Original Article

Clinical and molecular characteristics of periampullary carcinoma based on pathological subtypes

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Background: Periampullary carcinoma (PAC) is a relatively rare but highly aggressive malignancy, posing challenges to the determination of the optimal therapeutic approach. The objective of this study was to clarify the potential of histopathological typing in guiding chemotherapy selection for patients with advanced PAC and to characterize the distinct molecular features, underlying functional changes, and regulatory mechanisms associated with the different subtypes.

Methods: We conducted a retrospective analysis of clinical data from patients with advanced PAC admitted to the Oncology Department of Xijing Hospital between January 2015 and May 2022. These patients received first-line chemotherapy with either FOLFOX (folinic acid, fluorouracil, and oxaliplatin) or gemcitabine-based regimens. Certain patients were divided into the pathological typing group and the control group. The pathological typing group received subtype-specific chemotherapy regimens, while the control group received chemotherapy regimens based on the primary tumor site. We compared the median progression-free survival (PFS) and overall survival (OS) between the two groups. Using publicly available databases (GSE60980), we conducted differential gene screening, enrichment analysis, and immune cell infiltration assessment. A protein-protein interaction (PPI) network was constructed based on the differentially expressed genes, resulting in the identification of 60 node genes. Subsequently, a core gene selection using the least absolute shrinkage and selection operator (LASSO) regression machine learning algorithm was performed to identify the key genes specific to PAC-PB subtype.

Results: The pathological typing group consisted of 46 patients, with 26 classified as the PB subtype and 20 as the IN subtype, while the control group comprised 40 patients. Compared to those in the control group, patients in the pathological typing group demonstrated significant improvements in overall response rate (20.5% *vs.* 12.9%; P=0.04), median PFS (8.1 *vs.* 5.4 months; P=0.04), and median OS (34 *vs.* 25.9 months; P=0.02). Multivariate Cox regression analysis revealed that pathological typing independently influenced PFS [hazard ratio (HR) =0.20, 95% confidence interval (CI): 0.10–0.44; P=0.009] and OS (HR =0.21, 95% CI: 0.17–0.71; P=0.02). Using a publicly available PAC cohort (GSE60980), we selected 154 differentially expressed genes, which were significantly enriched in signaling pathways related to the cell cycle, fibroblasts, and epithelial-mesenchymal transition. Analysis of immune cell infiltration indicated a significant increase in the abundance of fibroblast cells and a significant decrease in that of B cells and γδ T cells in the PAC-PB subtype. Furthermore, we identified core genes specific to the PAC-PB subtype and used them to construct a PAC-PB diagnostic model.

Conclusions: Pathologic typing-guided individualized chemotherapy resulted in prolonged survival for patients with advanced PAC. The PB and IN subtypes of PAC exhibit distinct molecular regulatory

mechanisms and immune infiltration microenvironments. These findings underscore the importance of considering subtype-specific factors in the development of a PAC-PB diagnostic model.

Keywords: Periampullary carcinoma (PAC); pathological subtype; individualized treatment; molecular characteristics

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Introduction

Periampullary carcinoma (PAC) is a rare gastrointestinal malignancy, accounting for 0.5–2% of these cases, and encompasses ampullary carcinoma (AC), pancreatic head carcinoma (PC), distal common bile duct carcinoma (DCC), and duodenal carcinoma (DC) (1-3). Pancreaticoduodenectomy (PD) is the established treatment for patients with resectable PAC. However, the 5-year survival rate following surgery remains disappointingly low, ranging from 5% to 68% (4-8). Recurrence and metastasis significantly contribute to this unfavorable prognosis (9-11). Moreover, the prognosis for metastatic and advanced PAC is notably poor, with a 2-year overall survival (OS) rate of only 5% to 10%, primarily due to the limitation in treatment options (11-13).

Patients with advanced PAC have few choices for therapy, but there is considerable variation in the selection of chemotherapeutic regimens. Moreover, there is a scarcity of comprehensive clinical studies involving large sample sizes in real-world contexts pertaining to the selection of different chemotherapeutic regimens. It remains a challenging condition to diagnose and treat due to its nonspecific symptoms and complex anatomical location. Moreover, there is a lack of research on the molecular characteristics of different histological classifications. This study thus aimed to assess the treatment outcomes of patients with advanced PAC according to pathological type via an analysis of real-world clinical data from our institution, Department of Oncology, Xijing Hospital. Given the heterogeneous tissue origin of PAC tumors, it is plausible that different tumor-associated gene mutations and alterations in tissue composition exist between the subgroups that are collectively classified. We present this article in accordance with the STROBE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-2025-14/rc).

Highlight box

Key findings

- Our findings indicated that the molecular mechanisms related to the genetic pathology subtypes have considerable implications for better understanding the characteristics of periampullary carcinoma (PAC). The pancreatic-biliary (PB) and intestinal (IN) subtypes of PAC have differentially expressed genes, which we found were significantly enriched in signaling pathways related to the cell cycle, fibroblasts, and epithelial-mesenchymal transition. Analysis of immune cell infiltration indicated a significant increase in fibroblast cells and a significant decrease in B cells and γδ T cells in the PAC-PB subtype.

What is known and what is new?

- PAC is a rare and heterogeneous tumor, and due to its special anatomical location, drug treatment for advanced disease is mainly based on histological classification.
- We identified core genes specific to the PAC-PB subtype and used them to construct a PAC-PB diagnostic model. And we found that there is a significant increase in the abundance of fibroblast cells and a significant decrease in that of B cells and γδ T cells in the PAC-PB subtype.

What is the implication, and what should change now?

- The PB and IN subtypes of PAC have differentially expressed genes and differential immune cell infiltration. Therefore, individualized therapy based on subtype is critical for the treatment of patients with PAC.
- Other methods for accurately distinguishing different subtypes and selecting individualized treatments according to molecular differences should be developed in the future.

Methods

Patient inclusion and data collection

We retrospectively analyzed clinical data from patients with PAC who were treated at the Department of Oncology in Xijing Hospital between January 2015 and May 2022. The inclusion criteria were as follows: (I) histologically confirmed PAC, including AC, PC, DCC, and DC; (II)

age ≥18 years; (III) clinical stage IV disease; (IV) presence of at least one measurable lesion; and (V) completion of at least two cycles of FOLFOX (folinic acid, fluorouracil, and oxaliplatin) or gemcitabine-based chemotherapy. Patients were excluded if they met any of the following criteria: (I) no chemotherapy; (II) other concurrent malignancies; (III) incomplete data; (IV) administration of FOLFOX or gemcitabine-based chemotherapy in combination with targeted therapy, immunotherapy, or radiotherapy; and (V) loss to follow-up. This study was approved by the Ethics Committee of Xijing Hospital (No. KY20233268-1) and was conducted in accordance with the principles of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all study participants. For analysis, we obtained the GSE60980 PAC dataset from the Gene Expression Omnibus (GEO) database, including the transcriptomic and clinical data of patients.

Evaluation of clinical oncological response

The data collection encompassed various patient-related factors, tumor characteristics, laboratory findings, and treatment outcomes. Patient demographics, such as age, gender, smoking history, alcohol consumption, and comorbidities, were recorded. Tumor pathology, including histological differentiation, immunohistochemical results, presence of jaundice, nerve and vascular invasion, and tumor markers, were documented. The first-line chemotherapy regimen and prechemotherapy test results were noted. Follow-up data, including clinical oncological response and objective response rate (ORR), were also included in the analysis.

Specifically, the prechemotherapy tests and examinations consisted of a complete blood cell count, liver function assessment, tumor marker analysis, and chest and abdominal enhanced computed tomography (CT) scans conducted within 1 week prior to the initiation of first-line chemotherapy. The radiologists evaluated the clinical oncological response based on the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1) and classified it as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The ORR was calculated as the proportion of patients achieving CR or PR (14).

Survival analysis

The primary outcome measure of this study was progression-free survival (PFS), which was considered to be the time interval from the initiation of first-line systemic therapy until the earliest occurrence of radiologically confirmed disease progression or death from any cause. OS was considered to be the time of diagnosis until death from any cause or for patients who were still alive, the final follow-up date. We conducted follow-up assessments through outpatient reviews or telephone interviews, and the last recorded follow-up date for this study was December 20, 2022.

Identification of differentially expressed genes and pathway enrichment analysis

To identify suitable datasets, we used the search term "(periampullary[All Fields] AND ('adenocarcinoma'[MeSH Terms] OR adenocarcinomas[All Fields])) AND 'gse'[Filter]", which retrieved seven studies: GSE123377 (27 samples), GSE123375 (27 samples), GSE117687 (14 samples), GSE60980 (182 samples), GSE60979 (93 samples), GSE60978 (89 samples), and GSE39409 (32 samples). GSE60980 is a merged cohort of GSE60979 and GSE60978. Given its largest sample size, we selected GSE60980 for further analysis to explore the potential mechanisms underlying periampullary adenocarcinomas. The transcriptomic data analysis of PAC involved the use of the "limma" package. Differential expression analysis was conducted under a threshold of "$|\log_2$ fold change (FC)$|$ >1 and false discovery rate (FDR) <0.05" to identify genes that were significantly differentially expressed. In our study, we used Fragments Per Kilobase of transcript per Million mapped reads (FPKM) for gene expression quantification, followed by log transformation using "$\log_2(x+1)$". Batch effects were corrected using the "sva" package. For differential expression analysis, we applied the commonly used thresholds of $|\log_2FC|$ >1 and FDR <0.05, which are frequently applied in multiple studies. A total of 154 genes met these criteria and were selected for further investigation via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

Estimation of immune cell infiltration

In order to examine the variances in the immune microenvironment between the two subtypes of PAC, we employed two immune cell algorithms, Microenvironment Cell Population (MCP)-counter and CIBERSORT (15,16), to quantify and assess the relative proportions and abundances of different immune cell populations within the

tissue. The differences in immune cell composition between the two groups were evaluated using the Wilcoxon rank-sum test, and the results were visually represented using violin plots.

Construction of a protein-protein interaction (PPI) network and PAC pancreatic-biliary (PAC-PB) diagnostic model

Using the initial set of 154 differentially expressed genes, we constructed a PPI network using the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/). We used a minimum required interaction score of 0.700 (high confidence) to define the strength of interactions. The "evidence" of the network edges was also considered. This network analysis yielded 60 nodal genes (proteins) of interest. Subsequently, the least absolute shrinkage and selection operator (LASSO) machine learning algorithm was applied to identify the core genes specifically associated with the PAC-PB subtype. Through this process, a diagnostic model for PAC-PB was established using logistic regression, and the receiver operating characteristic (ROC) curve was drawn. The final model was comprised of 10 genes: *GRIN2D*, *FLRT2*, *RADIL*, *BEX2*, *C6orf222*, *CRH*, *IGFBP3*, *COL1A1*, *KRT16P2*, and *GLI1*. For additional details including the list of genes and their corresponding regression coefficients, please refer to Table S1.

Statistical analysis

Data analysis was conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as the mean ± standard deviation or as the median and range depending on their distribution. Categorical variables were compared using the χ^2 test (2), while the Mann-Whitney test was employed for the comparison of continuous variables. The OS was estimated using Kaplan-Meier product-limited method, and survival curves were compared between groups using the log-rank test. Univariate and multivariate Cox regression analyses were performed to identify variables associated with PFS or OS. A P value <0.05 was considered statistically significant.

Results

Patient characteristics

We conducted a retrospective analysis of clinical data from 118 patients with PAC who received treatment at the Oncology Department of Xijing Hospital between January 2015 and May 2022. After exclusion of 32 patients who did not meet the eligibility criteria, a total of 86 patients were included in the final analysis. Pathological subtyping was performed based on tumor location, pathological morphology [hematoxylin and eosin (H&E) morphology] and immunohistochemical staining patterns. Patients with tumor staining positive for MUC1 alone independent of CK20 and without CDX2 and MUC2 expression were classified as having the PB subtype. Patients with tumor staining positive for MUC1 and MUC2 or positive for CK20 (independent of MUC1) were classified with the intestinal (IN) subtype. Patients with unavailable immunohistochemical data were included in the control group. Among the 86 patients, 46 belonged to the pathological typing group, with 26 classified as the PB subtype and 20 as IN subtype. The remaining 40 patients were included in the control group. The baseline clinicopathological characteristics of all included patients are presented in *Table 1*. There were no significant differences in baseline characteristics between the two groups.

Efficacy

In the pathological typing group, patients with the PB subtype received gemcitabine-based combination chemotherapy as their first-line treatment, while patients with the IN subtype received FOLFOX chemotherapy. Conversely, the control group could not be classified due to the unavailability of immunohistochemical data. The control group received FOLFOX or gemcitabine-based chemotherapy as their first-line treatment based on the site of the primary tumor. The ORR was evaluated according to RECIST 1.1, and the pathological typing group exhibited a significantly higher ORR compared to the control group (20.5% *vs.* 12.9%; P=0.04). Kaplan-Meier survival analysis demonstrated that compared to the control group, the pathological typing group had a significantly longer median PFS [8.1 *vs.* 5.4 months; hazard ratio (HR) =0.59; P=0.04] and median OS (34 *vs.* 25.9 months; HR =0.57; P=0.02) (*Figure 1A*,*1B*. Further analysis within the pathological typing group revealed that compared to those in the PB subtype group, patients in the IN subtype group had a significantly longer median PFS (12 *vs.* 5.5 months; HR =0.46; P=0.04) and median OS (40 *vs.* 27.8 months; HR =0.70; P=0.03) (*Figure 1C*,*1D*).

Table 1 Pathological subtypes and clinical characteristics

Characteristics	Pathological typing (n=46)	Control (n=40)	P
Age			0.38
<65 years	30	22	
≥65 years	16	18	
Gender			0.19
Male	22	25	
Female	24	15	
Smoking history			0.52
Yes	20	21	
No	26	19	
Primary tumor			0.99
AC	15	14	
PC	10	9	
DCC	8	7	
DC	13	10	
Diagnosis of stage			0.89
I–II	10	11	
III	26	20	
IV	10	9	
Lymphatic metastasis			0.74
N0	17	15	
N1–2	29	25	
Number of metastasis sites			0.99
≤2	22	20	
>3	24	20	
Histological differentiation			0.91
High	8	7	
Middle	32	29	
Low	6	4	
NLR			0.95
>3	21	18	
≤3	25	22	
CA199			0.20
>35 IU/mL	26	23	
≤35 IU/mL	20	17	
CEA			0.19
>5 ng/mL	20	19	
≤5 ng/mL	26	21	
With jaundice			0.24
Yes	26	22	
No	20	18	

AC, ampullary carcinoma; PC, pancreatic head carcinoma; DCC, distal common bile duct carcinoma; DC, duodenal carcinoma; NLR, neutrophil-to-lymphocyte ratio; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen.

Prognostic value of the pathological typing

To assess the prognostic value of pathological typing in patients with PAC, univariate and multivariate Cox regression analyses were performed to evaluate the associations with PFS and OS. The univariate analysis of PFS revealed significant associations between several factors, including primary tumor site, number of metastasis sites, levels of carbohydrate antigen 199 (CA199) and CEA, carcinoembryonic antigen (CEA), pathological typing, and neutrophil-to-lymphocyte ratio (NLR), with PFS. Similarly, primary tumor site, stage at diagnosis, pathological typing, number of metastasis sites, presence of lymph node metastasis, and NLR demonstrated significant associations with OS (*Tables 2,3*). Significant factors identified in the univariate analysis were included in the subsequent multivariate analysis. The multivariate analysis confirmed that pathological typing was an independent prognostic factor for both PFS [HR =0.20, 95% confidence interval (CI): 0.10–0.44; P=0.009] and OS (HR =0.21, 95% CI: 0.17–0.71; P=0.02) (*Tables 4,5*).

Identification of differentially expressed genes and pathway enrichment analysis

To identify the characteristic genes and potential functional changes associated with different PAC types, we analyzed the differentially expressed genes in the pancreatic type, IN type, and normal pancreatic tissue type using the GSE60980 dataset from GEO database (*Figure 2A,2B*). We identified 154 differentially expressed genes by taking the

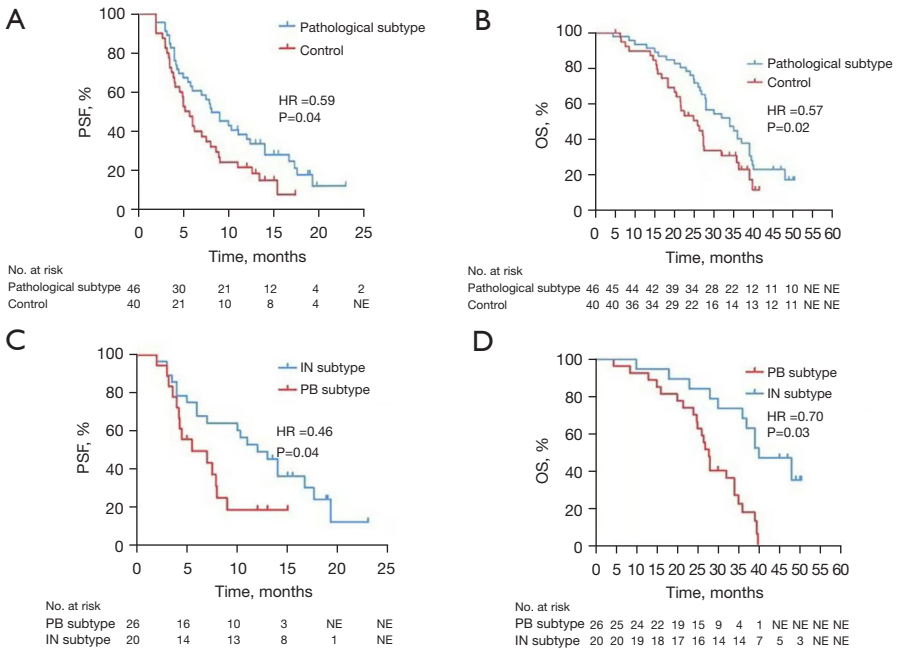

Figure 1 Survival analysis. (A,B) Kaplan-Meier curves comparing (A) PFS and (B) OS between the pathological typing group and the control group. (C,D) Kaplan-Meier curves comparing (C) PFS and (D) OS between the IN subtype and PB subtype in the pathological typing group. OS, overall survival; PFS, progression-free survival; HR, hazard rate; NE, not estimable; PB, pancreatic-biliary; IN, intestinal.

Table 2 Univariate Cox analysis of clinicopathological variables and pathological typing for the mPFS of patients with periampullary carcinoma

Factor	Cases (n)	mPFS (months)	P
Gender			0.22
Male	47	7.2	
Female	39	6.3	
Age			0.35
<65 years	52	6.5	
≥65 years	34	7	
Smoking history			0.59
Yes	41	6.2	
No	45	6.9	
Diagnosis of primary tumor			0.03*
AC	29	7.3	
PC	19	1.9	
DCC	15	4.5	
DC	23	9	
Diagnosis of stage			0.30
I–II	21	6.3	
III	46	5.9	
IV	19	5.4	

Table 2 (*continued*)

Table 2 (*continued*)

Factor	Cases (n)	mPFS (months)	P
Lymphatic metastasis			0.13
N0	32	7.2	
N1–2	54	5.3	
Number of metastasis sites			0.02*
≤2	42	6.9	
>3	44	5.2	
CA199			0.04*
>35 IU/mL	49	4.9	
≤35 IU/mL	37	7.1	
CEA			0.04*
>5 ng/mL	39	4.3	
≤5 ng/mL	47	7.6	
Histological type			0.04*
Pathological subtype	46	8.1	
Control	40	5.4	
NLR			0.04*
>3	39	4	
≤3	47	7.6	
With jaundice			0.67
Yes	48	6.4	
No	38	7.2	

*, P<0.05. PFS, progression-free survival; mPFS, median progression-free survival; AC, ampullary carcinoma; PC, pancreatic head carcinoma; DCC, distal common bile duct carcinoma; DC, duodenal carcinoma; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; NLR, neutrophil-to-lymphocyte ratio.

Table 3 Univariate Cox analysis of clinicopathological variables and pathological typing for the mOS of patients with periampullary carcinoma

Factors	Cases (n)	mOS (months)	P
Gender			0.27
Male	47	28	
Female	39	26	
Age			0.24
<65 years	52	27	
≥65 years	34	25.5	
Smoking history			0.33
Yes	41	26.9	
No	45	29.7	

Table 3 (*continued*)

Table 3 (*continued*)

Factors	Cases (n)	mOS (months)	P
Diagnosis of primary tumor			0.003*
AC	29	26.4	
PC	19	14.8	
DCC	15	20	
DC	23	30.5	
Diagnosis of stage			0.002*
I–II	21	40.4	
III	46	35.2	
IV	19	12.6	
Lymphatic metastasis			0.01*
N0	32	29	
N1–2	54	23	
Number of metastasis sites			0.02*
≤2	42	32.8	
>3	44	24.6	
CA199			0.22
>35 IU/mL	49	29.3	
≤35 IU/mL	37	31.6	
CEA			0.65
>5 ng/mL	39	30.1	
≤5 ng/mL	47	32.4	
Histological type			0.02*
Pathological subtype	46	34	
Control	40	25.9	
NLR			0.04*
>3	39	27.9	
≤3	47	34.2	
With jaundice			0.33
Yes	48	26.5	
No	38	28.7	

*, P<0.05. OS, overall survival; mOS, median overall survival; AC, ampullary carcinoma; PC, pancreatic head carcinoma; DCC, distal common bile duct carcinoma; DC, duodenal carcinoma; NLR, neutrophil-to-lymphocyte ratio.

intersection of these datasets. Gene set enrichment analysis (*Figure 3A*,*3B*) revealed significant enrichment of these 154 genes in signaling pathways related to the cell cycle, fibroblasts, and epithelial-mesenchymal transformation. This suggests different signaling regulation mechanisms exist between the pancreatic and IN types of PAC. To clarify

Table 4 Multivariate Cox analysis of clinicopathological variables and pathological typing for the PFS of patients with periampullary carcinoma

Factor	HR	95% CI	P
Diagnosis of primary tumor			0.04*
AC	1.00		
PC	1.73	1.37–2.17	
DCC	1.48	0.76–1.89	
DC	0.57	0.36–0.84	
CA199			0.35
>35 IU/mL	1.00		
≤35 IU/mL	0.89	0.53–1.17	
CEA			0.38
>5 ng/mL	1.00		
≤5 ng/mL	0.74	0.46–0.99	
Number of metastasis sites			0.13
≤2	1.00		
>3	1.82	0.86–3.22	
Histological type			0.009*
Control	1.00		
Pathological subtype	0.20	0.10–0.44	
NLR			0.46
>3	1.00		
≤3	0.82	0.78–1.35	

*, P<0.05. PFS, progression-free survival; HR, hazard rate; CI, confidence interval; AC, ampullary carcinoma; PC, pancreatic head carcinoma; DCC, distal common bile duct carcinoma; DC, duodenal carcinoma; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; NLR, neutrophil-to-lymphocyte ratio.

Table 5 Multivariate Cox analysis of OS by clinicopathological variables and pathological typing in patients with periampullary carcinoma

Factors	HR	95% CI	P
Diagnosis of primary tumor			0.03*
AC	1.00		
PC	1.63	0.61–2.24	
DCC	1.38	0.56–1.96	
DC	0.38	0.27–0.73	
Stage			0.02*
I–II	1.00		
III	1.45	1.23–1.92	
IV	2.35	2.22–2.85	
Lymphatic metastasis			0.31
N1–2	1.00		
N0	0.79	0.61–1.22	
Number of metastasis sites			0.13
≤2	1.00		
>3	1.82	0.86–3.22	
Histological type			0.02*
Control	1.00		
Pathological subtype	0.21	0.17–0.71	
NLR			0.46
>3	1.00		
≤3	0.82	0.78–1.35	

*, P<0.05. OS, overall survival; HR, hazard rate; CI, confidence interval; AC, ampullary carcinoma; PC, pancreatic head carcinoma; DCC, distal common bile duct carcinoma; DC, duodenal carcinoma; NLR, neutrophil-to-lymphocyte ratio.

the regulatory relationships between the differentially expressed genes, we constructed a protein interaction network, which consisted of 60 node genes (*Figure 4*).

Estimation of immune cell infiltration

Tumor immunity plays a significant role in tumor progression and classification. To further explore this aspect, we used two immune cell infiltration algorithms to analyze the differences in immune cell abundance between pancreatic PAC, IN PAC, and normal pancreatic tissue. The results demonstrated a significant increase in fibroblast abundance in pancreatic PAC, along with a significant reduction in B cells and γδ T cells (*Figure 5A*,*5B*).

Construction of the PPI interaction network and PAC-PB diagnostic model

The results presented above highlight the distinct clinical and pathological characteristics, patient prognosis, and regulatory mechanisms between the IN and pancreatic PB subtypes of PAC. Identifying these two subtypes is of great value in guiding the clinical diagnosis and treatment of PAC. From our screened 60 PPI network node genes, we

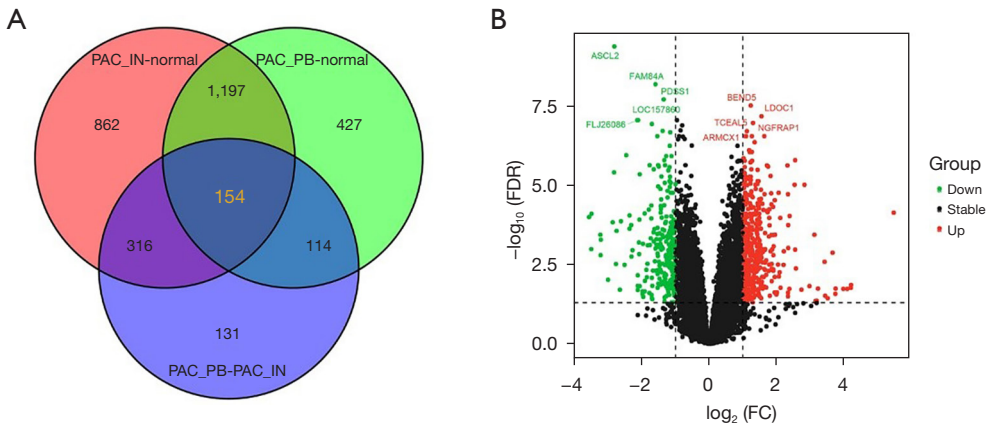

Figure 2 Venn and volcano plots of the differential genes. (A) Venn diagram of differential genes between the pancreatic type, intestinal type, and normal pancreatic tissue. (B) Volcano map of the pancreatic type and intestinal type. Red indicates highly expressed genes of pancreatic type tumor tissue. PAC, periampullary carcinoma; IN, intestinal; PB, pancreatic-biliary; FDR, false discovery rate; FC, fold change.

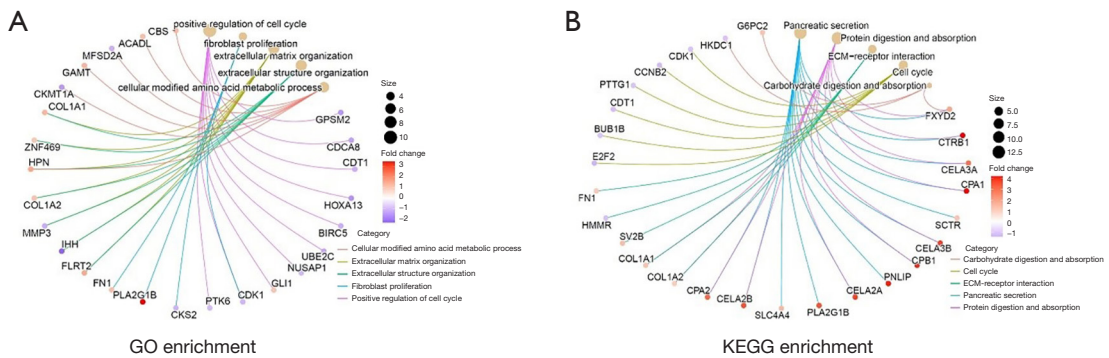

Figure 3 Enrichment analysis of the differentially expressed genes between the different subtypes. (A,B) Enrichment analysis of the differentially expressed genes: (A) GO and (B) KEGG enrichment in the pancreatic type, intestinal type, and normal pancreatic tissues. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

selected 10 core genes using the LASSO regression model (*Figure 6A*,*6B*). Logistic regression was then applied to construct a PAC differential diagnosis model based on the expression levels of each core gene and their respective regression coefficients (Table S1). The ROC curve demonstrated that the PB score achieved a diagnostic accuracy of 95.1% (*Figure 6C*), indicating its high diagnostic accuracy and clinical value. The heatmap of core gene expression (*Figure 6D*) showed that *KRT16P2*, *GLI1*, *IGFBP3*, *COL1A*, *CRH*, *RADIL*, *BEX2*, and *FLRT2* were highly expressed in the PAC-PB subtype, while *GRIN2D* and *C6orf222* had lower expression in the PAC-PB subtype. Additionally, the PB score was significantly lower in the PAC-PB subtype (*Figure 6E*).

Discussion

PAC is a distinct subgroup of tumors that is relatively rare, and as a result, research on its treatment primarily consists of retrospective and single-center studies. Currently, the management of advanced PAC lacks standardized treatment options and typically involves the use of fluorouracil or gemcitabine-based combination regimens (17).

PAC can be classified into two distinct histological subtypes: the IN subtype and the PB subtype, each with its own unique pathological and clinical characteristics. Previous studies have shown that patients with the PB subtype generally have a worse prognosis compared to those with the IN subtype (17,18). Patients with the IN subtype

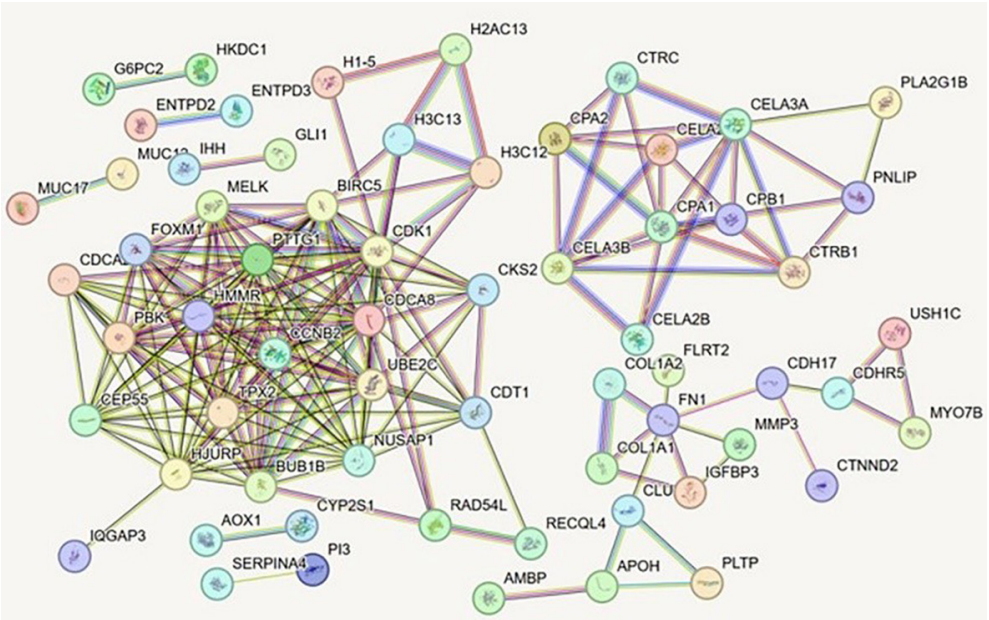

Figure 4 Protein interaction network construction of differentially expressed genes among different subtypes.

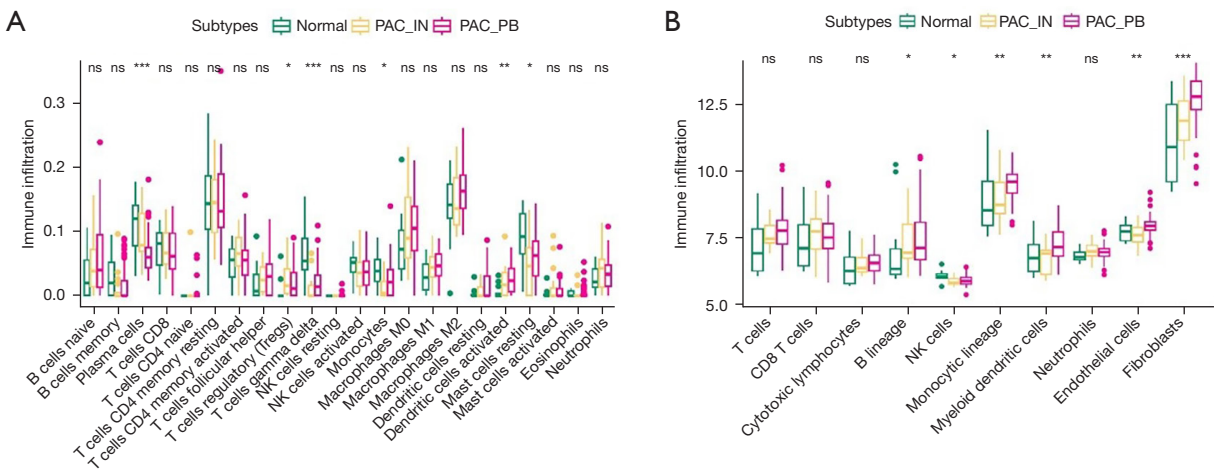

Figure 5 Analysis of the differences in immune cell infiltration among the different subtypes. (A,B) Boxplot of the differential analysis of immune cell infiltration abundance assessed by the (A) CIBERSORT and (B) MCP-counter algorithms in the pancreatic type, intestinal type, and normal pancreatic tissue. *, P<0.05; **, P<0.01; ***, P<0.001; ns, P≥0.05. PAC, periampullary carcinoma; IN, intestinal; PB, pancreatic-biliary; MCP, Microenvironment Cell Population; CD, cluster of differentiation; NK, natural killer.

tend to be more responsive to various chemotherapy regimens compared to those with the PB subtype. In this study, we conducted a real-world analysis by integrating clinical features, tumor markers, and immunohistochemistry to investigate whether pathological typing can guide the treatment approach for PAC. Given the limited treatment options available for patients with advanced disease, we examined the impact of chemotherapy regimens based on pathological classification in a cohort of 86 patients with PAC from our institution. The results revealed that patients who received chemotherapy based on histopathological classification experienced a significant improvement in

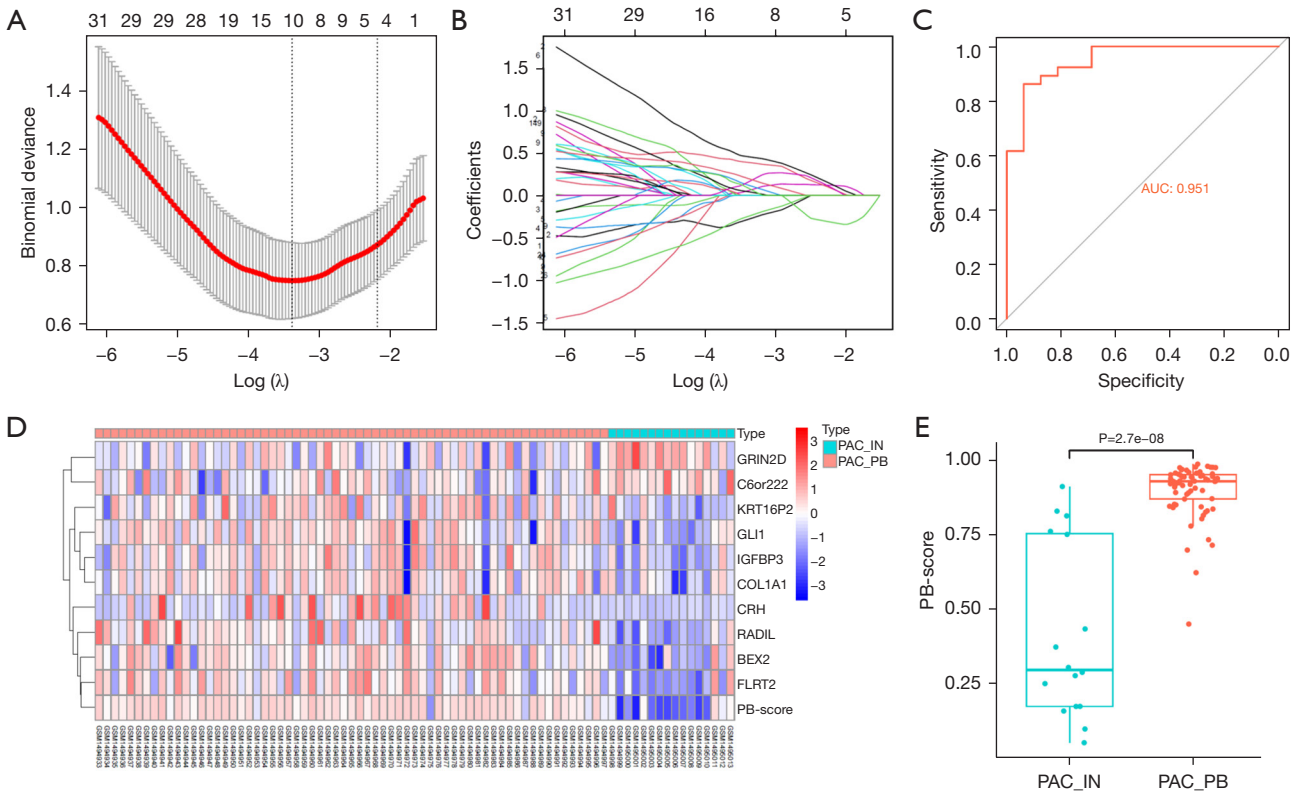

Figure 6 Identification of the PB and IN subtype in PAC and core gene screening and diagnostic model construction. (A) Cross-validation and (B) variable screening map of intestinal and pancreatic PAC. (C) ROC curve of pancreatic PAC (PB score) based on 10 core genes. (D) Heatmap of expression of 10 core genes in different samples of intestinal and pancreatic types and (E) boxplot of the PB score in the PB and pancreatic type. AUC, area under the curve; PAC, periampullary carcinoma; IN, intestinal; PB, pancreatic-biliary; ROC, receiver operating characteristic.

survival compared to those who could not be classified, demonstrating the potential survival benefit associated with tailored treatment strategies based on pathological typing.

In this study, the univariate analysis of PFS demonstrated significant associations with several factors, including primary tumor site, number of metastasis sites, CA199 level, CEA level, pathological typing, and NLR. Multivariate analysis revealed that pathological typing was an independent prognostic factor for both PFS and OS. These findings align with previous studies (18-23). Previous multivariate analyses have identified other independent factors associated with OS in PAC (24-27). These factors include high CEA level (>5 ng/mL), poor cell differentiation, absence of chemotherapy, presence of relapse, and specific pathological types. Similarly, studies have shown that a high lymph node ratio (>0.2) has an adverse impact on both PFS and OS, while lymphovascular invasion and advanced T stage affect PFS and OS, respectively. A large study involving 2,564 patients identified tumor type, vein resection rate, margin status, and nodal status as factors associated with poorer survival (25,28).

However, the molecular genetic mechanisms underlying PAC according to subtype classification remain poorly studied. Moreover, due to the rarity of this tumor, there are challenges in collecting samples on a large scale. To address this limitation and gain insights into gene differences in gene expression and regulatory networks between the different subtypes of PAC, we performed bioinformatics analyses using publicly available data. Our results revealed that 154 differentially expressed genes were significantly enriched in signaling pathways related to the cell cycle, fibroblasts, and epithelial-mesenchymal transformation. These findings suggest the involvement of distinct signaling regulation mechanisms between the PB and IN subtypes of PAC. Notably, our findings align with previous studies that have implicated these molecular pathways in the

development of PAC (29).

Numerous studies have provided evidence indicating that immune responses affect tumorigenesis at all stages, including initiation, invasion, malignant transformation, and metastasis (30,31). Immune cell infiltration plays a crucial role in controlling tumor growth and regulating the tumor microenvironment (31). In our study, we analyzed immune cell infiltration to examine the differences in the immune microenvironment between the two subtypes of PAC. The results revealed a significant increase in fibroblast infiltration in the PB subtype, while B cells and γδ T cells were significantly reduced. These findings indirectly suggest a potential explanation for the limited effectiveness of pancreaticobiliary therapy.

The limitations in our study are as follows: (I) its retrospective design; (II) the relatively small sample size; (III) the lack of molecular typing data; and (IV) the limited investigation into the molecular mechanisms associated with the two different subtypes.

Conclusions

Our findings indicate that pathologic typing-guided individualized chemotherapy contributes to the prolonged survival of patients with advanced PAC. The identification of the PB subtype and IN subtype is crucial, as they are associated with distinct clinicopathological characteristics, patient prognoses, and regulatory mechanisms. Therefore, recognizing these two subtypes is highly valuable in guiding the clinical diagnosis and treatment of PAC. Nevertheless, we acknowledge the need for prospective clinical trials involving different molecular subtypes. These trials would provide more standardized guidance for the clinical management of PAC, thereby facilitating further clinical advancements.

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Footnote

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References

1. Hester CA, Dogeas E, Augustine MM, et al. Incidence and comparative outcomes of periampullary cancer: A population-based analysis demonstrating improved outcomes and increased use of adjuvant therapy from 2004 to 2012. J Surg Oncol 2019;119:303-17.

2. Hu W, Duan Z, Zhang Y, et al. Remission from the 5-Fu-Based Chemotherapy to Gemcitabine-Based Chemotherapy-Based on the Pathological Classification of Periampullary Carcinoma: A Case Report and Literature Review. Onco Targets Ther 2022;15:891-6.
3. Hugenschmidt H, Labori KJ, Brunborg C, et al. Circulating Tumor Cells are an Independent Predictor of Shorter Survival in Patients Undergoing Resection for Pancreatic and Periampullary Adenocarcinoma. Ann Surg 2020;271:549-58.
4. Berberat PO, Künzli BM, Gulbinas A, et al. An audit of outcomes of a series of periampullary carcinomas. Eur J Surg Oncol 2009;35:187-91.
5. Riall TS, Cameron JL, Lillemoe KD, et al. Resected periampullary adenocarcinoma: 5-year survivors and their 6- to 10-year follow-up. Surgery 2006;140:764-72.
6. O'Connell JB, Maggard MA, Manunga J Jr, et al. Survival after resection of ampullary carcinoma: a national population-based study. Ann Surg Oncol 2008;15:1820-7.
7. Schnelldorfer T, Ware AL, Sarr MG, et al. Long-term survival after pancreatoduodenectomy for pancreatic adenocarcinoma: is cure possible? Ann Surg 2008;247:456-62.
8. Tang N, Chen ZY, Yang Z, et al. Development and verification of prognostic nomogram for ampullary carcinoma based on the SEER database. Front Oncol 2023;13:1197626.
9. Tella SH, Mahipal A. The future of adjuvant therapy in ampullary cancer: should we offer it to our patients? Hepatobiliary Surg Nutr 2020;9:368-70.
10. Seo HK, Hwang DW, Lee JH, et al. Role of systemic inflammation in predicting the prognosis of ampulla of Vater carcinoma. Surg Oncol 2019;29:33-40.
11. Kim HS, Heo CM, Choi YS, et al. Prognostic significance of histologic phenotype in periampullary adenocarcinomas. Front Oncol 2024;14:1407828.
12. Schneider M, Büchler MW. Periampullary carcinoma. Chirurg 2021;92:769-70.
13. Chandrasegaram MD, Gill AJ, Samra J, et al. Ampullary cancer of intestinal origin and duodenal cancer - A logical clinical and therapeutic subgroup in periampullary cancer. World J Gastrointest Oncol 2017;9:407-15.
14. Schwartz LH, Litière S, de Vries E, et al. RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer 2016;62:132-7.
15. Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol 2016;17:218.
16. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12:453-7.
17. Farid SG, Falk GA, Joyce D, et al. Prognostic value of the lymph node ratio after resection of periampullary carcinomas. HPB (Oxford) 2014;16:582-91.
18. El Nakeeb A, El Sorogy M, Ezzat H, et al. Predictors of long-term survival after pancreaticoduodenectomy for peri-ampullary adenocarcinoma: A retrospective study of 5-year survivors. Hepatobiliary Pancreat Dis Int 2018;17:443-9.
19. Sunil BJ, Seshadri RA, Gouthaman S, et al. Long-Term Outcomes and Prognostic Factors in Periampullary Carcinoma. J Gastrointest Cancer 2017;48:13-9.
20. Kamarajah SK. Pancreaticoduodenectomy for periampullary tumours: a review article based on Surveillance, End Results and Epidemiology (SEER) database. Clin Transl Oncol 2018;20:1153-60.
21. Papai E, Nevler A, Solomides C, et al. Intraoperative Cytologic Sampling for Resected Pancreatic and Periampullary Adenocarcinoma with Implications for Locoregional Recurrence-Free Survival. J Am Coll Surg 2022;234:48-53.
22. Bezrodnyi BH, Kolosovych IV, Hanol IV, et al. Comparison of the clinical effectiveness of hepaticojejunostomy and self-expanding metal stents for bypassing the bile ducts in patients with unresectable pancreatic head cancer complicated by obstructive jaundice. Wiad Lek 2024;77:629-34.
23. Narita M, Hatano E, Kitamura K, et al. Identification of patients at high risk for recurrence in carcinoma of the ampulla of Vater: Analysis in 460 patients. Ann Gastroenterol Surg 2023;8:190-201.
24. Neoptolemos JP, Moore MJ, Cox TF, et al. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine vs observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. JAMA 2012;308:147-56.
25. Zhu L, Kim K, Domenico DR, et al. Adenocarcinoma of duodenum and ampulla of Vater: clinicopathology study and expression of p53, c-neu, TGF-alpha, CEA, and EMA. J Surg Oncol 1996;61:100-5.
26. Nakagohri T, Takahashi S, Ei S, et al. Prognostic Impact of Margin Status in Distal Cholangiocarcinoma. World J Surg 2023;47:1034-41.
27. Skórzewska M, Kurzawa P, Ciszewski T, et al.

Controversies in the diagnosis and treatment of periampullary tumours. Surg Oncol 2022;44:101853.
28. Park SJ, Shin K, Hong TH, et al. Histologic subtype-based evaluation of recurrence and survival outcomes in patients with adenocarcinoma of the ampulla of Vater. Sci Rep 2023;13:16547.
29. Apurva, Abdul Sattar RS, Ali A, et al. Molecular pathways in periampullary cancer: An overview. Cell Signal 2022;100:110461.
30. Youssef R, Maniar R, Khan J, et al. Metabolic Interplay in the Tumor Microenvironment: Implications for Immune Function and Anticancer Response. Curr Issues Mol Biol 2023;45:9753-67.
31. Gao D, Fang L, Liu C, et al. Microenvironmental regulation in tumor progression: Interactions between cancer-associated fibroblasts and immune cells. Biomed Pharmacother 2023;167:115622.

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