of death. The cases with PDAC PanIN lesions were also divided into two group, i.e., individuals ≥64 and those <64 years of age at the time of death. All PanIN lesions were examined for MUC expression.

Statistical analysis

Chi-square and Fisher's exact tests were used to evaluate the significance of differences. A p value <0.05 was considered to be statistically significant.

Results

PanIN grading in normal pancreata

After completely examining all 54 normal pancreata, from zero to eight PanIN lesions were found in each pancreatic section for a total of 378 normal PanIN lesions detected. The percentages of normal PanIN-1A, PanIN-1B and PanIN-2 lesions were 70.9% (268/378), 26.2% (99/378) and 2.9% (11/378), respectively. No PanIN-3 lesions were found in any of the normal pancreata. As for the frequency of normal PanIN in the five parts of the pancreas (head, neck, body, body-tail and tail), there was no significant difference among the five pancreatic parts, although PanIN-2 lesions tended to be found more often in the pancreatic tail (Fig. 1).

MUC1 and MUC5AC expression in normal PanIN by grades

MUC1 was expressed in the apical and basolateral membranes of centroacinar cells and in small and large ductal cells (Fig. 2a–c), while MUC5AC was expressed in the perinuclear region of ductal cells (Fig. 2d–f). In normal PanIN-1A, PanIN-1B and PanIN-2 specimens, MUC1 was expressed in 2.6% (7/268), 10.1% (10/99) and 9.1% (1/11) of the lesions, respectively, while MUC5AC was expressed in 41.0% (110/268), 65.7% (65/99) and 36.4% (4/11) of the lesions, respectively. There were no significant differences in the expression of either MUC1 or MUC5AC regardless of PanIN grade between individuals ≥71 and <71 years of age. Also, there were no significant differences in the expression of either MUC1 or MUC5AC regardless of PanIN grade in the PDACs between individuals ≥64 and <64 years of age.

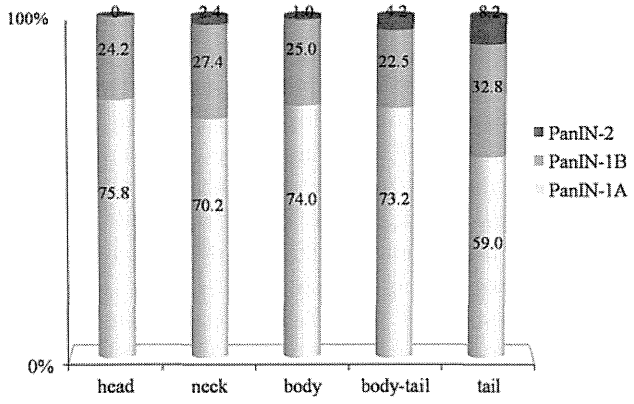

Fig. 1 Distribution of PanINs in normal pancreata. PanIN-2 lesions tended to be more frequent in pancreatic tail (×100)

PanIN grades in pancreata with PDAC

From zero to six PanIN lesions were found in each pancreatic section and 106 PanIN lesions were detected in total after completely examining all eight pancreata with PDACs. No morphological differences were detected between normal PanIN and PDAC PanIN lesions. The percentages of PDAC PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3 lesions were 44.3% (47/106), 27.4% (29/106), 21.7% (23/106) and 5.9% (7/106), respectively.

MUC1 and MUC5AC expression in PanIN lesions in pancreata with PDAC by grade

MUC1 was expressed in the cytoplasm and on the apical and basolateral membranes of cells in PDAC PanIN lesions (Fig. 3a–d), while MUC5AC was expressed in the perinuclear region of ductal cells, mucin vacuoles and luminal content in PDAC PanIN lesions (Fig. 4a–d). The percentages of PDAC PanIN-1A, PanIN-1B, PanIN-2 and PanIN-3 lesions with MUC1 expression were 19.1% (9/47), 27.6% (8/29), 13.0% (3/23) and 57.1% (4/7), respectively. There were significant differences in the frequency of MUC 1 expression between normal PanIN-1A and PDAC PanIN-1A lesions ($p < 0.0001$) and between normal PanIN-1B and PDAC PanIN-1B lesions ($p < 0.05$) (Fig. 5a). The percentages of PDAC PanIN-1A, PanIN-1B, PanIN-2 and PanIN-3 with MUC5AC expression were 80.9% (38/47), 75.8% (22/29), 78.3% (18/23) and 71.4% (5/7), respectively. There were significant differences in MUC5AC expression between normal PanIN-1A and PDAC PanIN-1A ($p < 0.0001$) and between normal PanIN-2 and PDAC PanIN-2 ($p < 0.05$) (Fig. 5b).

Discussion

An important new finding in this study was that PanIN lesions in normal pancreata are different from PDAC PanIN lesions with regard to MUC protein expression.

A number of articles have previously reported MUC expression in normal pancreata and PDACs. MUC1 was

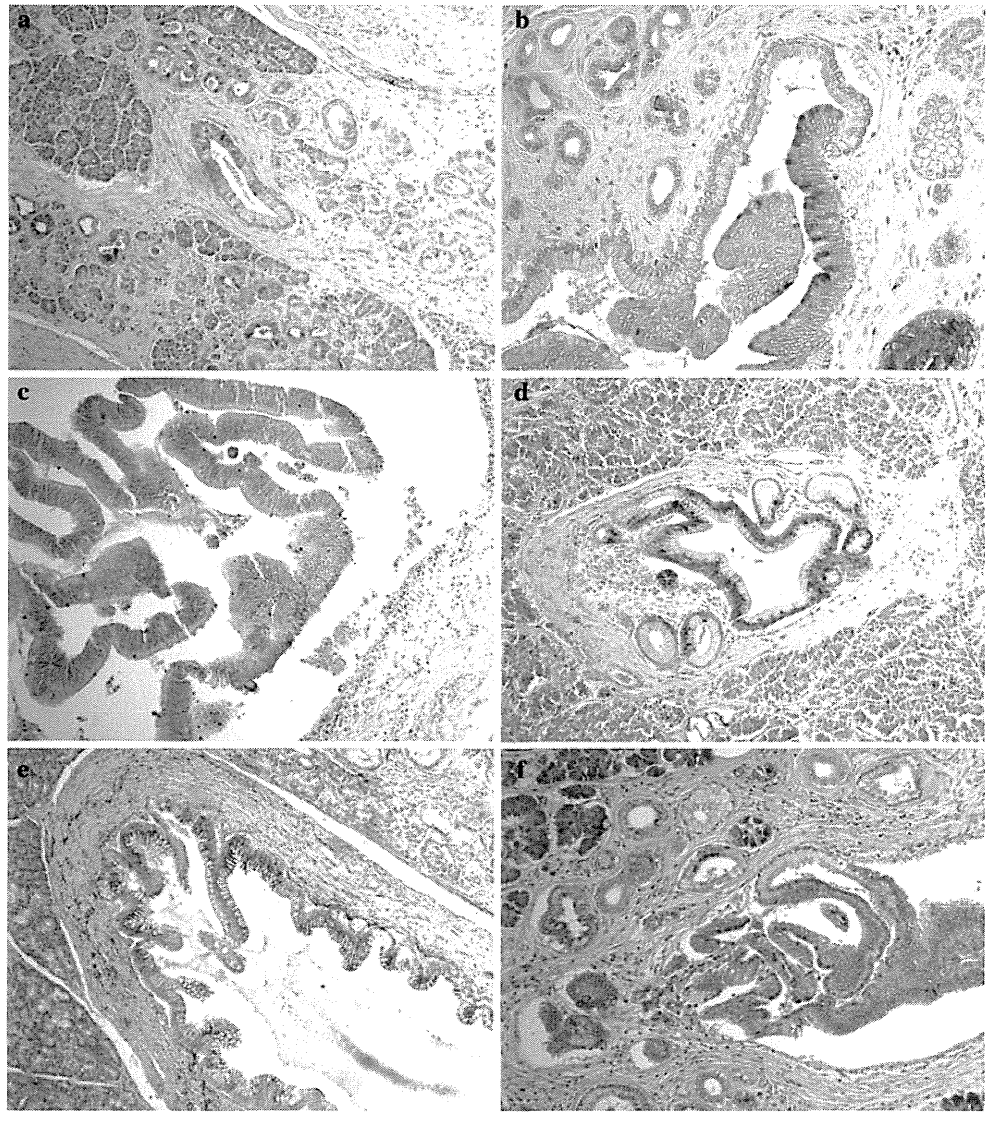

Fig. 2 MUC1 expression in normal pancreata. **a** PanIN-1A, **b** PanIN-1B, **c** PanIN-2. MUC1 expressed in apical and basolateral membranes of centroacinar cells and in small and large ductal cells in normal PanIN lesions (×100). **d** PanIN-1A, **e** PanIN-1B, **f** PanIN-2. MUC5AC expressed in the perinuclear region of ductal cells in normal PanIN lesions (×100)

expressed in ductal cells in normal pancreata, PanIN lesions and PDACs [10–12, 16–21], while MUC5AC was almost negative in normal pancreatic ducts, but highly positive in PanIN lesions and PDACs [16, 17, 20–22].

In normal pancreata, dysplastic ductal changes that morphologically resemble the PanIN lesions in pancreata with PDACs, have been observed; however, few studies have investigated MUC expression in PanIN lesions found in clinically and macroscopically normal pancreata. Using MUC monoclonal antibodies, we decided to examine and compare MUC expression to clarify whether normal PanIN lesions are precursors of PDAC PanIN lesions.

In normal pancreata, we were unable to find any significant differences in the distribution of PanIN lesions among the five anatomical sections of the pancreas, although PanIN-2 lesions were detected more frequently in the tail. Most pancreatic cancers, however, occur in the head of the pancreas [23, 24]. If there is a relationship between PanIN lesions and PDAC, PanIN lesions should have been found more frequently in the head of the pancreas. Kozuka, et al. [13] reported that most carcinomas in situ were found in the head of the pancreas. There may be differences between normal PanIN and PDAC lesions in view of our distribution findings.

Confirming the findings of previous studies, [13, 14] we did not detect any morphological differences between normal PanIN and PDAC PanIN lesions. As for MUC staining, we did not find any differences in the staining sites in epithelial cells, but the MUC1 expression rate was significantly higher among PDAC PanIN-1A and PanIN-1B lesions than among normal PanIN-1A and PanIN-1B lesions. Likewise, the MUC5AC expression rate was significantly higher among PDAC PanIN-1A and PanIN-2 lesions than among normal PanIN-1A and PanIN-2 lesions. The MUC expression rates among PDAC PanIN lesions were not significantly different from those reported earlier

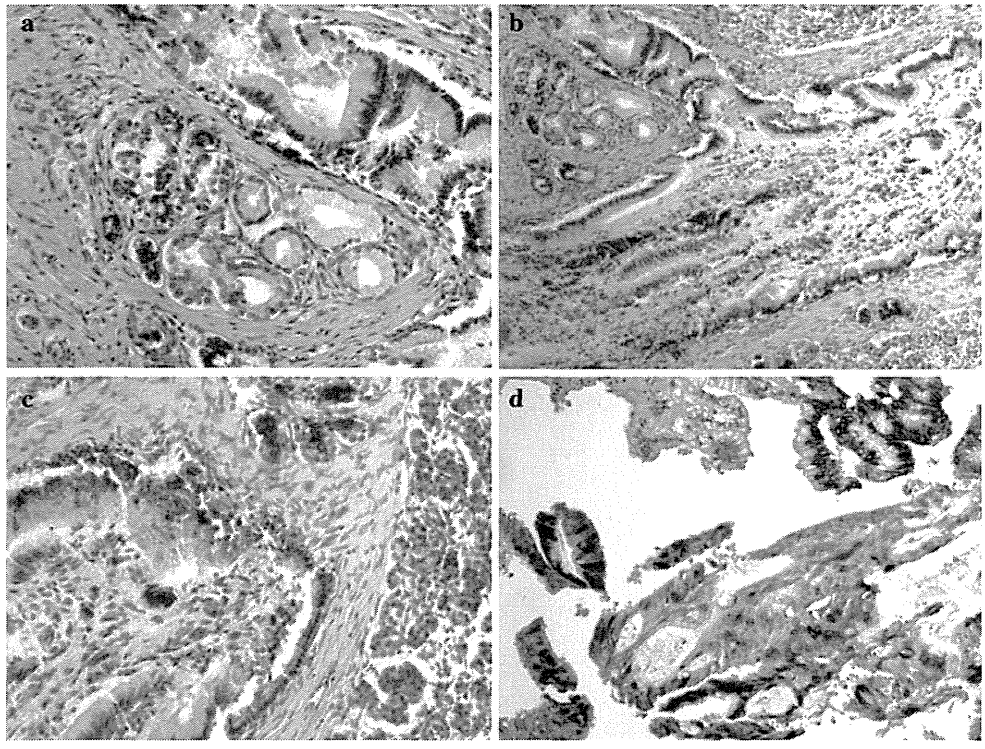

Fig. 3 MUC1 expression in PDACs. **a** PanIN-1A, **b** PanIN-1B, **c** PanIN-2, **d** PanIN-3. No significant differences compared with normal PanIN regarding MUC1 expression (×100)

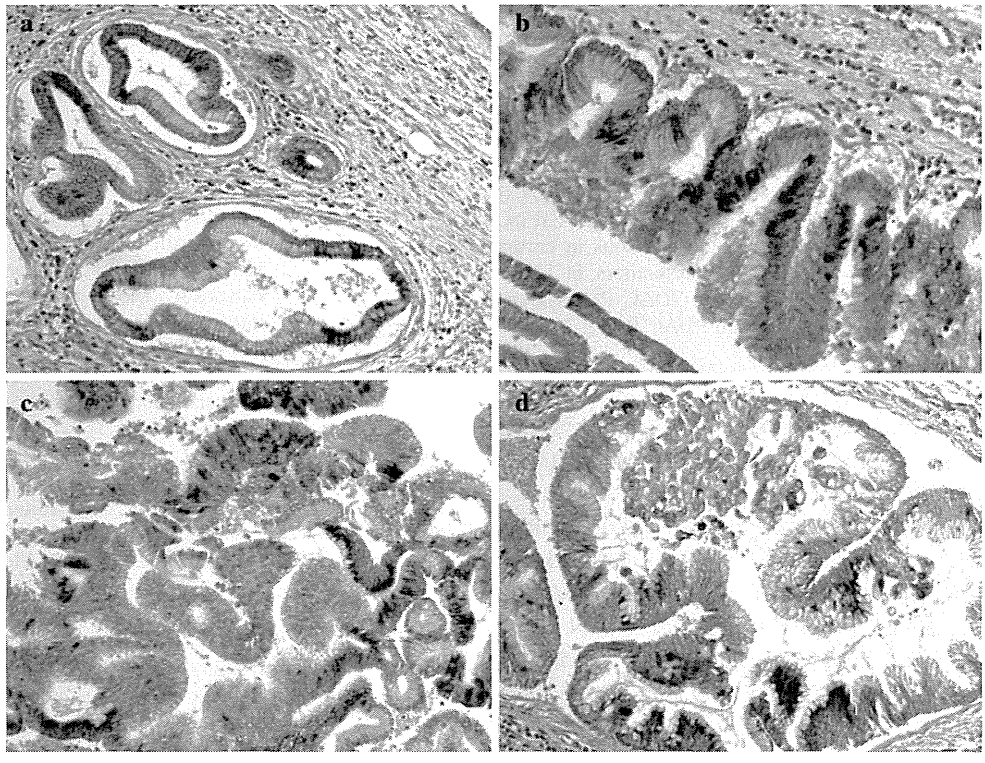

Fig. 4 MUC5AC expression in PDACs. **a** PanIN-1A, **b** PanIN-1B, **c** PanIN-2, **d** PanIN-3. No significant differences compared with normal PanIN regarding MUC5AC expression (×100)

[16–20]. The results of our study indicate that although normal PanIN lesions are morphologically similar to PDAC PanIN lesions, they have different characteristics concerning MUC expression.

Consequently, we hypothesize that advanced-stage PDAC affects MUC expression in PDAC PanIN lesions. The PDAC specimens in our study were all obtained from autopsied cases, most of them involving large tumor

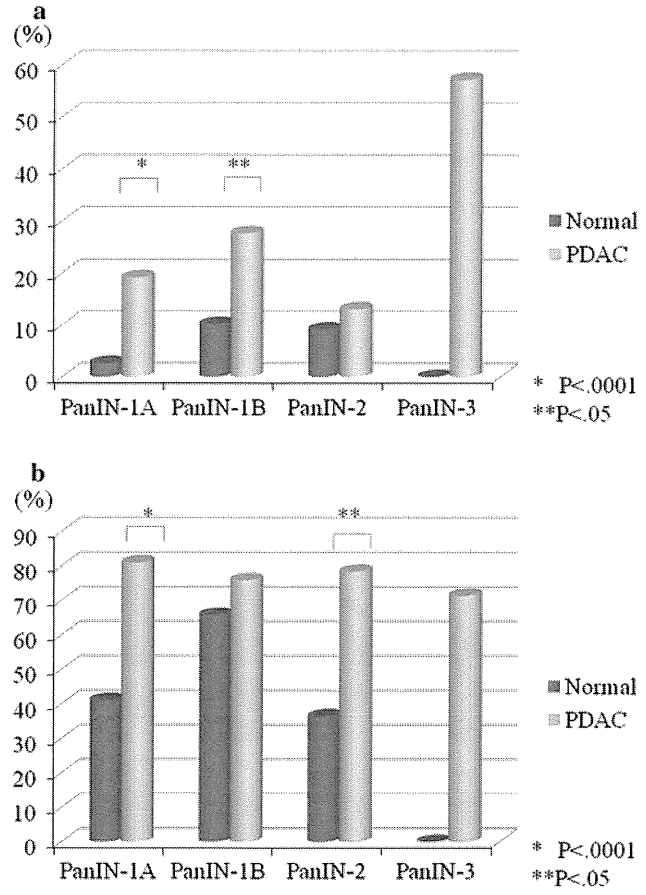

Fig. 5 **a** MUC1 expression in PanIN lesions in normal pancreata and PDACs by grade; significant differences in MUC1 expression between normal PanIN-1A and PDAC PanIN-1A ($p < 0.0001$) and between normal PanIN-1B and PDAC PanIN-1B ($p < 0.05$) (×100). **b** MUC5AC expression in PanIN lesions in normal pancreata and PDACs by grade; significant differences in MUC5AC expression between normal PanIN-1A and PDAC PanIN-1A ($p < 0.0001$) and between normal PanIN-2 and PDAC PanIN-2 ($p < 0.05$) (×100)

masses. Carcinogenesis of such large masses probably affects the entire pancreatic environment as reflected by the differences observed in MUC expression. Mucin glycosylation is altered by pro-inflammatory signaling in pancreatic cancer cells [25] so certain kinds of cytokines could conceivably affect MUC expression.

Another possibility is that PanIN lesions in normal pancreata could represent an early phase of pancreatic carcinogenesis and PDAC PanIN lesions could have a high potential for carcinogenesis in the pancreas. In other words, normal PanIN and PDAC PanIN lesions may represent different carcinogenic stages. Such a hypothesis is dependent on PanIN lesions actually being precursors of PDAC [1, 2], with MUC1 and MUC5AC expression being indicative of pancreatic carcinogenesis [19, 21].

The MUC staining results in our study differ from those previously reported by Kim et al. [16] with the frequency of MUC1 staining among PDAC PanIN lesions being lower than that reported earlier, although it was almost the same as that in other studies [23, 26]. Regardless of this, there have been no previously published comparative reports on MUC staining in PanIN lesions between normal PanIN and PDAC PanIN; therefore, our results are quite interesting and informative.

The primary limitation of this study was that we did not conduct in situ hybridization (ISH) for MUC expression, although we did perform immunohistochemical examinations (IHC). Both ISH and IHC could be important for verifying the results of our PanIN evaluation. In addition, we conducted pathological examinations of the pancreata of a relatively small number of patients who died of pancreatic cancer. In the future, we intend to examine a larger sample of pancreatic carcinoma cases using both IHC and ISH.

In conclusion, we have demonstrated that PanIN lesions found in clinically and macroscopically normal pancreata differ from PanIN lesions in PDACs with respect to MUC1 and MUC5AC expression.

Conflict of interest None of the authors currently has or had in the past any financial support, sponsorship or conflict of interest with respect to this manuscript.

References

1. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. 2001;25:579–86.
2. Wilentz RE, Iacobuzio-Donahue CA, Argani P, McCarthy DM, Parsons JL, Yeo CJ, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res. 2000;60:2002–6.
3. Kim YS, Gum JR Jr. Diversity of mucin genes, stricture, function, and expression. Gastroenterol. 1995;109:999–1001.
4. Kim YS, Gum J Jr, Brockhausen I. Mucin glycoproteins in neoplasia. Glycoconj J. 1996;13:693–707.
5. Kim YS, Gum J Jr, Crawley SC, Deng G, Ho JJ. Mucin gene and antigen expression in biliopancreatic carcinogenesis. Ann Oncol. 1999;10(Suppl 4):51–5.
6. Byrd JC, Bresalier RS. Mucins and mucin binding proteins in colorectal cancer. Cancer Metastasis Rev. 2004;23:77–99.
7. Chen Y, Zhao YH, Kalaslavadi TB, Hamati E, Nehrke K, Le AD, et al. Genome-wide search and identification of a novel gel-forming mucin MUC19/Muc19 in glandular tissues. Am J Respir Cell Mol Biol. 2004;30:155–65.
8. Higuchi T, Orita T, Nakanishi S, Katsuya K, Watanabe H, Yamasaki Y, et al. Molecular cloning, genomic structure, and expression analysis of MUC20, a novel mucin protein, up-regulated in injured kidney. J Biol Chem. 2004;279:1968–79.
9. Sasaki M, Ikeda H, Nakanuma Y. Expression profiles of MUC mucins core protein in the intrahepatic biliary system: physiological distribution and pathological significance. Acta Histochem Cytochem. 2005;38:295–303.
10. Balagué C, Gambús G, Carrato C, Porchet N, Aubert JP, Kim YS, et al. Altered expression of MUC2, MUC4, and MUC5 mucin

genes in pancreatic cancer cell lines and tissues. Gastroenterology. 1994;106:1054–61.
11. Balagué C, Audie JP, Porchet N, et al. In situ hybridization shows distinct patterns of mucin gene expression in normal, benign, and malignant pancreas tissues. Gastroenterology. 1995;109:953–64.
12. Andrianifahanana M, Moniaux N, Schmied BM, Ringel J, Friess H, Hollingsworth MA, et al. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. Clin Cancer Res. 2001;7:4033–40.
13. Kozuka S, Sassa R, Taki T, Masamoto K, Nagasawa S, Saga S, et al. Relation of pancreatic duct hyperplasia to carcinoma. Cancer. 1979;43:1418–28.
14. Ito R, Kondo F, Yamaguchi T, Kato K, Sakai Y, Saisho H, et al. Pancreatic intraepithelial neoplasms in the normal appearing pancreas: on their precise relationship with age. Hepatogastroenterology. 2008;55(84):1103–6.
15. Hruban RH, Takaori K, Klimsrtra DS, Adsay NV, Albores-Saavedra J, Biankin AV, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasms (IPMN). Am J Surg Pathol. 2004;28:977–87.
16. Kim GE, Bae HI, Park HU, Kuan SF, Crawley SC, Ho JJ, et al. Aberrant expression of MUC5AC and MUC6 gastric mucins and sialyl Tn antigen in intraepithelial neoplasms of the pancreas. Gastroenterology. 2002;123:1052–60.
17. Maitra A, Adsay NV, Argani P, et al. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. Mod Pathol. 2003;16:902–12.
18. Osako M, Yonezawa S, Siddiki B, Huang J, Ho JJ, Kim YS, et al. Immunohistochemical study of mucin carbohydrates and core proteins in human pancreatic tumors. Cancer. 1993;71:2191–9.
19. Masaki Y, Oka M, Ogura Y, Ueno T, Nishihara K, Tangoku A, et al. Sialylated MUC1 mucin expression in normal pancreas, benign pancreatic lesions, and pancreatic ductal adenocarcinoma. Hepatogastroenterology. 1999;46:2240–5.
20. Horinouchi M, Nagata K, Nakamura A, Goto M, Takao S, Sakamoto M, et al. Expression of different glycoforms of membrane mucin (MUC1) and secretory mucin (MUC2, MUC5AC and MUC6) in pancreatic neoplasms. Acta Histochem Cytochem. 2003;36:443–53.
21. Terada T, Ohta T, Sasaki M, Nakanuma Y, Kim YS. Expression of MUC apomucins in normal pancreas and pancreatic tumours. J Pathol. 1996;180:160–5.
22. Yonezawa S, Horinouchi M, Osako M, Kubo M, Takao S, Arimura Y, et al. Gene expression of gastric type mucin (MUC5AC) in pancreatic tumors: its relationship with biological behavior of the tumors. Pathol Int. 1999;49:45–54.
23. Alexakis N, Halloran C, Raraty M, Ghaneh P, Sutton R, Neoptolemos JP. Current standards of surgery for pancreatic cancer. Br J Surg. 2004;91:1410–27.
24. Shyr YM, Su CH, Tsay SH, Lui WY. Mucin-producing neoplasms of the pancreas: Intraductal papillary and mucinous cystic neoplasms. Ann Surg. 1996;223:141–6.
25. Wu YM, Nowack DD, Omenn GS, Haab BB, et al. Mucin glycosylation is altered by pro-inflammatory signaling in pancreatic-cancer cells. J Proteome Res. 2009;8:1876–86.
26. Nagata K. Analysis of mucins and CD10 expression in pancreatic intraductal neoplasia. Kagoshimadaigaku Igakuzashi (Med J Kagoshima Univ). 2005;57:7–17 (in Japanese, with English abstract).

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ORIGINAL ARTICLE

Whereabouts of an internal short stent placed across the pancreaticojejunostomy following pancreatoduodenectomy

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Abstract
Background/Purpose It is generally thought that an internal short stent placed across the pancreaticojejunostomy (PJ) following pancreatoduodenectomy (PD) usually passes spontaneously through the rectum thereafter; however, we experienced some patients who presented with pancreatitis and cholangitis owing to delayed defecation of the stent. The purpose of this study was to clarify when the stent eventually became detached from the PJ and how it passed through the body until it was finally defecated. In addition, we also investigated the factors that may prevent such detachment and defecation.
Methods This study retrospectively analyzed 57 patients who had had internal short stents placed across the PJ following PD. Defecation from the body, detachment from the PJ, and distal migration of the stent was confirmed by X-ray or computed tomography (CT) during the postoperative course. The cumulative rates of defecation and detachment of the stents, complications in relation to delayed defecation of the stents, and factors predictive of the delayed defecation, delayed detachment, and distal migration of the stents were analyzed.
Results Defecation of the stent was confirmed in 35 patients. The median time to defecation after PD and the cumulative defecation rate at 1 year were 454 days and 41 %, respectively. Acute pancreatitis occurred in 2 patients with the stent remaining in the pancreatic duct. One patient experienced acute cholangitis owing to migration of the stent to the bile duct. Multivariate analysis showed that ≥5 stitches in the duct-to-mucosa anastomosis, stent size of ≥5 Fr, and pancreatic fistula classified as either Grade B or C were independent predictive factors for delayed defecation of the stent. Five or more stitches in the duct-to-mucosa anastomosis was an independent predictive factor for delayed detachment of the stent. A stent size of ≥5 Fr was a risk factor for distal migration of the stent.
Conclusion In more than half of the study patients, internal short stents were not defecated within 1 year. Retrieval of the stent should be considered following the migration of an internal short stent. A stent size of ≥5 Fr was an independent predictive factor for delayed defecation and distal migration of a stent. Five or more stitches in the duct-to-mucosa anastomosis was an independent predictive factor for delayed defecation and detachment of a stent.

Keywords Stent · Pancreaticojejunostomy · Pancreatoduodenectomy

Introduction

Pancreatoduodenectomy (PD) is a standard surgical treatment for various diseases of the pancreatic head region. In spite of recent progress in surgical techniques and perioperative management, pancreatic fistula (PF) still occurs in 5–40 % of patients after PD [1–4] and the mortality rate of PD is about 5 % [5, 6]. PF can cause life-threatening conditions such as intra-abdominal abscess and intra-abdominal arterial hemorrhage [7]. Many surgeons have explored various tactics to reduce the problems associated with PF [8]. One of these tactics performed commonly is the use of a short stent (internal drainage) with a pancreaticojejunostomy (PJ). The use of a short stent with the PJ

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appears to reduce leakage of the PJ and the length of the hospital stay after PD [9–11].

Recently we experienced a patient with an internal short stent that had migrated into the bile duct, causing acute cholangitis with hepatolithiasis after PD. There have so far been few reports describing the long-term outcome of patients with short stents placed in the PJ following PD [9]. In this study, we retrospectively analyzed the data of 57 patients who had had internal short stents placed across the PJ following PD to clarify the whereabouts of the internal short stents.

Patients and methods

Patients

From April 1981 through October 2010, PD was performed in 637 patients at the Department of Surgery, Teikyo University Hospital. Eighty-eight of these 637 patients had an internal short stent placed across the PJ following PD. Most of the patients in whom an internal short stent was used underwent PD between 2004 and 2007. Thirty-one of the above 88 patients were excluded because they were lost to follow up. Therefore, the remaining 57 patients were included in this study.

Surgical procedure

The gallbladder, distal common bile duct, head of the pancreas, duodenum, and distal half of the stomach were removed during Whipple's PD. The whole stomach and the proximal duodenum 2–3 cm distal to the pylorus ring were preserved in pylorus-preserving PD (PPPD). Lymph nodes from the following areas were removed in patients with malignant disease: hepatoduodenal ligament, circumferentially around the common hepatic artery, and the right-half circumference of the superior mesenteric artery (in patients with pancreatic head carcinoma).

Reconstruction following Whipple's PD was performed by the modified Child method. Reconstruction following PPPD was performed by the Traverso method. The PJ was constructed with an end-to-side anastomosis, using 2 layers, the inner duct-to-mucosa layer and the outer pancreatic parenchyma-to-the jejunal seromuscular layer. The pancreatic duct with a little pancreatic parenchyma was anastomosed to the jejunal mucosal and seromuscular layer using interrupted 5-0 absorbent monofilament polydioxanone (PDS II; Ethicon, New Jersey, USA) sutures. The knots in both the anterior and posterior walls were outside the lumen. An internal short stent: either a polyvinyl chloride tube with a knot (pancreatic drainage tube; Sumitomo Bakelite, Tokyo, Japan) or a polyvinyl chloride tube without a knot (Atom Multipurpose Tube; Atom Medical, Tokyo, Japan) was inserted through the duct-to-mucosa anastomosis from the pancreatic duct to the jejunal lumen. The size and length of the stent were selected by the surgeon. The internal short stent was fixed to the anastomosis with 5-0 PDS II additional single sutures with a knot inside the lumen. The outer pancreatic parenchyma-to-the jejunal seromuscular layer approximation was created with interrupted penetrating 3-0 nonabsorbable monofilament polypropylene sutures (Prolene; Ethicon; Fig. 1a), or with anterior and posterior row 4-0 PDS II sutures (Fig. 1b). A closed drain was placed near the upper edge of the PJ.

Evaluation

Clinical status was determined by examinations at the Department of Surgery, Teikyo University Hospital, and by the referring physician. The length of the stent was measured by X-ray or CT during the postoperative follow up. The diameter of the main pancreatic duct was measured on CT. Defecation of the stent from the body, detachment from the PJ, and distal migration after detachment from the PJ was confirmed by X-ray or CT during the postoperative course. Abdominal X-ray was performed at least once a week during hospitalization. CT was performed every 3 or 4 months for 3 years after discharge, and every 6 months after that for patients with malignancy and every 6 months or every year for those with benign disease. During hospitalization, close attention was paid to the positional relationship between the internal stent and closed drain placed near the upper edge of the PJ. The retrieval of the stent was considered after discharge when complications associated with the stent were recognized or the patients without stent defecation requested the retrieval of the stent. Pancreatic texture was evaluated by intraoperative palpation by surgeons. The pancreatic duct in the remnant pancreas was measured on CT and was defined as dilated when the diameter was greater than 5 mm at the last evaluation before confirmation of stent detachment from the PJ. Postoperative PF was defined by the definition of the International Study Group on Pancreatic Fistula (ISGPF) [12]. Delayed gastric emptying (DGE) was defined according to a consensus definition, and the clinical grading of postoperative DGE was defined according to the criteria proposed by the International Study Group of Pancreatic Surgery (ISGPS) [13]. The severity of other early complications was classified according to Clavien's system (Dindo et al. [14]). Acute cholangitis was defined according to the diagnostic criteria for acute cholangitis in the Tokyo Guidelines [15]. Acute pancreatitis was defined according to the JPN diagnostic criteria for acute pancreatitis [16].

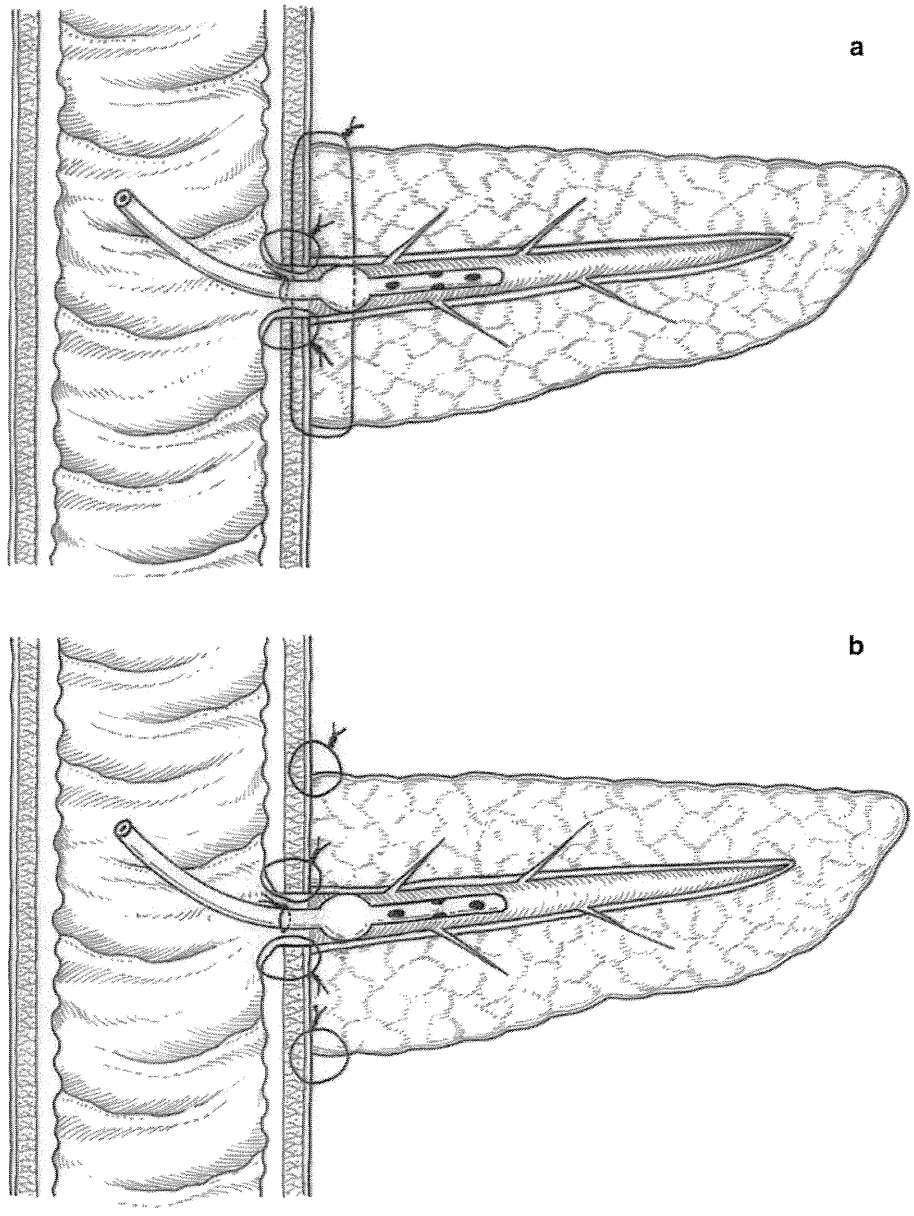

Fig. 1 **a** Schematic illustration of duct-to-mucosa pancreaticojejunostomy with an internal short stent held with interrupted penetrating 3-0 non-absorbable monofilament polypropylene sutures (Prolene; Ethicon). **b** Schematic illustration of duct-to-mucosa pancreaticojejunostomy with an internal short stent held with interrupted sutures composed of 2 rows of 4-0 PDS II sutures (anterior and posterior rows)

Statistical analysis

The stent defecation time was calculated as the interval between the operation and confirmation of defecation of the stent from the body. The stent detachment time was calculated as the interval between the operation and confirmation of stent detachment from the PJ. The cumulative stent defecation and detachment rates were calculated using the Kaplan–Meier method, supplemented with the log-rank test. Data for stent defecation and detachment were censored when a patient died without stent defecation and detachment or when the patient was alive without defecation or detachment of the stent. The stent migration rate was calculated as the number of patients with distal migration of the stent per the number of patients with stent detachment from the PJ. A univariate log-rank analysis and multivariate analysis using the Cox proportional hazard model with the backward stepwise procedure were performed to determine factors predictive of delayed defecation and delayed detachment of the stent. Factors were compared by the χ^2 test or Fisher's exact test where appropriate, and a multivariate analysis using the logistic analysis with the backward stepwise procedure was performed to determine the risk factors for distal migration of the stent. All factors tested by the univariate analysis were used for the multivariate analysis. The level of statistical significance was set at $P < 0.05$. Statistical evaluation was performed using the SPSS 18.0 software package (SPSS Japan, Tokyo, Japan) for Windows.

Results

There were 37 male and 20 female patients, with a mean age of 65.4 ± 10.8 years (range, 37–86 years). Indications for PD were pancreatic head carcinoma (19 patients), bile duct carcinoma (14 patients), chronic pancreatitis (6 patients), intraductal papillary mucinous adenoma (7 patients), ampullary carcinoma (4 patients), serous cystadenoma (2 patients), mucinous cystadenocarcinoma (1 patient), intraductal papillary mucinous carcinoma (1 patient), bile duct adenoma (1 patient), duodenal carcinoma (1 patient), and cystic duct carcinoma (1 patient). Dilatation of the main pancreatic duct was seen in 26 patients. PPPD and Whipple's PD were performed in 50 and 7 patients, respectively. Combined resection of the portal vein was performed in 13 patients. Normal soft pancreatic texture and hard pancreatic texture were found in 39 and 18 patients, respectively. Interrupted penetrating sutures were used in 40 patients and interrupted 2-row sutures in 17 patients. The mean size of the stent was 4.60 ± 1.14 Fr (range 3–7.5 Fr). The mean length of the stent was 56.4 ± 26.2 mm (range 30.9–125 mm). PF classified as either Grade B or C occurred in 22 patients. Dilatation of the remnant pancreatic duct was seen in 5 patients. Other early complications classified as ≥Grade II occurred in 7 patients; these complications were: hypoxic encephalopathy (1 patient), liver failure (1 patient), heart failure (1 patient), renal failure (1 patient), pulmonary edema (1 patient), leakage of gastrojejunostomy (1 patient), and mycotic infection (1 patient).

The mean postoperative observation period was 14.0 ± 15.2 months (range 0.3–61.4 months). Defecation of the stent was confirmed in 35 patients. Seven patients died within 1 year without stent defecation. The median defecation interval from PD and the cumulative defecation rate at 1 year were 454 days and 41 %, respectively (Fig. 2). Nine patients defecated the stent within 3 months after the operation, and seven patients defecated the stent within 1 month. Stent detachment from the PJ was confirmed in 43 patients. The median detachment interval from the PD and cumulative detachment rate at 1 year were 394 days and 46 %, respectively. Distal migration of the stent was observed in 8 of the 43 patients with stent detachment from the PJ: the overall stent migration rate was 18.6 %. The location of the stent migration was the bile duct in 4 patients and the afferent limb in 4 patients.

Complications associated with delayed defecation of the stent

Defecation of the stent had not occurred in 15 patients at the last follow-up evaluation (Table 1). The stent remained in the pancreatic duct, causing acute pancreatitis, in 2 patients, after 9 months and 36 months. The stent had migrated into the bile duct, thus causing cholangitis (1 patient) and liver abscess (1 patient) after 18 and 12 months, respectively, during the postoperative course. The two patients who presented with pancreatitis were treated conservatively. One patient (patient 10) underwent percutaneous transhepatic cholangial drainage (PTCD) based on a diagnosis of acute cholangitis after the stent had migrated into the bile duct. Cholangiography showed migration of the internal short stent into the left hepatic duct, as well as intrahepatic stones. The acute cholangitis resolved with the PTCD, and percutaneous transhepatic cholangioscopy (PTCS) was performed. The stent and hepatolithiasis were not revealed on PTCS; the stent and intrahepatic stones may have fallen into the jejunal limb, perhaps during dilatation of the PTCD fistula. The patient is alive without recurrence or symptoms 2 years after the PTCS. One patient (patient 12) presented with biliary obstruction owing to migration of the stent into the bile duct, and local recurrence of carcinoma at the hepatic hilum with a liver abscess. He underwent percutaneous transhepatic abscess drainage, but died owing to multiple organ failure.

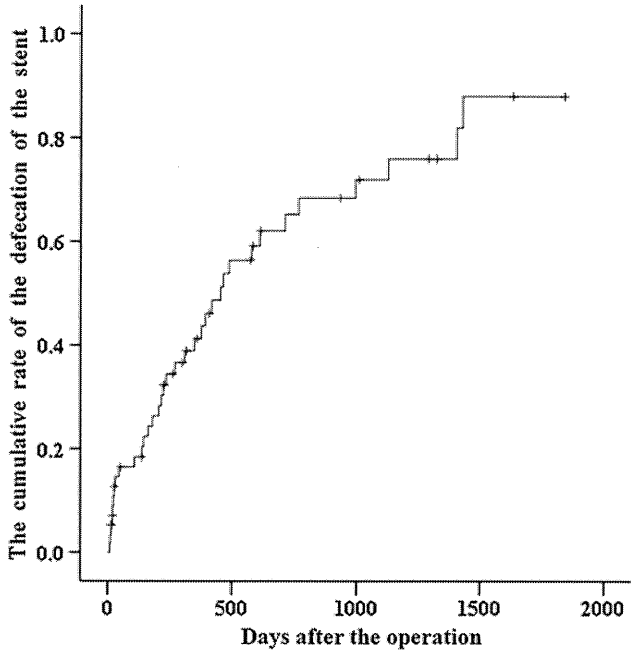

Fig. 2 Cumulative stent defecation rate

Analysis of factors predicting delayed defecation, delayed detachment, and distal migration of the stent

Patient characteristics and perioperative and postoperative parameters were reviewed, including patient age, gender, diameter of the main pancreatic duct, surgical procedure (pylorus preservation or not), portal vein resection, pancreatic texture, approximation of the jejunal wall and the

Table 1 Profiles and results of 15 patients without defecated stent at last follow-up evaluation

Patient	Age (years)/ gender	Diagnosis	Length of stent (mm)	Size of stent (Fr)	Number of stitches in duct-to-mucosa anastomosis	Approximation of the jejunal wall and the pancreatic stump	Status of the stent	Postoperative observation period (months)	Stent-associated complication	Retrieval of stent
1	80/F	MCC	60	4	6	Two-row suture	Remaining in the pancreatic duct	66	No	No
2	67/F	IPMA	53	6	6	Penetrating suture	Remaining in the pancreatic duct	58	No	No
3	60/M	Pancreatic carcinoma	60	5	6	Penetrating suture	Remaining in the pancreatic duct	47	Pancreatitis	No
4	63/M	Pancreatic carcinoma	65	6	6	Penetrating suture	Remaining in the pancreatic duct	21	No	No
5	76/M	Pancreatic carcinoma	72	4	7	Two-row suture	Remainingin the pancreatic duct	21	No	No
6	78/F	Bile duct carcinoma	125	6	7	Two-row suture	Remaining in the pancreatic duct	14	No	No
7	75/M	Pancreatic carcinoma	49	5	4	Penetrating suture	Remaining in the pancreatic duct	13	No	No
8	39/F	SCA	46	4	8	Penetrating suture	Remaining in the pancreatic duct	13	Pancreatitis	No
9	73/M	Ampullary carcinoma	67	4	5	Penetrating suture	Migration into the left hepatic duct	36	No	Single-balloon enteroscopy
10	77/M	Bile duct carcinoma	58	6	5	Penetrating suture	Migration into the left hepatic duct	22	Acute cholangitis, hepatolithiasis	PTCS
11	65/M	Pancreatic carcinoma	37	4	4	Penetrating suture	Migration into the left hepatic duct	16	No	No
12	61/M	Cystic duct carcinoma	87	7.5	7	Penetrating suture	Migration into the anterior segmental bile duct	12	Liver abscess	No
13	86/M	Bile duct carcinoma	47	6	8	Penetrating suture	Migration into the afferent limb	47	No	Single-balloon enteroscopy
14	57/M	IPMA	120	5	5	Penetrating suture	Migration into the afferent limb	42	No	No
15	63/M	Duodenal carcinoma	74	5	6	Penetrating suture	Migration into the afferent limb	33	No	No

MCC mucinous cystadenocarcinoma, *IPMA* intraductal papillary mucinous adenoma, *SCA* serous cystadenoma, *PTCS* percutaneous transhepatic cholangioscopy

Table 2 Univariate and multivariate analyses of risk factors for the delayed defecation of stents

	Number of patients (%)	Cumulative defecation rate at 1 year	Median defecation time (days)	Univariate *P* value	Multivariate *P* value (HR 95 % CI)
Age (years)					
<75	46 (81)	48	377	0.103	
≥75	11 (19)	11	769		
Gender					
Male	37 (65)	47	465	0.985	
Female	20 (35)	31	419		
Diameter of main pancreatic duct (mm)					
<3	31 (54)	43	394	0.377	
≥3	26 (46)	39	465		
Surgical procedure					
PPPD	50 (88)	44	394	0.188	
Whipple's PD	7 (12)	17	1127		
Portal vein resection					
Yes	13 (23)	24	465	0.779	
No	44 (77)	46	394		
Pancreatic texture					
Soft	39 (68)	37	489	0.429	
Hard	18 (32)	50	377		
Approximation of the jejunal wall and the pancreatic stump					
Penetrating suture	40 (70)	39	377	0.623	
Two-row suture	17 (30)	41	465		
Number of stitches in duct-to-mucosa anastomosis					
<5 stitches	19 (33)	70	181	0.002	0.010 (2.51: 1.25–5.05)
≥5 stitches	38 (67)	27	613		
Size of stent (Fr)					
<5	37 (65)	54	310	0.003	0.011 (3.03: 1.29–7.14)
≥5	20 (35)	17	1127		
Length of stent (mm)					
<50	24 (42)	45	454	0.433	
≥50	33 (58)	39	489		
Stent type					
With knot	41 (72)	38	581	0.466	
Without knot	16 (28)	47	377		
Ratio of diameters of stent and main pancreatic duct					
<0.5	30 (53)	35	581	0.476	
≥0.5	27 (47)	48	377		
Pancreatic fistula					
No or Grade A	35 (61)	51	349	0.074	0.033 (2.29: 1.07–4.90)
Grade B or C	22 (39)	28	1127		
Delayed gastric emptying					
No or Grade A	48 (84)	42	419	0.960	
Grade B or C	9 (16)	35	489		
Other early complications					
No or Grade I	50 (88)	42	454	0.419	
≥Grade II	7 (12)	32	419		

Table 2 continued

	Number of patients (%)	Cumulative defecation rate at 1 year	Median defecation time (days)	Univariate *P* value	Multivariate *P* value (HR 95 % CI)
Postoperative stool frequency					
<4 times per day	52 (91)	42	454	0.867	
≥4 times per day	5 (9)	40	1127		
Dilatation of the remnant pancreatic duct					
Yes	5 (9)	40	581	0.776	
No	52 (91)	42	454		

PD pancreatoduodenectomy, *PPPD* pylorus-preserving pancreatoduodenectomy, *HR* hazard ratio, *CI* confidence interval

pancreatic stump, stitches in the duct-to-mucosa anastomosis, diameter of the stent, length of the stent, stent type, ratio of the diameter of stent and that of the main pancreatic duct, PF, delayed gastric emptying, other early complications, postoperative stool frequency per day, and dilatation of the remnant pancreatic duct. Univariate analysis showed that significant prognostic factors for delayed defecation of the stent were ≥5 stitches in the duct-to-mucosa anastomosis, and stent size of ≥5 Fr (Table 2). The incidences of PF classified as either Grade B or C in patients who underwent duct-to-mucosa anastomosis with ≥5 stitches and <5 stitches were 31.6 % (6/19) and 42.1 % (16/38), respectively ($P = 0.442$).

Multivariate analysis was carried out to determine which factors were significantly predictive of delayed defecation of the stent. Stent size of ≥5 Fr, ≥5 stitches in the duct-to-mucosa anastomosis, and PF classified as either Grade B or C were independent prognostic factors for delayed defecation of the stent (Table 2).

Univariate analysis showed that the significant prognostic factors for delayed detachment of the stent were ≥5 stitches in the duct-to-mucosa anastomosis and stent size of ≥5 Fr (Table 3). Multivariate analysis was carried out to determine which factors were significantly predictive of delayed detachment of the stent. The use of five or more stitches in the duct-to-mucosa anastomosis was an independent predictive factor for delayed detachment of the stent (Table 3).

Univariate analysis showed that the only significant risk factor for distal migration of the stent was stent size of ≥5 Fr (Table 4). Multivariate analysis revealed that stent size of ≥5 Fr was an independent risk factor for distal migration of the stent (Table 4).

Discussion

PD is a complex procedure that is commonly performed for both benign and malignant diseases of the pancreas and periampullary region. Resection of the pancreatoduodenal specimen requires three anastomoses to reestablish gastrointestinal continuity: a pancreatic-enteric anastomosis, a biliary-enteric anastomosis, and a gastric or duodenal-enteric anastomosis [8]. PF is one of the most common complications after PD, causing significant morbidity and mortality. Many modifications of the PJ have been proposed to avoid fistula formation [17].

Stent placement across the PJ following PD may be useful for the diversion of pancreatic juice from the pancreatic anastomotic site, decompression of the remnant pancreas, and maintaining patency of the main pancreatic duct [11]. An external drainage stent can provide more complete diversion of pancreatic juice from the anastomosis and prevent activation of pancreatic enzymes by bile in comparison to an internal short stent [18]. However, external stents are uncomfortable for patients and have the potential for inadvertent removal; as well, there may be problems including twisting, bending, and occlusion of the stent [19, 20]. At our institution, the Department of Surgery, Teikyo University Hospital, an external stent is usually removed more than 5 weeks after the operation to avoid the occurrence of local peritonitis and pseudocysts; therefore, the hospital stay tends to be extended. Four randomized controlled trials (RCTs) have focused on the use of a stent in the pancreatic duct after PD [11, 21–24]. Two studies comparing the use of an external stent with no stent concluded that the use of an external stent through the pancreatic anastomosis reduced the PF rate [23, 24]. The Queen Mary Hospital group found that external drainage of the pancreatic duct with a stent reduced the leakage rate of the PJ after PD, and a multivariate analysis revealed that no stenting and a pancreatic duct diameter of <3 mm were significant risk factors for PF [23]. The French Surgery Research Group reported that the overall PF rate was 26 % in the external-stent group and 42 % in the no-stent group ($P < 0.03$) [24]. There are no RCTs that have proven the inferiority of an internal stent to an external stent or no stent, in terms of the rate of PF. The Johns Hopkins group reported that the rates of PF in their stent and no-stent groups were 11.3 and 7.6 %, respectively ($P = 0.3$) [22].

Table 3 Univariate and multivariate analyses of risk factors for the delayed detachment of stents from the pancreaticojejunostomy

	Number of patients (%)	Cumulative detachment rate at 1 year (%)	Median detachment time (days)	Univariate *P* value	Multivariate *P* value (HR: 95 % CI)
Age					
<75 years	46 (81)	52	349	0.174	
≥75 years	11 (19)	19	769		
Gender					
Male	37 (65)	51	349	0.640	
Female	20 (35)	37	419		
Diameter of main pancreatic duct (mm)					
<3	31 (54)	45	394	0.413	
≥3	26 (46)	48	454		
Surgical procedure					
PPPD	50 (88)	48	377	0.177	
Whipple's PD	7 (12)	33	714		
Portal vein resection					
Yes	13 (23)	38	454	0.868	
No	44 (77)	49	377		
Pancreatic texture					
Soft	39 (68)	40	454	0.264	
Hard	18 (32)	58	262		
Approximation of the jejunal wall and the pancreatic stump					
Penetrating suture	40 (70)	44	377	0.984	
Two-row suture	17 (30)	50	454		
Number of stitches in duct-to-mucosa anastomosis					
<5 stitches	19 (33)	72	181	0.012	0.014 (2.18: 1.17–4.05)
≥5 stitches	38 (67)	33	581		
Size of stent (Fr)					
<5	37 (65)	58	262	0.021	
≥5	20 (35)	25	934		
Length of stent (mm)					
<50	24 (42)	48	454	0.646	
≥50	33 (58)	46	394		
Stent type					
With knot	41 (72)	43	454	0.796	
Without knot	16 (28)	53	349		
Ratio of diameters of stent and main pancreatic duct					
<0.5	30 (53)	42	454	0.430	
≥0.5	27 (47)	50	349		
Pancreatic fistula					
No or Grade A	35 (61)	58	271	0.082	
Grade B or C	22 (39)	28	615		
Delayed gastric emptying					
No or Grade A	48 (84)	48	377	0.635	
Grade B or C	9 (16)	35	489		
Other early complications					
No or Grade I	50 (88)	48	377	0.487	
≥Grade II	7 (12)	32	1291		

Table 3 continued

	Number of patients (%)	Cumulative detachment rate at 1 year (%)	Median detachment time (days)	Univariate *P* value	Multivariate *P* value (HR: 95 % CI)
Postoperative stool frequency					
<4 times per day	52 (91)	47	394	0.608	
≥4 times per day	5 (9)	40	1127		
Dilatation of remnant pancreatic duct					
Yes	5 (91)	40	581	0.473	
No	52 (9)	47	394		

PD pancreatoduodenectomy, *PPPD* pylorus-preserving pancreatoduodenectomy, *HR* hazard ratio, *CI* confidence interval

The Wakayama Medical University group found that the median postoperative hospital stay in their group with internal drainage using a short stent was shorter than that in the group with external drainage using a long stent, although there was no difference in the incidence of PF [11]. Against this background, placement of an internal short stent across the PJ has been attracting increasing attention.

It is generally thought that a pancreatic internal stent usually passes spontaneously through the rectum. The median interval between stent placement and stent defecation was 454 days in the present series. There has been only one report describing the timing of the defecation of a pancreatic internal stent placed across the PJ. Yoshimi et al. [9] reported that all internal short stents placed in 11 patients who underwent PD followed by end-to-side PJ (in which 2 layers–an inner duct-to-mucosa layer and an outer pancreatic parenchyma-to-jejunal seromuscular layer–had been used, with 2 rows of sutures) had fallen out spontaneously by the 176th postoperative day. They used stents of about 3–5 cm in length and 6–12 Fr in diameter, composed of either a either silicon tube (internal stent; Create Medic, Tokyo Japan) or a polyvinyl chloride tube (pancreatic duct tube; Sumitomo Bakelite, Tokyo, Japan). Their uneventful results might have been due to the small number of patients and the short length of the stent.

The appropriate timing of stent defecation is unclear. A pancreatojejunal anastomosis may require 6 months to ''mature''; thus, a prolonged stent stay may ensure patency [25]. However, the potential benefit of prolonged stent stay must be balanced against the potential risks, as long-term transanastomotic stent stay is of no benefit and is potentially harmful. In the literature 9 cases of migration of a pancreatic internal short stent placed across a PJ following PD have been reported [17, 26–29]. The locations of the migration were the proximal pancreatic duct in 5 cases, afferent limb in 2 cases, and small intestine and bile duct in 1 case each. Four of the 5 patients experienced symptoms after the pancreatic stent had migrated into the proximal pancreatic duct: steatorrhea in 3 and pancreatitis in one patient [7, 11, 26]. Two patients had abdominal pain after the pancreatic stent had migrated into the afferent limb [27]. Stent migration into the small intestine and bile duct caused ileus [28] and liver abscess [29]. The intervals between PD and the occurrence of symptoms or recognition of the stent migration in these 9 patients ranged from 6 weeks to 7 years (median 1 year). In the present series, defecation of the stents had not occurred in 15 patients at the last follow-up evaluation. Two of these 8 patients experienced acute pancreatitis with the stent remaining in the pancreatic duct. Acute cholangitis with hepatolithiasis and liver abscess occurred in 2 patients after the stent had migrated into the bile duct. We did not use the expression "proximal migration into the pancreatic duct" and in this study, because it is difficult to differentiate between proximal migration into the pancreatic duct and "a stent remaining in the pancreatic duct" (delayed detachment from PJ), and the stent was not completely located in the pancreatic duct in any of our patients. There may have been confusion and overlap of these 2 expressions in the literature. Smyrniotis et al. reported that the stent was wedged into the pancreatic stump in 6 of 41 patients (14.7 %) who underwent PD with internal stenting, and 4 patients required analgesic treatment with opioids for severe back pain [30]. Stents that remain in the pancreatic duct may be common in clinical practice although the number of reported cases is small. Clinicians may not recognize or observe stent migration into the proximal pancreatic duct because the associated complications are mild. Retrieval of the stent should be considered when an internal short stent migrates.

Pancreatic duct stents are inserted endoscopically for a variety of pancreatic conditions including ductal obstruction arising from stricture or malignancy, ductal disruption, drainage of pancreatic pseudocysts, recurrent pancreatitis associated with pancreas divisum, obstructing ductal stones, and the prevention of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis [31]. Proximal migration of pancreatic stents inserted endoscopically for ductal strictures occurs at the rate of 5–6 %

Table 4 Univariate and multivariate analyses of risk factors for the distal migration of stents

	No. of patients	Migration rate	Univariate *P* value	Multivariate *P* value (HR: 95 % CI)
Age (years)				
<75	37	6 (16.2 %)	0.318	
≥75	6	2 (33.3 %)		
Gender				
Male	29	7 (24.1 %)	0.180	
Female	14	1 (7.1 %)		
Diameter of main pancreatic duct (mm)				
<3	24	4 (16.7 %)	0.714	
≥3	19	4 (21.1 %)		
Surgical procedure				
PPPD	39	7 (17.9 %)	0.730	
Whipple's PD	4	1 (25.0 %)		
Portal vein resection				
Yes	10	2 (20.0 %)	0.897	
No	33	6 (18.2 %)		
Pancreatic texture				
Soft	30	5 (16.7 %)	0.620	
Hard	13	3 (23.1 %)		
Approximation of the jejunal wall and the pancreatic stump				
Penetrating suture	30	7 (23.3 %)	0.226	
Two-row suture	13	1 (7.7 %)		
Number of stitches in duct-to-mucosa anastomosis				
<5 stitches	17	1 (5.9 %)	0.083	
≥5 stitches	26	7 (26.9 %)		
Size of stent (Fr)				
<5	31	3 (9.7 %)	0.016	0.025 (6.67: 1.28–34.84)
≥5	12	5 (41.7 %)		
Length of stent (mm)				
<50	17	2 (11.8 %)	0.351	
≥50	26	6 (23.1 %)		
Stent type				
With knot	30	7 (23.3 %)	0.226	
Without knot	13	1 (7.7 %)		
Ratio of diameters of stent and main pancreatic duct				
<0.5	22	4 (18.2 %)	0.942	
≥0.5	21	4 (19.0 %)		
Pancreatic fistula				
No or Grade A	27	4 (14.8 %)	0.407	
Grade B or C	16	4 (25.0 %)		
Delayed gastric emptying				
No or Grade A	38	8 (21.1 %)	0.255	
Grade B or C	5	0 (0.0 %)		
Other early complications				
No or Grade I	40	7 (17.5 %)	0.497	
≥Grade II	3	1 (33.3 %)		

Table 4 continued

	No. of patients	Migration rate	Univariate *P* value	Multivariate *P* value (HR: 95 % CI)
Postoperative stool frequency				
<4 times per day	40	8 (20.0 %)	0.391	
≥4 times per day	3	0 (0.0 %)		
Dilatation of remnant pancreatic duct				
Yes	4	0 (0.0 %)	0.315	
No	39	8 (20.5 %)		

PD pancreatoduodenectomy, *PPPD* pylorus-preserving pancreatoduodenectomy, *HR* hazard ratio, *CI* confidence interval

[32, 33]. Such migration may cause ductal damage, obstruction, and pancreatitis, and necessitate stent retrieval [34]. The frequency of distal migration of pancreatic and biliary plastic stents inserted endoscopically is 8–12 % [32, 33]. Stents that migrate distally usually pass spontaneously through the rectum; however, bowel wall penetration resulting in enteroenteric fistula formation, free perforation, and obstruction has been reported [35]. Two articles [36, 37] reported an extremely unusual complication of acute appendicitis due to appendiceal orifice obstruction by a migrated biliary stent. These complications are often associated with stent entrapment in areas of bowel fixation owing to adhesions and hernia or in colonic diverticula.

The present study analyzed the predictive factors for delayed defecation of the stent to clarify a strategy for avoiding delayed defecation. This study found that stent size of ≥5 Fr, ≥5 stitches in the duct-to-mucosa anastomosis, and PF classified as either Grade B or C were independent factors predictive of delayed defecation of the stent. The relationship between stent size of ≥5 Fr and ≥5 stitches in the duct-to-mucosa anastomosis and delayed defecation/detachment of the stent may reflect the strength of resistance against the stream of the stent. Stent size of ≥5 Fr was a predictive factor not only for delayed defecation but also for distal migration of the stent. The strength of resistance against the stream of the stent could account for the mechanism of these results. We also found that PF classified as Grade B or C was an independent predictive factor for delayed defecation of the stent. Hypoperistalsis owing to PF might be responsible for the delayed defecation of a stent. Therefore, the use of a narrow stent and the use of <5 stitches in the duct-to-mucosa anastomosis could prevent the delayed defecation of a stent. However, many surgeons might fear that the risk of PJ leakage would be increased if these parameters were employed, though there is no definite evidence that a narrow stent and small number of stitches in the duct-to-mucosa anastomosis are risk factors for PF. Reconstruction in which the hepaticojejunostomy is not distal to the the PJ, including Whipple's method, might be an effective method to avoid migration of the stent into the bile duct following PD.

One limitation of the present study is the limited number of patients. Additional investigation with a larger patient population in a multicenter study is needed before definitive conclusions can be drawn. In conclusion, this study suggests that retrieval of the stent should be considered when an internal short stent migrates. Stent size of ≥5 Fr, ≥5 stitches in the duct-to-mucosa anastomosis, and PF classified as either Grade B or C are strong risk factors for delayed stent defecation. Stent size of ≥5 Fr was a predictive factor not only for delayed defecation but also for distal migration of the stent.

References

1. Lowy AM, Lee JE, Pisters PW, et al. Prospective, randomized trial of octreotide to prevent pancreatic fistula after pancreaticoduodenectomy for malignant disease. Ann Surg. 1997;226:632–41.
2. Roder JD, Stein HJ, Bottcher KA, et al. Stented versus nonstented pancreaticojejunostomy after pancreatoduodenectomy: a prospective study. Ann Surg. 1999;229:41–8.
3. Cameron JL, Pitt HA, Yeo CJ, et al. One hundred and forty-five consecutive pancreaticoduodenectomies without mortality. Ann Surg. 1993:217:430–5 (discussion 435–8).
4. Montorsi M, Zago M, Mosca F, et al. Efficacy of octreotide in the prevention of pancreatic fistula after elective pancreatic resections: a prospective, controlled, randomized clinical trial. Surgery. 1995;117:26–31.
5. Yeo CJ, Cameron JL, Sohn TA, et al. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. Ann Surg. 1997:226:248–57 (discussion 257–60).
6. Poon RT, Fan ST, Lo CM, et al. Pancreaticoduodenectomy with en bloc portal vein resection for pancreatic carcinoma with suspected portal vein involvement. World J Surg. 2004;28:602–8.
7. Miura F, Takada T, Amano H, et al. Repeated pancreatectomy after pancreatoduodenectomy. J Gastrointest Surg. 2007;11:179–86.
8. Kennedy EP, Yeo CJ. Dunking pancreaticojejunostomy versus duct-to-mucosa anastomosis. J Hepatobiliary Pancreat Sci. 2011;18:769–774.
9. Yoshimi F, Ono H, Asato Y, et al. Internal stenting of the hepaticojejunostomy and pancreaticojejunostomy in patients

undergoing pancreatoduodenectomy to promote earlier discharge from hospital. Surg Today. 1996;26:665–7.
10. Shibuya T, Uchiyama K, Imai S, et al. Improvement of pancreaticojejunostomy in pancreatoduodenectomy. Int Surg. 1995;80:57–60.
11. Tani M, Kawai M, Hirono S, et al. A prospective randomized controlled trial of internal versus external drainage with pancreaticojejunostomy for pancreaticoduodenectomy. Am J Surg. 2010;199:759–64.
12. Bassi C, Dervenis C, Butturini G, et al. Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery. 2005;138:8–13.
13. Wente MN, Bassi C, Dervenis C, et al. Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the International Study Group of Pancreatic Surgery (ISGPS). Surgery. 2007;142:761–8.
14. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240:205–13.
15. Wada K, Takada T, Kawarada Y, et al. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007;14:52–8.
16. Koizumi M, Takada T, Kawarada Y, et al. JPN Guidelines for the management of acute pancreatitis: diagnostic criteria for acute pancreatitis. J Hepatobiliary Pancreat Surg. 2006;13:25–32.
17. Levy MJ, Chari S, Adler DG, et al. Complications of temporary pancreatic stent insertion for pancreaticojejunal anastomosis during pancreaticoduodenectomy. Gastrointest Endosc. 2004;59:719–24.
18. Poon RT, Fan ST. Decreasing the pancreatic leak rate after pancreaticoduodenectomy. Adv Surg. 2008;42:33–48.
19. Imaizumi T, Hatori T, Tobita K, et al. Pancreaticojejunostomy using duct-to-mucosa anastomosis without a stenting tube. J Hepatobiliary Pancreat Surg. 2006;13:194–201.
20. Suzuki S, Kaji S, Koike N, et al. Pancreaticojejunostomy of duct to mucosa anastomosis can be performed more safely without than with a stenting tube. Am J Surg. 2009;198:51–4.
21. Schulick RD. Use of pancreatic duct stents after pancreaticoduodenectomy. J Hepatobiliary Pancreat Sci. 2011;18:775–8.
22. Winter JM, Cameron JL, Campbell KA, et al. Does pancreatic duct stenting decrease the rate of pancreatic fistula following pancreaticoduodenectomy? Results of a prospective randomized trial. J Gastrointest Surg. 2006;10:1280–90 (discussion 1290).
23. Poon RT, Fan ST, Lo CM, et al. External drainage of pancreatic duct with a stent to reduce leakage rate of pancreaticojejunostomy after pancreaticoduodenectomy: a prospective randomized trial. Ann Surg. 2007;246:425–33 (discussion 433–5).
24. Pessaux P, Sauvanet A, Mariette C, et al. External pancreatic duct stent decreases pancreatic fistula rate after pancreaticoduodenectomy: prospective multicenter randomized trial. Ann Surg. 2011;253:879–85.
25. Lillemoe KD, Pitt HA, Cameron JL. Current management of benign bile duct strictures. Adv Surg. 1992;25:119–74.
26. Ammori BJ, White CM. Proximal migration of transanastomotic pancreatic stent following pancreaticoduodenectomy and pancreaticojejunostomy. Int J Pancreatol. 1999;25:211–5.
27. Layec S, D'Halluin PN, Pagenault M, et al. Removal of transanastomotic pancreatic stent tubes after pancreaticoduodenectomy: a new role for double-balloon enteroscopy. Gastrointest Endosc. 2010;72:449–51.
28. Biffl WL, Moore EE. Pancreaticojejunal stent migration resulting in "bezoar ileus". Am J Surg. 2000;180:115–6.
29. Rezvani M, O'Moore PV, Pezzi CM. Late pancreaticojejunostomy stent migration and hepatic abscess after Whipple procedure. J Surg Educ. 2007;64:220–3.
30. Smyrniotis V, Arkadopoulos N, Kyriazi MA, et al. Does internal stenting of the pancreaticojejunostomy improve outcomes after pancreatoduodenectomy? A prospective study. Langenbecks Arch Surg. 2010;395:195–200.
31. Price LH, Brandabur JJ, Kozarek RA, Gluck M, Traverso WL, Irani S. Good stents gone bad: endoscopic treatment of proximally migrated pancreatic duct stents. Gastrointest Endosc. 2009;70:174–9.
32. Johanson JF, Schmalz MJ, Geenen JE. Incidence and risk factors for biliary and pancreatic stent migration. Gastrointest Endosc. 1992;38:341–6.
33. Smits ME, Badiga SM, Rauws EA, Tytgat GN, Huibregtse K. Long-term results of pancreatic stents in chronic pancreatitis. Gastrointest Endosc. 1995;42:461–7.
34. Kozarek RA. Pancreatic stents can induce ductal changes consistent with chronic pancreatitis. Gastrointest Endosc. 1990;36:93–5.
35. Mastorakos DP, Milman PJ, Cohen R, Goldenberg SP. An unusual complication of a biliary stent-small bowel perforation of an incarcerated hernia sac. Am J Gastroenterol. 1998;93:2533–5.
36. Tzovaras G, Liakou P, Makryiannis E, et al. Acute appendicitis due to appendiceal obstruction from a migrated biliary stent. Am J Gastroenterol. 2007;102:195–6.
37. Schwab D, Baum U, Hahn EG. Colonoscopic treatment of obstructive appendicitis caused by dislocation of a biliary stent. Endoscopy. 2005;37:606.

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Targeting of MAPK-associated molecules identifies SON as a prime target to attenuate the proliferation and tumorigenicity of pancreatic cancer cells

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Abstract

Background: Pancreatic cancer is characterized by constitutive activation of mitogen-activated protein kinase (MAPK). Activation of MAPK is associated with the upregulation of genes implicated in the proliferation and survival of pancreatic cancer cells. We hypothesized that knockdown of these MAPK-associated molecules could produce notable anticancer phenotypes.

Methods: A RNA interference-mediated knockdown screening of 78 MAPK-associated molecules previously identified was performed to find molecules specifically associated with proliferation of pancreatic cancer cells *in vitro*. Expression of an identified molecule in pancreatic cancer tissues was examined by immunohistochemistry. *In vivo* tumorigenicity of cancer cells with stable knockdown of the molecule was assayed by using xenograft models. Flow cytometry and live cell imaging were employed to assess an association of the molecule with cell cycle.

Results: The knockdown screening revealed that knockdown of *SON*, the gene encoding SON, which is a large serine/arginine-rich protein involved in RNA processing, substantially suppressed pancreatic cancer cell proliferation and survival *in vitro* and tumorigenicity *in vivo*. SON expression was higher in ductal adenocarcinomas than in cells of normal ducts and precursor lesions in pancreatic cancer tissues. Knockdown of *SON* induced G2/M arrest and apoptosis in cultured cancer cells. The suppressive effect of *SON* knockdown on proliferation was less pronounced in cultured normal duct epithelial cells. SON formed nuclear speckles in the interphase of the cell cycle and dispersed in the cytoplasm during mitosis. Live cell imaging showed that SON diffusely dispersed in the early mitotic phase, accumulated in some foci in the cytoplasm in the late mitotic phase, and gradually reassembled into speckles after mitosis.

Conclusion: These results indicate that SON plays a critical role in the proliferation, survival, and tumorigenicity of pancreatic cancer cells, suggesting that SON is a novel therapeutic molecular target for pancreatic cancer.

Keywords: SON, MAPK, RNA interference, Speckle, Cell cycle

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Background

Pancreatic cancer is a leading cause of cancer-related deaths [1,2]. Despite advancements in diagnostic and therapeutic modalities, the 5-year survival rate of patients with pancreatic cancer is less than 10% [3]. This poor prognosis elicits an urgent need for the development of effective diagnostic and therapeutic measures to improve patient survival. Molecular medicine may be able to fulfill this need, as exemplified by imatinib in the treatment of chronic myeloid leukemia [4]. Pancreatic cancer is characterized by constitutive activation of mitogen-activated protein kinase (MAPK), due to gain-of-function mutations in *KRAS* or *BRAF* and loss-of-function of dual specificity phosphatase 6 (DUSP6) [5-7]. Active MAPK translocates to the nucleus, activates transcription factors, and induces the expression of a variety of genes [8]. In a previous study, we screened the genome for downstream targets of MAPK and identified 78 molecules specifically associated with MAPK activity in pancreatic cancer cells [9]. These MAPK-associated molecules include molecules implicated in DNA replication, RNA editing, spindle formation, mitosis, signal transduction, and membrane trafficking. These biological processes play critical roles in the survival, maintenance, and proliferation of pancreatic cancer cells. We hypothesized that molecular targeting of these MAPK-associated molecules could result in notable anticancer phenotypes, as we previously observed by targeting *AURKA* [9,10]. In this study, we performed a systematic knockdown screening of MAPK-associated molecules in pancreatic cancer cells.

Results

Knockdown screening of MAPK-modulated genes in pancreatic cancer cells

We performed knockdown screening using a pancreatic cancer cell line, MIA PaCa-2, and custom-designed short interfering RNAs (siRNAs) targeting all the 78 MAPK-modulated genes that were previously identified and isolated in the cell line (Additional file 1: Table S1) [9]. The cells were transiently transfected with each of the 78 siRNAs, and *in vitro* proliferation was subsequently examined for 5 consecutive days. This screening showed that proliferation of cancer cells was suppressed to variable degrees depending on the individual gene targeted (Figure 1). Knockdown of *AURKB, CENPA, EBNA1BP2, GOLT1A, KIF11, NEDD4L, SON, TPX2,* or *WDR5* suppressed proliferation by more than 50% compared with control. Among these targets, we focused on *SON* for further study because it showed the most substantial suppressive effect. This gene encodes a nuclear speckle protein, SON, which is involved in RNA processing.

Knockdown of *SON* attenuates proliferation in vitro, considerably in pancreatic cancer cells but less remarkably in normal phenotype cells

The *in vitro* suppressive effect of siRNA targeting *SON* on proliferation was reanalyzed in detail by using MIA PaCa2; PCI-35, a pancreatic cancer cell line with an aggressive phenotype; and HPDE, an immortalized normal human pancreatic duct epithelial cell line [7,11-13]. The suppressive effects of *SON* knockdown on cell proliferation appeared to be fatal in MIA PaCa-2, static in PCI-35, and insignificant in HPDE (Figure 2A). The effects of siRNA on SON expression were assayed by an immunoblotting method, which showed 77%, 10%, and 48% reduction of SON expression in MIA PaCa-2, PCI-35, and HPDE, respectively (Figure 2B). These results indicated that *SON* knockdown attenuated the *in vitro* proliferation of pancreatic cancer cells. The attenuation of proliferation depended on the efficiency of SON knockdown in pancreatic cancer cells, but was less remarkably affected in normal phenotype cells.

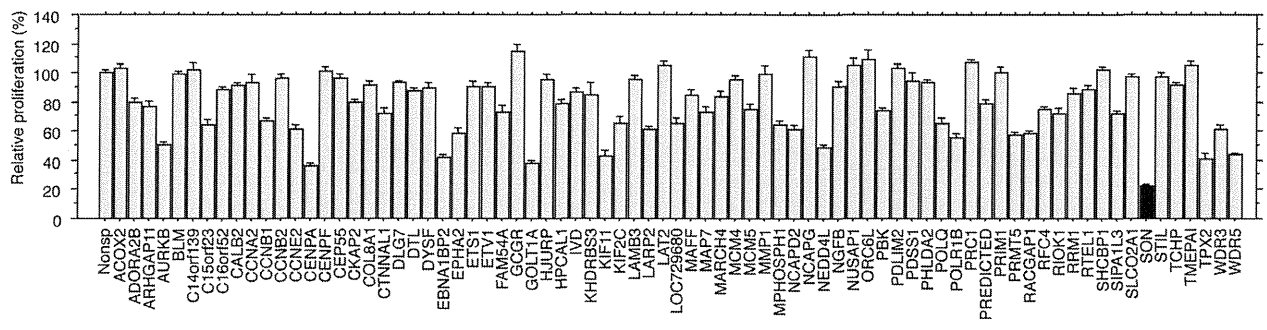

Figure 1 Knockdown screening of MAPK-associated genes in pancreatic cancer cells. Proliferation of MIA PaCa-2, a pancreatic cancer cell line, transfected with siRNA targeting various genes associated with MAPK (indicated on the horizontal axis), was determined by MTT assay on day 5 post-transfection. Plotted values are expressed relative to cells infected with control siRNA to a nonspecific sequence (Nonsp). Plots represent an average of 2 independent experiments; each experiment includes data from 8 independent transfection wells. Knockdown of *SON* (closed column) showed the most remarkable anti-proliferative phenotype.

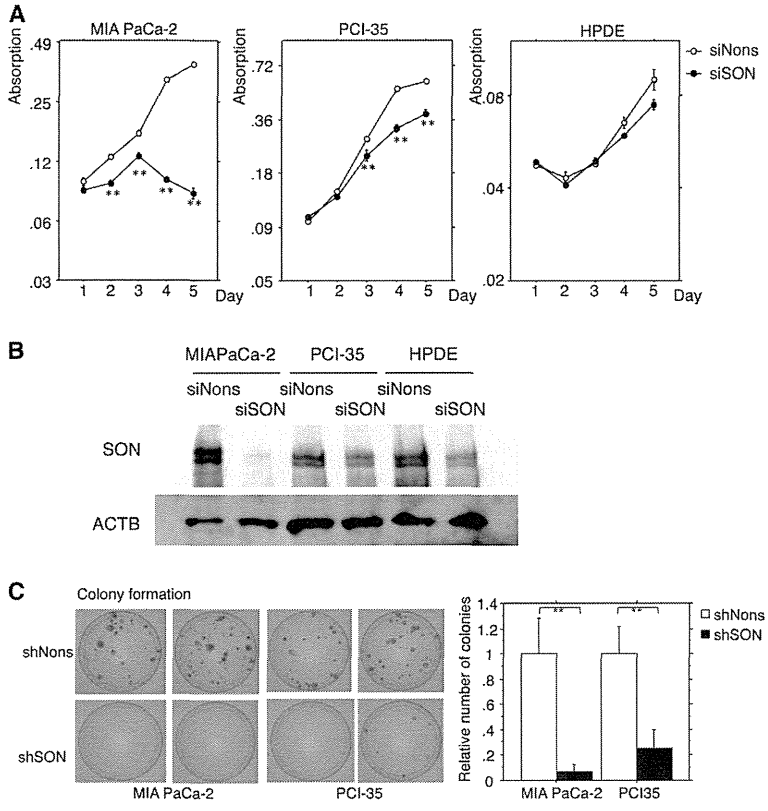

Figure 2 A. Proliferation of pancreatic cancer cells (MIA PaCa-2 and PCI-35) and normally phenotypic duct epithelial cells (HPDE) transfected with siRNA against *SON* (siSON) or a nonspecific sequence (siNons) and measured by MTT assay. The plots represent an average of 2 independent experiments; experiment includes data from 8 independent transfection wells. **B.** Expression of *SON* in cells transfected with siSON or siNons is shown in immunoblots probed with anti-SON antibody (SON) or anti-beta actin antibody (ACTB). **C.** Colony formation assay of pancreatic cancer cells transfected with vectors expressing shRNA targeting *SON* (shSON) or a non-specific sequence (shNons).

Stable knockdown of *SON* reduces the survival of pancreatic cancer cells in vitro

We next constructed a vector expressing short hairpin RNA (shRNA) identical to the *SON* siRNA when processed. We examined the effect of stable knockdown of *SON* on the survival of pancreatic cancer cells *in vitro* using a colony formation assay. We found that stable knockdown of *SON* strongly attenuated the survival of cancer cells, even in PCI-35 cells, in which transient transfection of siRNA targeting *SON* modestly suppressed proliferation (Figure 2C).

SON is overexpressed in pancreatic ductal adenocarcinomas

To establish the native expression of SON in pancreatic cancer, we examined 34 tissues with pancreatic ductal adenocarcinoma that were surgically resected. Immunohistochemistry showed that SON was strongly expressed in the nuclei of cancer cells in most ductal adenocarcinomas significantly more obviously than in the nuclei of non-neoplastic ducts or pancreatic intraepithelial neoplasia (PanIN), a precursor lesion of ductal adenocarcinoma ($p < 0.001$ by ANOVA) (Figure 3 and Table 1). This result indicates that SON is specifically overexpressed in pancreatic cancer.

Knockdown of *SON* retards the tumorigenicity of pancreatic cancer cells in vivo

We then performed a tumorigenicity assay using stably transfected pancreatic cancer cell clones carrying the shRNA vector targeting *SON*. Several stably transfected clones of MIA PaCa-2 and PCI-35 cells were obtained, and expression of *SON* was determined by real-time quantitative PCR. *SON* expression was lowest, reduced by 50%, in an MIA PaCa-2 clone (Figure 2D). We could not obtain any stably transfected PCI-35 clones in which *SON* expression was obviously reduced. This was probably because PCI-35, unlike MIA PaCa-2, could not survive modest knockdown of *SON*, which strongly suppresses the survival of cancer cells *in vitro*. The stably transfected clone of MIA PaCa-2 was inoculated into the subcutis of nude mice, and tumorigenicity was monitored. After 4 weeks, tumorigenicity was significantly retarded (Figure 4A).