorescence staining was used to detect the expression of ITGA2, E-cad, and PD-L1 in both the tumor and stroma areas of pancreatic cancer tissue. (scale bar used was 50 µm). A: The colocalization tumor map of ITGA2, E-cad, and PD-L1 expression was obtained along with DAPI staining. Itga2-positive cells were represented in purple. Red color represents E-cad positive cells. Green represents PD-L1 positive cells; Blue is DAPI. B: This study analyzed the colocalization stroma map of ITGA2, E-cad, and PD-L1 expression with DAPI staining. C: The average density of cells expressing ITGA2 in the tumor was 79.156%, while E-cad and PD-L1 had densities of 19.973% and 20.042%, respectively. D: In the stroma, the average density of cells expressing ITGA2 was 70.752%, while E-cad and PD-L1 had densities of 25.827% and 34.346%, respectively. Abbreviations used in the study include DAPI for 4,6-diamidino-2-phenylindole, E-cad for E-cadherin, PD-L1 for programmed cell death-Ligand 1, and ITGA2 for integrin subunit alpha2.
Correlation between the expression levels of ITGA2+ cell density, E-cad+ cell density in tumor and PD-L1+ cell density in tumor and stroma in pancreatic cancer tissues.

A: Scatter plot graph illustrating that tumor ITGA2+ cell density (%) directly correlates with tumor E-cad+ cell density (%) ($r=0.55, P<0.001$).

B: Scatter plot graph illustrating that tumor ITGA2+ cell density (%) directly correlates with tumor PD-L1+ cell density (%) ($r=0.51, P<0.001$).

C: Scatter plot graph illustrating that tumor PD-L1+ cell density (%) directly correlates with tumor E-cad+ cell density (%) ($r=0.95, P<0.001$).

D: Scatter plot graph illustrating that tumor ITGA2+ cell density (%) directly correlates with stroma PD-L1+ cell density (%) ($r=0.51, P<0.001$).

E: Scatter plot graph illustrating that tumor E-cad+ cell density (%) directly correlates with stroma PD-L1+ cell density (%) ($r=0.91, P<0.001$).

F: Tumor ITGA2+ cell density (%) VS stroma PD-L1+ cell density (%) $P=0.012$.
The study found that the expression of ITGA2, CD4 and CD8 in pancreatic cancer tissues and adjacent tissues were analyzed using HIC. The left scar bar represents 100µm and the right scar bar represents 20µm. A: The results showed that ITGA2 had low, medium and high expression in pancreatic cancer and the H-score between adjacent and cancer was significant (P<0.05). B: In pancreatic cancer, CD4 exhibit low, medium, and high expression levels. The H-score between adjacent and cancer is significant (P<0.05). C: In pancreatic cancer, CD8 exhibit low, medium, and high expression levels. The H-score between adjacent and cancer is significant (P<0.05).
Figure 4

Correlation and Prognostic Significance between the expression levels of ITGA2, CD4, and CD8 in Pancreatic Cancer. A: Scatter plot graph illustrating that area of ITGA2 positive cells (%) directly correlates with number of CD4 positive cells (r = -0.344, P<0.05). B: Scatter plot graph illustrating that area of ITGA2 positive cells (%) directly correlates with number of CD8 positive cells (r = -0.398, P<0.05). C: Patients with ITGA2+++ had a shorter survival than patients with ITGA2++ and ITGA2+ (median survival: 9.5 vs 9 vs 9 months, P<0.05). D: Patients with CD4+++ had a shorter survival than patients with CD4++ and CD4+ (median survival: 16 vs 11 vs 9 months, P<0.05). E: Patients with CD8+++ had a shorter survival than patients with CD8++ and CD8+ (median survival: 10 vs 13 vs 9 months, P<0.05).

Supplementary Files

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- TableS1.docx