Original Article

The clinicopathological features and prognosis of pancreatic ductal adenocarcinoma with concurrent carcinoma *in situ*

Ziyao WANG[1], Yoshihiro HAMADA[2], Yuzo OYAMA[2], Daisuke KATO[3] and Toshiharu UEKI[4]

[1]Graduate School of Medical Sciences, Fukuoka University, Fukuoka, Japan

[2]Department of Pathology, School of Medicine, Fukuoka University, Fukuoka, Japan

[3]Department of Surgery, Yamamoto Memorial Hospital, Saga, Japan.

[4]Department of Gastroenterology, Chikushi Hospital, Fukuoka University, Fukuoka, Japan.

Short running title: Pancreatic carcinoma with carcinoma *in situ*

Abstract

Background/Objectives: The prognosis of pancreatic cancer is poor. Although some case reports have revealed that the prognosis of pancreatic ductal adenocarcinoma (PDAC) with carcinoma *in situ* (CIS) is better than that of PDAC without CIS, this finding has not been fully validated. Therefore, this retrospective study investigated the prognosis of PDAC with CIS (CIS^+ PDAC) at our institution.

Methods: Of the 267 patients with PDAC (January 1981-December 2011), we retrospectively analyzed the clinicopathological data of eight patients with CIS^+ PDAC. We compared the prognosis of these patients 8 with that of 79 patients with PDAC without CIS (CIS^- PDAC).

Results: The overall survival of CIS^+ PDAC patients was significantly higher than that of CIS^- PDAC patients (P=0.0068). Patients with CIS^+ PDAC had multiple favorable prognostic factors, including well differentiated adenocarcinoma (P=0.0267), smaller tumor sizes (P=0.0299) and R0 surgery (P=0.0005). Furthermore, the incidences of retroperitoneum tissue invasion and lymph node metastasis were lower in CIS^+ PDAC than in CIS^- PDAC. A multivariate analysis revealed that CIS was an independent prognostic factor.

Conclusions: CIS^+ PDAC is associated with a significantly better prognosis than CIS^- PDAC.

Keywords: pancreatic ductal adenocarcinoma, pancreatic cancer, carcinoma in situ, prognosis

Corresponding author:

Yoshihiro Hamada, Department of Pathology, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka, Japan. Tel: +81-92-801-1011; Fax: +81-92-863-8383; E-mail: yhamada@fukuoka-u.ac.jp

Introduction

Pancreatic cancer has the fourth-highest cancer-related death rate in the world, and this rate has increased in recent years.[1] Surgical resection is essential for curing this neoplasm; however, 80%-85% patients cannot undergo radical surgery because of local invasion and metastasis.[2] The mean postoperative survival duration of pancreatic cancer is only 10-20 months.[3]

Although the early salvage of pancreatic ductal adenocarcinoma (PDAC) based on specific pathophysiological findings may help prolong the prognosis, it is poorly understood. PDAC has morphologies that include intraductal spreading-type and intraductal non-spreading-type. The intraductal spreading-type is further classified into two subtypes. One subtype is pancreatic intraepithelial neoplasia (PanIN), which is defined as a non-invasive, flat or micropapillary, epithelial neoplasm, and is considered to be a precursor lesion being further classified into high- and low-grade PanIN according to the degree of architectural and cytologic atypia. High-grade lesions are recognized as carcinoma.[4–6] The diagnosis of PanIN has become challenging recently and is generally only possible when screening high-risk populations.[3] The other subtype is carcinoma in situ (CIS), defined as intraductal carcinoma associated with invasive ductal adenocarcinoma of the pancreas.[7] It exists in the pancreatic duct connected to invasive carcinoma. Ikeda et al. classified CIS into the low-papillary-type, flat-type, and mixed-type.[8]

Several case reports have described instances of PDAC patients with CIS (CIS^+ PDAC) achieving a long-term survival.[9,10] Regarding the characteristics of these long-surviving patients, Conlon et al. reported that 7 of 12 patients with a long-term survival

had intraductal components.[9] Fukuba et al. also reported a patient with PDAC with a concurrent intraductal component who survived for five years.[10]

We hypothesized that components of CIS might be a favorable prognostic factor in cases of advanced PDAC; however, assessments using a larger sample size are limited. Therefore, in the present study, we elucidated the prognosis of CIS^+ PDAC in our institution and compared the findings with those of PDAC without CIS (CIS^- PDAC).

Methods

Patients

We retrospectively reviewed the clinical records of 297 patients with invasive PDAC treated surgically at Fukuoka University (Fukuoka, Japan) between January 1981 and December 2011. Patients who had a concurrent CIS component of ≥20 mm in the long-axis direction were defined as CIS^+ PDAC. We excluded 30 cases of intraductal papillary mucinous neoplasms whose pathological findings included cystic lesions with a diameter of >5 mm, grossly visible structures, and a large amount of mucus.[11]

Clinicopathologic characteristics

Of the 267 patients with PDAC, 8 (3.0%) met the criteria for CIS. Clinical information was obtained from each patient's records, including histopathologic features, such as histological differentiation and retroperitoneal tissue invasion (pRP). In addition, the primary tumor (pT) and regional lymph node (pN) were evaluated according to the WHO Classification of Tumors of the Digestive System.[3] The presence of residual cancer was assessed; a tumor present within 1 mm of the resected circumferential margin, including locoregional spreading or metastasis, was defined as a residual tumor status of R1 in previous reports.[12,13] We compared the clinicopathologic parameters of the 8 CIS^+ PDAC patients to those of the 79 CIS^- PDAC patients out of among the 267 patients who

underwent surgery. Twenty-eight patients of the 79 CIS^- PDAC patients obtained an R0 surgical resection status.

Immunohistochemistry

The Ki-67 (MIB-1; 1: 100 dilution Glostrup, Denmark) labeling index was evaluated in both CIS^+ PDAC and CIS^- PDAC. In CIS^+ PDAC, the CIS component and invasive carcinoma were separately calculated MUC1 (ab15481; 1: 50 dilution Novocastra, UK), MUC5AC (CLH2; 1: 100 dilution Novocastra, UK) and MUC6 (CLH5; 1: 100 dilution Novocastra, UK). Staining method conducted as follows: in brief, 3μm-thick paraffin sections were deparaffinized. Endogenous peroxidase was inactivated with 3% hydrogen peroxide, and antigen retrieval was performed. After incubation of the sections with a primary antibody (for Ki-67, MUC1, MUC5AC or MUC6) for 1 hour at 37°C, the sections were treated with the secondary antibody (MAX-PO; Nichirei Biosciences, Tokyo, Japan). Diaminobenzidine (DAB) was used as a chromogen. Finally, the sections were counterstained with hematoxylin. Positive control slides were included. The percentage of Ki67-positive tumor cells was counted among 1000 tumor cells per tumor in hotspot areas. More than 10% positivity for MUC1, MUC5AC and MUC6 was defined as being positive for the factor.

Statistical analyses

Categorical variables were compared using Fischer's exact test and Wilcoxon test. Survival rates were calculated using the Kaplan–Meier methods and differences were calculated using the log-rank test. A multivariate survival analysis was performed using the Cox proportional hazards model, including calculations of relative risks and 95% confidential intervals. Analyses were performed with the StatView 5.0 statistical software package (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as $p< 0.05$.

Results

Clinical findings

Table 1 showed the characterization of the eight patients (three men and five women) with CIS^+ PDAC. The mean age was 63.0 years old (median: 67 years old; range: 48–73 years old). The time from the onset of symptoms to surgical resection ranged from three months to three years. Of the eight patients, three underwent pylorus-preserving pancreatoduodenectomy (PPPD), three underwent total pancreatectomy (TP), and two underwent distal pancreatectomy (DP). No significant differences in the age or sex were seen between the two groups (Table 2).

No patients received preoperative chemoradiation therapy. After surgical resection, eight patients with CIS^+ PDAC received adjuvant chemotherapy. Initially, 4-8 mg of mitomycin C was injected intravenously. After 1 week, 4-8 mg of mitomycin C and 5-fluorouracil were intravenously injected for 3 consecutive days. Orally 1, 500 mg/day of Tegafur (5-fluoro-1-(oxolan-2-yl) pyrimidine-2,4-dione)/uracil treatment was taken simultaneously. The dose of the drugs was modified according to patient's condition.

Postoperative course

Three of the eight patients died of peritoneal carcinomatosis due to local recurrence. The pancreatic surgical margins in three cases were changed to positive for CIS^+ on a re-evaluation of sections after being indicated as negative in the intraoperative frozen sections. In these cases, CIS was not present in the intraoperative section. The intraoperative frozen sections cut from the pancreatic surgery tissue did not include the main pancreatic duct (MPD) and surrounding tissues due to heat artifact and defects, and no cancer cells were observed, so it was diagnosed as R0. After, the pancreatic surgery tissue was embedded in paraffin and subjected to H&E Staining, cancer cells were found on the pancreatic surgery tissue connected to the intraoperative frozen sections. Therefore,

we speculated that there might have been cancer cells in the previous intraoperative frozen sections. Furthermore, one patient (case 1) experienced leakage of the pancreaticojejunostomy after operation; in this case, invasive carcinoma had formed at the posterior aspect of a the pancreaticojejunostomy. The remaining five patients survived for three to nine years without recurrence.

A Kaplan–Meier analysis of the CIS^+ PDAC group (mean survival: 62 months) showed a significantly better prognosis than that for the CIS^- PDAC group (mean survival: 49 months; Figure 1A; P=0.0068). Furthermore, a multivariate analysis revealed that CIS was the only promising parameter for the prognosis (Table 3; P=0.0388). In summary, CIS components were considered useful pathophysiological findings for predicting a better prognosis. Kaplan–Meier analysis of the R0-resection CIS^+ PDAC group showed a significantly better prognosis than the R0-resection CIS^- PDAC group (Figure 1B; P= 0.0004).

Pathological findings

The CIS^+ PDAC lesions were located at the pancreas head in one (case 5), body in one (case 8) and tail in one (case 6). The remaining five patients had a tumor at the border between the head and body (case 1-4, 7; Figure 2, Table 1). Six cases had pRP. However, all invasive sites were within 500 μm of the pancreatic tissue margin. The CIS^+ PDAC cases had CIS located mostly on the MPD side (Figure 3, Figure 4a, d). The morphologies between the invasive carcinoma of CIS^+ PDAC group and CIS^- PDAC group were not markedly different (Figure 4). In the CIS^+ PDAC group, tumor differentiation as follows: well differentiated tubular adenocarcinoma (six cases), moderately differentiated tubular adenocarcinoma (one case) and poorly differentiated adenocarcinoma (one case) (Figure 4e, f, Table 1).

The CIS^+ PDAC group had significantly more well differentiated tubular adenocarcinoma cases than the CIS^- PDAC group (Table 2; *P*=0.0267). All CIS components were low-papillary-type, and the mean longitudinal length was 36mm (Figure 4d; longitudinal size range: 24–68 mm). The CIS^+ PDAC group had significantly smaller tumors than the CIS^- PDAC group (Table 2; *P*=0.0299). In addition, the CIS^+ PDAC group had lower lymph node metastasis rates than the CIS^- PDAC group; however, there was no significant difference in these rates between the two groups (Table 2; *P*=0.0561). All CIS^+ PDAC patients had a significantly higher rate of R0 than those with CIS^- PDAC (*P*=0.0005; Table 2). Ki-67-positive cells in the CIS component of CIS^+ PDAC group were significantly fewer than in the CIS^+ PDAC group (*P*=0.0038). However, there was no significant difference between the number of Ki-67 positive cells in the invasive carcinoma component of the groups (Figure 4h-j, Table 4; *P*=0.2113). In the CIS^+ PDAC group, MUC1 was 100% (6/6) expressed in CIS and invasive carcinoma, MUC5AC was 100% (6/6) expressed in CIS and 67% (4/6) expressed in invasive carcinoma, and MUC6 was 67% (4/6) expressed in CIS and 33% (2/6) expressed in invasive carcinoma (Figure 5; Table 5).

Discussion

Our results showed that PDAC cases with concurrent CIS had more favorable prognoses than PDAC cases without concurrent CIS. In addition, the CIS^+ PDAC group had multiple favorable prognostic factors, including well differentiated adenocarcinoma (*P*=0.0267) and a smaller tumor size (*P*=0.0299). Recently, a few cases of patients with CIS^+ PDAC who achieved a long-term survival have been reported. Conlon et al. reported that seven cases of PDAC with intraductal components survived for more than five years.[10] Fukuba et al. reported a case of pancreatic cancer with 15-mm intraductal spreading without recurrence for 5 years.[11] Oda et al. showed that PDAC with high-grade PanIN (PDAC-H-PanIN) had a

better prognosis than PDAC with low-grade PanIN (PDAC-L-PanIN).[14] As the mechanism, Miyazaki et al. suggested that the carcinogenic pathways might differ based on the immunohistochemical expression profiles of p53, p16, and SMAD4: the PanIN-carcinoma sequence for PDAC-H-PanIN and *de novo* carcinoma for PDAC-L-PanIN. The frequency of p53 high expression, loss of p16 expression, and loss of SMAD4 expression was similar between high-grade PanIN and invasive carcinoma in the PDAC-H-PanIN group, whereas differentces existed between low-grade PanIN and invasive carcinoma in PDAC-L-PanIN group.[15] CIS and high-grade PanIN have been considered synonymous.[3] Therefore, the better prognosis for CIS^+ PDAC than for CIS^- PDAC might be due to differences in the carcinogenic pathway. This may also be the reason for the differences in the prognosis of CIS^+ PDAC and CIS^- PDAC. Furthermore, Miyazaki et al. found that the rate of loss of SMAD4 expression in PDAC-H-PanIN was lower than that in PDAC-L-PanIN.[15] Wang et al. reported that PDAC with intact SMAD4 was associated with well differentiation and smaller tumors.[16] A smaller tumor sizes and well differentiation have been reported to be good prognostic factors for PDAC.[17-24] The statistically significant differences in these two parameters in the CIS^+ PDAC group might be attributed to the intact SMAD4 status.

In breast cancer studies, invasive ductal carcinoma (IDC) with ductal carcinoma in situ (DCIS) have shown a more favorable prognosis than IDC without DCIS.[25, 26] Chagpar et al. showed that IDC with DCIS generally showed a smaller tumor size and well-differentiated characteristics.[26] The associated genetic changes differ between IDC with and without DCIS; therefore, these tumors might have different carcinogenic pathways,[27] a finding similar to that for pancreatic cancer.

In the present study, the CIS$^+$ PDAC group was characterized by promising factors associated with a good prognosis including higher R0 rates (P=0.0005) and a smaller tumor sizes. In their experiments with mice, Tsutsumi et al. revealed that invasive ductal carcinoma results from CIS of the MPD and of the branch pancreatic duct (BPD). CIS of the BPD progresses to IDC more rapidly than CIS of the MPD. Furthermore, after developing into IDC, the IDC originating from BPD-type CIS infiltrates the surrounding pancreatic tissue more rapidly than that originating from MPD-type CIS.[28] Ikeda et al. speculated that the low-papillary-type CIS would infiltrate the surrounding tissues after progressing to the pancreatic duct, whereas flat-type CIS would directly infiltrate the surrounding tissues.[8] In our study, the eight evaluated CIS$^+$ PDAC cases had cancer originating from the MPD, and all had the low papillary type. The smaller tumor sizes in CIS$^+$ PDAC cases might have been due to the slower infiltration of IDC from the MPD. In PDAC, the presence of residual cancer cells at the surgical margin is considered to be significantly related to recurrence and the survival.[19-24] In PDAC with R1 resection, the posterior resection margin is the most frequently infiltrated (65.6%) by cancer cells.13 Although pRP rates did not differ significantly between the CIS$^+$ PDAC and CIS$^-$ PDAC groups, in the CIS$^+$ PDAC group, the pRP depths were all within 500 μm of the pancreatic edge, and vertical invasion fronts were not present at the margin. Lower pRP values may be caused by slower infiltration of CIS of the MPD into the surrounding tissues. As our CIS$^+$ PDAC cases had shallow invasion depths of the posterior peritoneum, it was easier to achieve an R0 resection margin.

In our study, all CIS$^+$ PDAC cases were diagnosed as R0, which is an important prognostic factor for PDAC, [19–24] but R0-resection CIS$^+$ PDAC cases had more favorable prognoses than did R0-resection CIS$^-$ PDAC cases, showing that CIS still has an effect on the prognosis in cases of R0 resection. Smaller tumor sizes and well differentiated tumors

are considered to be favorable prognostic factors.[17, 18] In our multivariate analysis, only CIS showed a favorable prognostic factor, indicating that CIS affects the prognosis without any the influence of other factors. Therefore, the presence of CIS may be a new prognostic factor of PDAC.

In the CIS^+ PDAC group, the MUC1 expression of CIS was the same as that of invasive carcinoma, and the MUC5AC and MUC6 expression of CIS was higher than that of invasive carcinoma. The mucin expression profile was associated with the progression to the precursor lesions of invasive carcinoma of sequence: the expression of MUC5AC and MUC6 for early-stage carcinogenesis and MUC1 for late-stage carcinogenesis.[29] The MUC1 showed that CIS was similar to invasive carcinoma. The higher expression of MUC5AC and MUC6 may indicate that CIS was at an earlier stage than invasive carcinoma.

The result of Ki67 proved that CIS has a weaker proliferation ability than invasive carcinoma. However, the Ki67 of invasive carcinoma was not markedly different between the CIS^+ PDAC group and CIS^- PDAC groups. This was not inconsistent with the more favorable prognosis of the CIS^+ PDAC group, as Ki67 had no correlation with the survival in previous studies of PDAC.[30, 31, 32] Lüttges et al. showed that the malignancy of ductal adenocarcinoma depended on the tumor differentiation and rarely depended on the proliferation ability.[33]

Usually, PDAC is located in the pancreas head, body or tail. However, >50% of CIS^+ PDAC cases had tumors in the pancreas neck, where a part of the anterior superior mesenteric artery and portal vein are located. In cases of PDAC located in the pancreas neck on preoperative imaging, we should examine the possibility of CIS extending to the surgical margin.

Assessment the surgical margins in CIS$^+$ PDAC cases is puzzling because the optimal pancreatic surgical margin is often unclear. More than half of the invasive areas were located in the pancreas neck or along the surgical resection line for PPPD and DP. A previous study showed that the type of CIS also affects surgical choices; papillary-type CIS can spread widely in the pancreatic duct compared with flat or flat-and-low papillary types.[8] All of our CIS$^+$ PDAC patients had papillary-type CIS.

Interestingly, Matthaei et al. reported that pancreatic intraepithelial neoplasia 3 (PanIN-3), including CIS, did not affect the prognosis over a follow-up period of 1.6–64.5 months.[34] However, we recommend that the pancreatic duct surgical margin be negative for carcinoma, including CIS. CIS$^+$ PDAC appears to have a relatively slow progression. Two patients who were positive for CIS at the pancreas surgical margin died from peritoneal carcinomatosis after local recurrence in the remnant pancreas, two and six years after surgical resection. Brockie et al. reported that the natural history of CIS is to progress to infiltrating adenocarcinoma in the remaining pancreas; they examined 9 patients over 29 years from surgical resection to recurrence.[35] Brat et al. also showed a long latent period (17 months to 29 years) between the diagnosis of atypical ductal hyperplasia and appearance of invasive carcinoma.[36] We should therefore strive for CIS$^-$ surgical margins.

At present, CIS can be detected by pancreatic fluid cytology.[37] However, as its precise location is difficult to determine by preoperative imaging, surgeons rely on the intraoperative frozen diagnosis. Frozen-section diagnoses come with two risks: pancreatic fluid leakage and an inaccurate diagnosis. One patient (Case 1) experienced pancreatic fluid leakage, causing peritoneal carcinomatosis after local recurrence. Intraoperative diagnoses for cases 1–3 were negative for carcinoma, but their specimens had few foci that degenerated thermally. Re-examination might have revealed CIS in the deficit area.

Surgeons should be mindful of spilling pancreatic fluid and submit the entire cut-margin section to pathology.

CIS^+ PDAC operative procedure can also be difficult to determine because of the location in the pancreas. We considered TP to be a suitable procedure for CIS^+ PDAC as it solves the pancreatic surgical-line problem and avoids the complications associated with the intraoperative diagnosis.[38] However, the incidence of diabetes after TP was 100%.[39] The quality of life (QOL) of patients with TP was significantly decreased, because of diarrhoea, fatigue, dyspnoea, etc.[40] Recently, insulin glargine U-300 (Lantus; Sanofi-Aventis, Bridgewater, NJ, USA) and insulin degludec (Tresiba; Novo Nordisk, Bagsvaerd, Denmark), new long-acting basal insulin formulations, demonstrated the characteristics of longer acting time and lower incidence of hypoglycemia than insulin glargine U-100 (Lantus; Sanofi-Aventis, Bridgewater, NJ, USA).[41] An alternative treatment include continuous subcutaneous insulin infusion combined with continuous glucose monitoring, which achieved stable glycemic control and improved the QOL in diabetic patients after TP.[42] Long-term follow-up by endocrinologists and diabetes nurses might improve the QOL of diabetic patients.[43] TP may become a viable surgical methods for CIS^+ PDAC treatment in the future. The need for additional lymphadenectomy for curative CIS^+ PDAC treatment is unclear as carcinoma deposits were not seen in additional lymphadenectomy specimens. Optimal surgical treatment for CIS^+ PDAC will be able to be developed with the further examination of accumulated cases and follow-up of previous patients.

Although our results showed a favorable prognosis of CIS^+ PDAC, this study had several limitations, including a small number of patients. In addition, because all cases of CIS^+ PDAC were R0 resection, the prognosis of CIS^+ PDAC cases with R1 resection could not be studied. Furthermore, we were unable to evaluate flat-type CIS^+ PDAC because all cases in this study were of the low papillary type.

In conclusion, CIS^+ PDAC showed a better prognosis than CIS^- PDAC.

Acknowledgments

The authors wish to especially thank and fully honor the memory of Dr. K. Maeshiro. The authors also thank Dr. Y. Nakayama, Ms. M. Ishiguro and Ms. Ohnaka for their assistance. We thank Katrina Krogh, MD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. The authors declare no conflicts of interest.

References

1. Cronin KA, Lake AJ, Scott S, et al. Annual Report to the Nation on the Status of Cancer, part I: national cancer statistics. Cancer. 2018; 124: 2785-2800.
2. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet. 2011; 378: 607–620.
3. Classification of tumours Editorial Board (ed): WHO classification of tumours: Digestive System Tumors, 5th ed., IARC Press, Lyon, 2019.
4. Yanagisawa A, Kato Y, Ohtake K, Kitagawa T, Ohashi K, Hori M, et al: c-Ki-ras point mutation in ductectatic type mucinous cystic neoplasm of the pancreas. Jpn J Cancer Res 1991; 82: 1057-1060.
5. Yanagisawa A, Ohtake K, Ohashi K, Hori M, Kitagawa T, Sugano H, et al: Frequent c-Ki-ras oncogene activation in mucous cells hyperplasia of pancreas suffering from chronic inflammation. Cancer Res 1993; 53: 953-956.
6. Yanagisawa A, Ohashi K, Hori M, Takagi K, Kitagawa T, Sugano H, et al: Ductectatic-type mucinous cystadenoma and cystadenocarcinoma of the human pancreas: a novel clinicopathological entity. Jpn J Cancer Res 1993; 84: 474-479.
7. Yamasaki S, Suda K, Nobukawa B, Sonoue H: Intraductal spread of pancreatic cancer. Clinicopathologic study of 54 pancreatectomized patients. Pancreatology. 2002; 2: 407-412.
8. Ikeda M, Yanagisawa A, Seki M, Sasaki K, Takano K, Kato Y: The early state of invasive pancreatic ductal adenocarcinomas. Characteristics of the low papillary type and flat type intraductal carcinoma. Pancreas 2006; 33: 135-141.
9. Conlon K, Klimstra DS, Brennan MF: Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. Ann Surg 1996; 223: 273-279.

10. Fukuba N, Fujita K, Nakayama S, Takenaka M, Matsui S, Ozaka M, et al: A case of small pancreatic cancer with intraductal progress. Nihon Shokakibyo Gakkaishi 2010; 107: 792-797 (in Japanese with English abstract).
11. Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Blankin AV, et al: An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasm. Am J Surg Pathol 2004; 28: 977-987.
12. Esposito I, Kleeff J, Bergmann F, Reister C, Herpel E, Friess H, et al: Most pancreatic cancer resections are R1 resections. Ann Surg Oncol 2008; 15: 1651-1660.
13. Verbeke CS, Leitich D, Menon KV, McMahon MJ, Guillow PJ, Anthoney A: Redefining the R1 resection in pancreatic cancer. Br J Surg 2006; 93: 1232-1237.
14. Oda Y, Aishima S, Morimatsu K, Shindo K, Fujino M, Mizuuchi Y, Hattori M, Miyazaki T, Tanaka M, Oda: Pancreatic intraepithelial neoplasia in the background of invasive ductal carcinoma of the pancreas as a prognostic factor. Histopathology 2014; 65: 389-397.
15. Miyazaki T, Ohishi Y, Miyasaka Y, Oda Y, Aishima S, Ozono K, Abe A, Nagai E, Nakamura M, Oda Y: Molecular Characteristics of Pancreatic Ductal Adenocarcinomas with High-Grade Pancreatic Intraepithelial Neoplasia (PanIN) Are Different from Those without High-Grade PanIN. Pathobiology; 2017; 84: 192-201.
16. Wang Z, Li Y, Zhan S, Zhang L, Zhang S, Tang Q, Li M, Tan Z, Liu S, Xing X: SMAD4 Y353C promotes the progression of PDAC. BMC Cancer 2019; 19: 1037.
17. Geer RJ, Brennan MF: Prognostic indicators for survival after resection of pancreatic adenocarcinoma. Am J Surg 1993; 165; 68-73.
18. Fortner JG, Klimstra DS, Senie RT, Maclean BJ: Tumor size is the primary prognosticator for pancreatic cancer after regional pancreatectomy. Ann Surg 1996; 223: 147-153.

19. Wasif N, Ko CY, Farrell J, Wainberg Z, Hines OJ, Reber H, et al: Impact of tumor grade on prognosis in pancreatic cancer: should we include grade in AJCC staging? Ann Surg Oncol 2010; 17: 2312-2320.

20. Rochefort MM, Ankeny JS, Kadera BE, Donald GW, Isacoff W, Wainberg ZA, et al: Impact of tumor grade on pancreatic cancer prognosis: validation of a novel TNMG staging system. Ann Surg Oncol 2013; 20: 4322-4329.

21. Howard TJ, Krug JE, Yu J, Zyromski NJ, Schmidt CM, Jacobsen LE, et al: A margin-negative R0 resection accomplished with minimal postoperative complications is the surgeon's contribution to long-term survival in pancreatic cancer. J Gastrointest Surg 2006; 10: 1338-1346.

22. Sohn TA, Yeo CJ, Cameron JL, Kaniaris L, Kaushal S, Abrams RA, et al: Resected adenocarcinoma of the pancreas—616 patients: results, outcomes, and prognostic indicators. J Gastrointest Surg 2000; 4: 567-579.

23. Yeo CJ, Cameron JL, Lilemoe KD, Sitzmann JV, Hruban RH, Goodman SN, et al: Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. Ann Surg 1995; 221: 721-731.

24. Neoptolemos JP, Stocken DD, Dunn JA, Almond J, Beger HG, Pederzoli P, et al: Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001; 234: 758-768.

25. Wong H, Lau S, Yau T, Cheung P, Epstein RJ. Br J Cancer. Presence of an in situ component is associated with reduced biological aggressiveness of size-matched invasive breast cancer. 2010; 102: 1391-1396.

26. Chagpar AB, McMasters KM, Sahoo S, Edwards MJ. Does ductal carcinoma in situ accompanying invasive carcinoma affect prognosis? Surgery. 2009; 146: 561-568.

27. Gao Y, Niu Y, Wang X, Wei L, Lu S. Genetic changes at specific stages of breast cancer progression detected by comparative genomic hybridization. J Mol Med (Berl). 2009; 87: 145-152.

28. Tsutsumi M: Development of exocrine pancreatic tumor. Byori to Rinsyo 2004; 22: 768-774 (in Japanese).

29. Nagata K, Horinouchi M, Saitou M, Higashi M, Nomoto M, Goto M, Yonezawa S. Mucin expression profile in pancreatic cancer and the precursor lesions. J Hepatobiliary Pancreat Surg. 2007; 14: 243-254.

30. K.J. Stanton, R.A. Sidner, G.A. Miller, O.W. Cummings, C.M. Schmidt, T.J. Howard, E.A. Wiebke. Analysis of Ki-67 antigen expression DNA proliferative fraction, and survival in resected cancer of the pancreas. Am. J. Surg., 186; 2003: 486-492.

31. Lebe B, Sağol O, Ulukuş C, Coker A, Karademir S, Astarcioglu H, Küpelioğlu A, Astarcioğlu I, Obuz F. The importance of cyclin D1 and Ki67 expression on the biological behavior of pancreatic adenocarcinomas. Pathol Res Pract. 2004; 200: 389-96.

32. Myoteri D, Dellaportas D, Lykoudis PM, Apostolopoulos A, Marinis A, Zizi-Sermpetzoglou A. Prognostic evaluation of vimentin expression in correlation with Ki67 and CD44 in surgically resected pancreatic ductal adenocarcinoma. Gastroenterol Res Pract 2017; 2017: 9207616.

33. J. Luttges, S. Schemm, I. Vogel, J. Hedderich, B. Kremer, G. Kloppel, The grade of pancreatic ductal carcinoma is an independent prognostic factor and is superior to the immunohistochemical assessement of proliferation, J. Pathol. 2000; 191: 154–161.

34. Matthaei H, Hong SM, Mayo SC, dal Molin M, Olino K, Venkat R, et al: Presence of pancreatic intraepithelial neoplasia in the pancreatic transection margin does not influence outcome in patients with R0 resected pancreatic cancer. Ann Surg Oncol 2011; 18: 3493-3499.

35. Brockie E, Anand A, Albores-Saavedra J: Progression of atypical ductal hyperplasia/carcinoma in situ of the pancreas to invasive adenocarcinoma. Ann Diang Pathol 1998; 2: 286-292.

36. Brat DJ, Lillemoe KD, Yeo CJ, Warfield PB, Hruban RH: Progression of pancreatic intraductal neoplasia to infiltrating adenocarcinoma of the pancreas. Am J Surg Pathol 1998; 22: 163-169.

37. Ishikawa O, Ohigashi H, Imaoka S, Takenaka A, Uehara H, Nakaizumi A: Surgical techniques of cytology-guided pancreatectomy for occult neoplasm of the pancreas. Hepatogastroenterology 1995; 42: 724-729.

38. Barbier L, Jamal W, Dokmak S, Aussilhou B, Corcos O, Ruszniewski P, et al: Impact of total pancreatectomy: short- and long-term assessment. HPB 2013 Jan 29. doi: 10.1111/hpb.12054.

39. Maeda H, Hanazaki K. Pancreatogenic diabetes after pancreatic resection. Pancreatology. 2011; 11: 268-276.

40. Scholten L, Stoop TF, Del Chiaro M, Busch OR, van Eijck C, Molenaar IQ, de Vries JH, Besselink MG; Dutch Pancreatic Cancer Group. Systematic review of functional outcome and quality of life after total pancreatectomy. Br J Surg. 2019; 106: 1735-1746.

41. Gallegos Aragon K, Elmaoued AA, Pham NT, Conklin JR, Ray GM. Long-Acting Basal Insulins: A Review of the More Recently Approved Agents. Cardiol Rev. 2019 ; 27: 260-266.

42. Scott ES, Fulcher GR, Clifton-Bligh RJ. Sensor-augmented CSII therapy with predictive low-glucose suspend following total pancreatectomy. Endocrinol Diabetes Metab Case Rep. 2017; 17-0093.

43. Stoop TF, Ateeb Z, Ghorbani P, Scholten L, Arnelo U, Besselink MG, Del Chiaro M. Impact of Endocrine and Exocrine Insufficiency on Quality of Life After Total Pancreatectomy. Ann Surg Oncol. 2020; 27: 587-596.

Figure legends

Figure 1. A. Kaplan–Meier survival curves of patients with pancreatic intraductal carcinomas with and without carcinoma *in situ* (CIS). The dotted line shows pancreatic ductal adenocarcinoma (PDAC) with CIS, and the solid line shows CIS^- PDAC. The CIS^+ PDAC group has a significantly longer survival duration than the CIS^- PDAC group (P=0.0068).

B. Kaplan–Meier survival curves of R0 resection cases with pancreatic intraductal carcinomas with and without carcinoma *in situ* (CIS). The dotted line shows pancreatic ductal adenocarcinoma (PDAC) with CIS, and the solid line shows CIS^- PDAC. The CIS^+ PDAC group has a significantly longer survival duration than the CIS^- PDAC group in R0 resection cases (P= 0.0004).

Figure 2. Schematic illustration of CIS^+ PDAC cases. The stars indicate invasive carcinoma, and the dotted line indicates CIS.

Figure 3. A. Whole specimen slices of CIS^+ PDAC (case 4). An ill-defined tumor is seen in slices I-K (arrow heads). Slices S-Z show the normal main pancreatic duct (black arrows), with CIS originating from the main pancreatic duct. The CIS lesion is extends

from slice F, and from slice L to slice Q (white arrows). B. A schematic illustration of the total pancreatectomy specimen cutting (case 4).

Figure 4. Histology of representative CIS^+ PDAC and CIS^- PDAC. H&E staining of a specimen slice shows that the low-papillary CIS covers the lumen of the main pancreatic duct. Tumor cells are atypical epithelial cells of different sizes, arranged in irregular papillary structures. Tumor cells lose their polarity and proliferate in multiple layers (a: white arrowheads; d). H&E staining of a specimen slice shows poorly differentiated adenocarcinoma composed of small cell, nest-like structures and individual cells, surrounded by fibrous stroma (b: white arrows; f). Cancer cells have polymorphisms and an increased nuclear size. Partially well to moderately differentiated tubular adenocarcinoma are tubular structures composed of mild atypical columnar epithelial cells, accompanied by fibrous stroma (b: black arrowheads; e). Histology of representative photos of CIS^- PDAC (c: white arrowheads). H&E staining of specimen slices show poorly differentiated adenocarcinoma (g: white arrows) and well to moderately differentiated tubular adenocarcinoma (g: black arrows) with the a similar morphology to the CIS^+ PDAC group. Ki-67 expression in CIS (h). Ki-67 expression in invasive carcinoma (i, j).

Figure 5. Representative immunopositive cells in CIS^+ PDAC. MUC1, MUC5AC, and MUC6 expression in CIS (a-c). MUC1, MUC5AC, and MUC6 expression in invasive carcinoma (d-f).

Table 1. Clinicopathological findings of pancreatic ductal adenocarcinoma with carcinoma *in situ*.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age (years)	62	67	61	68	48	71	73	54
Sex	F	F	M	M	M	F	F	F
Term	2.8 yr	3 yr	3 mo	1yr	8 mo	2 yr	3 yr	6 mo
Location (h/b/t)	h	hb	hb	hb	hb	bt	hb	bt
Procedure	PPPD	PPPD	PPPD	TP	TP	DP	TP	DP
pT	1	3	3	3	3	3	3	2
pN	0	1	1	0	0	0	0	0
pS	–	–	–	–	–	–	–	–
pRP	–	+	+	+	+	+	+	–
Differentiation	Well	Mod	Well	Por Well	Well	Well	Well	Well
CIS length (mm)	40	>24	>24	38	28	24	40	68
MUC 1 (C/I)	+/+	+/+	+/+	+/+	+/+		+/+	
MUC 5AC (C/I)	+/–	+/+	+/+	+/–	+/+		+/+	
MUC 6 (C/I)	+/–	–/–	–/–	+(part)/–	+/+		+/+	
Prognosis	3 yr Dead	6 yr Dead	2 yr Dead	10 yr Alive	8 yr Alive	7 yr Alive	6 yr Alive	4 yr Alive

DP: distal pancreatectomy; pN: regional lymph node; PPPD: pylorus-preserving pancreatoduodenectomy; pRP: retroperitoneal tissue invasion; pS: anterior pancreatic capsule invasion; pT: primary tumor; term: from the onset of symptoms to operation; TP: total pancreatectomy; Mod: moderately differentiated type; Por: poorly differentiated type; INF: growth patterns of tumors infiltrating the surrounding tissue; ly: lymphatic invasion; v: venous invasion; ne: invasion of intrapancreatic nerves; C: carcinoma *in situ* component; I: invasive carcinoma component.

Table 2. The comparison of clinicopathological parameters of PDAC with and without CIS.

	PDAC with CIS n=8	PDAC without CIS n=79	*P*
Age (years)	54–73	40–76	0.5419
Sex (M/F)	3/5	55/24	0.1106
Procedure			<0.001
PPPD	3	58	
DP	2	21	
TP	3	0	
Location			<0.0001
Ph/Phb/Pbt	1/5/2	58/0/21	
Tumor size (mm)	3–32	8–60	0.0299
Differentiation			0.0267
Well/mod/poor	6/1/1	23/44/12	
pT			0.3624
1/2/3	1/1/6	3/1/75	
pN			0.0561
0/1	2/6	50/29	
pS (+/–)	0/8	26/53	0.0988
pRP (+/–)	6/2	61/18	>0.9999
R status			0.0005
0/1	8/0	28/51	

PDAC: pancreatic ductal adenocarcinoma; CIS: carcinoma *in situ*; DP: distal pancreatectomy; Phb: pancreas head and body (the invasive carcinoma is present above the portal vein); Pbt: pancreas body and tail; Ph: pancreas head; pN: regional lymph node; PPPD: pylorus-preserving pancreatoduodenectomy; pRP: retroperitoneal tissue invasion; pS: anterior pancreatic capsule invasion; pT: primary tumor; TP: total pancreatectomy.

Table 3. Results of a multivariate analysis of the clinicopathologic parameters and prognosis.

	P	95% CI
PDAC with CIS	0.0388	0.080–0.935
Tumor size	0.8543	0.974–1.022
R0	0.9547	0.559–1.730
Differentiation		
Well	0.0944	0.180–1.145
Moderate	0.8564	0.494–2.399

PDAC: pancreatic ductal adenocarcinoma; CIS: carcinoma in situ; CI: confidence interval

Table 4. Ki-67 labeling index in the CIS$^+$ PDAC group and CIS$^-$ PDAC group.

	CIS$^+$ PDAC group			CIS$^-$ PDAC group	
	CIS	invasive carcinoma	*P*	invasive carcinoma	*P*
Ki-67	5-15%	26-52%	0.0038	25%-59%	0.2113

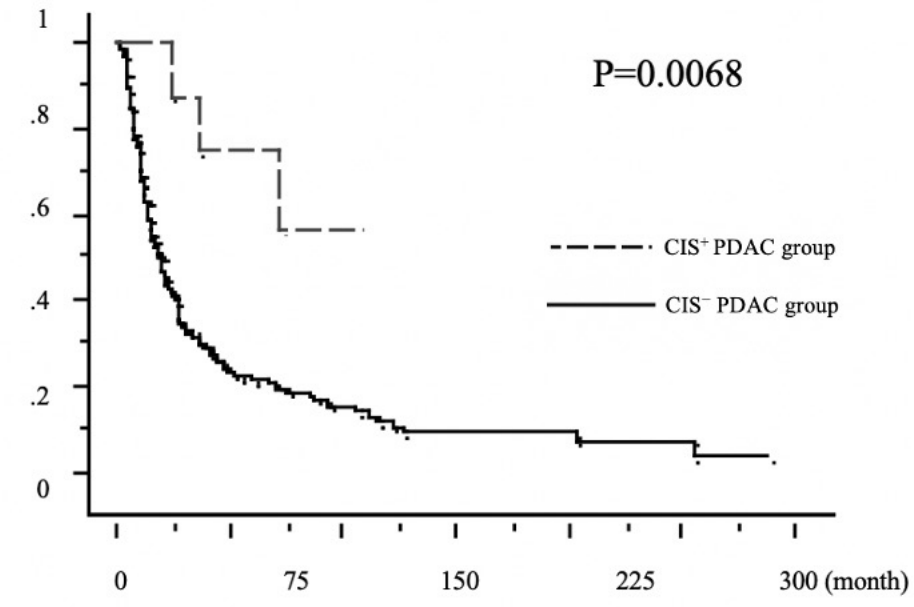
PDAC: pancreatic ductal adenocarcinoma; CIS: carcinoma *in situ*

Table 5. Pancreatic immunophenotypes in the CIS$^+$ PDAC group.

	CIS$^+$ PDAC group	
	CIS	invasive carcinoma
MUC 1	100% (6/6)	100% (6/6)
MUC 5AC	100% (6/6)	67% (4/6)
MUC 6	67% (4/6)	33% (2/6)

PDAC: pancreatic ductal adenocarcinoma; CIS: carcinoma *in situ*

A


1
.8
.6
.4
.2
0
P=0.0068
CIS+ PDAC group
CIS− PDAC group
0
75
150
225
300 (month)

B

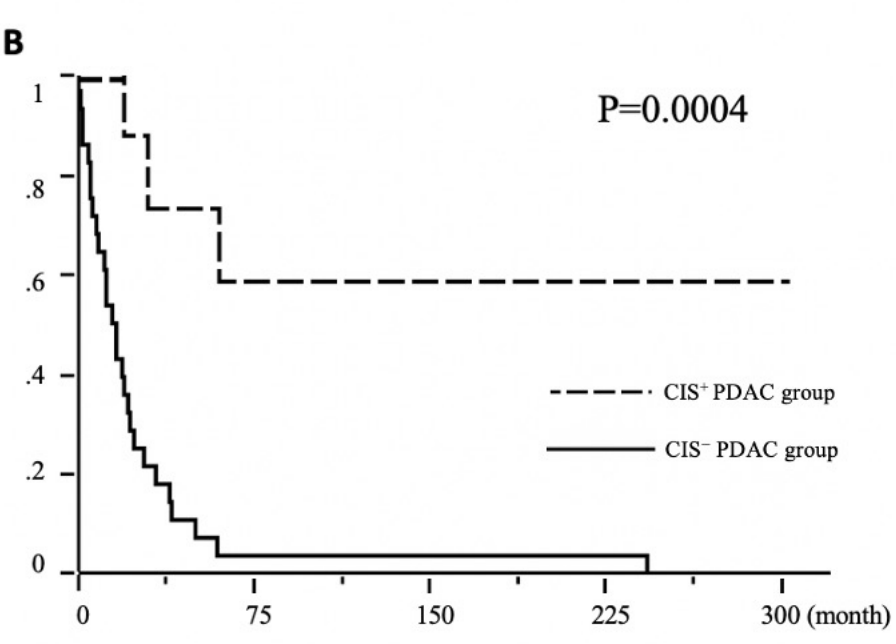

1
.8
.6
.4
.2
0
P=0.0004
CIS+ PDAC group
CIS− PDAC group
0
75
150
225
300 (month)

Figure 1

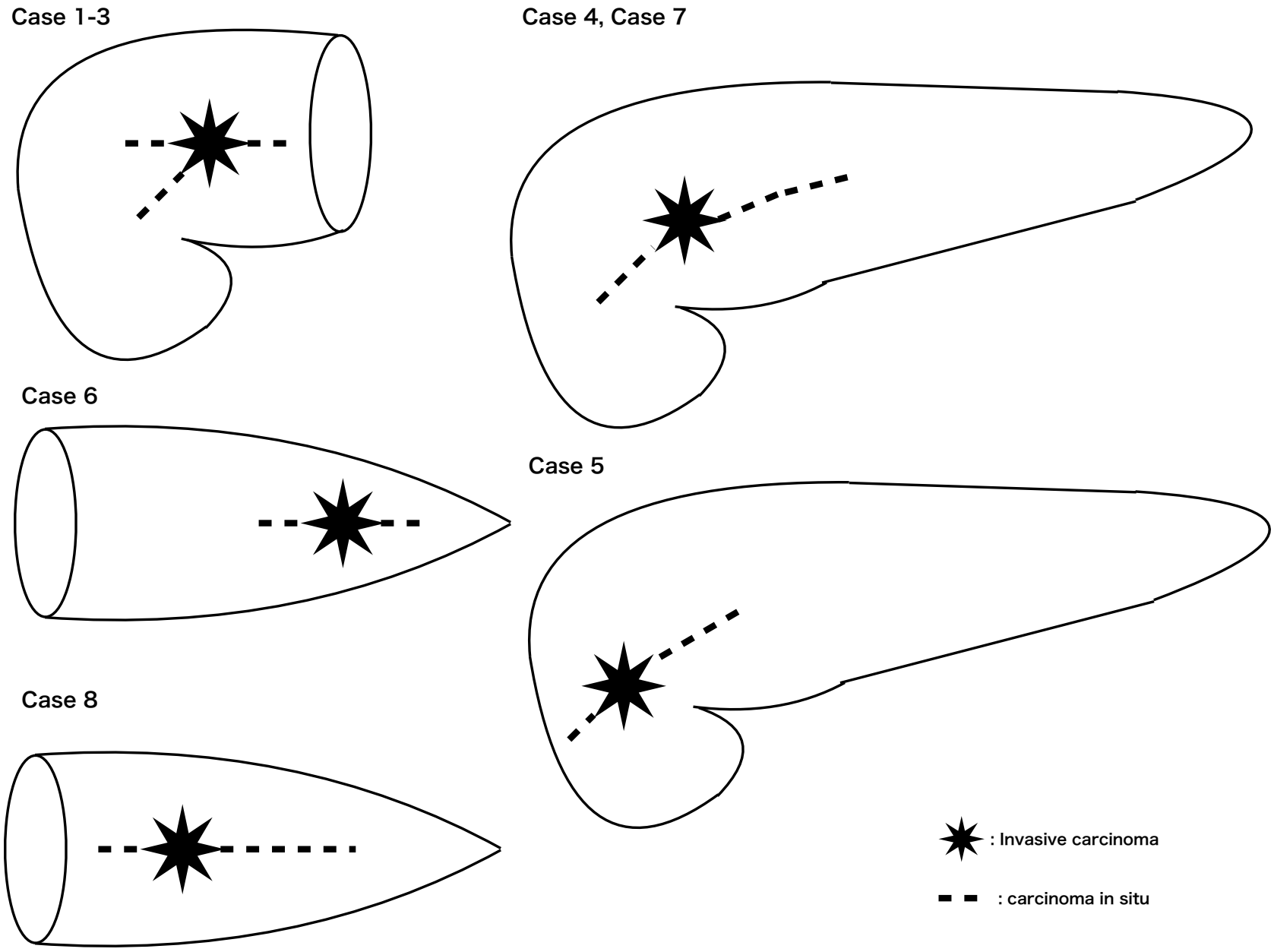
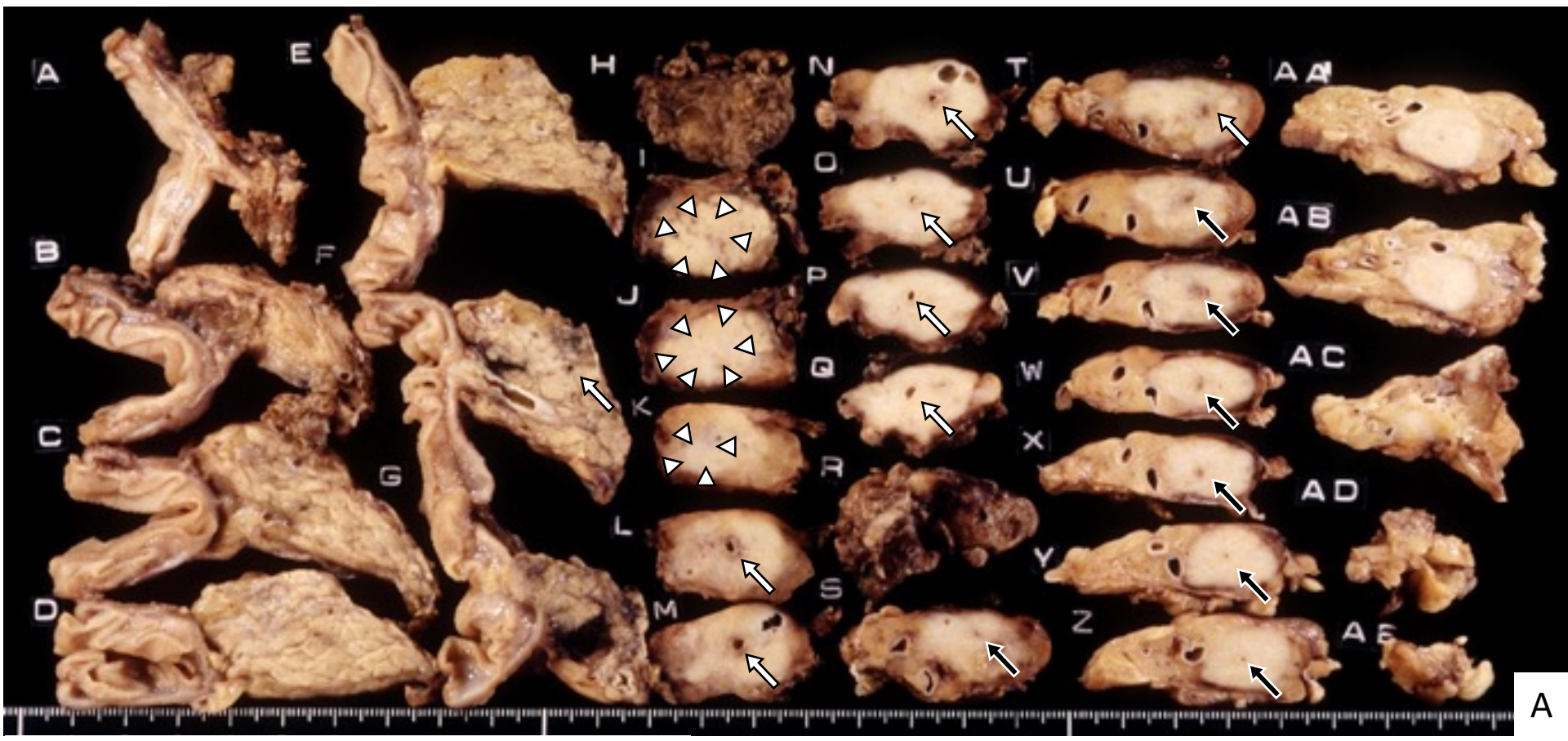
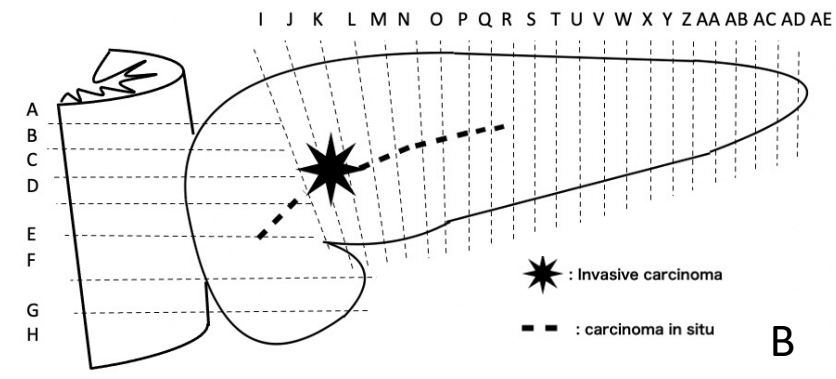

Case 1-3
Case 4, Case 7
Case 6
Case 5
Case 8
: Invasive carcinoma
: carcinoma in situ

Figure 2


A
E
H
N
T
AA
B
F
I
O
U
AB
J
P
V
C
G
K
Q
W
AC
X
R
AD
L
Y
D
M
S
Z
AE

A


I J K L M N O P Q R S T U V W X Y Z AA AB AC AD AE
A
B
C
D
E
F
G
H
: Invasive carcinoma
: carcinoma in situ

B

Figure 3

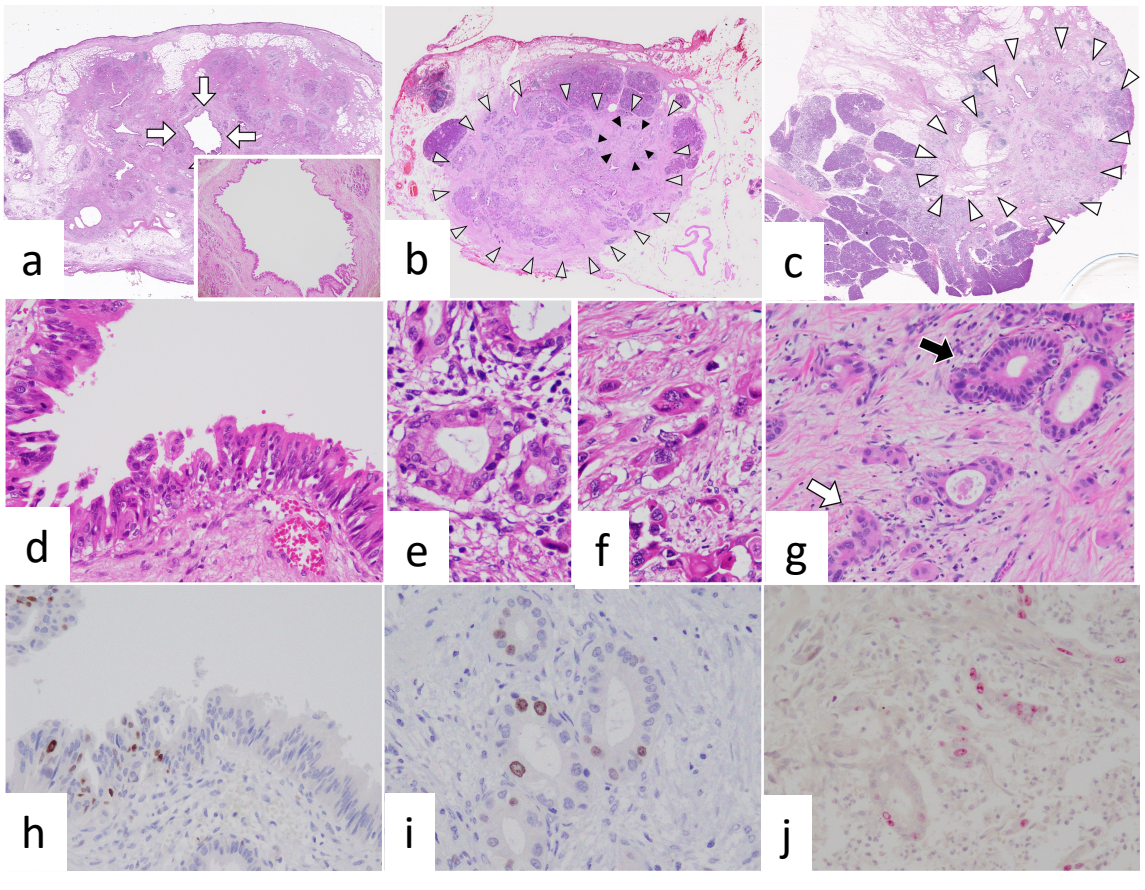
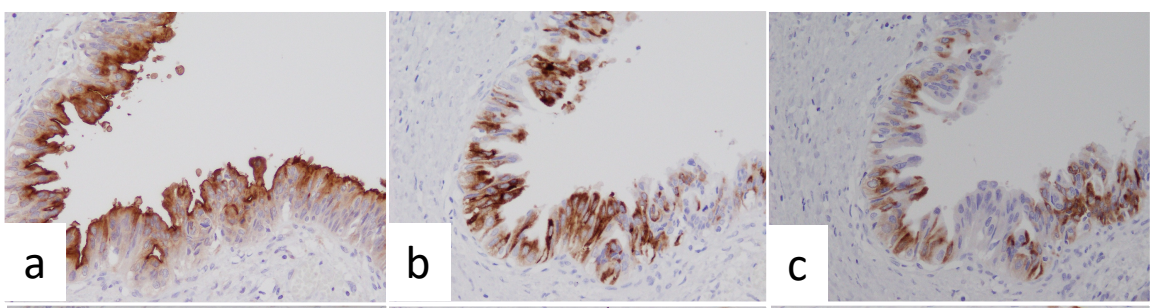


Figure 4



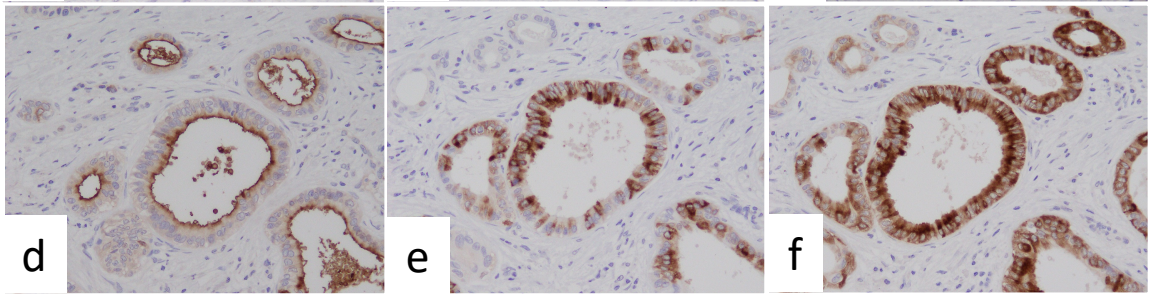


Figure 5