Prognostic value of protein expression, tumor morphology and location within the pancreas in pancreatic ductal adenocarcinoma

HARUTO NISHIDA, KENGO ANDO, RYO KAIMORI, KAZUHIRO KAWAMURA and TSUTOMU DAA

Department of Diagnostic Pathology, Faculty of Medicine, Oita University and Oita University Hospital, Yufu, Oita 879-5593, Japan

Received November 11, 2024; Accepted January 24, 2025

DOI: 10.3892/ol.2025.15034

Abstract. Pancreatic ductal adenocarcinoma (PDA) of the head (hPDA) is more frequently diagnosed than PDA of the body/tail (btPDA) due to prevalent biliary obstruction symptoms, such as jaundice. hPDA is diagnosed and treated at an earlier stage than btPDA, leading to an improved prognosis. Data from 60 patients with PDA (30 patients with hPDA and 30 patients with btPDA) were analyzed, investigating tumor location (hPDA/btPDA) and clinical information [tumor size, lymph node metastasis, tumor stage and overall survival (OS)] depending on histological patterns [large duct pattern (PDA-L) and small duct pattern (PDA-S)], fibrotic focus (FF) and protein expression [GATA binding protein 6 (GATA6), cytokeratin 5/6, hepatocyte nuclear factor-1β (HNF1β), S100 calcium binding protein A4 (S-100A4), keratin 81 and transforming growth factor-β]. hPDA was significantly associated with tumor size, lymph node metastasis and more advanced stage. The worse OS was not related to tumor location, tumor size, lymph node metastasis or more advanced stage; however, GATA6 positivity was related to poor OS. Except for FF, PDA-L/PDA-S and immunostaining results were not associated with tumor location. PDA-L was related to S-100A4^{low}, GATA6^{+} and HNF1β^{+}. In the present study, tumor location did not influence tumor prognosis and histological pattern; otherwise, protein expression could influence PDA-L/PDA-S and OS. Therefore, histological classification may be useful in hPDA treatment.

Correspondence to: Dr Haruto Nishida, Department of Diagnostic Pathology, Faculty of Medicine, Oita University and Oita University Hospital, 1-1 Idaigaoka, Hasamamachi, Yufu, Oita 879-5593, Japan
E-mail: nharuto@oita-u.ac.jp

Abbreviations: btPDA, body/tail pancreatic ductal adenocarcinoma; CK5/6, cytokeratin 5/6; EMT, epithelial-mesenchymal transition; FF, fibrotic focus; G0, grade 0; G1, grade 1; G2, grade 2; G3, grade 3; hPDA, head pancreatic ductal adenocarcinoma; HNF1β, hepatocyte nuclear factor-1β; IHC, immunohistochemical; KRT81, keratin 81; PDA-L, large duct pattern; NKX6.1, NK6 homeobox 1; PDX1, pancreatic and duodenal homeobox 1; PDA, pancreatic ductal adenocarcinoma; PDA-S, small duct pattern

Key words: PDA, tumor location, histological feature, protein expression, overall survival

Introduction

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related deaths in Japan as of 2021 (1). Although surgical resection is necessary for curative treatment, approximately 80% of patients with PDA present with an unresectable disease. Postoperative chemotherapy is performed with TS-1, gemcitabine, and other agents; however, tumor recurrence or metastasis typically occurs within 1 year of treatment (2). PDA of the head (hPDA) is more frequently diagnosed than PDA of the body/tail (btPDA) owing to the higher incidence of clinical biliary obstruction symptoms such as jaundice. Consequently, hPDA is identified at an earlier, more treatable stage than btPDA, leading to a more favorable prognosis (3). Birnbaum *et al* (4) reported differences in gene expression between the PDA types. Specifically, the expression of genes associated with epithelial cell development and differentiation, smooth muscle cells, and transforming growth factor β (TGFβ) is higher in patients with btPDA than in those with hPDA (4,5). Moffitt *et al* (6) classified PDA into classical and basal cell types based on gene expression analysis findings and reported that the basal cell types were more likely to respond to chemotherapy. Additionally, Moll *et al* (7) reported that the Moffitt classification can be based on the expression levels of GATA6, a transcriptional regulator required for normal pancreatic development, and keratin 5, expressed in basal cells (8). Therefore, we evaluated the levels of not only GATA6 and cytokeratin 5/6 (CK5/6) but also hepatocyte nuclear factor-1β (HNF1β, expressed in the intestinal tract), S-100A4 (expressed in cholangiocarcinoma), keratin 81 (KRT81, a marker of basal cells), pancreatic and duodenal homeobox 1 (PDX1), NK6 homeobox 1 (NKX6.1, responsible for pancreatic development), TGFβ, and SMAD family member 4 (SMAD4, associated with the development of PDA) using immunostaining (9-13).

Regarding the histological and morphological classification of PDA, the large duct type (PDA-L) exhibits a more favorable overall prognosis than the small duct type (PDA-S), even if the tumor diameter is larger in the former type (14). Patients with colon cancer exhibiting more severe fibrosis reportedly exhibit a poorer prognosis (15). Existing literature indicates a stark contrast in gene expression between hPDA and btPDA.

However, the comprehensive evaluation of clinical data and protein expression to elucidate differences between these PDA types remains limited. In this study, we aimed to investigate the difference in tumor location (hPDA/btPDA) or prognosis based on protein expression levels or histological features (PDA-L/PDA-S and fibrotic focus [FF]).

Materials and methods

Case selection. We retrospectively collected data on 60 patients with PDA who underwent resection at Oita University Hospital, between November 2014 and February 2021. Surgery was performed in all operable cases. The patients who underwent neochemoradiotherapy were omitted. Cases were selected sequentially, starting with the newest case, retrospectively to reduce bias. Based on clinical, imaging, and macroscopic findings, the patients were stratified into two groups by tumor origin within the pancreas (hPDA and btPDA). We obtained clinical data from patient records, including sex, age, tumor location, tumor size (20 mm or not; pT1 or not), lymph node metastasis status (positive or negative), tumor stage (1 or over), overall survival (OS) (dead or alive), and histological diagnosis.

Histological staining. Hematoxylin and eosin (HE) staining and immunohistochemical (IHC) staining were performed manually. Formalin-fixed paraffin-embedded tissue blocks showing representative histology were selected for each case from our internal tissue archive and sliced into 4-μm-thick sections. Briefly, the sections were deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was quenched by incubating the sections with 3% hydrogen peroxide for 20 min at 25˚C. The subsequent process was determined according to the antibodies used (Table I) (9-13). Immunoreaction was visualized using the Histofine Simple Stain (MULTI) (Nichirei Biosciences, Tokyo, Japan). The nuclei were counterstained with Mayer's hematoxylin.

Histopathological evaluation. We evaluated the histological patterns and FF using HE staining. The histological patterns of PDA-L and PDA-S were evaluated following a previously reported method as a binary variable (14). Tumor glands >0.5 mm and occupying >50% of the total tumor area were defined as PDA-L, and those not meeting these criteria were defined as PDA-S (Fig. 1A and B) in the representative largest cut surface (14). FF was evaluated using a four-grade system (0, 1, 2, and 3) (Fig. 1C-E) (16). Grade 0 (G0) specimens primarily comprised infiltrating carcinoma without FF, and grade 1 (G1) specimens exhibited abundant fibroblasts arranged in a storiform pattern. Grade 2 (G2) specimens comprised intermediate fibroblasts mixed with collagen fibers and grade 3 (G3) specimens primarily comprised hyalinized collagen fibers. Tumor cells were rarely observed in FF. Based on assessment results, G1 and G2 specimens were categorized as having low fibrosis and G3 specimens were categorized as exhibiting high fibrosis (17).

During immunohistochemistry, reactivity was scored using three grades of colorimetric intensity (0, 1+, and 2+) and four grades of positive cell population (0, 1+, 2+, and 3+) (Figs. 1F and G, and S1). According to intensity, specimens were scored as follows: strong staining, 2+; weak staining, 1+; and no staining, 0. For the positive cell population grading, a staining grade of 3+ was detected in 50%, 2+ in 49-10%, and 1+ in 10-1% of the cells. Furthermore, specimens exhibiting negative staining were assigned a score of 0 (18). If positive intensity score was 2+ and 1+, we classified by the population grade (S-100A4, GATA6, HNF1β, PDX1, NKX6.1, KRT81, SMAD4, and TGFβ). For S-100A4, cases in which the gland duct epithelium was partially stained were defined as S-100A4^{low}, whereas those in which the entire epithelium was stained were defined as S-100A4^{high} (Fig. 1H and I). Additionally, the stromal staining was evaluated separately for the presence of TGFβ expression (Fig. 1J and K).

Statistical analysis. The χ^2 test, Welch's t-test and Fisher's exact test were performed using R (version 4.4.2), with the significance level set at 95%. If the calculated P-value was smaller than the one-sided P-value, it indicated a significant difference and warranted evaluation. The association between proteins was assessed using Phi coefficient, with a >0.2 indicating significance. The Mann-Whitney U test was performed to determine the association between PDA-L/PDA-S and S-100A4 expression, OS and lymph node metastasis, and OS and tumor stage. The Wilcoxon Signed Rank test was used to determine the association between GATA6 and HNF1β expression.

Results

Clinical characteristics and morphological findings of hPDA and btPDA. The clinical and pathological characteristics of patients, clinical relationships, histological and IHC findings, and clinical and histopathological relationships are shown in Tables II-IV. The study involved 60 patients with PDA: 30 cases each of hPDA and btPDA. In the hPDA group, the follow-up duration ranged from 6 to 70 (mean: 28.1) months, and that in the btPDA group ranged from 4 to 60 (mean: 22.3) months. Follow-up data regarding prognosis were not available for six patients with hPDA and one patient with btPDA. Morphologically, 24 and 36 patients were classified as having PDA-L and PDA-S, respectively. Regarding FF grading, 42 patients had a low FF grade (G1 and G2), whereas 18 had a high FF grade (G3). No differences were observed in sex, age, or histological patterns between the hPDA and btPDA groups (Tables II and III).

The hPDA group had smaller tumors than the btPDA group, and lymph node metastasis was more frequent in the hPDA group than in the btPDA group (P=0.020 and P=0.0061, respectively). Additionally, the hPDA group had a more advanced tumor stage than the btPDA group (P=0.030). OS was analyzed according to tumor location and histological patterns; the OS of patients was not significantly different between the groups (Table II). In contrast, the degree of fibrosis differed between the hPDA and btPDA groups, and the btPDA group had a higher FF grade than the hPDA group (P=0.0001).

IHC results of the hPDA and btPDA groups. The immunostaining results are shown in Table III, and Figs. 1 and S1.

Table I. List of antibodies used.

Antigen	Cat. no.	Dilution	Antigen retrieval	Temperature,˚C	Reaction time	Other	Source
S-100A4	ab133554	1:500	pH 9, autoclave	4	16 h	-	Abcam
GATA6	AF1700	1:40	pH 6, autoclave	25	30 min	Simple stain	R&D Systems, Inc.
HNF1β	12533-1-AP	1:500	pH 6, autoclave	25	30 min	Simple stain	Proteintech Group, Inc.
PDX1	ab47383	1:1,000	pH 6, autoclave	4	16 h	-	Abcam
NKX6.1	ab221549	1:500	pH 6, autoclave	4	16 h	-	Abcam
CK5/6	D5/16 B4	1:50	pH 6, autoclave	4	16 h	Simple stain	Dako; Agilent Technologies, Inc.
KRT81	sc-100929	1:50	pH 6, autoclave	4	16 h	Simple stain	Santa Cruz Biotechnology, Inc.
SMAD4	sc-7966	1:50	-	4	16 h	Simple stain	Santa Cruz Biotechnology, Inc.
TGFβ	21898-1-AP	1:50	-	25	30 min	-	Proteintech Group, Inc.

S-100A4, S100 calcium binding protein A4; GATA6, GATA binding protein 6; HNF1β, hepatocyte nuclear factor-1β; PDX1, pancreatic and duodenal homeobox 1; NKX6.1, NK6 homeobox 1; CK5/6, cytokeratin 5/6; KRT81, keratin 81.

S-100A4 positivity was observed in all patients; furthermore, S-100A4^{high} was observed in 33 and S-100A4^{low} in 27 of the 60 patients. S-100A4^{high} was observed in 13 of the 30 patients with hPDA and 20 of the 30 patients with btPDA. All were negative for PDX1, NKX6.1 and SMAD4, but some cells were positive for CK5/6 and KRT81 (Fig. S1). No differences were observed in the expression levels of all proteins between the hPDA and btPDA groups.

IHC results of the PDA-L and PDA-S groups. Variations in protein expression levels were observed between the PDA-L and PDA-S groups (Table IV). In the PDA-L group, S-100A4 ^{high} was observed in 3 and S-100A4^{low} was observed in 21 of the 24 patients. In the PDA-S group, S-100A4^{high} was observed in 29 and S-100A4^{low} was observed in 7 of the 36 patients. S-100A4^{high} was significantly associated with PDA-S (Table IV). S-100A4^{high} showed a PDA-S pattern and S-100A4^{low} showed a PDA-L pattern. GATA6 positivity was observed in 56 and GATA6 negativity was observed in 4 of the 60 patients. Of the 56 GATA6-positive patients, 24 patients were categorized under PDA-L and 32 under PDA-S. HNF-1β expression was positive in 22 of the 60 patients and negative in 38 of the 60 patients. In the PDA-L group, 14 of the 24 patients showed HNF-1β positivity, whereas in the PDA-S group, 8 of the 36 patients showed HNF-1β positivity. Patients with PDA-L showed GATA6 and HNF1β positivity (P=0.0350 and P=0.0010, respectively). Of the 53 patients who showed GATA6 positivity, 22 showed HNF1β positivity, 14 of whom had PDA-L. A significant association was observed between histological pattern and GATA6 expression (P=0.0350), and between histological pattern and HNF-1β expression (P=0.0010) (Table IV). All 27 patients with S-100A4^{low} showed GATA6 positivity; of the 33 patients with S-100A4^{high}, 27 showed GATA6 positivity. GATA6 expression was associated with HNF-1β expression (Table SI).

Relationship among clinical information, histological findings, and IHC results. With regard to OS, 9 of the 20 patients with PDA-L died of the disease; in contrast, 23 of the 33 patients with PDA-S died of the disease (P=0.136) (Table SII). However, OS was significantly related to GATA6 positivity (P=0.0001) (Table SII). Tumor size had no relation with HNF1β or GATA6 positivity (P=0.267 and P>0.999, respectively) (Table SIII). Furthermore, no positive association was found between FF and TGF-β expression in the stroma (P>0.999) (Table SIV).

Association between factors. We investigated the association between various factors, with a particular focus on OS (Table SII). There was a significant association between poor OS and positive lymph node metastasis (P=0.016) and advanced stage (P=0.016) (Table SII). Furthermore, there were no significant associations between tumor location and histological subtypes (PDA-L and PDA-S), nor between tumor location and protein expression (Table III).

Discussion

hPDA was significantly associated with small tumors, increased lymph node metastasis, advanced stages, and low FF grade tendency in this study. Patients with hPDA exhibited advanced stages owing to increased lymph node metastasis, even though a small tumor can trigger the onset of clinical symptoms and early detection. This observation may be attributed to the presence of immature fibrosis (low FF grade), facilitated by early detection. However, there was no relationship between hPDA and OS; instead, it was associated with increased lymph node metastasis and

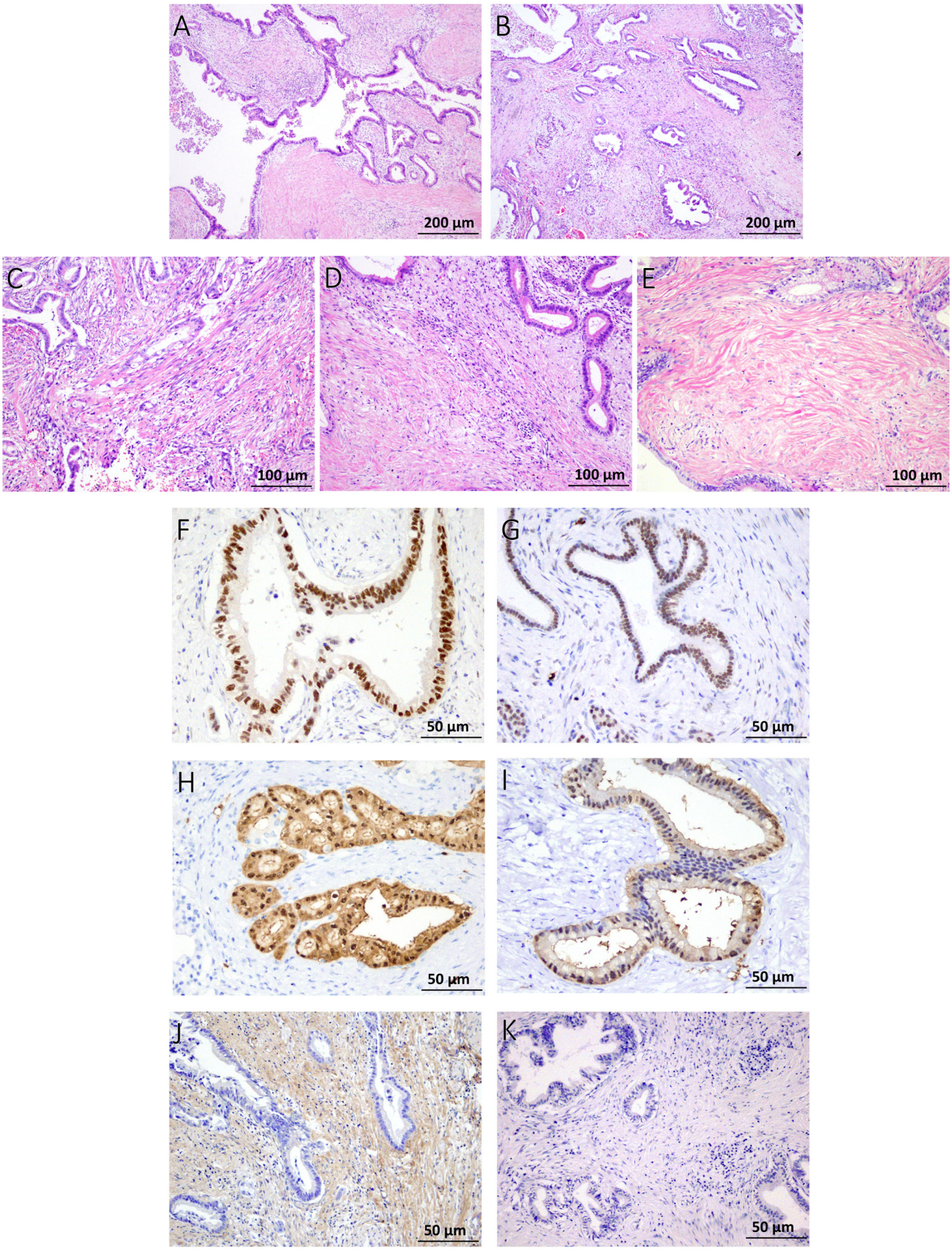

Figure 1. Evaluation of the histological patterns in PDA-L and PDA-S. (A) Tumor glands >0.5 mm and occupying >50% of the total tumor area were defined as PDA-L, and (B) the others were defined as PDA-S. Assessment of FF. (C) Samples rated grade 1 had abundant fibroblasts arranged in a storiform pattern, (D) those rated grade 2 consisted of intermediate fibroblasts mixed with collagen fibers and (E) those rated grade 3 consisted mainly of hyalinized collagen fibers. (E) Tumor cells were seldom observed in FF. (A-E) Hematoxylin and eosin stain. (F and G) Immunohistochemistry results for GATA6 and HNF1β. (F) GATA6 positivity is shown, displaying an intensity score of 2+ and a population score of 3+ (positive). (G) HNF1β positivity is also shown (intensity score of 2+; population score of 3+). Immunohistochemistry results for S-100A4. (H) Sections showing complete staining across the entire gland duct epithelium were designated as S-100A4^{high}, whereas (I) sections showing partial staining of the gland duct epithelium were designated as S-100A4^{low}. Immunohistochemistry results for TGFβ. The stroma exhibited (J) positive and (K) negative staining for TGFβ. FF, fibrotic focus; GATA6, GATA binding protein 6; HNF1β, hepatocyte nuclear factor-1β; PDA-L, large duct pattern; PDA-S, small duct pattern; S-100A4, S100 calcium binding protein A4.

Table II. Clinical characteristics of patients with head pancreatic ductal adenocarcinoma and patients with body/tail pancreatic ductal adenocarcinoma.

	Tumor location		
Characteristics	Head (n=30)	Body/tail (n=30)	P-value
Sex, n (male/female)	14/16	16/14	0.261
Mean age, years	72.4	70.2	0.229
Tumor size, n			0.030[a]
<20 mm	9	2	
≥20 mm	21	28	
Lymph node metastasis, n			0.030[a]
Positive	21	15	
Negative	9	15	
Stage, n			0.058[a]
1	9	15	
2, 3 and 4	21	15	
Follow-up, months			0.110
Range	6-70	4-60	
Mean	28.1	22.3	
Overall survival, n			0.715
Dead	18	14	
Alive	6	15	

[a]P<0.05. The χ^2 test (sex, tumor size, lymph node metastasis, stage and overall survival) and Welch's t-test (mean age and follow-up) were used to analyze the data and comparisons between the head and the body/tail were performed.

advanced tumor stage. This finding may be owing to the limited number of cases. A high FF grade tended to be also associated with poor OS, as reported by a previous study on colon cancer; nonetheless, in our study, a low FF grade was not associated with poor OS (15). The study findings did not establish a definitive relationship between each clinical factor and poor OS. However, other studies have suggested that HNF1β is an indicator of poor prognosis in pancreatic cancer, and our results are consistent with this finding (19). These findings suggest that protein expression is related to the morphology rather than tumor location, elucidating the notable association between HNF1β expression and OS. Therefore, the expressed protein may affect the choice of drug to be used. For example, high HNF1β expression may be associated with drug resistance in colorectal adenocarcinoma, and HNF1β expression may affect cisplatin nephrotoxicity (20,21).

The histological PDA-L pattern was related to S-100A4^{low}, GATA6 positivity, and HNF1β positivity. GATA6 is an important transcriptional regulator during pancreatic development, particularly in the formation of exocrine glands in the pancreas (22-24). It is required for gland development and is considered an oncogene in pancreatic cancer pathogenesis (22,24). GATA6 is reportedly highly expressed in classical type pancreatic cancer based on Moffitt's classification (6). Compared with low GATA6 expression in the basal cell type, high GATA6 expression has been observed in the classical type, which suppresses the basal cell-like changes, resulting in a better prognosis (6,14,25,26). This observation may be related to factors of epithelial-mesenchymal transition (EMT), such as decreased expression of E-cadherin and increased expression of vimentin that occur with decreased GATA6 expression. Tumors with high GATA6 expression have ductal structures (26). In the present study, high GATA6 expression was associated with PDA-L, which may be a crucial factor for the formation of ductal structures. Increased GATA6 expression reportedly increases HNF1β expression during pancreatic development (26). Although genetic alterations were not discussed in this study, PDA-L was regarded as the classical type and PDA-S as the basal cell type in the Moffitt's classification (6,14,15). Future studies should examine the relationship among tumor location, morphological features, and genetic alterations.

Our findings revealed that GATA6 and HNF1β expression was high, whereas S-100A4 expression was low in PDA-L. S-100A4 is expressed in various cancer types other than PDA and acts by inhibiting p53 release and increasing epidermal growth factor receptor expression (27,28). S-100A4 is associated with EMT, and the prognosis of S-100A4-positive PDA is poorer than that of S-100A4-negative PDA (27,28). Furthermore, S-100A4 promotes cell invasion and metastasis by degrading the extracellular matrix, which is involved in the upregulation of matrix metalloproteinases (MMPs), especially MMP-9, MMP-13, and MMP-2. They play important roles in tumor metastasis (28). Additionally, increased MMP9 expression decreases the expression of the long noncoding RNA *GATA6-AS*, a cancer suppressor gene, resulting in repressive feedback in endometrial cancer (29). Based on

Table III. Morphological and immunohistochemistry positivity findings in the head pancreatic ductal adenocarcinoma and body/tail pancreatic ductal adenocarcinoma groups.

Morphological and immunohistochemical findings	Head, n (%)	Body/tail, n (%)	P-value
Histological pattern			
Large pattern	13 (43)	11 (37)	0.792
Small pattern	17 (57)	19 (63)	0.347
Fibrotic focus			
Low (G1, G2)	27 (90)	15 (50)	0.323
High (G3)	3 (10)	15 (50)	0.0001
S-100A4 positivity			
High	13 (43)	20 (67)	0.125
Low	17 (57)	10 (33)	0.792
GATA6 positivity	24 (80)	29 (97)	0.999
HNF1β positivity	14 (47)	8 (27)	0.599
KRT81 positivity	17 (57)	22 (73)	0.425
NKX6.1 positivity	0 (0)	0 (0)	
PDX1 positivity	5 (17)	8 (27)	0.759
SMAD4 positivity	1 (3)	5 (17)	0.999
TGFβ (stroma positivity)	10 (33)	20 (67)	0.667

The χ^2 test (except for GATA6 and SMAD4) and Fisher's exact test (GATA6 and SMAD4) were used to analyze all morphological and immunohistochemical findings between the head and the body/tail were performed. G1, grade 1; G2, grade 2; G3, grade 3; S-100A4, S100 calcium binding protein A4; GATA6, GATA binding protein 6; HNF1β, hepatocyte nuclear factor-1β; KRT81, keratin 81; NKX6.1, NK6 homeobox 1; PDX1, pancreatic and duodenal homeobox 1.

Table IV. Relationship between histological findings and immunohistochemistry results.

	Histological pattern		
Immunohistochemistry results	Large, n	Small, n	P-value
S-100A4			0.0001[a]
High	3	29	
Low	21	7	
GATA6			0.0350
Positive	24	32	
Negative	0	4	
HNF1β			0.0010[a]
Positive	14	8	
Negative	10	28	

[a]P<0.05. The χ^2 test (except for GATA6) and Fisher's exact test (GATA6) were used to analyze all immunohistochemical results and comparisons between histological patterns were performed. S-100A4, S100 calcium binding protein A4; GATA6, GATA binding protein 6; HNF1β, hepatocyte nuclear factor-1β.

these findings, the characteristics of patients with GATA6 positivity, HNF-1β positivity, and S-100A4^{low} are consistent with those of patients with PDA-L. High GATA6 positivity in treatment-naive PDA favors good survival and can manifest a potent anti-tumor immune microenvironment (30). These protein expressions are linked to drug choice, and histological analysis becomes important. Furthermore, btPDA was associated with a high FF grade tendency and S-100A4^{high} in this study. This finding is consistent with the results of a previous study, which suggested that fibrosis is more pronounced in btPDA and that S-100A4 expression plays an important role in PDA fibrosis (4).

This study has some limitations. First, it included a small cohort of 60 Japanese patients with pancreatic cancer, employing a retrospective study design. Although the cohort size was small, this study represents a promising preliminary investigation. Most patients were recruited before 2019, and a few who underwent neochemoradiotherapy were omitted. Additionally, variations in the disease course resulted in incomplete follow-up for some patients. Moreover, we focused on histopathological aspects, using limited clinical data and without taking into account potential differences in treatment response among the groups. Future investigations should focus on larger cohorts and comprehensive clinical data.

Despite the limitations, our findings suggest that hPDA was significantly associated with small tumors, more frequent lymph node metastasis, more advanced stages, and low FF grade trend. The tumor origin could not influence tumor prognosis, but GATA6 was related to poor OS. In terms of histological findings, the PDA-L pattern was related to S-100A4^{low}, GATA6 positivity, and HNF1β positivity. We conclude that protein expression shows a stronger association with tumor morphology than with its location. The combination of morphology and protein expression could serve as an indicator for PDA prognosis depending on the choice of certain

drugs used in treatment. In addition, histological classification may be useful in PDA treatment.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study are not publicly available as they could compromise the privacy of research participants but may be requested from the corresponding author.

Authors' contributions

HN conceived and designed the experiments. HN and KA performed the experiments. HN, KA, RK, KK and TD analyzed the data. HN and KA wrote the manuscript. HN, KA and TD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the tenets of The Declaration of Helsinki (2013). The study protocol was reviewed and approved by the Institutional Ethics Committee and Review Board of Oita University (approval no. 2641; Yufu, Japan). The need for informed consent was waived due to the retrospective nature of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ushio J, Kanno A, Ikeda E, Ando K, Nagai H, Miwata T, Kawasaki Y, Tada Y, Yokoyama K, Numao N, et al: Pancreatic Ductal Adenocarcinoma: Epidemiology and Risk Factors. Diagnostics (Basel). 11: 562, 2021.
2. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, Faluyi O, O'Reilly DA, Cunningham D, Wadsley J, *et al*: Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): A multicentre, open-label, randomised, phase 3 trial. Lancet 389: 1011-1024, 2017.
3. Lee M, Kwon W, Kim H, Byun Y, Han Y, Kang JS, Choi YJ and Jang JY: The role of location of tumor in the prognosis of the pancreatic cancer. Cancers (Basel) 12: 2036, 2020.
4. Birnbaum DJ, Bertucci F, Finetti P, Birnbaum D and Mamessier E: Head and body/tail pancreatic carcinomas are not the same tumors. Cancers (Basel) 11: 497, 2019.
5. Ling Q, Xu X, Zheng SS and Kalthoff H: The diversity between pancreatic head and body/tail cancers: Clinical parameters and in vitro models. Hepatobiliary Pancreat Dis Int 12: 480-487, 2013.
6. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SG, Hoadley KA, Rashid NU, Williams LA, Eaton SC, Chung AH, *et al*: Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 47: 1168-1178, 2015.
7. Moll R, Franke WW, Schiller DL, Geiger B and Krepler R: The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. Cell 31: 11-24, 1982.
8. O'Kane GM, Grünwald BT, Jang GH, Masoomian M, Picardo S, Grant RC, Denroche RE, Zhang A, Wang Y, Miller JK, *et al*: Correction: GATA6 expression distinguishes classical and basal-like subtypes in advanced pancreatic cancer. Clin Cancer Res 28: 2715, 2022.
9. Bártů M, Dundr P, Němejcová K, Tichá I, Hojný H and Hájková N: The role of HNF1B in tumorigenesis of solid tumours: A review of current knowledge. Folia Biol (Praha) 64: 71-83, 2018.
10. Chen H, Xu C, Jin Q and Liu Z: S100 protein family in human cancer. Am J Cancer Res 4: 89-115, 2014.
11. Zhou X, Hu K, Bailey P, Springfeld C, Roth S, Kurilov R, Brors B, Gress T, Buchholz M, An J, *et al*: Corrigendum: clinical impact of molecular subtyping of pancreatic cancer. Front Cell Dev Biol 11: 1179559, 2023.
12. Zhao M, Mishra L and Deng CX: The role of TGF-β/SMAD4 signaling in cancer. Int J Biol Sci 14: 111-123, 2018.
13. Roy N, Takeuchi KK, Ruggeri JM, Bailey P, Chang D, Li J, Leonhardt L, Puri S, Hoffman MT, Gao S, *et al*: PDX1 dynamically regulates pancreatic ductal adenocarcinoma initiation and maintenance. Genes Dev 30: 2669-2683, 2016.
14. Kim SJ, Choi SJ, Yang J, Kim D, Kim DW, Byun JH and Hong SM: Pancreatic ductal adenocarcinoma with a predominant large duct pattern has better recurrence-free survival than conventional pancreatic ductal adenocarcinoma: A comprehensive histopathological, immunohistochemical, and mutational study. Hum Pathol 127: 39-49, 2022.
15. Ueno H, Ishiguro M, Nakatani E, Ishikawa T, Uetake H, Murotani K, Matsui S, Teramukai S, Sugai T, Ajioka Y, *et al*: Prognostic value of desmoplastic reaction characterisation in stage II colon cancer: Prospective validation in a Phase 3 study (SACURA Trial). Br J Cancer 124: 1088-1097, 2021.
16. Fujitani Y: Transcriptional regulation of pancreas development and β-cell function (Review). Endocr J 64: 477-486, 2017.
17. Ding JH, Xiao Y, Zhao S, Xu Y, Xiao YL, Shao ZM, Jiang YZ and Di GH: Integrated analysis reveals the molecular features of fibrosis in triple-negative breast cancer. Mol Ther Oncolytics 24: 624-635, 2022.
18. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, *et al*: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 38: 1346-1366, 2020.
19. Yu DD, Guo SW, Jing YY, Dong YL and Wei LX: A review on hepatocyte nuclear factor-1beta and tumor. Cell Biosci 5: 58, 2015.
20. Gao F, Gong W, He H, Zhang Z, Yang H, Shao F, Gao Y and He J: Integrated multi-omics analysis reveals clinical significance of hepatocyte nuclear factor-1β in tumor immune microenvironment, immunotherapy and prognostic prediction for colon adenocarcinoma. Cancer Immunol Immunother 74: 38, 2024.
21. Zhang Y, Hao J, Du Z, Li P, Hu J, Ruan M, Li S, Ma Y and Lou Q: Inhibition of hepatocyte nuclear factor 1β contributes to cisplatin nephrotoxicity via regulation of nf-κb pathway. J Cell Mol Med 25: 2861-2871, 2021.
22. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, *et al*: Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 531: 47-52, 2016.
23. Kloesch B, Ionasz V, Paliwal S, Hruschka N, Martinez de Villarreal J, Öllinger R, Mueller S, Dienes HP, Schindl M, Gruber ES, *et al*: A GATA6-centred gene regulatory network involving HNFs and ΔNp63 controls plasticity and immune escape in pancreatic cancer. Gut 71: 766-777, 2022.
24. Martinelli P, Carrillo-de Santa Pau E, Cox T, Sainz B Jr, Dusetti N, Greenhalf W, Rinaldi L, Costello E, Ghaneh P, Malats N, *et al*: GATA6 regulates EMT and tumour dissemination, and is a marker of response to adjuvant chemotherapy in pancreatic cancer. Gut 66: 1665-1676, 2017.
25. Yang MX, Coates RF, Ambaye A, Gardner JA, Zubarick R, Gao Y, Skelly J, Liu JG and Mino-Kenudson M: Investigation of HNF-1B as a diagnostic biomarker for pancreatic ductal adenocarcinoma. Biomark Res 6: 25, 2018.

26. Che P, Yang Y, Han X, Hu M, Sellers JC, Londono-Joshi AI, Cai GQ, Buchsbaum DJ, Christein JD, Tang Q, *et al*: S100A4 promotes pancreatic cancer progression through a dual signaling pathway mediated by Src and focal adhesion kinase. Sci Rep 5: 8453, 2015.
27. Martinelli P, Cañamero M, del Pozo N, Madriles F, Zapata A and Real FX: Gata6 is required for complete acinar differentiation and maintenance of the exocrine pancreas in adult mice. Gut 62: 1481-1488, 2013.
28. de Andrés MP, Jackson RJ, Felipe I, Zagorac S, Pilarsky C, Schlitter AM, Martinez de Villareal J, Jang GH, Costello E, Gallinger S, *et al*: GATA4 and GATA6 loss-of-expression is associated with extinction of the classical programme and poor outcome in pancreatic ductal adenocarcinoma. Gut 72: 535-548, 2023.
29. Zhao Y, Zou X, Wang G, Liu Y, Zhang C, Lu W and Li Q: Effects of GATA6-AS/MMP9 on malignant progression of endometrial carcinoma. J BUON 26: 1789-1795, 2021.
30. van Eijck CWF, Real FX, Malats N, Vadgama D, van den Bosch TPP, Doukas M, van Eijck CHJ and Mustafa DAM; Dutch Pancreatic Cancer Group (DPCG): GATA6 identifies an immune-enriched phenotype linked to favorable outcomes in patients with pancreatic cancer undergoing upfront surgery. Cell Rep Med 5: 101557, 2024.