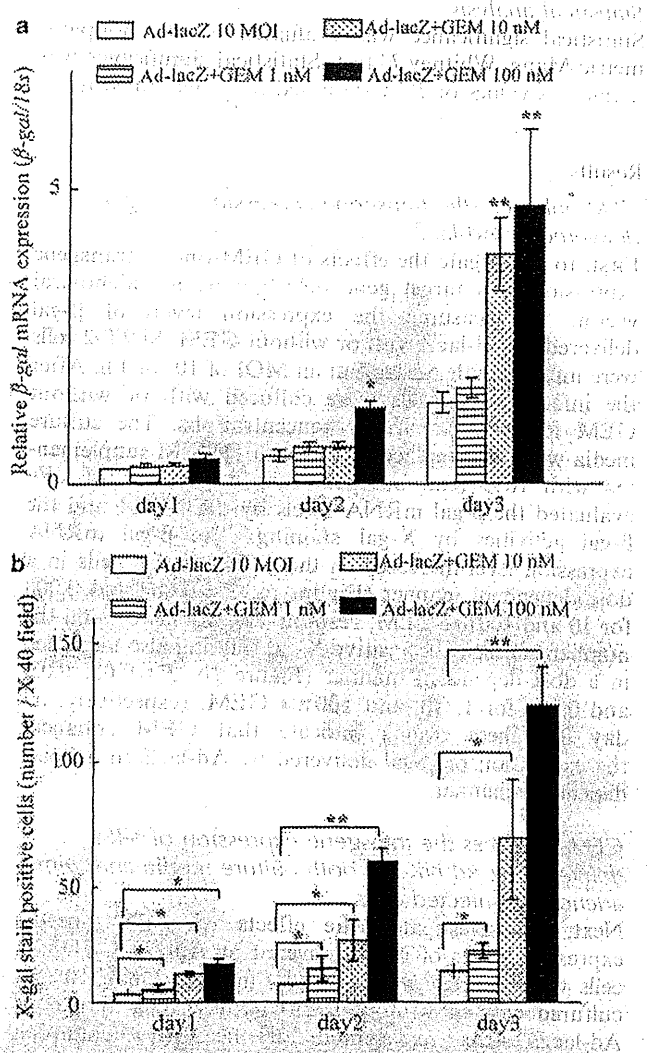

Figure 1 GEM enhances the transgene expression of β-gal delivered by Ad-lacZ. SUIT-2 cells were infected with Ad-lacZ (MOI of 10) for 1 h and treated with GEM (0, 1, 10, and 100 nM) for 24 h. The culture media were then replaced with fresh media without GEM. (**a**) Total RNA samples were extracted on the indicated days. The expression levels of *β-gal* mRNA were measured by qRT–PCR and normalized by the corresponding expression level of *18S rRNA*. Bars represent relative expression levels as the fold changes in comparison with untreated cells. Each value represents the mean ± s.d. of three independent samples. (**b**) β-gal activity was assessed by X-gal staining and counted numbers of β-gal-positive cells (magnification, ×100). Each value represents the mean ± s.d. of five independent fields. $^{*}P<0.05$, $^{**}P<0.01$.

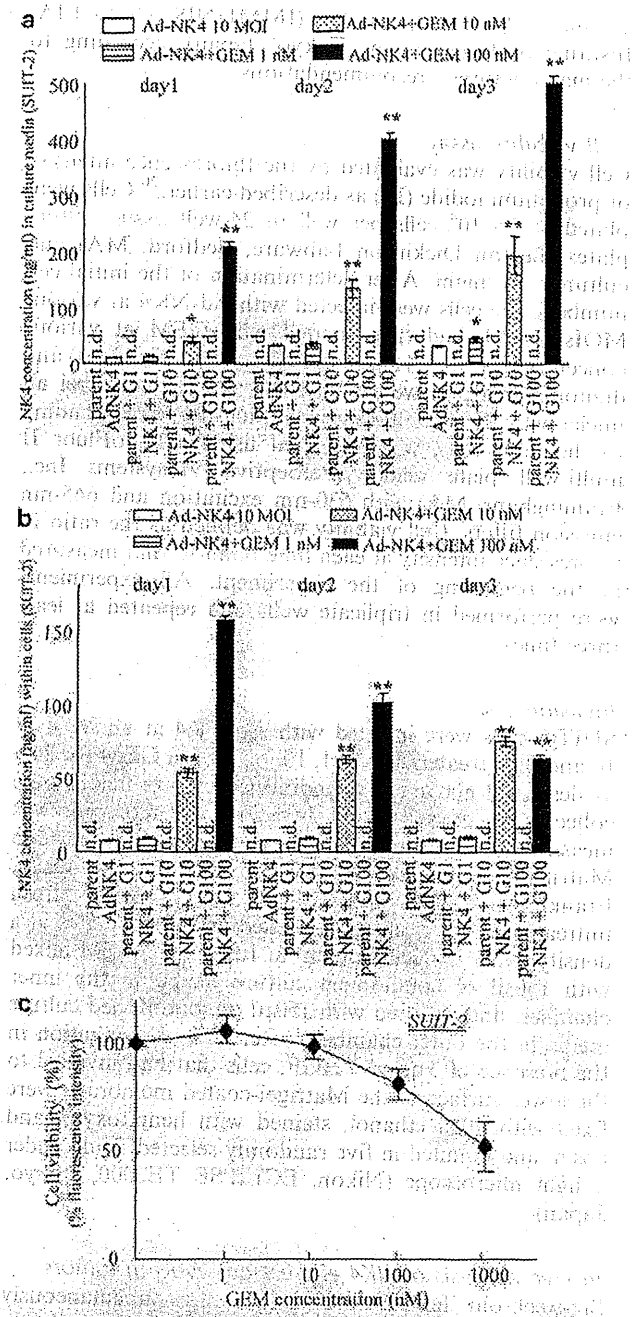

Figure 2 GEM enhances the transgene expression of NK4 delivered by Ad-NK4 in both the culture media and within adenovirus-infected SUIT-2 cells *in vitro*. (**a**, **b**) SUIT-2 cells were infected with Ad-NK4 (MOI of 10) for 1 h and treated with GEM (0, 1, 10, and 100 nM) for 24 h. The culture media were then replaced with fresh media without GEM. The NK4 expression levels were measured in the culture media (**a**) and within cells (**b**) on days 1, 2, and 3. (**c**) SUIT-2 cells were treated with GEM for 24 h, followed by *replacement of the culture media with fresh media without GEM*. After 72 h, the cell viabilities were determined by PI assays as the ratio of the fluorescence intensity. 'n.d.' in the graphs means 'not detectable'. Bars represent relative cell viabilities as the fold changes in comparison with control cells. Each value represents the mean ± s.d. of three independent samples. $^{*}P<0.05$, $^{**}P<0.01$.

18.8 ± 6.9% reduction in viability on day 3 (Figure 2c). Interestingly, the increase in intracellular NK4 expression in SUIT-2 cells began to be notable on day 1 (Figure 2b), although GEM did not kill the cells at any of the concentrations examined on day 1 (Figure 2c). Furthermore, low doses of GEM, such as 10 nM, did not kill SUIT-2 cells even on day 3 (Figure 2c), but still

remarkably increased NK4 expression in the culture media ($P=0.0014$). These data suggest that the increased levels of NK4 in the culture media in the presence of GEM were caused not by release of NK4 from dead cells alone, and also, parental SUIT-2 cells did not express NK4 both in culture media and within cells, even in the presence of GEM. These data suggested that GEM induced only increased expression of the transgene, but not the endogenous expression of NK4. In other pancreatic cancer cell lines, namely KP-2 and MIA PaCa-2 cells, which did not also express NK4, we found similar effects of GEM on the transgene expression of Ad-NK4 in culture media (Figure 3a1, a2, KP-2: $P=0.0025$ and 0.0018 for 100 nM and 1 μM GEM, respectively, on day 3; Figure 3b1, b2, MIA PaCa-2: $P=0.0086$ and 0.0002 for 10 and 100 nM GEM, respectively, day 3).

GEM synergistically enhances the inhibitory effects of Ad-NK4 on cancer cell invasion in a dose-dependent manner

NK4, which inhibits biological events driven by HGF-Met signaling, inhibits invasion, but has no effects on the proliferation and survival of pancreatic cancer cells.[16,22] As shown in Figure 4, we found that Ad-NK4 alone at an MOI of 10 did not inhibit the proliferation of SUIT-2 cells and that 10 nM GEM alone or in combination with Ad-NK4 at an MOI of 10 did not affect the proliferation for 3 days. Next, we investigated whether the enhanced NK4 expression mediated by GEM had biological effects on cancer cell invasion. Ad-NK4-infected SUIT-2 cells were treated with GEM at various concentrations for 24 h. After replacement of the GEM-containing media with fresh media without GEM, the cells were cultured for 1 or 3 days and the culture media were collected.

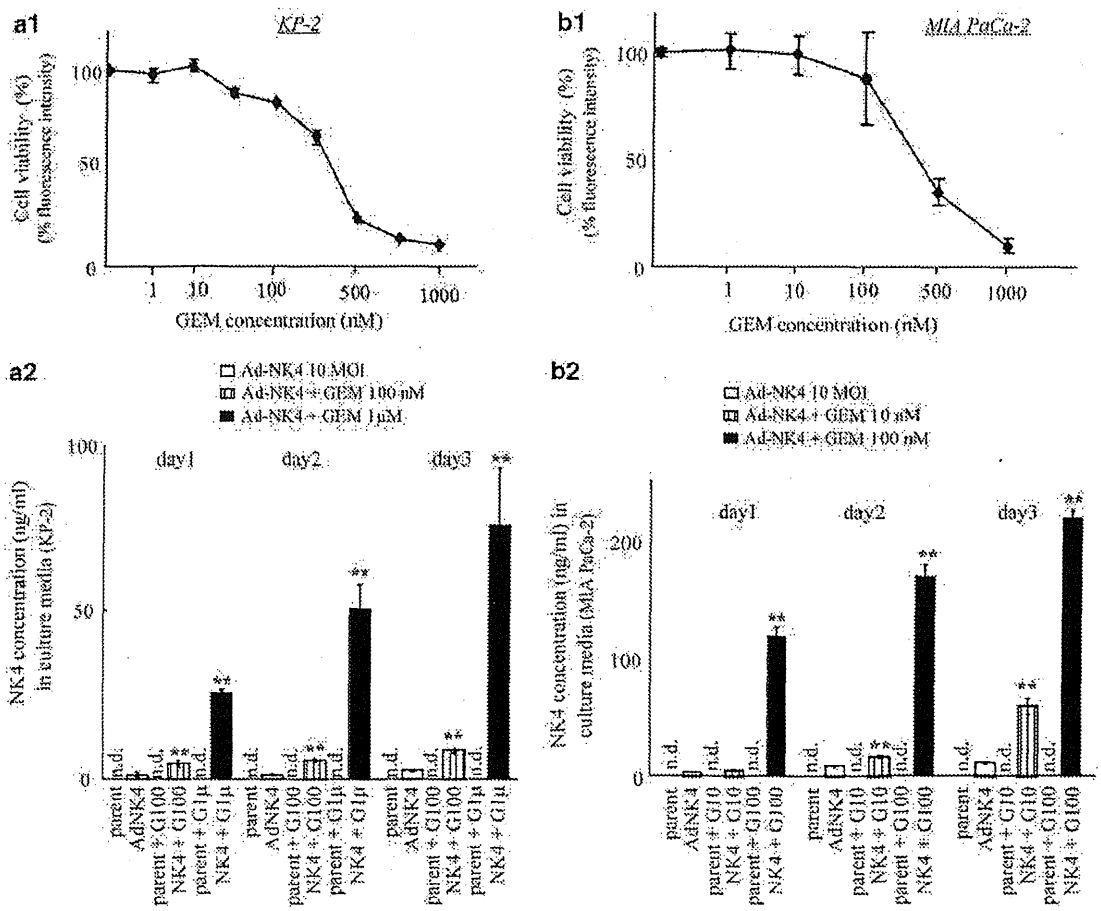

Figure 3 GEM enhances the transgene expression of NK4 delivered by Ad-NK4 in culture media of pancreatic cancer cell lines *in vitro*. (**a1**, **a2**, **b1**, **b2**) The pancreatic cancer cell lines KP-2 (**a1**, **a2**) and MIA PaCa-2 (**b1**, **b2**) were infected with Ad-NK4 (MOI of 10) and cultured with or without GEM (100 nM and 1 μM for KP-2; 10 and 100 nM for MIA PaCa-2). The NK4 expression levels in the culture media were measured. For viability assays, the cells were treated with GEM for 24 h, followed by replacement of the culture media with fresh media without GEM. After 72 h, the cell viabilities were determined by the PI assays as the ratio of the fluorescence intensity. 'n.d.' in the graphs means 'not detectable'. Bars represent relative cell viabilities as the fold changes in comparison with control cells. Each value represents the mean ± s.d. of three independent samples. $^{**}P<0.01$.

The culture media were used for invasion assays. The GEM-free culture media derived from the Ad-NK4-infected cells treated with GEM, even if they were collected at the early phase of these treatment, significantly inhibited the number of invading cancer cells in a dose-dependent manner (Figure 5a and b, $P=0.017$ for 100 nM GEM on day 1, $P=0.017$ and 0.0002 for 10 and 100 nM GEM, respectively, on day 3). These findings suggest that the enhanced levels of NK4 expression by Ad-NK4 mediated by low doses of GEM have biological effects in a dose-dependent manner.

GEM increases CMV promoter activity and leads to increased NK4 expression by Ad-NK4

As shown in Figure 2, we found that the NK4 expression levels within Ad-NK4-infected cells were increased in the early phase after GEM treatment. Ad-NK4 contains the CMV promoter, a strong viral promoter, to drive expression of its target gene, NK4. Several studies have shown that genotoxic stresses, such as those induced by irradiation and chemotherapy, enhance transgene expression under the control of the CMV promoter in cancer cells.[12–15] Therefore, we hypothesized that GEM may also enhance CMV promoter activity. To investigate this hypothesis, we transfected SUIT-2 cells, which did not express NK4 (Figure 2), with a plasmid-expressing

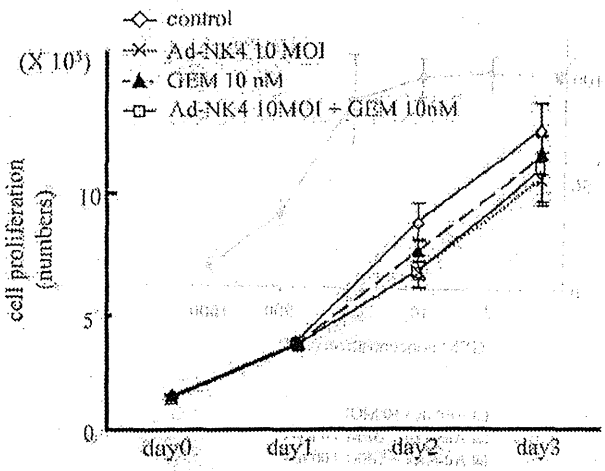

Figure 4 GEM does not affect the inhibitory effect of Ad-NK4 on cell proliferation. SUIT-2 cells were infected with Ad-NK4 (MOI of 10) and/or treated with GEM (10 nM). Cell viability was measured by cell numbers on the indicated days. All experiments were performed in triplicate wells and repeated at least three times.

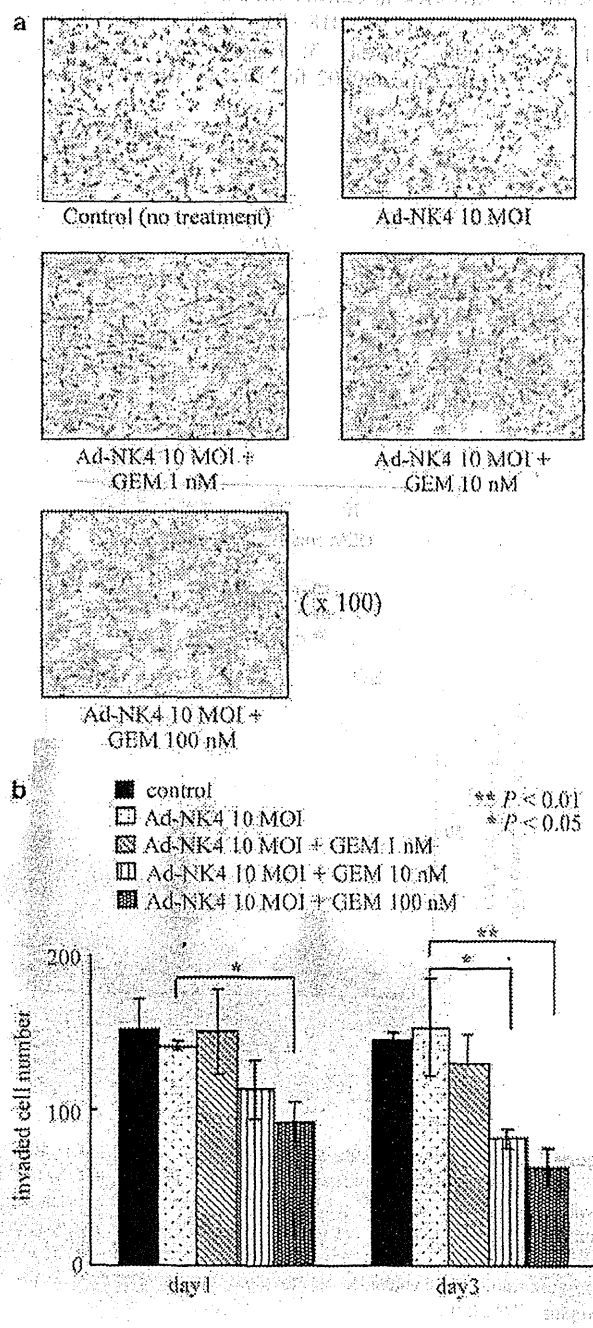

Figure 5 GEM synergistically enhances the inhibitory effect of Ad-NK4 on cancer cell invasion in a dose-dependent manner. SUIT-2 cells were infected with Ad-NK4 (MOI of 10) and treated with GEM (0, 1, 10, and 100 nM) for 24 h. The culture media were then replaced with fresh media without GEM. The culture media were collected on days 1 and 3. Fresh untreated SUIT-2 cells were seeded in 24-well plates at a density of 5×10^4 cells per cm^2 in DMEM mixed with conditioned culture media in the inner chamber and cultured with conditioned culture media in the outer chamber. After 48 h of culture in the presence of 3 ng ml^{-1} HGF, cells that had invaded to the lower surface of the Matrigel-coated membrane were fixed with 70% ethanol, stained with hematoxylin and eosin, and counted in five randomly selected fields under a light microscope. (a) Photomicrographs of SUIT-2 cells that have invaded to the lower surface of the Matrigel-coated membrane. (b) Numbers of SUIT-2 cells that have invaded to the lower surface of the Matrigel-coated membrane. Each value represents the mean ± s.d. of five randomly selected fields. $^*P<0.05$, $^{**}P<0.01$.

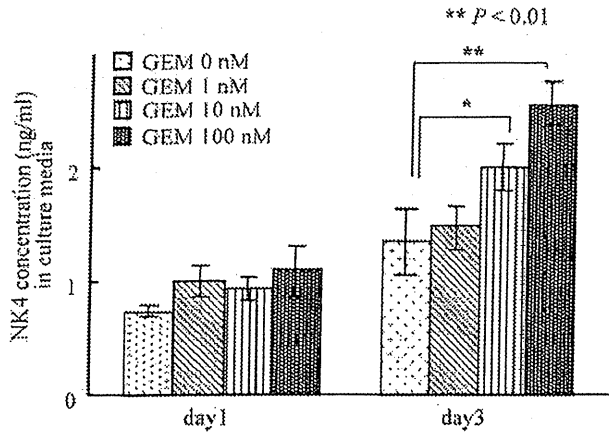

Figure 6 GEM increases NK4 expression by an NK4-expressing plasmid through enhancement of CMV promoter activity. SUIT-2 cells were transfected with an NK4-expressing plasmid and treated with GEM (0, 1, 10, and 100 nM) for 24 h. The culture media were then replaced with fresh media without GEM. The NK4 expression levels in the culture media were measured on the indicated days. Each value represents the mean ± s.d. of three independent samples. $^{*}P<0.05$, $^{**}P<0.01$.

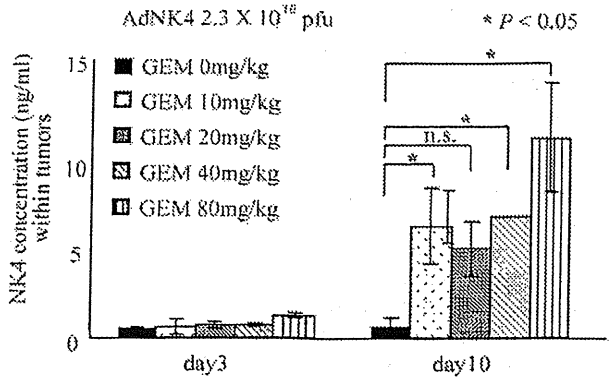

Figure 7 GEM enhances the transgene expression levels of NK4 delivered by Ad-NK4 within tumors *in vivo*. Six-week-old female nude mice were subcutaneously injected with SUIT-2 cells. After 7 days (day 0), the mice were administered 2×10^9 pfu of Ad-NK4 peritumorally, with intraperitoneal administration of GEM (0, 10, 20, 40, and 80 mg kg^{-1}). At 3 days after the administration, three mice in each group were killed and their tumors were excised. On day 7, the other three mice in each group were administered the same treatment and were killed on day 10, and their tumors were also excised for protein extraction. NK4 was measured by ELISA. Each value represents the mean ± s.d. of three independent samples. $^{*}P<0.05$.

NK4 under the control of the CMV promoter, cultured the cells with or without GEM for 24 h, and measured the NK4 expression levels in culture media after 1 and 3 days. As shown in Figure 6, GEM-treated cells showed significantly higher levels of NK4 expression than untreated cells on day 3 ($P=0.04$ and 0.007 for 10 and 100 nM GEM, respectively). These data suggest that GEM enhances CMV promoter activity, thereby leading to increased expression of the NK4 transgene under the control of the CMV promoter in these pancreatic cancer cells, similar to findings for other chemotherapeutic agents in diverse cell types.

Earlier studies have reported that several chemotherapeutic agents and irradiation enhance NF-κB activity and the MAPK pathway,[23,24] and that the CMV promoter contains binding sites for NF-κB in its enhancer region.[25–27] Therefore, we investigated the effects of GEM on NF-κB activity by measuring the NF-κB protein levels in the nuclei of GEM-treated cells. GEM-treated cells expressed significantly higher levels of NF-κB in their nuclei than untreated cells ($P=0.03$), but the difference was small (data not shown). These data suggest that GEM enhances CMV promoter activity partially through activation of some transcriptional factors, including NF-κB.

GEM enhances the expression levels of NK4 delivered by Ad-NK4 in tumors in vivo

We have shown that GEM enhances transgene expression of NK4 delivered by Ad-NK4, thereby leading to inhibitory effects of Ad-NK4 on cancer cell invasion *in vitro*. We earlier showed that peritumoral injection of Ad-NK4 combined with GEM suppressed the growth of pancreatic cancer cells implanted orthotopically into nude mice,[11] but did not examine the effects of GEM on the expression levels of NK4 *in vivo*. Therefore, we investigated whether the enhanced effects of the combination therapy were partly due to increased levels of NK4 expression within the tumors. We administered Ad-NK4 peritumorally, with or without GEM intraperitoneally at the same time on days 0 and 7. The subcutaneously implanted tumors were excised and measured for their NK4 expression levels on days 3 and 10. As shown in Figure 7, the levels of NK4 expression in the tumors did not show significant differences among the mice on day 3, whereas the levels of NK4 expression in GEM-treated mice were significantly increased in comparison with untreated control mice on day 10 ($P<0.03$, 0.06, 0.01, and 0.02 for 10, 20, 40, and 80 mg kg^{-1} GEM, respectively). These results strongly suggest that GEM enhances the effects of Ad-NK4-mediated gene therapy by increasing the expression of its NK4 transgene.

Discussion

In this study, we have shown that GEM enhanced adenovirus-delivered transgene expression of β-gal within infected cells and NK4 in culture media as well as within infected cells *in vitro*. In addition, GEM increased adenovirus-mediated NK4 expression within subcutaneously implanted tumors in nude mice *in vivo*. Furthermore, these increases in NK4 enhanced the inhibitory effects of Ad-NK4 on cancer cell invasion *in vitro*. These data indicate that GEM enhances the transgene

expression of target genes delivered by adenoviral vectors as well as the biological effects of the transgenes.

Ad-NK4-infected cells produce NK4 in their cytoplasm and then secrete it. When high doses of GEM kill Ad-NK4-infected cells, the NK4 proteins within these cells may be released after cell lysis, leading to increased levels of NK4 in the culture media. Interestingly, however, we found that even low doses of GEM without cytotoxic effects enhanced adenovirus-mediated NK4 expression in both culture media and within infected cells at the early phase after the GEM treatment. These findings suggest that there are some mechanisms by which GEM enhances adenovirus-mediated transgene expression. Earlier studies have reported that several chemotherapeutic agents and irradiation can enhance the expression of transgenes under the control of the CMV promoter by increasing CMV promoter activity.[13,14] In this study, we also found that GEM increased CMV promoter activity, leading to increased levels of adenovirus-mediated transgene expression. Nevertheless, despite the fact that the GEM-enhanced transgene expression was increased by factors of tens to hundreds, the CMV promoter activity was only increased by 1.2–2.0-fold by GEM compared with controls, suggesting that other mechanisms may exist. Further studies are, therefore, required to clarify the more detailed mechanisms of the GEM-induced enhancement of adenovirus-mediated gene transfer.

Adenovirus-mediated gene therapy has some problems in clinical settings.[28–30] The main problems are considered to be poor induction of target genes in clinical settings and poor penetration within tumors compared with those expected based on laboratory experiments.[31] In this study, we found that GEM enhanced the expression levels of transgenes within tumors *in vivo* as well as *in vitro*. We earlier reported that Ad-NK4 combined with GEM suppressed the growth and metastasis of human pancreatic cancer cells implanted orthotopically into nude mice.[11] In the study, we did not examine the detailed mechanism of the enhanced suppression of tumor growth and metastasis, because we considered that the suppression effect of the combination therapy was simply induced by the combined effects of GEM-mediated growth inhibition and NK4-mediated invasion inhibition. However, our earlier data also revealed remarkable inhibitory effects on the invasive potential of cells, such as tumor invasive growth and metastasis. Therefore, in this study, we focused on the effects of GEM on adenovirus-mediated expression of NK4, which inhibits cancer cell invasion.

The present data suggest that the remarkable inhibitory effects of the combination of GEM and Ad-NK4 may be partially due to GEM-enhanced expression of the NK4 transgene delivered by Ad-NK4. In addition, we found that adenovirus-mediated gene therapy might have other two advantages in clinical use when we use with GEM. One advantage is due to the cell killing effect of GEM. We found that GEM-treated mice did not show any significant increases in intratumoral NK4 expression at 3 days after the first administration of GEM and Ad-NK4, but showed significant increases in its expression compared with untreated mice after the second administration. These findings suggest that Ad-NK4 functions more effectively after treatment with GEM than during simultaneous treatment with GEM. Nagano *et al.*[31] reported that cancer cell death enhances the penetration and efficacy of oncolytic adenoviruses in tumors.[32] Therefore, pretreatment with GEM may be advantageous for improving the effects of adenoviral vectors *in vivo*. The other advantage is that we can reduce the doses of adenovirus administered when we use with GEM. Some studies have reported that the reduction of the amount of adenovirus decreased the risks of adenovirus-related side effects such as hepatotoxicity.[33] In this study, we found that even low doses of GEM enhanced adenovirus-mediated NK4 expression. Therefore, when we use adenovirus with low doses of GEM, we can decrease the side effects of both treatments while maintaining the antitumor effect of adenovirus gene therapy, especially for the patients suffering from fatal diseases such as advanced pancreatic cancer.

In conclusion, the present data suggest that GEM enhances the effects of adenovirus-mediated gene therapy partially through enhancement of CMV promoter activity. Therefore, adenovirus-mediated gene therapy with GEM may be a promising approach for advanced pancreatic cancer, and this combination therapy may be a tolerable and suitable treatment for the patients of advanced pancreatic cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Cyst Size Indicates Malignant Transformation in Branch Duct Intraductal Papillary Mucinous Neoplasm of the Pancreas Without Mural Nodules

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Objectives: In branch duct intraductal papillary mucinous neoplasm (IPMN) of the pancreas, the importance of the cyst size to predict malignancy is still controversial. Our aim was to elucidate the malignant potential of branch duct IPMN without mural nodules (flat branch duct IPMN).

Methods: Seventy-three patients with flat branch duct IPMNs were studied in our institution.

Results: There were 6 malignant IPMNs in this series, all of which were 30 mm or more in size, whereas there was no malignancy in IPMNs of less than 30 mm. Statistically significant predictors of malignancy were atypical cytological condition and main pancreatic duct (MPD) diameter of 5 mm or more. The cyst size of 30 mm or more tended to be associated with malignancy. The frequency of malignancy in flat branch duct IPMNs with the size of 30 mm or more and MPD diameter of less than 5 mm was 3.6%, whereas there were 5 malignant cases (26.3%) in flat branch duct IPMNs with the size of 30 mm or more and MPD diameter of 5 mm or more.

Conclusions: We conclude that the size criteria (≥30 mm) to predict malignancy proposed in the international consensus guidelines is appropriate and resection or meticulous follow-up using cytological examination and MPD dilatation is needed in patients with flat branch duct IPMNs.

Key Words: branch duct IPMN, mural nodule, cyst size, malignant potential

(*Pancreas* 2010;39: 232–236)

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is one of the rare pancreatic diseases. Since the World Health Organization (WHO) defined intraductal papillary mucinous tumor[1] in 1996 and renamed it as IPMN in 2000,[2] there has been increased detection of IPMN with increased awareness and use of imaging technology. The international consensus guidelines published by the International Association of Pancreatology in 2006 recommended that all main duct IPMNs should be resected.[3] In branch duct IPMNs, surgical resection is indicated in patients with symptoms attributable to the IPMN, mural nodules, cyst size of 30 mm or more, and dilatation of the main pancreatic duct (MPD). The guidelines stated that more pathological data of branch duct IPMNs of 30 mm or more in size without MPD dilatation or mural nodules were needed to determine if all branch duct IPMNs of 30 mm or more should be resected immediately. There have been few previous studies reported in this regard. In this study, therefore, we retrospectively analyzed the pathological findings of branch duct IPMNs without mural nodules diagnosed and resected in a single center.

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MATERIALS AND METHODS

Study Population

From January 1987 to May 2008, 238 patients had a diagnosis of IPMN at the Kyushu University Hospital. Based on imaging findings, the IPMNs were classified into 2 types, that is, main and branch duct IPMNs. The main duct type was defined as an IPMN predominantly dilating the MPD with or without branch duct dilatation, and the branch duct type was defined as an IPMN exclusively involving branch ducts and consisting of a grapelike collection of small cysts. Basically, computed tomography (CT) and magnetic resonance cholangiopancreatography were performed in all patients to classify the subtype of IPMN and precisely determine the cyst and MPD diameters and existence of mural nodules. In addition, endoscopic ultrasonography, endoscopic retrograde pancreatography, and intraductal ultrasonography were performed as appropriate. Of them, 203 IPMNs were diagnosed as branch duct type, whereas 35 were classified as main duct type. Of these 203 patients with branch duct IPMN, 170 (83.3%) had no mural nodules. Seventy-seven of the 170 patients with flat branch duct IPMN underwent surgical treatments. Four of them were excluded from the analysis (1 exploratory laparotomy and 3 pancreatectomies only for a coexistent pancreatic cancer). Ninety-three of the 170 patients were followed up without operation. Flat branch duct IPMNs of less than 30 mm in size have been reported to carry a low risk of cancer.[3,4] Therefore, cutoff value of the cyst diameter was decided as 30 mm. Pancreatic juice was obtained for cytological analysis during endoscopic retrograde pancreatography and classified as benign or atypical. Clinical characteristics including sex, age, symptom (present or absent), presence or absence of coexistent pancreatic cancer or malignant neoplasms of the other organs, location of the IPMN (pancreatic head, body-tail, or diffuse), serum concentrations of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA)-19-9, and maximal cyst and MPD diameters measured on CT scans were recorded. Patients' data were collected retrospectively, including their present and past histories, physical examination findings, and radiologic parameters (Table 1).

Pathological Parameters

All specimens were analyzed by 2 pathologists experienced in histopathological classification of IPMN and characterized according to the WHO histological classification of IPMNs (IPM adenoma [IPMA], borderline IPMN [IPMB], IPM carcinoma in situ [noninvasive IPMC], or invasive IPMC).[2] Patients

TABLE 1. Patients' Data Divided by Cyst Diameters of Less Than 30 mm and 30 mm or More

Factor	Population (N = 73 Patients)	D < 30 mm (N = 26)	D ≥ 30 mm (N = 47)
Age, median ± SD (range), yr	66.0 ± 8.0 (46–82)	63.2 ± 9.0 (46–79)	67.5 ± 6.9 (51–82)
Sex			
Male	48	19	29
Female	25	7	18
Symptomatic, n (%)	26 (35.6)	10 (38.5)	16 (34.0)
Abdominal or back pain	20	8	12
Abdominal fullness	4	1	3
General fatigue	2	1	1
Abnormal glucose tolerance	5	2	3
Tumor location, n (%)			
Head	38 (52.0)	13 (50.0)	25 (53.2)
Body-tail	27 (37.0)	12 (46.2)	15 (31.9)
Diffuse	8 (11.0)	1 (3.8)	7 (14.9)
Cyst diameter, mean ± SD, mm	32.9 ± 12.7 (10–70)	20.2 ± 4.5 (10–27)	39.9 ± 10.0 (30–70)
MPD diameter, mean ± SD, mm	4.8 ± 7.6 (2–15)	4.6 ± 2.2 (2–10)	4.9 ± 2.8 (2–15)
Comorbidity, n (%)			
Pancreas cancer	11 (15.1)	5 (19.2)	6 (12.8)
Others	20 (27.4)	5 (19.2)	15 (31.9)
Operation, n (%)			
PpPD or PD	29 (39.7)	8 (30.8)	21 (44.7)
DP or DP + SP	19 (26.0)	9 (34.6)	10 (21.3)
DPRHP	5 (6.9)	4 (15.4)	1 (2.1)
PHRSD	9 (12.3)	2 (7.7)	7 (14.9)
TP	1 (1.4)	0 (0.0)	1 (2.1)
Seg	7 (9.6)	3 (11.5)	4 (8.5)
Cystic resection	3 (4.1)	0 (0.0)	3 (6.4)

D indicates cyst diameter; PpPD, pylorus preserving pancreatoduodenectomy; PD, pancreatoduodenectomy; DP, distal pancreatectomy; SP, splenectomy; DPRHP, duodenum-preserving resection of the head of the pancreas; PHRSD, pancreatic head resection with segmental duodenectomy; TP, total pancreatectomy; Seg, segmentectomy of the pancreas.

were categorized as benign (IPMA and IPMB) or malignant IPMN (noninvasive and invasive IPMCs) on the basis of the pathological diagnosis.

Statistical Analysis

Statistical significance was analyzed by the Student *t* and Fisher exact probability tests using JMP 7.0.1 (SAS Institute, Incorporation, Cary, NC). Differences were considered significant when $P < 0.05$.

RESULTS

Clinical Characteristics of Flat Branch Duct IPMN Resected

Clinical and radiological characteristics of the 73 patients with flat branch duct IPMN are demonstrated in Table 1. There were 48 men and 25 women with the mean age of 66.0 years (range, 46–82 years). Twenty-six patients (35.6%) were symptomatic (abdominal and back pains, abdominal fullness, and general fatigue). Overt diabetes or impaired glucose tolerance was present in 5 patients (6.8%). The IPMN was located in the head of the pancreas in 38 patients (52.0%) or body and tail in 27 (37.0%). In the remaining 8 patients (11.0%), multiple IPMNs were diffusely present in the whole pancreas. Twenty (27.4%) of the 73 patients had synchronous (11 patients) or metachronous (9 patients) malignant neoplasms such as colon cancer (8 patients), gastric cancer (6 patients), breast cancer (2 patients), and miscellaneous (n = 4). Eleven (15.1%) of the 73 patients had concurrent pancreatic ductal adenocarcinoma. According to the cyst size, the 73 patients were divided into 2 groups: 26 patients with IPMNs of less than 30 mm and 47 with IPMNs of 30 mm or more. There were no significant differences in each parameter except for the age and cyst diameter between the 2 groups (*P* values are not presented in Table 1).

Clinical Outcomes of Flat Branch Duct IPMN Left Unresected

The 93 patients with flat branch duct IPMN were followed up without operation for 31.6 ± 30.1 months. The mean ± SD values of the MPD diameter and cyst size were 3.7 ± 1.9 and 21.5 ± 10.9 mm, respectively. Of them, 24 IPMNs were 30 mm or more in size and 69 IPMNs were less than 30 mm. During

TABLE 2. Pathological Details Divided by the Cyst Diameters of Less Than 30 mm and 30 mm or More

Pathological Details	Population (N = 73 Patients)	D < 30 mm (N = 26)	D ≥ 30 mm (N = 47)
IPMA	48	19	29
IPMB	19	7	12
IPMC	6	0	6
Noninvasive	5	0	5
Invasive	1	0	1

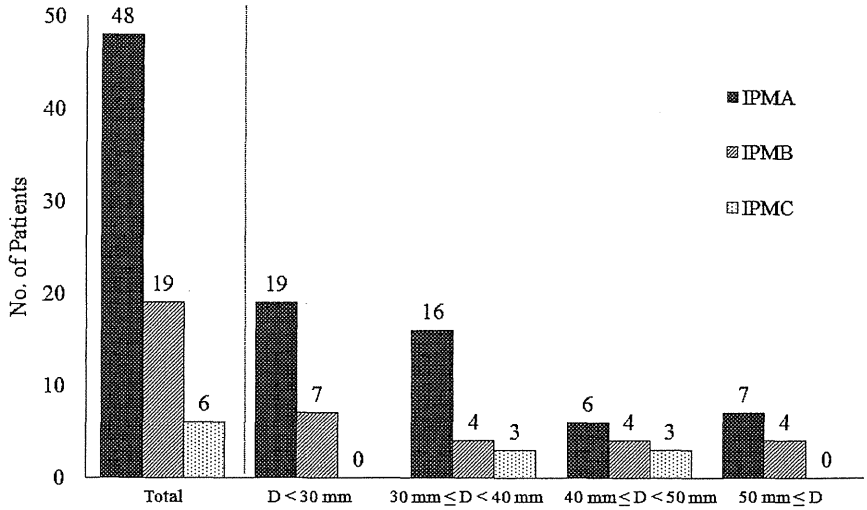

FIGURE 1. Relationship between size cutoffs of the cyst diameter (D) of less than 30 mm, diameter of 30 mm or more and less than 40 mm, the cyst diameter of 40 mm or more and less than 50 mm, and the cyst diameter of 50 mm or more and the final pathological diagnosis of IPMNs. There was no significant difference in the distribution of the diagnosis between the size categories. However, all malignant cases were more than 30 mm in size.

the follow-up, the cyst size increased from 35.0 ± 7.7 to 36.3 ± 7.9 mm in the former subgroup and from 15.9 ± 6.6 to 16.4 ± 6.0 mm in the latter subgroup ($P = 0.91$). Eight of the 24 IPMNs of 30 mm or more in size were associated with the MPD diameter of 5 mm or more at the beginning of the follow-up. However, these patients did not undergo operation owing to old age, renal failure, and patients' reluctance. Three patients died of concurrent pancreatic ductal adenocarcinoma at 3, 6, and 7 months after the diagnosis of IPMN.

Histological Characteristics of Flat Branch Duct IPMN Resected

Table 2 shows the histological findings of flat branch duct IPMN in this series. Histological classification identified 48 IPMAs (65.8%), 19 IPMBs (26.0%), and 6 IPMCs (8.2%) including 5 noninvasive (6.8%) and 1 invasive (1.4%) IPMCs. All 26 branch duct IPMNs of less than 30 mm in size were benign IPMN (IPMA/IPMB), whereas 47 IPMNs of 30 mm or more in size included all 6 malignant cases, one of which were invasive IPMC. However, the diagnosis of the invasive case was made in 1989 only using CT, and no magnetic resonance cholangiopancreatographical and endoscopic ultrasonographical findings were available.

In addition to the continuous size variables, we also examined the association of histological diagnoses and the cyst diameter by 10-mm increments (Fig. 1). In patients with the cyst diameter of 30 mm or more, the increasing diameter was not correlated to the tendency of malignancy.

Predictors of Malignant Disease in Patients With Flat Branch Duct IPMN Resected

The clinical characteristics of benign and malignant groups are compared in Table 3. The cyst diameter of the lesions (<30 and ≥30 mm) tended to be correlated with malignant IPMN pathological condition, although the difference did not reach statistical significance ($P = 0.063$). The MPD diameter (<5 and ≥ 5 mm) showed significant correlation with benign or malignant IPMN ($P = 0.034$). Cytological analysis was performed in 56 patients. The other 17 patients were excluded because of coexistent pancreatic cancer or inappropriate volume of the pancreatic juice obtained. Pancreatic juice cytological examination result was classified as benign in 51 patients and malignant in the remaining 5. Atypical cytological diagnoses were obtained in 10 of the 51 patients with benign IPMNs and 4 of the 5 patients

TABLE 3. Predictors of Malignant IPMN

Parameter	Benign IPMN (N = 67 Patients)	Malignant IPMN (N = 6)	*P*
Age, yr			
≥ 65	39	4	
< 65	28	2	0.52
Sex			
Male	44	4	
Female	23	2	0.67
Symptomatic			
Absent	42	5	
Present	25	1	0.97
Tumor location			
Head	35	3	
Body-tail	25	2	
Diffuse	7	1	0.89
Cyst diameter, mm			
<30	26	0	
≥30	41	6	0.063
MPD diameter, mm			
<5	43	1	
≥5	24	5	0.034*
Serum CEA, ng/mL			
<4.0	44/46	5/6	
≥4.0	2/46	1/6	0.31
Serum CA-19-9, ng/mL			
<37	45/47	5/6	
≥37	2/47	1/6	0.31
Cytological condition			
Benign	41/51	1/5	
Atypical	10/51	4/5	0.012*

The *P* values with the asterisk (*) indicate statistical significance.

TABLE 4. Multivariate Analysis in Benign and Malignant IPMNs

Parameter	Odds Ratio (95% CI)	*P*
MPD diameter ≥ 5 mm, mm	3.66 (0.41–76.7)	0.29
Atypical cytological condition	13.0 (1.61–275.5)	0.031*

The *P* value with the asterisk (*) indicates statistical significance.
CI indicates confidence interval.

with malignant IPMNs ($P = 0.012$). Regarding tumor markers, coexistent pancreatic cancer and other synchronous cancer were eliminated to exclude the effect of the concurrent diseases. As a result, the role of tumor markers (serum CEA and CA-19-9) was investigated in 53 of the 73 patients. The tumor markers had no association with the diagnosis of malignant IPMNs.

In this study, atypical cytological condition and MPD diameter of 5 mm or more were significant indicators of malignant IPMN, whereas age, sex, presence of symptoms, and tumor markers were not diagnostic of malignant IPMN. Of these factors, cytological condition remained significant by the multivariate analysis (Table 4).

DISCUSSION

In this study, we report a large retrospective series of flat branch duct IPMN that were surgically resected. Although Tanno et al[5] reported a follow-up result of 83 patients with flat branch duct IPMNs, their series included only 3 surgical patients. Our study reviewed 73 surgical patients with flat branch duct IPMNs consisting of 67 benign and 6 malignant IPMNs. To the best of our knowledge, this is the largest series of flat branch duct IPMNs that were surgically resected at a single institution.

Since the first report by Ohashi et al[6] in 1982, IPMNs have been recognized with increasing frequency. There were many reports that attempted to identify prognostic factors that might influence the management of these patients.[7–13] There has been a general agreement that mural nodules and dilated MPD are suggestive of malignancy in IPMNs.

The size of the cyst of branch duct IPMN had also been analyzed for its malignant transformation. In the analysis of 82 patients with branch duct IPMN of less than 30 mm in size, Schmidt et al[14] reported 16 cases of malignancy. On the other hand, Sugiyama et al[15] reported that only 1 of 15 patients with branch duct IPMN of less than 30 mm was malignant. They did not mention whether this patient had a mural nodule or not. The guidelines suggested that asymptomatic IPMNs of less than 30 mm in size can be safely observed.[3] In this study, all 26 flat branch duct IPMNs of less than 30 mm in size were benign, although 10 of them were symptomatic. Our data confirmed the recommendation of the guidelines regardless of the existence of symptoms. All 6 malignant IPMNs were 30 mm or more, and 5 of them were asymptomatic in this study. We could not prove the high frequency of malignancy in symptomatic flat branch duct IPMNs.

The management of large flat branch duct IPMNs is still controversial. Recently, some articles have reported that the cyst size of branch duct IPMNs is not related to a malignant IPMN pathological diagnosis.[12,14,16] Meanwhile, some morphological studies have shown that a large cyst diameter is a malignant sign of branch duct IPMN; the threshold value being 20,[17] 30,[15,18] and 60 mm.[19] However, none of these articles provided separate comparison of the cyst diameter and the presence/absence of mural nodules in branch duct IPMN. The present study was designed to determine whether flat branch duct IPMNs of 30 mm or more in size should be resected immediately or not by analyzing the 73 patients with flat branch duct IPMN, including 47 patients with IPMN of 30 mm or more in size. In this study, we showed 12.8% (6/47) malignancy in flat branch duct IPMNs of 30 mm or more in size, whereas all branch duct IPMNs of less than 30 mm in size were benign. This finding proved the validity of the cutoff at 30 mm in size. Although we analyzed multiple cutoff sizes including 40 and 50 mm, neither of them was significantly associated with malignancy. It can be said that the cyst diameter of 30 mm or more is associated with a higher tendency toward malignancy.

Dilated MPD has been mentioned as one of the high-risk factors to predict malignant IPMN.[3,20,21] Our data confirmed the general understanding. However, 8 patients with flat branch duct IPMN of 30 mm or more in size and the MPD diameter of 5 mm or more are surviving without operation for 30.9 ± 28.4 months to date. These patients are at poor operative risk or reluctant to undergo operation but willing to be closely watched against the appearance of more obvious malignant findings. In addition, we examined the results of the cytological analysis in branch duct IPMN in 58 patients and identified cytological condition as a factor significantly associated with malignancy by univariate analysis. Other factors, such as the existence of symptoms, tumor location, and age were not significant predictors of malignancy. The sensitivity and specificity of the atypical cytological condition to diagnose malignant IPMN were 80.0% and 80.4%, respectively. However, we still need to improve the sensitivity and specificity of pancreatic juice cytological examination result, because cytological examination is one of the most reliable parameters to diagnose malignant IPMN.

In our series, the malignant rate of flat branch duct IPMNs with the diameter of the cyst of 30 mm or more and MPD diameter of less than 5 mm was only 3.6% (1/28). When all flat branch duct IPMNs with the diameter of the cyst of 30 mm or more and MPD diameter of 5 mm or more were resected, this subgroup included 7 IPMAs, 7 IPMBs, and 5 IPMCs, the malignant rate being 25.0%. Only 1 IPMC would have been missed and left unresected with these criteria. Actually, however, this patient underwent resection owing to repeated episodes of acute pancreatitis. Although this study has not shown the symptom as a predictor of malignant IPMN, further studies are needed because some previous articles have demonstrated that the symptom is one of the indicators of malignancy.[8,14,15] All flat branch duct IPMN of 30 mm or more in size should be resected if the additional criterion of MPD dilatation of 5 mm in diameter or more in diameter or atypical cytological condition is present.

CONCLUSIONS

About branch duct IPMNs without mural nodules, those of less than 30 mm in size can be observed safely, those of 30 mm or more in size with the MPD diameter of less than 5 mm need careful investigation, and those of 30 mm or more in size with the MPD diameter of 5 mm or more should have resection or strict surveillance for malignancy.

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Invasive carcinoma derived from the nonintestinal type intraductal papillary mucinous neoplasm of the pancreas has a poorer prognosis than that derived from the intestinal type

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Background. *Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is divided into 4 subtypes: an intestinal type, a gastric type, a pancreatobiliary type, and an oncocytic type. The purposes of this study were to clarify the outcomes and the characteristics of invasive carcinoma derived from IPMN (invasive IPMC) by focusing on these subtypes with a comparison to conventional invasive ductal carcinoma (IDC) of the pancreas.*

Methods. *A total of 30 patients with invasive IPMC were reviewed, and the tumors were divided into 2 pathologic subtypes, intestinal and nonintestinal type. The prognosis and characteristics of the 2 subtypes were evaluated. Furthermore, the prognosis of 119 patients with conventional IDC was compared with that of patients with invasive carcinoma derived from the intestinal or nonintestinal type IPMN.*

Results. *The 5-year survival rate of patients with the nonintestinal type (0.0%) was as poor as that of patients with conventional IDC (19.9%; P = .67). The patients with the intestinal type (66.7%) had a more favorable prognosis than patients with conventional IDC (P < .001). The nonintestinal type was characterized by positive lymphatic invasion and tubular invasive pattern.*

Conclusion. *Invasive carcinoma derived from the nonintestinal type IPMN characterized by lymphatic invasion and tubular invasive pattern is associated with a poor prognosis. (Surgery 2010;147:812-7.)*

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INTRADUCTAL PAPILLARY MUCINOUS NEOPLASM (IPMN) OF THE PANCREAS is characterized morphologically by an intraductal mucin-producing neoplasm with cystic dilatation of either the main pancreatic duct and/or the branch duct. Pathologically, papillary proliferations are formed by the mucin-producing columnar epithelium. Since the World Health Organization (WHO) reported the definition of intraductal papillary mucinous tumors in 1996,[1] heightened awareness of this entity has led to an increase in its detection.[2,3] The WHO also established 4 categories of IPMNs: IPMNs with slight or no dysplasia as adenoma (IPMA), IPMNs with moderate dysplasia as borderline neoplasm (IPMB), IPMNs with severe dysplastic epithelial change without invasion as carcinoma in situ (CIS), and invasive carcinoma derived from IPMN (invasive IPMC).[4] As to treatment of IPMNs, international consensus guidelines describing a therapeutic strategy were published by the International Association of Pancreatology in 2006.[5]

IPMN has been reported to be a multimodal entity.[6,7] Recently, Furukawa et al[8] reported a new subclassification of IPMNs based solely on the morphologic phenotypes versus the pathologic malignant grades. This subclassification categorizes IPMNs into 4 subtypes: an intestinal type, a gastric type, a pancreatobiliary type, and an oncocytic type. These categories are based on the morphologic characterization of the papillary formation

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and immunohistochemical variations, which are described below.

The intestinal type IPMN expresses 2 mucin genes, *MUC5AC* and *MUC2*, and is characterized by intestinal epithelium-like cells that form villous papillae with moderate to severe dysplasia in a manner similar to colonic adenoma.[9] The gastric type of IPMN consists of gastric foveolar epithelium-like cells that form thick finger-like papillae and expresses only 1 mucin gene (*MUC5AC*).[8,10,11] This subtype is often recognized in branch duct IPMNs[10,12]; the malignant grade is usually low, although severe cellular atypia is observed in some cases.[9,13] The pancreatobiliary type of IPMN is much rarer than the intestinal type and gastric type.[9] This subtype, like the intestinal type, also expresses 2 mucin genes, namely, *MUC5AC* and *MUC1* (rather than *MUC2*); it is characterized by the formation of thin, branching, complex papillae that are lined by cells containing enlarged hyperchromatic nuclei, and shows severe atypia corresponding to carcinoma. Finally, the oncocytic type of IPMN is also rare and characterized by the formation of thick, branching, complex papillae[8,9]; this subtype expresses *MUC5AC* and *MUC1*, and most cases are diagnosed as carcinoma.[14]

Thus, IPMN is a heterogeneous entity consisting of 4 subtypes, and immunohistochemical expression of *MUC2* is specific for the intestinal type IPMN only. Furthermore, some studies have described that caudal-related homeobox gene 2 (CDX2) and regenerating islet-derived family, member 4 (REG4) are specifically expressed in the intestinal type IPMN,[15,16] and this type frequently progresses to invasive IPMN with a colloid pattern.[10,17,18] According to these morphologic and immunohistochemical differentiations, Adsay et al[15] suggested that the progression sequence of the intestinal type IPMN to colloid carcinoma is a distinct pathway of carcinogenesis, namely, the "intestinal pathway."

The biologic malignancy of IPMN has been discussed in comparison with conventional invasive ductal carcinoma (IDC) of the pancreas.[2,19-21] Generally, patients with IPMA, IPMB, and CIS have a more favorable prognosis than patients with invasive IPMC.[2,22,23] The postoperative 5-year survival rate for invasive IPMC is reported to be from 31 to 58%,[2,19,23-25] which is better than that of conventional IDC.[2,19,23,26,27] Although IPMN is a heterogeneous entity, few previous studies have compared the outcomes of the individual subtypes of invasive IPMC with that of conventional IDC.

In the present study, considering the specific expression of *MUC2*, CDX2, and REG4 and the peculiar progression to colloid carcinoma of the intestinal type IPMN, we divided IPMNs into 2 main categories: intestinal type and nonintestinal type. Next, to investigate the biologic malignancies of invasive IPMCs derived from the intestinal type and nonintestinal type, we analyzed the prognosis and characteristics of patients with these 2 types of invasive IPMC and compared their prognosis with that of patients with conventional IDC.

PATIENTS AND METHODS

Between January 1987 and May 2008, a total of 117 patients underwent surgical treatment for IPMN at Kyushu University Hospital in Japan. All specimens were reviewed by 2 pathologists experienced in histopathologic classification of IPMN. Using the WHO histologic classification,[4] IPMNs were divided into IPMA, IPMB, CIS, and invasive IPMC. Of 117 patients, 30 with invasive IPMC were reviewed radiologically and pathologically. The demographic and clinical conditions including age, sex, presenting symptoms, history of diabetes mellitus (DM), and worsening glucose tolerance, as well as serum concentrations of carcinoembryonic antigen and carbohydrate antigen 19-9 were evaluated by reviewing the medical records. Worsening glucose tolerance was defined as worsening glycemic control in the course of DM or the first detection of DM.

Pancreatic juice was obtained for cytology during endoscopic retrograde pancreatography and classified as benign or atypical. IPMNs were morphologically classified as main duct IPMN or branch duct IPMN according to the position of the predominant ductal dilatation. As preoperative imaging examinations, computed tomography and magnetic resonance cholangiopancreatography were performed in all patients to classify the morphologic types of IPMN and determine the existence or absence of mural nodules.

According to the subclassification proposed by Furukawa et al,[8] specimens were categorized as intestinal type, gastric type, pancreatobiliary type, oncocytic type, or nonclassifiable. The types of subclassification were comprehensively examined by immunohistochemical staining and morphologic variations. When several subtypes existed in 1 case concurrently, the histologic subclassification was determined by the subtype represented by the area of the highest degree of atypia of the IPMNs.

In our study, the gastric type, pancreatobiliary type, oncocytic type, and nonclassifiable IPMNs were categorized as the nonintestinal type and compared with the intestinal type. As a result, there were 14 intestinal and 16 nonintestinal type

Table. Clinical and histologic characteristics of 30 patients with invasive carcinoma derived from IPMN (invasive IPMC)

	Invasive IPMC (n = *30*)	*Intestinal type* (n = *14*)	*Non-intestinal type* (n = *16*)	P *value*
Clinical parameters				
Age, y	67.1 ± 8.1	66.6 ± 8.1	67.6 ± 8.4	.63
Sex, male/female	19/11	11/3	8/8	.14
Presenting symptoms, *n* (%)	15/30 (50.0)	6 (42.9)	9 (56.3)	.72
DM, *n* (%)	6/30 (20.0)	3 (21.4)	3 (18.8)	.60
Worsening glucose tolerance, *n* (%)	7/30 (23.3)	4 (28.6)	3 (18.8)	.41
Type of IPMN (main duct/branch duct)	12/18	6/8	6/10	.53
CEA ≥4 ng/ml	2/26	0/12	2/14	.48
CA19-9 ≥37 ng/ml	11/27	5/13	6/14	.56
Existence of mural nodules, *n* (%)	21/30 (72.4)	12 (85.7)	10 (62.5)	.13
Atypical cytology, *n* (%)	19/26 (73.1)	8/13 (61.5)	11/13 (84.6)	.38
Histologic parameters, *n* (%)				
Lymph node metastasis	12/30	4 (28.6)	8 (50.0)	.21
Peripancreatic invasion	7/30	2 (14.3)	5 (31.3)	.26
Venous invasion	5/30	2 (14.3)	3 (18.8)	.56
Lymphatic invasion	9/30	1 (7.1)	8 (50.0)	.0013*
Invasive pattern (colloid/tubular)	7/23	7/7	0/16	.0017*

*Difference between the 2 subtypes is statistically significant.

IPMN, Intraductal papillary mucinous neoplasm *DM,* diabetes mellitus; *CEA,* carcinoembryonic antigen; *CA19-9,* carbohydrate antigen 19-9.

IPMNs (6 gastric type, 8 pancreatobiliary type, 1 oncocytic type, and 1 nonclassifiable). The invasive patterns were pathologically classified into 2 types: tubular and colloid patterns. Presence or absence of lymph node metastasis, peripancreatic invasion, venous invasion, and lymphatic invasion were also examined. The prognosis and characteristics were compared between intestinal and nonintestinal groups.

Survival analysis. Individual disease-specific survival (DSS) information was obtained from the medical records and a telephone survey. The end date of the surveillance was set as August 2008. One patient with invasive IPMC was lost to follow-up. Survival analyses were carried out between the patients with invasive IPMC and those with conventional IDC who were matched for their International Union Against Cancer (UICC) stage (I or II) and age. The series of conventional IDC included 12 patients with stage I carcinoma and 107 with stage II carcinoma who underwent pancreatectomy at our institution in the same period with IPMN.

Statistical analysis. Statistical analysis was performed with JMP statistical software (version 7.0.1; SAS Institute, Cary, NC). The Fisher exact probability test and the Student *t* test were used to evaluate differences in factors between noninvasive and invasive IPMCs. Survival analysis was performed using the Kaplan-Meier method[28] with log-rank univariate comparisons. Differences were considered significant when the *P* value was less than .05.

RESULTS

Clinical characteristics. The demographic and clinical characteristics of 30 invasive IPMCs are shown in the Table. There were 19 males and 11 females with the mean age of 67.1 ± 8.1 years. Of the 30 patients, 6 (20%) had DM; worsening glucose tolerance was present in 7 (23.3%). The 30 surgical procedures comprised 20 (66.7%) pylorus-preserving or standard pancreatoduodenectomies, 8 (26.7%) distal pancreatectomies, 1 (3.3%) segmentectomy of the pancreas, and 1 (3.3%) total pancreatectomy. There was no difference regarding clinical and morphologic characteristics between the intestinal and nonintestinal groups (Table).

Survival analysis. During the mean follow-up period of 40.8 ± 58.2 months (range, 1.4–255.4), only 1 patient had metachronous IPMNs despite negative surgical margins. A total of 14 patients died of IPMN, and 1 patient died of peri-operative pulmonary edema. As a result, the patients with invasive IPMC had 1-, 3- and 5-year DSS rates of 88.3%, 48.6%, and 32.4%, respectively. The 5-year DSS rate of patients with conventional IDC was 19.9%, which was significantly less than that of patients with invasive IPMC ($P = .027$; Fig 1).

When the tumors were divided into 2 subgroups, we found that the intestinal group had a

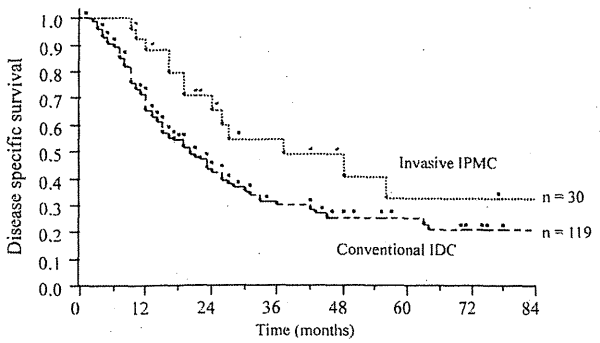

Fig 1. Kaplan-Meier actuarial survival curves comparing patients with invasive intraductal papillary mucinous neoplasm (invasive IPMC) of the pancreas (n = 30) and conventional invasive ductal carcinoma (IDC) (n = 119). Patients with invasive IPMC have a 5-year disease-specific survival rate of 32.4%. The 5-year disease-specific survival rate of patients with conventional IDC is 19.9%, which is significantly lower than that of patients with invasive IPMC (P = .027).

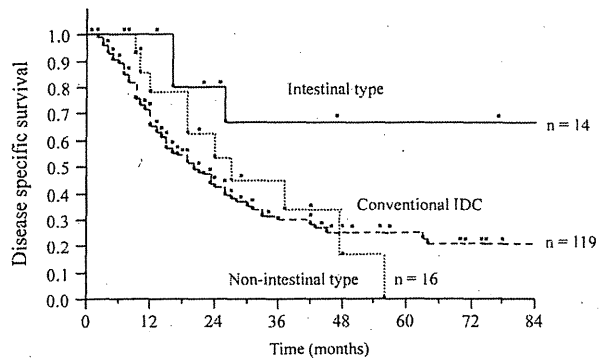

Fig 2. Kaplan-Meier actuarial survival curves comparing patients with invasive intraductal papillary mucinous neoplasm (invasive IPMC) derived from the intestinal type (n = 14), invasive IPMC derived from the nonintestinal type (n = 16), and conventional invasive ductal carcinoma (IDC) (n = 119). Patients in the nonintestinal group have a significantly more favorable prognosis compared with patients in the intestinal group (P = .029). The 5-year disease-specific survival rate of patients with conventional IDC is 19.9%, which is significantly lower than that of patients in the intestinal group (P < .001). Patients in the nonintestinal group, however, have a poor survival rate that is similar to that of patients with conventional IDC (P = .67).

favorable prognosis compared with the nonintestinal group. The 5-year DSS rates of patients of the intestinal group and nonintestinal group were 66.7% and 0.0%, respectively (P = .029; Fig 2). Furthermore, the patients in the intestinal group had a favorable survival rate compared with those with conventional IDC (P < .001), whereas patients in the nonintestinal group exhibited a poor survival rate that was similar to that of patients with conventional IDC (P = .67).

Pathologic features. To analyze the characteristics of the intestinal and nonintestinal groups, the pathologic parameters including lymph node metastasis, invasion to peripancreatic tissues, lymphatic and venous invasion, and invasive pattern are shown in the Table. All cases of the nonintestinal group showed a tubular invasive pattern (P = .0017). Lymphatic invasion was more frequently recognized in the nonintestinal group (P = .0013).

DISCUSSION

The present study demonstrated that patients with invasive IPMC derived from the nonintestinal type exhibited poor survival, which was similar to those with conventional IDC. Furthermore, patients with invasive IPMC derived from the nonintestinal type were characterized by tubular invasive pattern and lymphatic invasion.

Recent studies have indicated that patients with IPMN often develop extrapancreatic malignant neoplasms and conventional IDC.[29,30] Due to these characteristics of IPMN, analysis of the DSS rate is more appropriate than analysis of the overall survival rate. In the present study, the 5-year DSS rates of the 87 patients with IPMA, IPMB, and CIS were 100% (data not shown), whereas the rate was 32% for patients with invasive IPMC. These results indicate that all the noninvasive IPMNs were completely cured by surgical resection. In contrast, we observed a poor prognosis of patients of invasive IPMC derived from the nonintestinal type; this prognosis was similar to that of patients with conventional IDC. Moreover, we found that the patients with invasive IPMC derived from the intestinal type had a more favorable prognosis than those with conventional IDC. These findings suggest that, even in invasive IPMC, subclassification of the noninvasive component could predict the prognosis of the patients.

Previous studies[2,24] have reported that lymph node metastasis, vascular invasion, positive surgical margin, and tubular invasive pattern are the predictive factors for poor prognosis of patients with the invasive IPMCs. Our study reveals that the nonintestinal group has poor outcomes possibly because of the frequent presence of lymphatic invasion and tubular invasive pattern, which is consistent with previous reports. We also showed that lymph node metastasis and peripancreatic invasion were often observed in the nonintestinal group, although there were no statistical differences, which possibly was due to the small number of the study

population. Furthermore, invasive depth of IPMN might affect the outcomes, although we did not examine such issues in this study due to the difficulties associated with this type of evaluation.

It is difficult to distinguish the 2 subtypes of IPMNs by the pre-operative clinical factors. Recent articles[31,32] demonstrated that it is possible to evaluate the intestinal type IPMN preoperatively by immunohistochemical analysis using samples obtained from pancreatic juice and endoscopic ultrasonography–guided fine-needle aspiration. Even if a tumor seems to be large, preoperative diagnosis of invasive IPMC derived from the intestinal type would be 1 piece of critical information needed to aggressively perform pancreatectomy. Furthermore, postoperative diagnosis of invasive IPMC derived from the intestinal type might help guide the selection of clinical management, which may be different from that used for conventional IDC, although further investigation is needed.

Adsay et al[15] and Nakata et al[16] reported that atypical cells were often observed in the intestinal type IPMNs. We also observed that 38 (90%) of 42 intestinal type IPMNs, from among 177 resected IPMNs, were IPMB/IPMC (data not shown). Our results, however, demonstrated that patients with invasive IPMC derived from the intestinal type IPMN had favorable outcomes. A possible reason for this discrepancy could be that the intestinal type IPMN grows very slowly in both the late carcinogenic stage and the invasive stage, whereas the nonintestinal type IPMN grows rapidly in such stages.

In conclusion, our study demonstrated that patients with invasive carcinoma derived from the intestinal type IPMN had a favorable prognosis, whereas invasive carcinoma derived from nonintestinal type IPMN was significantly correlated with a poor outcome. The prognosis of patients with invasive carcinoma derived from the nonintestinal type IPMN was equivalent to that of patients with conventional IDC.

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OPINION

Controversies in the management of pancreatic IPMN

Masao Tanaka

Abstract | Although considerable progress has been made in our understanding of intraductal papillary mucinous neoplasm (IPMN) of the pancreas, some issues still remain to be resolved. Uncertainty exists regarding the classification of IPMNs. The necessity of the mixed-type category of IPMN and whether such lesions should be defined radiographically or histologically needs to be determined. The preoperative distinction of branch duct IPMNs from nonmucinous cysts should be further investigated so that potentially malignant lesions can be identified and management strategies guided effectively. The role and safety of cystic fluid analysis remains to be clarified in this context. With regard to the diagnosis of malignancy in branch duct IPMNs, criteria for identifying malignancy need to be re-evaluated. The presence of mural nodules is a very reliable predictor; however, controversy exists over the value of size as a reliable indicator. Criteria with increased specificity are needed, perhaps including histological subtype of lesion, to reduce the false-positive rate of the present criteria. Finally, the best modality and interval for surveillance of branch duct IPMNs requires determination because of its significance in terms of malignant transformation, development of distinct ductal adenocarcinoma and disease recurrence after resection.

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Introduction

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a relatively new entity that is being diagnosed with increasing frequency. The first case in the world was reported in 1980 in a patient with marked dilatation of the main pancreatic duct, profuse production of mucin and a pancreatobiliary fistula.[1] Approximately a decade later, IPMN became established as a clinical entity distinct from mucinous cystic neoplasm (MCN).[2] Since the publication of international consensus guidelines for the management of IPMN and MCN after the Sendai meeting of the International Association of Pancreatology, IPMN of the pancreas has been drawing increasing attention.[3] Although the guidelines contributed to increased awareness and better understanding of IPMNs and improved management of patients with this entity, many issues still remain to be resolved (Box 1). This Perspectives aims to discuss several aspects of this fascinating disease that deserve further attention, including the classification of mixed-type IPMNs, differential diagnosis of these lesions from other cystic neoplasms, criteria for the resection of branch duct IPMNs and surveillance.

Competing interests
The author declares no competing interests.

Classification

At present, IPMNs are classified into three types: branch duct, main duct and mixed or combined.[4] This classification is based on the dominant location of the IPMN where the ductal dilatation occurs. The vast majority of patients have either branch duct or main duct IPMNs. However, a small proportion of patients have mixed-type IPMNs, in which the ductal dilatation involves one or a few branch ducts and a large part of the main duct. Classification of IPMNs is based either on radiographic morphology or histology with preference for either differing between institutions. As histological examination of branch duct IPMNs often reveals involvement of the main duct, the proportion of patients with mixed-type IPMNs becomes very high if the classification is only based on histological findings. Indeed, some pathologists may be reluctant to classify IPMNs as one type or another knowing that most IPMNs when evaluated histologically fall into the mixed-type category. However, the preoperative classification of IPMNs is important because it affects therapeutic decision-making. The classification of IPMNs and the value of the mixed-type category need to be determined more clearly.

Analysis of the histological and clinical characteristics of a large number of IPMNs (comprising 159 branch duct, 81 main duct and 149 mixed) showed that the frequency of invasive carcinoma in branch duct, main duct and mixed IPMNs was 11%, 48% and 42%, respectively.[5] Therefore, mixed-type IPMNs seem to have similar clinicopathological features to main duct IPMNs. To eliminate uncertainty in the classification of IPMNs, the issue of whether the mixed type should be defined radiographically or histologically needs to be resolved.

Distinction of branch duct IPMNs

Branch duct IPMNs should be distinguished from a variety of cystic neoplasms of the pancreas, including MCNs, macrocystic or oligocystic serous cystic neoplasms, epidermoid cysts, lymphoepithelial cysts, chronic or acute pseudocysts and cystic varieties of some other neoplasms. Careful history taking, laboratory tests and imaging findings are helpful when considering differential diagnosis. However, even on the basis of a thorough understanding of the characteristics of each entity,[3] IPMNs, MCNs and macrocystic or oligocystic serous cystic neoplasms are sometimes difficult to differentiate preoperatively.

Cystic fluid analysis

The ability to distinguish mucinous cysts with malignant potential, such as IPMNs and MCNs, from nonmucinous cysts is particularly important. In this regard, the role of analyzing cystic fluid obtained by endoscopic ultrasonography (EUS)-guided fine-needle aspiration has been enthusiastically investigated (Supplementary Table 1 online).

Several studies that have investigated the validity of biomarkers to distinguish lesions

Box 1 | Unresolved issues with IPMNs

The following issues have not yet been resolved in the management of IPMNs:

- Classification of mixed-type IPMNs
- Differential diagnosis of these lesions from other cystic neoplasms
- Criteria for the resection of branch duct IPMNs
- Extent of resection of branch duct IPMNs
- Treatment of multifocal IPMNs
- Lymph node involvement and dissection
- Surveillance
- Genetic and histopathological relationship of IPMNs to pancreatic ductal adenocarcinoma
- Clinical outcome

Abbreviation: IPMN, intraductal papillary mucinous neoplasm.

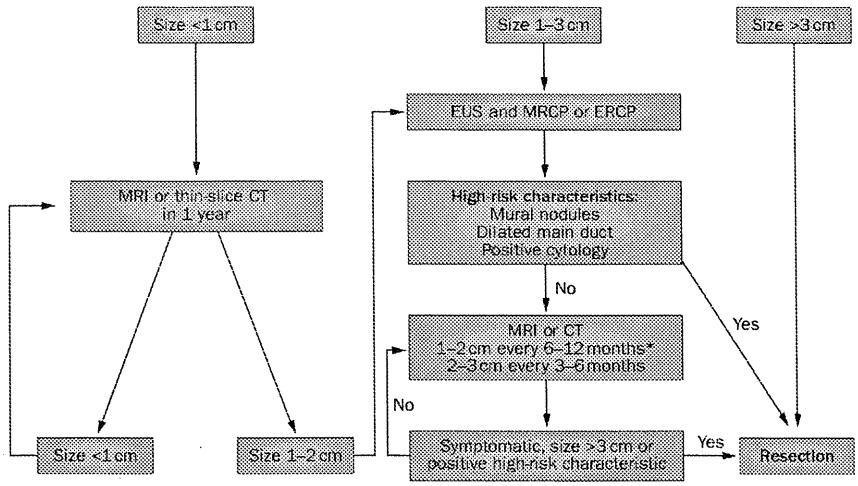

Figure 1 | Algorithm for the management of branch duct intraductal papillary mucinous neoplasms according to the 2006 international guidelines for the management of these neoplasms.[3] *The interval of follow-up examination can be lengthened after 2 years of no change. Abbreviations: ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; MRCP, magnetic resonance cholangiopancreatography. Permission obtained from Karger © Tanaka, M. *et al. Pancreatology* **6**, 17–32 (2006).

have reported that a high level of carcinoembryonic antigen (CEA) (>367 ng/ml,[6] >800 ng/ml,[7] ≥480 ng/ml[8] and >800 ng/ml[9]) in cystic fluid can be used to discriminate between mucinous and nonmucinous cysts. In a study of 112 patients with pancreatic cysts, the accuracy of a CEA level >192 ng/ml for the diagnosis of a mucinous cyst was 79%, and significantly better than the accuracy of either EUS imaging (51%), cytology (59%) or these two procedures combined ($P < 0.05$).[10] However, poor agreement between CEA levels and molecular analysis for the diagnosis of mucinous cysts has been reported, although the diagnostic sensitivity of such tests improved when the results were combined.[11] In addition, one study has reported that the CEA level in cystic fluid was higher (median 471 ng/ml) in 50 potentially malignant or malignant MCNs and IPMNs than in 29 benign cysts (median 1 ng/ml).[12]

Haab *et al.*[13] demonstrated that detection of a glycan variant of MUC-5AC discriminated IPMNs and MCNs from nonmucinous benign cysts with a sensitivity of 78% and a specificity of 80%. Furthermore, sensitivity and specificity improved to 87% and 86%, respectively, when this molecular test was combined with measurement of the level of CA (carbohydrate antigen) 19-9 in cystic fluid. By contrast, measurement of CEA level on its own had a low sensitivity (37%) and specificity (80%) in this study. Although mucin-like carcinoma-associated antigen levels, *KRAS* mutations and CA72-4 levels are claimed to be helpful in the diagnosis of mucinous cysts, the role of cystic fluid analysis and its biomarkers in preoperative differentiation between mucinous and nonmucinous pancreatic cysts requires further evaluation.[14–16]

Adequacy of the Sendai criteria

The international guidelines for the management of IPMN and MCN advocate resection of all main duct IPMNs and some branch duct IPMNs that meet one or more of five criteria for suspected malignancy: pancreatic juice cytology positive for malignancy, the presence of mural nodules, cyst size >3 cm, dilatation of the main pancreatic duct, and abdominal pain (Figure 1).[3] Besides positive pancreatic juice cytology, the presence of mural nodules is the most reliable predictor of malignant changes in branch duct IPMNs.[17–20] However, the size criterion is a matter of controversy and remains unresolved. Although Nagai *et al.*[21] reported that 4 of 49 patients with branch duct IPMNs or mixed-type IPMNs <3 cm without mural nodules had malignancy, several other studies have found no carcinomas among patients with purely branch duct IPMNs of <3 cm without mural nodules (Table 1).[17,22–24] In a large cohort of 145 patients with surgically resected branch duct IPMNs, malignancy was absent in patients with tumors <3 cm without mural nodules.[24] The Sendai criteria, therefore, seem to be adequate for identifying all malignancies, although only 22% (11% carcinoma *in situ*; 11% invasive) of resected tumors in the series were malignant.[24] Likewise, a study by Tang *et al.*[25] reported that 18 of 23 (78%) branch duct IPMNs recommended for surgical resection on the basis of the Sendai criteria were benign. Salvia *et al.*[26] employed different criteria: the presence of clinical symptoms, cyst size >3.5 cm, the presence of nodules, thick tumor walls, a serum CA19-9 level of >25 U/l, and recent onset or worsened diabetes. These authors still found that 17 of 20 (85%) branch duct IPMNs selected for surgery were benign. More specific criteria certainly need to be developed to exclude such false-positive results that result in unnecessary surgical intervention.

Since the introduction of histological subclassification of IPMNs into gastric, intestinal, pancreatobiliary and oncocytic types and the recognition of differences in mucin expression between these subtypes,[27,28] the possibility of adding such subclassification to criteria for resection of branch duct IPMNs has been anticipated. Two studies found that most branch duct IPMNs were of the gastric type, whereas main duct IPMNs were usually of the intestinal type. The prognosis of branch duct IPMNs is well known to be better than that of main duct IPMNs, and hence the histological subtypes correlate with the prognosis.[29,30] Furthermore, invasive intraductal papillary mucinous carcinomas derived from nonintestinal types of IPMNs ($n = 16$), that is gastric type ($n = 6$), pancreatobiliary type ($n = 8$), oncocytic type ($n = 1$) and nonclassifiable ($n = 1$), showed a poorer prognosis than those derived from intestinal-type IPMNs ($n = 14$).[31] The

Table 1 | Frequencies of malignancy in branch duct IPMNs

Study	Number of patients	Cyst size criteria (in cm)			
		≥3		<3	
		Mural nodules (+/−)	Malignancy (%)	Mural nodules (+/−)	Malignancy (%)
Bernard *et al.* (2002)[17]	12	0/3	0/0 (100/0)	2/7	2/0 (100/0)
Sugiyama *et al.* (1998)[22]	16	5/5	4/5 (80/100)	1/5	1/0 (100/0)
Rodrigues, *et al.* (2007)[23]	145	11/36	7/13 (63.6/36.1)	12/86	12/0 (100/0)
Sadakari *et al.* (2010)[24]	73	0/47	0/6 (0/12.8)	0/26	0/0 (0/0)
Total	246	16/91	11/24 (69/26)	15/124	15/0 (100/0)

Abbreviation: IPMN, intraductal papillary mucinous neoplasm.

Table 2 | Incidences of PDACs in patients with branch duct IPMNs

Study	Number of patients	Number of PDACs	Follow-up period	Incidence (%)
Yamaguchi *et al.* (2002)[15]	76	7	NA*	9.2
Uehara *et al.* (2008)[41]	60	5	Median 87 months	8.0
Ingkakul *et al.* (2010)[42]	236	22	NA	9.3
Kanno *et al.* (2010)[43]	159	7	NA	4.4
Tanno *et al.* (2010)[44]	168	9	NA	5.4
Ikeuchi *et al.* (2010)[45]	145	5	Mean 55.9 months	3.5

*Not applicable as study is a retrospective analysis. Abbreviations: IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma.

preoperative determination of histological subtypes by either immunohistochemical staining[32] or molecular marker analysis[33] of the pancreatic juice or aspirated cyst fluid samples would be of paramount interest and benefit.

Surveillance of branch duct IPMNs

The optimal modality for follow-up surveillance of branch duct IPMNs without malignant signs remains to be determined. Some investigators use EUS to observe changes in the size of the cyst and main pancreatic duct,[34] and others use ultrasonography, CT and/or MRI. EUS is more sensitive than the other techniques for identifying such changes but has inherent drawbacks of invasiveness, increased cost and intraobserver and interobserver variability. The sensitivities of ultrasonography, CT and MRI should be thoroughly examined with regard to the rate of detection of malignant changes.

The clinical significance of branch duct IPMN surveillance is threefold. First, these IPMNs are well known to exhibit malignant changes following the adenoma-carcinoma sequence;[35,36] the criteria for such malignant transformation were proposed in the 2006 guidelines and are described earlier.[3] Second, clinicians should be on guard against the development of pancreatic ductal adenocarcinoma (PDAC) at a different site in the pancreas from the IPMN. Third, even among patients who undergo partial pancreatectomy for noninvasive IPMNs, 10% are reported to experience disease recurrence.[37]

Since the occurrence of *in situ* or invasive PDAC concomitant with a benign branch duct IPMN was reported, this phenomenon has been drawing increasing attention.[38–40] Evidence indicates a relatively high incidence of PDAC in patients with branch duct IPMNs (Table 2).[40–45] A study reports that PDACs were found in 5 of 60 (8%) patients with branch duct IPMNs, the initial sizes of which were <1 cm, during a median follow-up of 87 months.[41] Thus the 5-year rate of PDAC development was 6.9% and the incidence was 1.1% per year. On the other hand, malignant changes of IPMNs were noted in just 2 of 60 (3%) patients.[41] Ingkakul and co-workers[42] detected concomitant synchronous or metachronous PDACs in 22 of 236 (9.3%) patients with IPMNs. All 22 IPMNs were branch duct IPMNs and histology indicated that 12 resected IPMNs were benign. Worsened diabetes and high levels of serum CA19-9 predicted the presence of PDAC on the basis of multivariate analyses. Increased serum levels of CA19-9 were also a predictor of PDACs in a study by Kanno *et al.*[43] in which PDACs were noticed in 7 of 159 (4%) patients with branch duct IPMNs. Tanno and colleagues[44] found that 9 of 168 (5.4%) patients with branch duct IPMNs also had PDACs. The patients who developed PDACs in this study were older and had smaller IPMN cysts and main pancreatic ducts compared with the patients who did not develop PDACs.

The main pancreatic duct should always be examined by cytology as well as frozen-section histology when a branch duct IPMN, with or even without main pancreatic duct dilatation, is resected just as in patients with main duct IPMNs.[46] The significance is twofold: to survey the presence of carcinoma concomitant with IPMN, but not detected during preoperative work-up; and to confirm the absence of malignant transformation of IPMN undetectable by preoperative imaging examinations.

The intervals for follow-up examinations of potentially benign branch duct IPMNs and the residual pancreas after resection of IPMNs must be reconsidered in this context. The 2006 guidelines suggest that branch duct IPMNs <1 cm, 1–2 cm and 2–3 cm in size should be examined by CT or MRI every year, every 6–12 months and every 3–6 months, respectively (Figure 1).[3] The guidelines also state that the interval of follow-up examination can be lengthened after 2 years of no change. However, this statement may need to be reconsidered in view of the relatively high incidence of PDACs that develop distinct from IPMNs in comparison with the incidence of malignant changes to the IPMN itself.

As stated above, all patients with branch duct IPMN should undergo meticulous surveillance examinations because of the alleged high risk of PDAC development, although the best interval and modality for surveillance remain to be determined. Whether patients with IPMN and a family history of PDAC have a higher risk of developing this carcinoma than patients with IPMN but without a family history of PDAC is as yet unknown. Furthermore, patients with a strong family history of PDAC are likely to develop IPMNs and subsequently a PDAC during follow-up.[47,48] Whether those patients with IPMN and a family history of