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Investigation of clinical application of claudin 18 isoform 2 in pancreatic ductal adenocarcinoma: A retrospective analysis of 302 chinese patients

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Summary. The malignancy of pancreatic ductal adenocarcinoma (PDAC) results from high frequency of recurrence and limited effective therapies. Targeted therapy is a promising treatment in multiple solid tumours. A new target, claudin 18 isoform 2 (CLDN18.2) was discovered in gastric and pancreatic adenocarcinoma, but more clinical evaluations of CLDN18.2 are still needed. Several CLDN18.2-targeted drugs have already been in procedure of clinical trials. Therefore, the present study aimed to explore the expression and clinical value of CLDN18.2 in PDAC by immunohistochemistry. A microarray cohort of 302 PDAC specimens and a whole-slide cohort of randomized 84 PDAC specimens were constructed. In total, 56.52% (171/302) of PDAC patients showed diverse positivity for CLDN18.2, especially in highly differentiated PDAC. About eighty-two percent (62/75) highly- and 62.61% (72/115) intermediate-differentiated PDAC showed positive for CLDN18.2, while only 10.16% (6/59) low differentiated PDAC was positive for CLDN18.2. Besides, CLDN18.2 positivity was associated with several clinicopathological characteristics, including sex (P=0.001), smoking (P=0.006), abdominal pain (P=0.021), jaundice (P=0.010), pathological differentiation (P=0.001), common bile duct invasion (P=0.010), and M stage (P=0.003). CLDN18.2-positive expression also predicts an improved survival (P=0.032) but not progression free survival (P=0.460). However, CLDN18.2 is not an independent prognostic predictor. In conclusion, CLDN18.2 may be a potential therapeutic target for PDAC and the study supplies persuasive pathological evidence for CLDN18.2-targeted therapy on PDAC patients.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most frequent malignant tumour of the exocrine pancreas (Siegel et al., 2021). Although several targeted drugs have already been used in clinical applications, the mortality remains stable. Moreover, several studies have shown that the recurrence rate remains high after pancreatectomy (Groot et al., 2019). Most patients with PDAC require postoperative therapy, even with an R0 surgical margin; however, the recurrence rate remains high (Tempero et al., 2021). This suggests that new treatment targets for PDAC are urgently needed.

Claudin 18 isoform 2 (CLDN18.2) is a claudin family protein and a transmembrane part of the tight junction (Kominsky, 2006; Zhu et al., 2019; Li, 2021). It is an alternative splicing product of the gene CLDN18 and multiple studies have identified its clinical and therapeutic value. Previous studies have also reported that it is positive in normal gastric tissue and gastric cancer (Dottermusch et al., 2019). A clinical trial (MONO study) has shown that CLDN18.2-targeted drugs, zolbetuximab (IMAB362), can be used as a single agent in the treatment of gastric adenocarcinoma (Türeci et al., 2019). Another clinical trial (FAST) has reported that zolbetuximab plus EOX (epirubicin, oxaliplatin, and capecitabine) can be applied as a first-line treatment for advanced CLDN18.2 positive gastric adenocarcinoma (Wöll et al., 2014; Lordick et al., 2021; Sahin et al.,

Abbreviations. CLDN18.2, claudin 18 isoform 2; EMT, epithelial mesenchymal transition; FFPE, formalin fixed paraffin embedded; HE, hematoxylin and eosin; OS, overall survival; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; PFS, progression free survival; PUMCH, Peking Union Medical College Hospital; TMA, tissue microarray; WHO, World Health Organization.

2021). Subsequent clinical trials have identified dozens of CLDN18.2-targeted drugs in several solid tumours, including gastric and oesophageal cancer (GOAC), ovarian cancer, and pancreatic cancer. Some studies have reported CLDN18.2-positivity in pancreatic cancer, including PDAC, neuroendocrine tumour, and metastatic cancer, suggesting zolbetuximab might be applied in pancreatic cancers (Wöll et al., 2014).

Although CLDN18.2-targeted drugs have been in process of clinical trials, the prognostic value of CLDN18.2 in PDAC has not been verified. A meta-analysis reported that CLDN18.2 was not associated with overall survival (OS) for gastric adenocarcinoma patients (Ungureanu et al., 2021). Two other studies also showed that CLDN18.2 was not related to OS in gastric oesophageal adenocarcinoma (GOAC) (Arnold et al., 2020; Moentenich et al., 2020). Therefore, this study aims to identify the positive ratio of CLDN18.2 in PDAC and clinicopathological association of CLDN18.2 to provide evidence that CLDN18.2 targeted drugs may be a potential treatment for PDAC.

Materials and methods

Cohort

In total, 1149 records of pancreatic lesions were identified from the Department of Pathology, Peking Union Medical College Hospital (PUMCH). After selection by inclusive and exclusive criteria, 302 patients were included in the cohort to be distributed in the tissue microarray (TMA) cohort. The inclusive criteria were: 1) pathological diagnosis is "pancreatic ductal adenocarcinoma"; 2) hematoxylin and eosin (HE) slides and corresponding paraffin blocks are acquired from the archive of the department of pathology; 3) all clinical information is available in the medical record; 4) follow-up information, including OS and progression-free survival (PFS), is intact. Other diagnoses (acinar cell carcinoma, neuroendocrine tumour, etc.) were excluded. The TMA cohort was used to identify the positive ratio of CLDN18.2 in PDAC and its clinicopathological and prognostic association. To verify CLDN18.2 expression mode in PDAC, 84 randomised PDAC specimens were enrolled for detection on whole slides (whole slide cohort). The relationship to the epithelial-mesenchymal transition (EMT) phenotype was also analysed as well. Ten pancreatic intraepithelial neoplasms (PanINs) attached to advanced PDAC were identified according to the criteria of World Health Organization (WHO) classification of tumours of the digestive system (Nagtegaal et al., 2020). These lesions were verified inconsecutively to the advanced area using serial sections. Therefore, the 10 cases were selected to evaluate the expression levels of CLDN18.2 in precancerous lesions. The flow chart is shown in Fig. 1.

Clinicopathological information was acquired from the medical records of the PUMCH archive, including general conditions (age, sex, smoking, drinking), diabetes mellitus, and digestive symptoms (abdominal

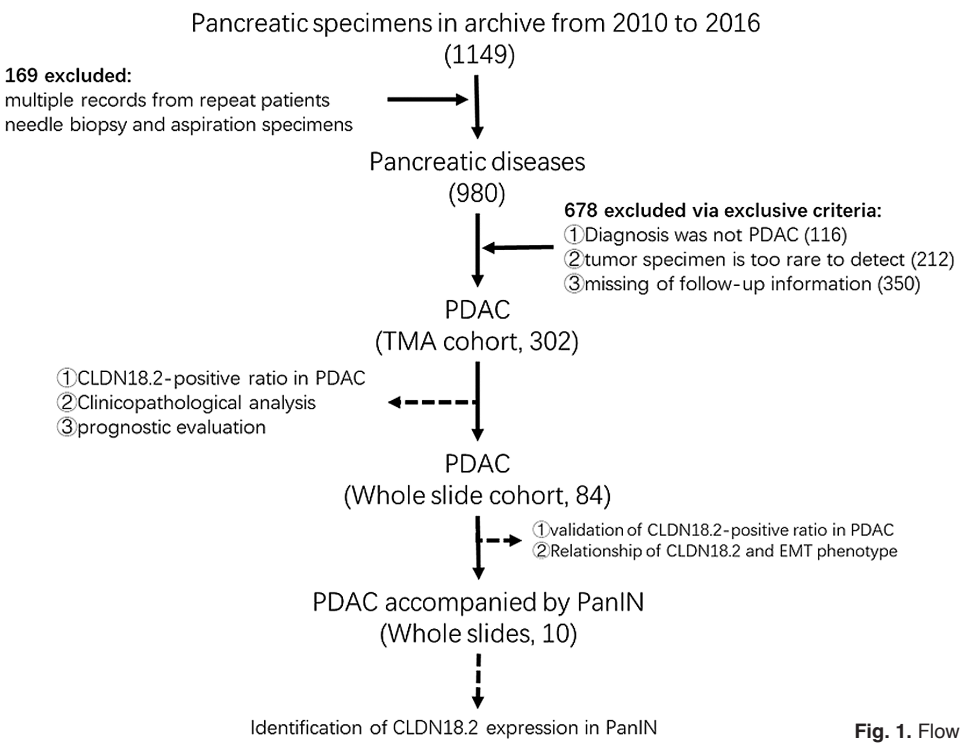

Fig. 1. Flow chart of the study of CLDN18.2 in PDAC.

pain, peritoneal irritation signs, lumbago, jaundice, abdominal mass, and weight loss). Prognostic information mainly included OS and PFS. OS was defined as the period from the day of diagnosis to the day of the patient's death. PFS was defined from the day of diagnosis to the day of tumour recurrence, metastasis, or patient death. The pathological interpretation was performed by two expert pathologists to validate the morphological characteristics. This included pathological differentiation (high, intermediate, and low differentiation), capsule invasion, endovascular embolus, common bile duct invasion, lymph node metastasis, and perineural invasion. The pathological TNM stage was also recorded. All patients provided informed consent at the beginning of the clinical intervention for research use of data or material. The present study was approved by the Ethics Committee of PUMCH.

Tissue Microarray (TMA)

Representative lesions of PDAC were annotated on HE slides and located on corresponding formalin-fixed paraffin-embedded (FFPE) blocks. The regions of the tumour and para-tumour were interpreted by two pathologists, and these regions were located as interesting sites for TMA manufacture. The TMAs were constructed using a semi-automated TMA construction system (Quick-Ray UT-06, UNITMA, Korea) according to the manufacturer's instructions.

Immunohistochemistry (IHC) and interpretation

FFPE slices were selected for immunohistochemical staining. Four-micrometre slices of PDAC were deparaffinised and hydrated. Then, the slices were subjected to antigen retrieval (pH=9.0, EDTA, high-pressure), and blocked with foetal bovine serum. The slices were then incubated with the primary antibody, including CLDN18.2 (Catalogue No. 2130WPI1A154A, WuxiDiagnostics, China), E-cadherin [Catalogue No. 3195S, Cell Signalling Technology (CST), USA], and vimentin [Catalogue No. 5741S, Cell Signalling Technology (CST), USA] at 4°C overnight. The slices were incubated with the secondary antibody at room temperature (RT) for one hour and detected using 3,30-diaminobenzidine as the chromogen. All procedures were performed according to the manufacturer's instructions (Catalogue No. PV-6000, ZSGB, Beijing, China).

The interpretation of CLDN18.2 expression was carried out by two pathologists. The staining of the tumour cell membrane was considered an authentic positive expression while other staining modes (nucleus or plasma) were nonspecific staining. A positive control of staining in gastric epithelial cells was used. The expression level of CLDN18.2 was classified into 0 (no staining), 1+ (1%–10% weakly stained cells), 2+ (10–50% intermediately stained cells), and 3+ (50–100% strongly stained cells) according to previous experience (Wöll et al., 2014). The positive ratio of CLDN18.2 was evaluated by two experienced pathologists without prior information. The 1+–3+ cases were considered positive because CLDN18.2-positive cells could be targeted for IMAB362.

Statistical analysis

All data were analysed using the SPSS software (version 22.0, IBM SPSS software). Clinicopathological characteristics were assessed using the Chi-square test and Fisher's exact test after examination with Gaussian distribution. Prognostic data were calculated using Kaplan-Meier and Cox regression tests, and survival curves were plotted using GraphPad (Version 7.0, GraphPad Software Inc., La Jolla, CA). Statistical significance was set at $p<0.05$.

Results

Demographics of PDAC patients

In the present TMA cohort, 302 patients were included in the analysis. Of all patients, the median age of the cohort was 61 years old, and 173 patients were male. One hundred and seven patients had a history of smoking, and 55 had a history of drinking. Forty patients had a previous diagnosis of diabetes mellitus. Weight loss was the most frequent manifestation in the cohort, and 168 patients complained of decrease of body weight. In addition, 150 patients complained of abdominal pain, and 35 had signs of peritoneal irritation. Sixty-three patients complained of lumbago, while 100 patients had jaundice. Radiologically, 173 tumours were located at the pancreatic head and neck, while 118 were in the body or tail. Microscopically, 59 were diagnosed as low-differentiation, 115 as intermediate, and 75 as high-differentiation. Invasion is frequent in PDAC, with 274 tumours invading the pancreatic capsule and 20 patients with endovascular tumour embolus. Common bile duct invasion was observed in 97 tumours, lymph node metastasis in 152 tumours, and perineural invasion in 55 patients. The American Joint of Cancer Committee (AJCC) pathological tumour-node-metastasis (pTNM) staging was also recorded. All clinicopathological distributions are presented in Table 1.

Clinicopathological association of CLDN18.2 in PDAC

Clinicopathological associations were analysed in the TMA cohort. The analytic results showed that sex ($P=0.001$), smoking ($P=0.006$), abdominal pain ($P=0.021$), jaundice ($P=0.010$), pathological differentiation ($P<0.001$), common bile duct invasion ($P=0.010$), and AJCC M staging ($P=0.003$) were significantly associated with CLDN18.2 expression. This showed that female patients and non-smokers tended to acquire CLDN18.2 expression. CLDN18.2-positive patients were more vulnerable to abdominal pain and jaundice. Pathologically, CLDN18.2-positive

Table 1. Clinicopathological characteristics and their relation to CLDN 18.2 expression.

Characteristics	N	CLDN 18.2 (-)	CLDN 18.2 (+)	P value
Total number	302	132 (43.71%)	171 (56.52%)	
Age				0.141
<65	199	93 (46.73%)	106 (53.27%)	
>65	103	39 (37.86%)	64 (62.14%)	
Sex				0.001
Male	173	92 (53.18%)	81 (46.82%)	
Female	129	40 (31.01%)	89 (68.99%)	
Smoking				0.006
no	195	74 (37.95%)	121 (62.05%)	
yes	107	58 (54.21%)	49 (45.79%)	
Drinking				0.234
no	247	104 (42.11%)	143 (57.89%)	
yes	55	28 (50.91%)	27 (49.10%)	
Diabetes Mellites				0.122
no	262	110 (41.98%)	152 (58.02%)	
yes	40	22 (55.00%)	18 (45.00%)	
Abdominal pain				0.021
no	147	54 (36.73%)	93 (63.27%)	
yes	150	75 (50.00%)	75 (50.00%)	
Peritoneal irritation sign				0.168
no	262	110 (41.98%)	152 (58.02%)	
yes	35	19 (54.29%)	16 (45.71%)	
Lumbago				0.107
no	234	96 (41.03%)	138 (58.97%)	
yes	63	33 (52.38%)	30 (47.62%)	
Jaundice				0.010
no	197	96 (48.73%)	101 (51.27%)	
yes	100	33 (33.00%)	67 (67.00%)	
Abdominal mass				0.527*
no	291	126 (43.30%)	165 (56.70%)	
yes	6	3 (50.00%)	3 (50.00%)	
Weight loss				0.159
no	129	62 (48.06%)	67 (28.68%)	
yes	168	67 (39.88%)	101 (60.12%)	
Pathological differentiation				<0.001
Low	59	53 (89.83%)	6 (10.17%)	
Intermediate	115	43 (37.39%)	72 (62.61%)	
High	75	13 (17.33%)	62 (82.67%)	
Tumor position				0.220
Head/Neck	173	71 (41.04%)	102 (58.96%)	
Body/Tail	118	57 (48.31%)	61 (51.69%)	
Capsule invasion				0.186
no	27	15 (55.59%)	12 (44.44%)	
yes	274	116 (42.34%)	158 (57.66%)	
Endovascular tumor embolus				0.802
no	254	107 (42.13%)	147 (57.87%)	
yes	20	9 (45.00%)	11 (55.00%)	
Common bile duct invasion				0.010
no	177	85 (48.02%)	92 (51.98%)	
yes	97	31 (31.96%)	66 (68.04%)	
Lymph node metastasis				0.410
no	122	55 (45.08%)	67 (54.92%)	
Yes	152	61 (40.13%)	91 (59.87%)	
Perineural invasion				0.191
no	219	97 (44.29%)	122 (55.71%)	
Yes	55	19 (34.55%)	36 (65.45%)	
pT stage				0.233
1	1	0 (0)	1 (100%)	
2	15	10 (66.67%)	5 (33.33%)	
3	276	119 (43.12%)	157 (56.88%)	
4	6	2 (33.33%)	4 (66.67%)	
N stage				0.175
No	123	57 (46.34%)	66 (53.66%)	
Yes	167	70 (41.92%)	97 (58.08%)	
M stage				0.003
No	248	100 (40.32%)	148 (59.68%)	
Yes	20	15 (75.00%)	5 (25.00%)	

* Calculated by Fisher exact test.

tumours were associated with high differentiation, lack of common bile duct invasion, and low frequency of distant metastasis (Table 1). The other clinicopathological characteristics showed no association with CLDN18.2 expression.

CLDN18.2 is a potential target for PDAC patients, especially for high-differentiated PDAC

The total positive ratio of CLDN18.2 in PDAC was 56.52% (171/302), suggesting that more than half of PDAC patients were suitable for CLDN18.2-targeted therapy (Fig. 2). Morphologically, all PDAC cases were classified as high-, intermediate-, or low-differentiation according to the WHO Classification of Tumours of the Digestive System. In the present cohort, 59 low-, 115 intermediate-, and 75 highly differentiated PDAC were identified. CLDN18.2 positivity was present mainly in highly differentiated PDAC (82.67%, 62/75), while the CLDN18.2-positive ratio gradually decreased in intermediate- and low-differentiated PDAC (62.61%, 72/115, and 10.17%, 6/59, respectively), with statistical significance (Fig. 3). Pathological differentiation was verified in whole slide cohort. It showed that highly differentiated PDAC patients had a high possibility of benefitting from CLDN18.2-targeted treatment.

Ten PanINs were discovered in the para-tumour area of PDAC. The results showed that all 10 PanINs were positive for CLDN18.2, while the nearby normal ductal epithelium was negative (Fig. 4). This demonstrated that CLDN18.2-targeted therapy was effective in the precancerous period.

Table 2. Prognostic value of clinicopathological characteristics in PDAC

Characteristics	OS		PFS	
	P (univariate)	P(multivariate)	P (univariate)	P(multivariate)
Age	0.253		0.984	
Sex	0.015	0.381	0.279	
Smoking	0.545		0.661	
Drinking	0.664		0.133	
Diabetes Melitus	0.589		0.481	
Abdominal pain	0.749		0.363	
Peritoneal irritation sign	0.530		0.029	0.153
Lumbago	0.190		0.034	0.046
Jaundice	0.545		0.551	
Abdominal mass	0.989		0.751	
Weight loss	0.075	0.015	0.749	
Pathological differentiation	0.014	0.139	0.561	
Tumor position	0.229		0.218	
pT stage	0.032	0.603	0.167	
N stage	0.172		0.151	
M stage	0.019	0.053	0.295	
Capsule invasion	0.477		0.226	
Endovascular thrombus	0.304		0.820	
Common bile duct invasion	0.155		0.169	
Lymph node metastasis	0.024	0.133	0.071	0.081
Perineural invasion	0.502		0.986	
CLDN18.2	0.032	0.460	0.406	

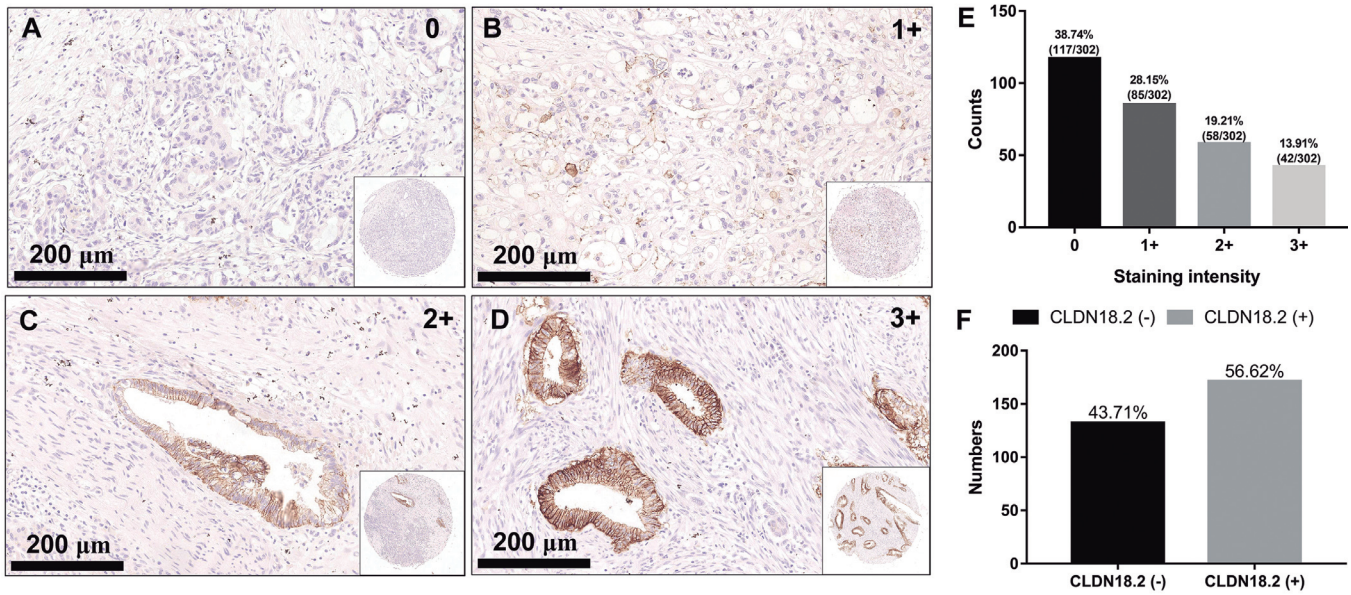

Fig. 2. The expression diversity of CLDN18.2 in PDAC. CLDN18.2 expression was divided into 0 **(A)**, 1+ **(B)**, 2+ **(C)**, and 3+ **(D)** according to a previous report(Wöll et al., 2014) and the number of each group is shown **(E)**. As the clinical trial reported that IMAB162 was effective for CLDN18.2-positive cases, so all 1+ ~3+ cases were considered positive and the ratio is shown **(F)**.

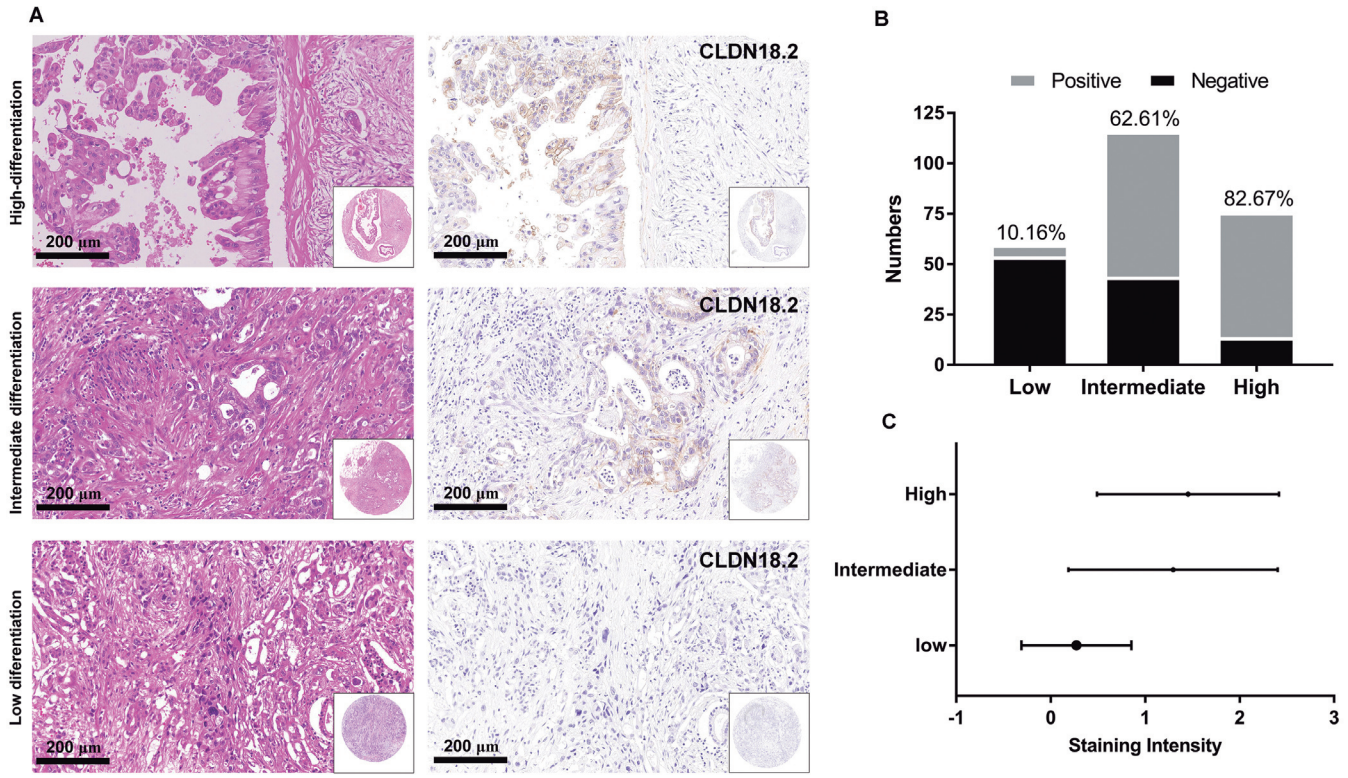

Fig. 3. Relationship of CLDN18.2 to pathological differentiation in PDAC. CLDN18.2 was associated with pathological association and the positivity can be observed in each group **(A)**. The positivity of CLDN18.2 was mainly in intermediate- and high-differentiated PDAC, with statistical significance **(B)**, and the staining intensity was stronger **(C)**.

CLDN18.2 was associated with the OS of PDAC patients but was not an independent prognostic predictor

Prognostic analysis was performed to determine the predictive value of CLDN18.2 in PDAC. The results of univariate Cox regression analysis showed that CLDN18.2 was associated with OS of PDAC patients (p=0.032), but was not a prognostic predictor for PFS in PDAC (p=0.406). This demonstrated that PDAC patients with CLDN18.2-positive expression had a longer survival (Fig. 5). Besides, sex (p=0.015), pathological differentiation (p=0.014), lymph node metastasis (p=0.024), AJCC pT stage (p=0.032), and M stage (p=0.019) were associated with OS in PDAC (Fig. 6). Peritoneal irritation signs (p=0.029) and lumbago (p=0.034) were associated with PFS in PDAC (Fig. 6). Based on the univariate analysis results, all characteristics (p≤0.100) were included in the multivariate analysis. However, CLDN18.2 was not an independent prognostic predictor of OS in PDAC (p=0.460). Only weight loss was an independent predictor of OS, and no characteristics were found to be

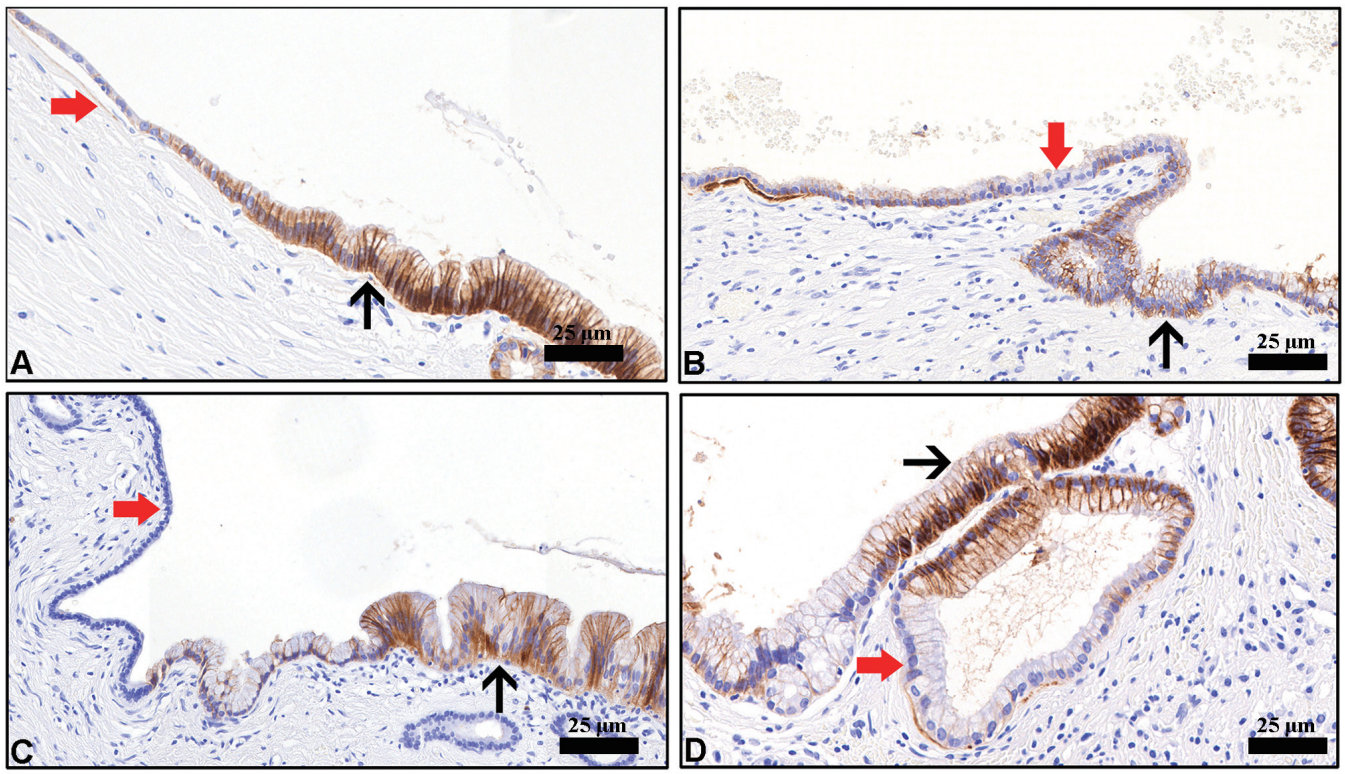

Fig. 4. The positivity of CLDN18.2 in PanIN. Two representative PanIN show that CLDN18.2 was positive for the region of PanIN (black arrow) but negative for normal ductal epithelial cells (red arrow).

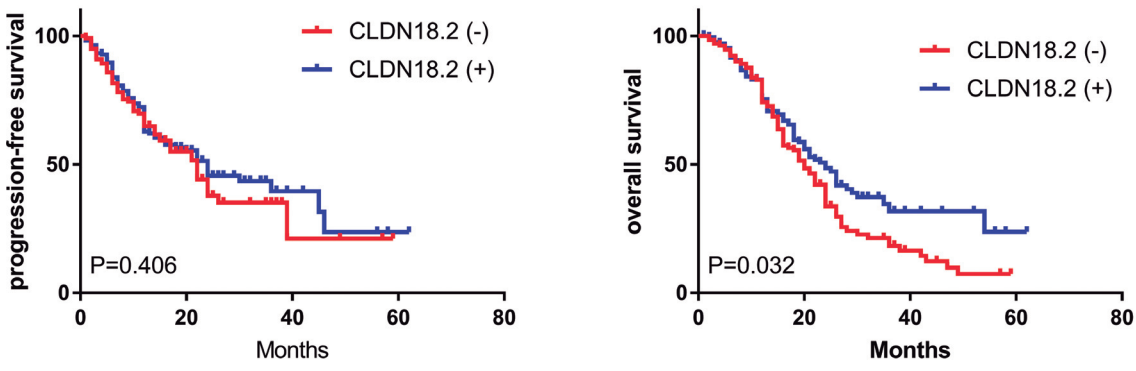

Fig. 5. The prognostic value of CLDN18.2 in PDAC. Survival curves show that CLDN18.2 was associated with OS **(B)** but not with PFS **(A)** of PDAC.

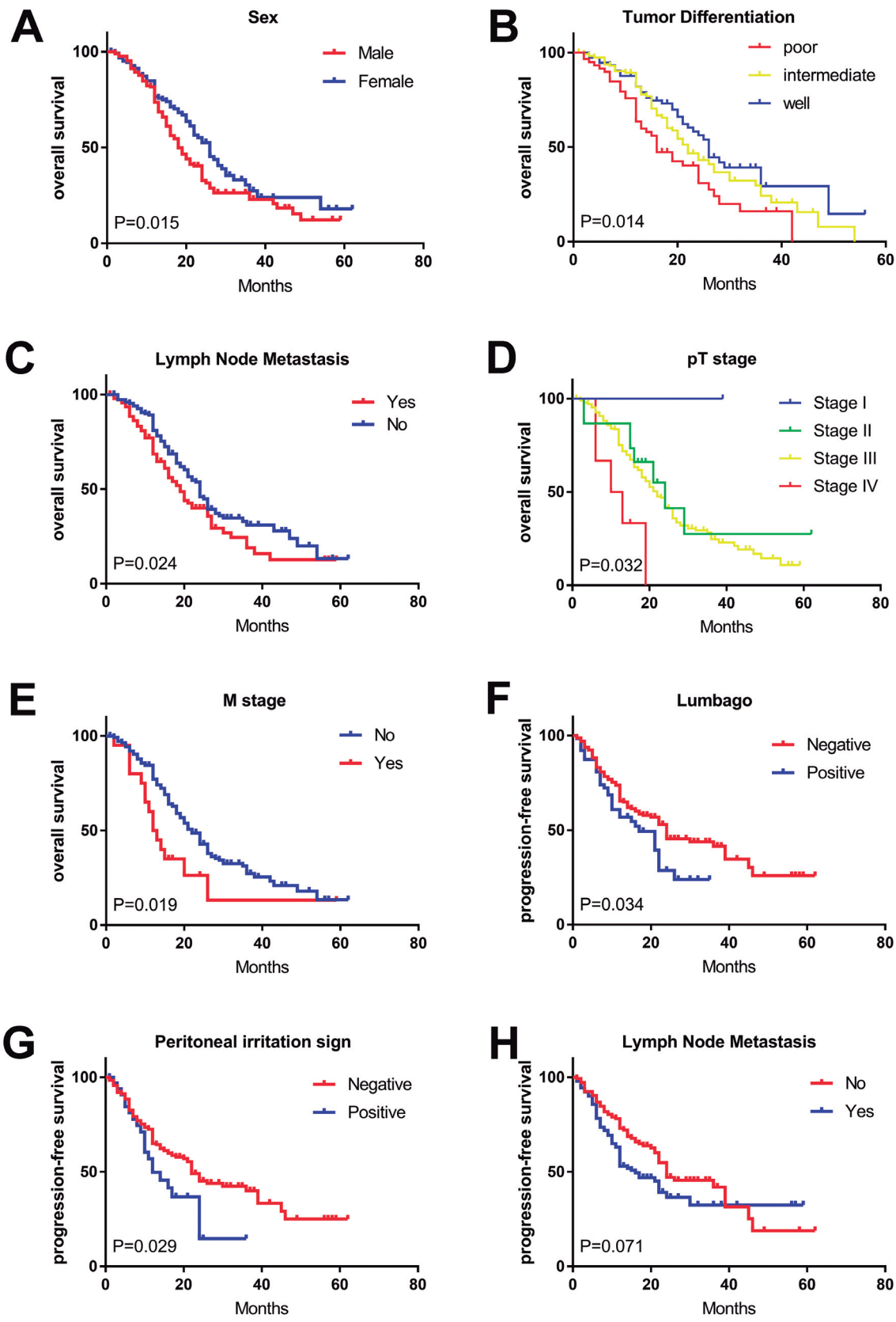

Fig. 6. Survival curves of clinicopathological characteristics. Prognostic value of clinicopathological characteristics was analyzed by Kaplan Meier analysis. Survival curves show that sex **(A)**, tumor differentiation **(B)**, lymph node metastasis **(C)**, pT stage **(D)** and M stage **(E)** were associated with OS, with statistical significance; lumbago **(F)**, peritoneal irritation sign **(G)** and lymph node metastasis **(H)** were associated with PFS.

independent predictors of PFS (Table 2).

CLDN18.2 was not expressed in the procedure of EMT phenotype

CLDN18.2 is a part of the tight junction on the cell membrane. Therefore, we examined the role of CLDN18.2 in the EMT phenotype. The EMT-related biomarkers E-cadherin and vimentin were detected by IHC. Six cases were positive for vimentin in the whole slide cohort (7.14%), which showed that those malignant cells were in the process of EMT. However, none of the six cases showed expression of CLDN18.2 (Fig. 7). This suggested that once cancer cells displayed the EMT phenotype, those cells would lose CLDN18.2 expression.

Discussion

To the best of our knowledge, this is the largest cohort to detect CLDN18.2 in PDAC. PDAC is the most frequent deadly malignant tumour of the exocrine pancreas, with a high rate of recurrence even after pancreatectomy with an R0 margin (Tempero et al., 2021). CLDN18.2 is a member of the claudin family and isoform of CLDN18.1, which has a highly conserved sequence (Türeci et al., 2011). CLDN18.2, a component of tight junctions, is mainly expressed in normal gastric epithelial cells (Türeci et al., 2011). Previous studies have shown that CLDN18.2 can be a potential solid tumour therapeutic target via RT-PCR and immunohistochemistry (Sahin et al., 2008; Micke et al., 2014; Iwaya et al., 2021). Recent studies have shown that CLDN18.2 is highly expressed in several cancers, mainly in gastric cancer and pancreatic cancer, which has brought new possibilities for treating PDAC (Micke et al., 2014; Zhu et al., 2019; Iwaya et al., 2021).

Some studies have reported that CLDN18.2 is a therapeutic target for pancreatic neoplasms (Wöll et al., 2014). The CLDN18.2-targeted antibody, IMAB362, has been used in several clinical trials for gastric adenocarcinoma (Baek et al., 2019). Some CLDN18.2-targeted drugs (claudiximab, TST001, CT041, etc.) have been approved in clinical trials to test their feasibility in clinical applications (Baek et al., 2019; Türeci et al., 2019; Sahin et al., 2021). IMAB362 plus EOX has already been tested as a first-line treatment for advanced CLDN18.2-positive gastric cancer (Lordick et al., 2021; Sahin et al., 2021). Several studies have reported that the antibody, IMAB362, might be a target for pancreatic neoplasms (Wöll et al., 2014; Zhu et al., 2019). Therefore, we constructed a large cohort to test for the expression of CLDN18.2 in PDAC. More than half of the patients showed positive expression, which suggested that those patients could benefit from CLDN18.2-targeted treatment.

However, not all patients with PDAC can benefit from the treatment. A previous study suggested a possible relationship between CLDN18.2 expression and AJCC pTNM stage that CLDN18.2 was mainly expressed in pT3/4 and pN1 stages (Wöll et al., 2014). No studies have focused on morphological differentiation. In the present study, we identified that CLDN18.2 expression was associated with tumour differentiation. We focused on pathological differentiation because it is the most relevant characteristic of cellular adhesion. The results proved our assumption and showed that CLDN18.2 was mainly positive for highly- (82.67%, 62/75) and intermediate-differentiated (62.61%, 72/115) PDAC, while low-differentiated PDAC had a significantly lower frequency of CLDN18.2 positivity (10.17%, 6/59). This suggests

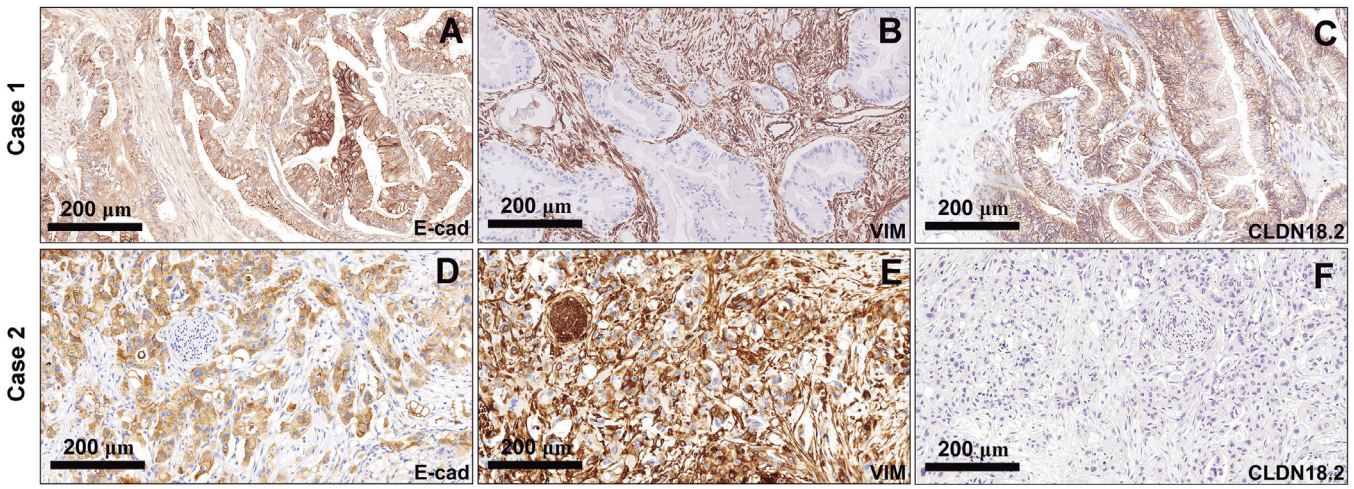

Fig. 7. Relationship of CLDN18.2 to EMT phenotype. Two representative cases of diverse EMT phenotype show that case 1 was not in the procedure of EMT and CLDN18.2 was positive **(A-C)**, while case 2 was positive for vimentin and negative for CLDN18.2 **(D-F)**. It shows that CLDN18.2 was lost in EMT phenotype.

that poorly differentiated PDAC patients might not benefit from CLDN18.2-targeted treatment as significantly as highly- and intermediate-differentiated PDAC patients.

Prognostic value of CLDN18.2 in PDAC has not been studied before and our study is the first prognostic evaluation study. Several studies have already evaluated the prognostic relevance of CLDN18.2 in gastric cancer. A meta analysis summarized three articles and showed that CLDN18.2 was not associated with OS for gastric cancer patients (Ungureanu et al., 2021). Arnold showed that CLDN18.2 expression was not associated with either OS or disease free survival (DFS) in GOAC (Arnold et al., 2020). A review showed that CLDN18.2 as regards meaningful prognosis was still ambiguous (Zhang et al., 2020). In the study, we found that CLDN18.2 high expression was associated with improved OS, with statistical significance, but not with PFS. However, multivariate cox Regression showed that CLDN18.2 was not an independent prognostic predictor. Our result also showed a relation between CLDN18.2 and tumour differentiation that higher CLDN18.2 expression was mainly observed in well-differentiated PDAC. It also suggested that CLDN18.2 was associated with a better outcome for PDAC patients.

Ten PanINs in the para-tumour area of PDAC were verified in the whole slide cohort, and all PanIN lesions were positive for CLDN18.2. Previous studies have regarded PanIN as a positive control, suggesting that PanIN was positive for CLDN18.2 (Wöll et al., 2014). Our study confirmed this. This suggests that CLDN18.2 can be used as a biomarker to distinguish precancerous lesions from normal ductal epithelial cells of the pancreas. Therefore, CLDN18.2-targeted drugs, such as IMAB362, might be valid for pancreatic precancerous lesions. Thus, the present study provides new evidence for the application of IMAB362 in PanIN.

We also explored the relationship between CLDN18.2 and the EMT phenotype of PDAC. We discovered six vimentin-positive PDAC cases, and the malignant cells showing positive for vimentin were in the EMT phenotype (Dongre et al., 2019). However, CLDN18.2-positive expression was not detected in all six cases. Claudins, as members of cellular tight junctions, are expressed by epithelial cells and are lost in the EMT phenotype (Kominsky, 2006). Previous studies have reported that claudin family proteins (e.g., CLDN4 in ovarian cancer and CLDN7 in breast cancer) were inactive in the EMT phenotype, resulting in the invasiveness of cancer cells. Our discovery that CLDN18.2 was negative in vimentin-positive cases proved CLDN18.2-inactivation in the EMT phenotype. It suggested that CLDN18.2 expression was only present before EMT phenotype. The mutual exclusion offered a possibility that CLDN 18.2 might be an early-stage marker and be a potential screening target for PDAC. However, the detailed mechanism still needs to be elucidated.

In summary, we constructed a large cohort to identify CLDN18.2-positive PDAC patients, especially for highly-differentiated PDAC, that could benefit from CLDN18.2-targeted therapies. Besides, CLDN18.2 was also associated with several clinicopathological characteristics, including sex, smoking, abdominal pain, jaundice, pathological differentiation, common bile duct invasion, and metastasis. Prognostically, higher expression of CLDN18.2 was associated with improved OS for PDAC patients, and these results suggest that CLDN18.2 may become a potential target for PDAC in the future.

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