

Original article

aPKC λ /I is a beneficial prognostic marker for pancreatic neoplasms

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ABSTRACT

Pancreatic cancer is a lethal disease. Overall survival is typically 6 months from diagnosis. Determination of prognostic factors in pancreatic cancer that would allow identification of patients who could potentially benefit from aggressive treatment is important. However, until date, there are no established reliable prognostic factors for pancreatic cancer patients. Herein, we propose a beneficial biomarker which is significantly correlated with the prognosis in pancreatic cancer patients. Atypical protein kinase C λ /I (aPKC λ /I) is overexpressed and has been implicated in the progression of several cancers. We tested the expression levels of aPKC λ /I in two types of pancreatic neoplasm, pancreatic ductal adenocarcinoma (PDAC) and intraductal papillary mucinous neoplasms (IPMNs), by immunohistochemistry. Examination of the aPKC λ /I expression levels in surgically resected specimens of PDCA ($n = 115$) demonstrated that the expression levels of aPKC λ /I in PDAC had prognostic implications, independent of the Tumor-Node-Metastasis classification and World Health Organization tumor grade. In the case of IPMNs ($n = 46$) also, the expression levels of aPKC λ /I in IPMN were found to be of prognostic importance, independent of the World Health Organization histological grade or morphological type. Interestingly, high expression levels of aPKC λ /I were significantly correlated with a worse histological grade ($p = 0.010$) and advanced stage of the tumor ($p = 0.0050$) in IPMN patients. These findings suggest that high expression levels of aPKC λ /I could be involved in the malignant transformation of IPMNs. Based on these observations, we propose the expression level of aPKC λ /I as a prognostic marker common to different types of pancreatic neoplasms.

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1. Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States and the fifth in Japan, with an estimated number of annual deaths from this disease of 34,100 and 22,000 in the two countries, respectively [1,2]. Because of its aggressive growth and potential for early metastatic dissemination, the average overall 5-year survival rate of patients with pancreatic cancer is <5%, and

only 20% of the patients can be treated by surgery with curative intent at the time of diagnosis [3].

To date, various treatments, including those that are highly invasive, have been tried to improve the poor prognosis of pancreatic cancer. Irradiation, chemotherapy, immunotherapy and other therapies, with or without surgery, have been applied to treat patients with pancreatic cancer [4,5]. Unfortunately, however, these modalities have failed to sufficiently control the aggressive behavior of invasive pancreatic cancer, and the overall survival of patients with pancreatic cancer remains grim, as assessed by global statistics [1].

Because pancreatic cancer is associated with a high mortality rate, aggressive treatment may be warranted, but the patients must

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be carefully chosen. Establishment of a prognostic scoring system could allow therapeutic management to be designed specifically for different sub-populations of the patients, and also identification of populations that might actually benefit from radical surgical treatment and/or chemotherapy. However, until date, there are no established reliable prognostic factors for pancreatic cancer patients whose usefulness has been proven by at least two examinations [6].

Isoforms of atypical protein kinase C, aPKC λ/ι and aPKC ζ , are PKC isozymes that are structurally and functionally distinct from other classes of this enzyme [7,8]. They play multifunctional roles in cellular maintenance and growth of epithelial cells, for example, signal transduction and cell polarity [9–15]. Studies on lung, ovary, gastric, colon, breast and prostate cancers have demonstrated a relationship between aPKC λ/ι expression and cancer progression, and have suggested that elevated aPKC λ/ι expression could be predictive of unfavorable outcomes [16–23]. Scotti ML et al. reported that high expression levels of aPKC λ/ι in pancreatic cancer were correlated with poor patient survival [24]. However, because only patients with pancreatic ductal adenocarcinoma (PDAC) were included in the aforementioned study, it remains unclear whether high aPKC λ/ι expression levels may also represent a poor prognostic factor in cases of intraductal papillary mucinous neoplasm (IPMN).

The pancreas is a complex organ comprised of three critical cell lineages; islet (endocrine), acinar, and ductal. Reflecting this variety of cell types, various types of neoplasms of the pancreas can occur in the pancreas. The most common type of pancreatic neoplasm among resected cases is PDAC, followed in frequency by IPMN, which accounts for up to 20% of all resected pancreatic neoplasms [25–27]. IPMN is characterized by the presence of dilated, frequently cystic, pancreatic ducts filled with mucus [28]. The prognosis of patients with non-invasive IPMN is excellent, and the reported 5-year survival rate is 77–100% [29–35]. Conversely, the reported 5-year survival rate of patients with IPMN-derived carcinoma ranges from 34% to 62% [25,31–33,35–40]. This survival is significantly worse than that of patients with non-invasive IPMN, but appears to be better than that of PDAC patients, which ranges from 9% to 21% [31,32,35–37,40]. It remains unclear, however, whether this difference in survival between IPMN-derived carcinoma and PDAC is related to their distinct biologies or might simply reflect the tendency of patients with IPMN-derived carcinoma to present at an earlier stage. To date, several studies have directly compared the clinicopathological features and long-term survival of patients of IPMN-derived carcinoma with those of PDAC, and conflicting results have been reported as to whether IPMN-derived carcinoma may be associated with improved survival, even in patients matched by the tumor stage [30,32,35–37,40,41].

In this study, we describe, for the first time, that the aPKC λ/ι expression level in the tumor tissue may have an important prognostic impact in IPMN. In addition, we confirmed that high aPKC λ/ι expression was correlated with a poor survival in patients with PDAC. These findings suggest that the aPKC λ/ι expression level in the tumor tissue can be a good biomarker common to two or more types of pancreatic neoplasm.

2. Material and method

2.1. Patients and samples

This study was conducted with the approval of the Ethics Committee of Yokohama City University (Yokohama, Japan). Clinical and pathologic data and the specimens used for the immunohistochemical analysis were obtained through a detailed retrospective review of the medical records of all 115 patients with PDAC and 46 patients with IPMN who had undergone initial surgical resection between 1991 and 2010 at the Yokohama City University Hospital

(Yokohama, Japan). In addition, 11 paired normal pancreatic tissue and pancreatic cancer tissue samples were obtained from patients who had undergone surgical resection in 2008. None of the patients had received any prior therapy. All the patients with PDAC and IPMN had received standard therapy appropriate for their clinical stages. Tumors were classified according to the WHO classification [42,43], the International Union Against Cancer Tumor-Node-Metastasis classification [44], and the classification of pancreatic carcinoma of the Japan Pancreas Society [45]. The medical records were complete for all patients, and all had been followed up by the tumor registries for survival and outcome. The latest survival data were collected on August 31, 2011. The mean follow-up was 20 months (range, 2–137 months) for patients with pancreatic ductal adenocarcinoma, and 45 months (range, 2–142 months) for patients with IPMN. The clinicopathologic features of the patients with PDAC and IPMN are summarized in Table 1 and Table 2, respectively.

2.2. Pathological examination of IPMN

Analysis of the IPMN background included the histological grade [42], type of duct involvement, and the epithelial subtype. The type of duct involvement by IPMN was determined by macroscopic and microscopic examinations, as well as by clinical imaging studies, and was classified into main-duct, branch-duct and mixed-duct type involvement [46]. Because the epidemiological and clinicopathologic characteristics of the patients with main-duct and mixed type IPMN have been reported to be similar [47], patients with the main-duct and mixed types were grouped together into the main-duct type for statistical analysis. The intraductal components were classified into four distinct epithelial subtypes (gastric, intestinal, pancreatobiliary and oncocytic) on the basis of their epithelial morphology in sections stained with hematoxylin and eosin (H&E), and where available, immunoreactivity against mucin glycoproteins, according to previously described criteria [48,49]. In cases exhibiting a heterogeneous epithelium type, the subtype was determined on the basis of the most prevalent type of epithelium of the highest malignancy grade.

Invasive carcinomas arising in the background of IPMN were classified into three histological subtypes: tubular adenocarcinoma, colloid carcinoma and oncocytic carcinoma [50,51]. In cases containing more than one histological subtype, the predominant one was considered for the purpose of this study.

2.3. RNA extraction and real-time quantitative PCR (qPCR)

Total RNA from the frozen tissues was extracted using ISOGEN (NipponGene, Tokyo, Japan), according to the manufacturer's instructions. After cDNA synthesis, qPCR was performed with an iCycler and TaqMan[®] Gene Expression Master Mix (Applied Biosystems, Carlsbad, CA). Primers for aPKC λ/ι were determined by the Primer-Express software (Applied Biosystems); 18S ribosomal RNA (rRNA) was used as the internal control. The primer sequences were as follows: aPKC λ/ι forward 5'-CCAATGGCCACACTTTCCA-3', reverse 5'-CGTCCAAGTCCCATATTCG-3', TaqMan[®] probe 5'-AAGCGTTCAA-CAGGCGT-3'. The reaction conditions consisted of preheating for 3 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C. The quantities of the amplified products were normalized to the quantity of 18S rRNA. All PCR products were confirmed using the melt curve method for specific amplification.

2.4. Immunohistochemistry

Immunohistochemical staining for aPKC λ/ι was performed according to the method described in a previous paper [20]. Formalin-fixed, paraffin-embedded sections (5- μ m thick) were

Table 1
Correlations between aPKC λ /i expression and the clinicopathological features in PDAC.

Clinical or pathological feature	Total (N)	aPKC λ /i		p value
		Low	High	
All cases	115	53	62	
Gender				
Male	68	30 (56.6%)	38 (61.3%)	0.61 ^d
Female	47	23 (43.4%)	24 (38.7%)	
Age (years)				
<65	45	24 (45.3%)	21 (33.9%)	0.21 ^d
≥65	70	29 (54.7%)	41 (66.1%)	
Pathologic tumor status ^a				
pTis	0	0 (0.0%)	0 (0.0%)	0.74 ^e
pT1	3	1 (1.9%)	2 (3.2%)	
pT2	9	4 (7.5%)	5 (8.1%)	
pT3	103	48 (90.6%)	55 (88.7%)	
Pathologic node status ^a				
pN0	23	10 (18.9%)	13 (21.0%)	0.78 ^d
pN1	92	43 (81.1%)	49 (79.0%)	
Pathologic metastasis status ^a				
pM0	98	45 (84.9%)	53 (85.5%)	0.93 ^d
pM1	17	8 (15.1%)	9 (14.5%)	
Stage ^a				
0	0	0 (0.0%)	0 (0.0%)	0.83 ^e
Ia	2	1 (1.9%)	1 (1.6%)	
Ib	4	2 (3.8%)	2 (3.2%)	
IIa	17	7 (13.2%)	10 (16.1%)	
IIb	75	35 (66.0%)	40 (64.5%)	
III	0	0 (0.0%)	0 (0.0%)	
IV	17	8 (15.1%)	9 (14.5%)	
Tumor grade ^b				
Grade 1	28	13 (24.5%)	15 (24.2%)	0.70 ^d
Grade 2	69	33 (62.3%)	36 (58.1%)	
Grade 3	18	7 (13.2%)	11 (17.7%)	
Lymphatic invasion ^c				
Negative	36	21 (39.6%)	15 (24.2%)	0.075 ^d
Positive	79	32 (60.4%)	47 (75.8%)	
Venous invasion ^c				
Negative	30	15 (28.3%)	15 (24.2%)	0.62 ^d
Positive	85	38 (71.7%)	47 (75.8%)	
Extrapancreatic nerve plexus invasion ^c				
Negative	65	31 (58.5%)	34 (54.8%)	0.69 ^d
Positive	50	22 (41.5%)	28 (45.2%)	
Tumor size				
<2.0 cm	17	5 (9.4%)	12 (19.4%)	0.14 ^d
≥2.0 cm	98	48 (90.6%)	50 (80.6%)	

^a Classified according to the International Union against cancer tumor-node-metastasis classification.

^b Classified according to the World Health Organization classification.

^c Classified according to the classification of pancreatic carcinoma of the Japan Pancreas Society.

^d χ^2 test.

^e Mann-Whitney's *U* test.

deparaffinized and dehydrated. Then, the sections were autoclaved in 10 mM citrate buffer (pH 6.0) and incubated with normal swine serum, exposed to anti-aPKC λ /i antibody (BD Biosciences) overnight at 4 °C, followed sequentially by incubation with biotinylated secondary antibody and avidin-biotinyl-peroxidase complex using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). The peroxidase reaction was developed using diaminobenzidine tetrahydrochloride plus 0.02% hydrogen peroxide as the chromogen, and nuclear counterstaining was performed with hematoxylin.

2.5. Immunohistochemistry scoring

Three investigators, including a board-certified pathologist, independently assessed the stained sections. The staining intensities of aPKC λ /i were graded as previously described, using a semiquantitative scale from 0 to 3, with 0 representing no staining,

1 representing light brown staining, 2 representing moderate brown staining, and 3 representing intense brown staining [20].

Nuclear and cytoplasmic aPKC λ /i expression levels were independently scored on a scale of 0–3 and added to obtain a total cellular expression score of 0–6. If there were multiple staining intensities on one section, the score that defined the largest area was determined as the score for each specimen. The scores for the IPMN cases were determined in the area showing the highest degree of dysplasia. Low aPKC λ /i expression level was defined as a total expression score of ≤ 3 , and high aPKC λ /i as a total expression score of > 3 . Sections stained with the secondary antibody alone served as the negative control.

2.6. Statistical analysis

All statistical analyses were performed using the SPSS for windows (SPSS Inc. Chicago, IL). The results on aPKC λ /i mRNA expression were analyzed by the Wilcoxon's *t* test. The correlations between the immunohistochemistry scoring and the clinicopathologic findings were analyzed by the chi-square test; if the expected value was less than 5 in more than 20% of the cells, Fisher's exact test was used instead. For the analysis of parameters showing a significant difference, we checked the absolute value of the adjusted residuals. Because we considered that $p < 0.05$ indicated statistical significance, the cutoff value of the absolute value of the adjusted residuals was set at 1.96 for this study. The parameters including multiple ordinal scales, such as the pathologic tumor status, tumor stage, and tumor/histological grade, were analyzed by the Mann-Whitney *U* Test. We constructed Kaplan-Meier curves and used the log-rank test to determine the prognostic significances of the variables.

A Cox proportional hazards regression model was used to simultaneously examine the effects of multiple covariates on the survival. Prior to the analysis, we prepared a correlation table of the variables and confirmed that none of the variables showed any significant correlation. The effect of each variable was assessed using the likelihood test and described by the hazard ratio with a 95% confidence interval. The stepwise Cox proportional hazards models initially included age, pathologic tumor status, pathologic lymph node status, pathologic metastasis status, tumor grade, tumor size, tumor growth pattern, presence/absence of a history of chemotherapy, as well as the aPKC λ /i expression for the PDAC patients. In the case of IPMN patients, the parameters included age, histological grade, macroscopic type, morphological types, and aPKC λ /i expression. The final model was developed by dropping each variable in turn from the model and conducting a likelihood ratio test to compare the full and nested models. A significance level of 0.1 was used as the cutoff to exclude a variable from the model. The results of the cell invasiveness assay were analyzed by the Mann-Whitney *U* Test. All statistical analyses were two-sided; $p < 0.05$ was considered to indicate statistical significance in all the analyses.

3. Results

3.1. Overexpression of aPKC λ /i mRNA in PDAC

To investigate the relationship between aPKC λ /i expression and pancreatic cancer, we evaluated the expressions of aPKC λ /i at the mRNA level in 11 pairs of surgically resected specimens of human PDAC and the surrounding non-tumor pancreatic tissue obtained from the same patients. The pathological findings in the H&E sections were confirmed by a board-certified pathologist. Quantitative real-time PCR analyses revealed that aPKC λ /i mRNA were significantly overexpressed in the PDAC tissues as compared with those in

Table 2
Correlations between aPKC λ/ι expression and the clinicopathological features in IPMN.

Clinical or pathological feature	Total(N)	aPKC λ/ι		p value
		Low	High	
All cases	46	35	11	
Gender				
Male	35	27	8	(72.7%)
Female	11	8	3	(27.3%)
Age (years)				0.42 ^c
<65	16	13	3	(37.1%)
≥65	30	22	8	(62.9%)
Histological grade ^a				
IPMN with low- or intermediate-grade dysplasia	20	19	1	(54.3%)
IPMN with high grade dysplasia	9	6	3	(17.1%)
IPMN with an associated invasive carcinoma	17	10	7	(28.6%)
Stage ^b				
0A	20	19	1	(54.2%)
0B	9	6	3	(17.1%)
0	0	0	0	(0.0%)
Ia	1	1	0	(2.9%)
Ib	11	7	4	(20.0%)
IIa	2	1	1	(2.9%)
IIb	1	0	1	(0.0%)
III	0	0	0	(0.0%)
IV	2	1	1	(2.9%)
Macroscopic type				
Branch	18	15	3	(42.9%)
Mixed	16	13	3	(37.1%)
Main	12	7	5	(20.0%)
Morphological types				
Gastric	19	18	1	(51.4%)
Intestinal	18	12	6	(34.3%)
Pancreatobiliary	4	2	2	(5.7%)
Oncocytic	5	3	2	(8.6%)
Invasion types				
None	29	25	4	(71.3%)
Tubular adenocarcinoma	10	8	2	(22.9%)
Colloid carcinoma	2	1	1	(2.9%)
Oncocytic carcinoma	5	1	4	(2.9%)

^a Classified according to the World Health Organization classification.

^b Classified according to the International Union Against Cancer Tumor-Node-Metastasis classification. Stage 0A and Stage 0B indicate low/moderate grade dysplasia and high grade dysplasia in the primary neoplasm, respectively.

^c Fisher's exact test.

^d Mann-Whitney's *U* test.

the paired non-tumor pancreatic tissues obtained from the same patients ($p = 0.0044$) (Fig. 1a).

3.2. Immunohistochemical analysis of aPKC λ/ι expression

Subsequent immunohistochemical analysis of the 115 specimens of PDAC was conducted to confirm the expressions of aPKC λ/ι at the protein level. In normal pancreatic duct epithelium,

expression of aPKC λ/ι protein was scarcely observed (Fig. 1b, white arrow). Although there were some specimens that showed faint aPKC λ/ι staining in the cytoplasmic region, no significant staining was observed in the membrane, junctional or nuclear regions in any of the specimens. On the other hand, in the cancer cells, aPKC λ/ι staining was present in various intensities in the cytoplasmic and nuclear regions (Fig. 1b, black arrow). Because the intensity of cytoplasmic and nuclear staining was not necessarily the same, we

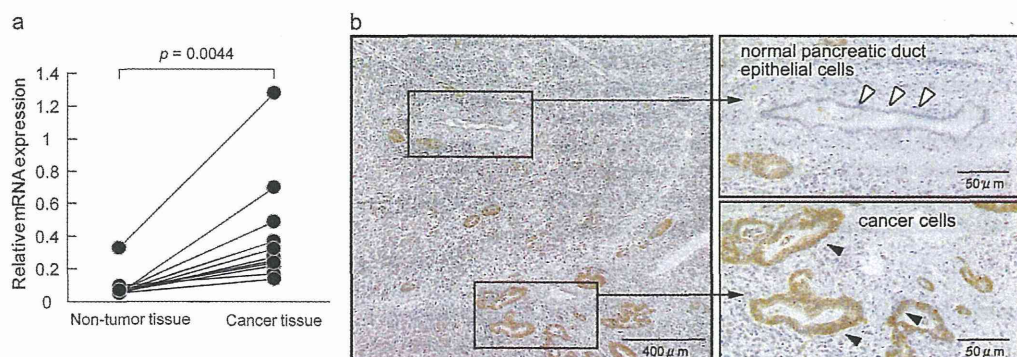


Fig. 1. **a** Quantitative real-time PCR analyses of aPKC λ/ι mRNA expression. aPKC λ/ι mRNA expression was compared between PDAC tissue and surrounding non-tumor pancreatic tissue specimens obtained from the same patients ($n = 11$ pairs). aPKC λ/ι mRNA was significantly overexpressed in the pancreatic cancer tissues ($p = 0.0044$). **b** Immunohistochemical analysis in a case of PDAC. aPKC λ/ι was overexpressed in the cancer cells (black arrow), but not in normal pancreatic duct epithelial cells (white arrow).

scored cytoplasmic and nuclear aPKC λ/ι expression levels separately on a scale of 0–3 (Fig. 2, upper row), and add the scores to obtain a total cellular expression score of 0–6. For the statistical analysis, low aPKC λ/ι expression level was defined as a total expression score of ≤ 3 ($n = 53$, 46.1%), and high aPKC λ/ι expression as a total expression score of >3 ($n = 62$, 53.9%).

In addition, we analyzed the protein expression of aPKC λ/ι in 46 specimens of IPMN. Consistent with the findings in the case of PDAC, protein expression of aPKC λ/ι was detected in only neoplastic lesion in the IPMN specimens (Fig. 2, lower row). The scores for the IPMN cases were determined in the areas with the highest degree of dysplasia, on the same scale as that for PDAC. In IPMN, there were 35 (76.1%) cases showing low aPKC λ/ι expression, and 11 (23.9%) showing high aPKC λ/ι expression.

3.3. Correlations between the clinicopathological features and the aPKC λ/ι expression level

In the PDAC patients, no correlations were observed between the aPKC λ/ι expression levels and any of the clinical parameters (Table 1). By contrast, the aPKC λ/ι expression levels were correlated with some clinical features in the IPMN patients (Table 2). As shown in Table 2, the aPKC λ/ι expression level was significantly correlated with the histological grade ($p = 0.010$), advanced tumor stage ($p = 0.0050$), morphological type ($p = 0.025$), and invasion type of the tumors ($p = 0.0026$). From a comparison of the standardized residuals, a significant difference of the aPKC λ/ι expression level was observed between the gastric type and other morphological types (absolute value of the adjusted residuals = 2.5). In regard to the differences among the invasion types, significant difference of the aPKC λ/ι expression level was observed between the tumors showing no invasion and oncocytic carcinoma (absolute value of the adjusted residuals = 2.1, 3.1, respectively).

3.4. Prognostic significance of the aPKC λ/ι expression level

In the analysis of the overall survival of the PDAC patients, 8 of the 115 patients (7.0%) survived for more than 5 years after the surgical resection, and 16 patients (13.9%) survived for more than 3 years. The median survival was 17.2 months for all patients. The group with low aPKC λ/ι expression showed better survival than the group with high aPKC λ/ι expression ($p = 0.00025$, Fig. 3a). The median survival was 29.6 months in the group showing low aPKC λ/ι expression, and 14.1 months in the group showing high aPKC λ/ι expression.

In the case of IPMN also, high aPKC λ/ι expression was correlated with a worse survival ($p = 0.020$; Fig. 3b). The median survival was only 16.1 months in the group showing high aPKC λ/ι expression. Because of the good prognosis, the median survival could not be calculated in the group showing low aPKC λ/ι expression in the tumors.

Furthermore, even when only patients with invasive IPMN ($n = 17$) were extracted, aPKC λ/ι expression was found to show prognostic significance. Among the patients with invasive IPMN, 4 of the 17 patients (23.5%) survived for more than 5 years after the surgical resection, and 5 patients (29.4%) survived for more than 3 years. The median survival was 25.9 months for all patients. The group with low aPKC λ/ι expression showed better survival than the group with high aPKC λ/ι expression ($p = 0.00094$, Fig. 3c). The median survival was 99.6 months in the group showing low aPKC λ/ι expression, and 10.6 months in the group showing high aPKC λ/ι expression.

3.5. Multivariable analysis for identifying prognostic factors

We applied the Cox proportional hazards model to our dataset to compare the prognostic risk factors. Our univariate analysis revealed that in PDAC patients, high aPKC λ/ι expression had the highest prognostic significance ($p = 0.00036$). In a stepwise multivariable Cox regression model with standard parameters, high aPKC λ/ι expression ($p = 0.0000056$), presence of distant metastasis ($p = 0.00041$), high tumor grade ($p = 0.0032$), and history of chemoradiotherapy ($p = 0.043$) were identified as significant factors affecting the prognosis in all patients (Table 3). The hazard ratio for a poor prognosis was 2.71 in patients showing high expression levels of aPKC λ/ι relative to other patients.

In a univariate analysis of IPMN patients, high aPKC λ/ι expression was identified as the factor with the second highest prognostic significance ($p = 0.0053$) after the histological grade ($p = 0.0010$). In a stepwise multivariable Cox regression model with standard parameters, only high aPKC λ/ι expression ($p = 0.010$) and histological grade ($p = 0.0012$) were identified as significant factors affecting the prognosis in all patients (Table 4). The hazard ratio for a poor prognosis was 5.23 in patients showing high expression levels of aPKC λ/ι , relative to other patients.

4. Discussion

It has been reported that high aPKC λ/ι expression is correlated with a poor prognosis in many types of cancers. In relation to

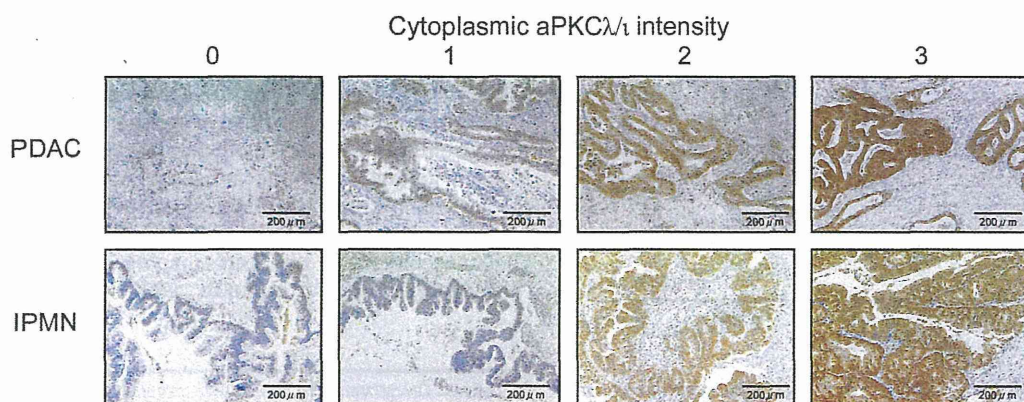


Fig. 2. Representative immunohistochemical micrographs of aPKC λ/ι expression in formalin-fixed specimens of human pancreatic ductal adenocarcinoma (PDAC; upper row) and intraductal papillary mucinous neoplasms of the pancreas (IPMN; lower row).

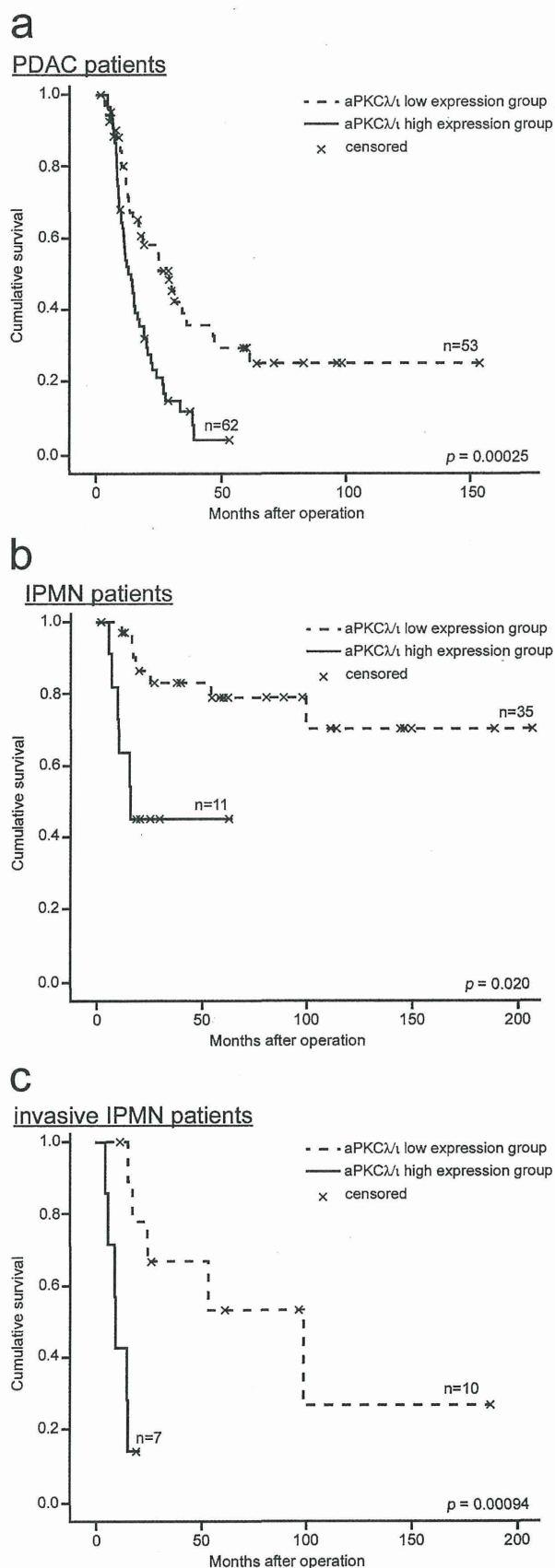


Fig. 3. a Prognostic significance of aPKCλ/ι expression in PDAC patients. The group with low aPKCλ/ι expression showed better survival than the group with high aPKCλ/ι expression ($p = 0.00025$). b Prognostic significance of aPKCλ/ι expression in IPMN

cancers of the digestive system, such an association has been reported for gastric cancer, colon cancer, cholangiocarcinoma, and PDAC [21,23,24,52]. In this study, we obtained evidence of the existence of a statistically significant correlation between high aPKCλ/ι expression and the prognosis in patients with two types of pancreatic neoplasms.

IPMN has been reported as the second common type of pancreatic neoplasm among resected pancreatic tumor specimens, after PDAC [25–27]. When the prognosis of patients with pancreatic neoplasms is analyzed, IPMN and PDAC can hardly be analyzed under comparable conditions. One reason is that the prognosis of patients with non-invasive IPMN is excellent. Even if patients with invasive IPMN are extracted, the prognosis is different between invasive IPMN and PDAC [32,35–37,40]. Thus, in this study, we analyzed the prognostic significance of the aPKCλ/ι expression level separately in PDAC and IPMN, and our results revealed the prognostic significance of aPKCλ/ι expression in PDAC, IPMN, as well as invasive IPMN. Moreover, multivariable analysis to identify significant prognostic factors revealed that the aPKCλ/ι expression level was a prognostic factor independent of standard clinical parameters. These findings suggest that the aPKCλ/ι expression level can be used as a common prognostic marker for different pancreatic neoplasms.

Although there were no correlations between high aPKCλ/ι expression and any clinical features in PDAC patients, high aPKCλ/ι expression was associated with some clinical features in IPMN patients. In IPMN patients, high aPKCλ/ι expression was significantly correlated with the histological grade ($p = 0.010$) and advanced stage of the tumor ($p = 0.0050$). IPMN is a rare neoplasm of the pancreas in which the course can be followed up from adenoma to carcinoma [42]. These findings suggest that aPKCλ/ι overexpression could be involved in the malignant transformation of IPMN.

In human PDAC, it is difficult to analyze the process of carcinogenesis in detail. One possible way is to analyze pancreatic intraepithelial neoplasia (PanIN), one of the distinct precursors to invasive pancreatic cancer that can be detected by pathological studies [53]. However, surgical specimens containing only PanIN are very rare. Scotti ML et al. reported that overexpression of aPKCλ/ι in human PDAC was correlated with poor patient survival [24]. They also demonstrated that aPKCλ/ι is required for the transformed growth of pancreatic cancer cells *in vitro* and their tumorigenesis *in vivo* [24].

In contrast to the case for PDAC, the course of malignant transformation of human IPMN can be easily analyzed in pathological specimens. In this study, we demonstrated the possible role of aPKCλ/ι in malignant transformation of human IPMN. Moreover, taken together with the results for PDAC, it is speculated that aPKCλ/ι may be involved in the carcinogenesis of several types of pancreatic cancers.

Although the mechanism remains unclear, two possibilities are speculated. One is K-ras mediated signaling, and the other is interleukin-6 (IL-6) mediated signaling. Oncogenic KRAS mutations are found in >90% of all advanced pancreatic cancers [54]. It has been demonstrated that antisense inhibition of oncogenic K-ras expression in pancreatic ductal adenocarcinoma (PDAC) cell lines blocks cellular transformation, suggesting continued requirement of oncogenic K-ras mediated signaling to maintain the transformed phenotype [55]. Furthermore, aPKCλ/ι has been reported as an

patients. The group with low aPKCλ/ι expression showed better survival than the group with high aPKCλ/ι expression ($p = 0.0020$). c Prognostic significance of aPKCλ/ι expression in patients with invasive IPMN. The group with low aPKCλ/ι expression showed better survival than the group with high aPKCλ/ι expression ($p = 0.00094$).

Table 3
Hazard ratios of clinicopathologic characteristics for overall survival in PDAC.

Variables	Categories	Overall survival	
		HR (95% CI)	p
Univariate analysis			
aPKC λ /i expression	high versus low	2.34 (1.46–3.74)	0.00036 ^a
Age, years	<65 versus \geq 65	1.07 (0.69–1.67)	0.77
Pathologic tumor status	pT1 + pT2 versus pT3	1.79 (0.73–4.44)	0.21
Pathologic node status	pN0 versus pN1	1.40 (0.79–2.45)	0.25
Pathologic metastasis status	pM0 versus pM1	2.61 (1.48–4.60)	0.00091 ^a
Tumor Grade	Grade 1 + Grade 2 versus Grade 3	1.74 (0.93–3.25)	0.82
Tumor Size (cm)	<2.0 versus \geq 2.0	1.78 (0.94–3.58)	0.077
Surgical margin	negative versus positive	1.81 (1.15–2.84)	0.011 ^a
Chemoradiotherapy	received versus not received	0.67 (0.34–1.20)	0.18
Multivariate analysis			
aPKC λ /i expression	high versus low	2.71 (1.68–4.40)	0.000056 ^a
Pathologic metastasis status	pM0 versus pM1	2.83 (1.59–5.03)	0.00041 ^a
Tumor Grade	Grade 1 + Grade 2 versus Grade 3	2.01 (1.06–3.79)	0.032 ^a
Chemoradiotherapy	received versus not received	0.54 (0.29–0.98)	0.041 ^a

The result of the model chi-square was significant ($p < 0.001$).

Variables were selected by the forward selection method (likelihood ratio test).

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

^a Significant.

important effector of oncogenic K-ras, both *in vitro* and *in vivo* [21,56–61]. Therefore, one possibility is that of aPKC λ /i playing a role as a critical downstream K-ras effector molecule.

The other possibility is involvement of IL-6 mediated signaling. IL-6, a cytokine involved in immune and hematopoietic activities, has been implicated in the progression of a variety of human cancers [62]. We demonstrated in an experiment conducted at our laboratory, that aPKC λ /i activates transcription of the IL-6 gene and promotes the growth prostate cancer cells [22,63]. In fact, significantly enhanced IL-6 production has been shown in pancreatic cancer patients with cachexia [64,65]. Thus, there is a possibility that aPKC λ /i induces tumor progression by stimulating IL-6 production in pancreatic cancer.

Table 4
Hazard ratios of clinicopathologic characteristics for overall survival in IPMN.

Variables	Categories	Overall survival	
		HR (95% CI)	p
Univariate analysis			
aPKC λ /i expression	high versus low	5.25 (1.63–16.89)	0.0053 ^a
Age, years	<65 versus \geq 65	1.71 (0.52–5.60)	0.37
Histological grade	IPMN with low, intermediate and high grade dysplasia versus IPMN with an associated invasive carcinoma	12.61 (2.79–57.26)	0.0010 ^a
Macroscopic type	Branch versus Mixed and Main	2.38 (0.66–8.67)	0.19
Morphological types	Gastric type versus others	4.22 (0.93–19.04)	0.61
Multivariate analysis			
aPKC λ /i expression	high versus low	5.23 (1.47–18.55)	0.010 ^a
Histological grade	IPMN with low, intermediate and high grade dysplasia versus IPMN with an associated invasive carcinoma	12.50 (2.71–57.70)	0.0012 ^a

The result of the model chi-square was significant ($p < 0.001$).

Variables were selected by the forward selection method (likelihood ratio test).

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

^a Significant.

One of the limitations of our study was the small number of cases of IPMN. Furukawa et al. analyzed the prognostic relevance of the morphological types of IPMN using a sufficiently large number of cases ($n = 283$), and reported that morphological type was significantly associated with the patient prognosis, and that gastric type IPMN was associated with the best prognosis amongst the morphological types [66]. However, in our study, we could not demonstrate any superior prognosis of the gastric type IPMNs as compared to the other morphological types of IPMN (Table 4). This finding could suggest that our sample may not have been correctly representative of the population of IPMN. Thus, further analyses of a large number of cases are needed to confirm our results.

In conclusion, our data show the expression level of aPKC λ /i as a promising prognostic marker common to different types of pancreatic neoplasms. Although further analysis is needed for clinical application, the accumulation of the knowledge of possible prognostic biomarkers that are independent of standard clinicopathological staging will help the decision of application of highly invasive treatment.

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Positioning of nasobiliary tube using magnet-loaded catheters

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Background and study aims: In endoscopic nasobiliary drainage (ENBD), repositioning the catheter from the mouth to the nose is complicated. We devised a method using catheters with magnets and verified its utility and safety.

Patients and methods: We prospectively enrolled 20 patients undergoing ENBD at Yokohama City University Hospital.

Results: The procedures were successful in all 20 cases and no case required a change of operators to a senior doctor. The mean time for the proce-

dures was 36.6 seconds. The emetic reflex was induced 0.5 times on average using the magnet method. The mean X-ray exposure time was 29.6 seconds. No complications occurred.

Conclusions: The magnet-loaded catheter method for positioning the ENBD catheter before finally leading it through the nose took little time and was performed successfully and safely. Therefore, the magnet method could become the first choice among techniques for ENBD catheter placement.

Introduction

Endoscopic nasobiliary drainage (ENBD) is used with endoscopic retrograde cholangiopancreatography (ERCP) and has been employed for over 30 years [1,2]. ENBD has recently been replaced by endoscopic retrograde biliary drainage (ERBD). Nevertheless, ENBD remains useful, for drainage in cholangitis; for cytological investigation, as it provides a continuous biliary sample [3,4]; in extracorporeal shock wave lithotripsy (ESWL) for biliary stones, to permit contrast injection and focusing on the stone [5]; and in treatment for perforation of the bile duct [6,7]. Also, the efficacy of preoperative ENBD for hilar and superior cholangiocarcinoma has recently been reported [8–10].

However, because leading the ENBD catheter from the mouth to the nose is painful for patients and complicated for operators, ENBD has been avoided. Currently, the finger method and the guide wire method [11] have been proposed as alternative placement techniques. Each method has some advantages, but also disadvantages. The finger method is speedy, but painful for patients and operators. The guide wire method is not painful, but is complicated for operators. We have devised a novel easier and quicker method using magnets, and have evaluated this technique for utility and safety.

Patients and methods

This was a prospective case series at a single center. Patients who underwent ENBD at Yokohama City University Hospital were included in the study, but patients were excluded if they had previously received a pacemaker. All patients gave written informed consent before the ENBD procedure and the study was performed in accordance with the ethical standards of the committees of the institute and the Declaration of Helsinki (Protocol B110901010, 1 October 2011), and was registered (ClinicalTrials.gov, ID UMIN000008118). For sedation and analgesia, 5 mg of midazolam and 7.5 mg of pentazocine were administered for induction, and additional amounts given as needed. The ENBD tubes were placed under fluoroscopic guidance using a duodenal endoscope (JF-260V; Olympus, Tokyo, Japan). The ENBD tube was in all cases a 6-Fr reverse alpha type, and we used a Nelaton tube with two side holes. A trainee physician with 1–3 years' experience attempted placement of the ENBD tubes using the magnet method. If they failed to lead the ENBD tube to the mouth within 3 minutes, a senior gastroenterologist took over.