Jpn J Clin Oncol 2002;32(4)146–151

Case Reports

Invasive Ductal Adenocarcinoma of the Remnant Pancreatic Body 9 Years after Resection of an Intraductal Papillary-Mucinous Carcinoma of the Pancreatic Head: a Case Report and Comparison of DNA Sequence in K-*ras* Gene Mutation

Takamichi Komori[1], Osamu Ishikawa[1], Hiroaki Ohigashi[1], Terumasa Yamada[1], Yo Sasaki[1], Shingi Imaoka[1], Akihiko Nakaizumi[2], Hiroyuki Uehara[2], Sachiko Tanaka[2], Yoshiyuki Mano[3] and Tsutomu Kasugai[3]

Departments of [1]Surgery, [2]Internal Medicine and [3]Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

Received November 15, 2001; accepted January 28, 2002

Recently, there have been a few case reports of invasive ductal adenocarcinoma (IDC) developed in the remnant pancreas after partial pancreatectomy for intraductal papillary-mucinous neoplasm (IPMN). It is necessary to clarify their histogenetic relationships among two sporadic tumors and their surrounding duct epithelium and it would be more reliable if genetic analysis is added to the conventional histology. We report a 76-year-old woman who received pancreaticoduodenectomy for IPMN with a focal *in situ* carcinoma (IPMC), which was transitional to the surrounding duct epithelium with papillary proliferation and a wide variety of dysplasia. Nine years after the operation, she died of IDC in the remnant pancreatic body and its surrounding duct epithelium consisted of hyperplastic mucous cells with slight–mild dysplasia. Analysis of K-*ras* mutation at codon 12 (wild-GGT) by direct sequencing after polymerase chain reaction indicated that their transitioning patterns differed from each other: CGT in IPMC; no mutation in the mildly dysplastic duct epithelium around IPMC; GAT in IDC of the remnant pancreas; and AGT in mucous cell hyperplasia with mild dysplasia close to the IDC. This is the first report in which the DNA sequence of K-*ras* mutation was determined for the two sporadic pancreatic cancers and surrounding duct changes. The following two suggestions are made: (1) the cell-origin might have differed between the two types of cancer (IDC and IPMC); and (2) no precursor lesion toward IDC or IPMC was identified in their surrounding duct epithelium.

Key words: pancreatic cancer – intraductal papillary-mucinous carcinoma – intraductal papillary-mucinous neoplasia – invasive ductal adenocarcinoma – K-ras point mutation

INTRODUCTION

Owing to the increased availability of endoscopic retrograde pancreatography (ERP) and magnetic resonance pancreatography (MRP), it has become easier to detect intraductal papillary-mucinous neoplasm (IPMN) of the pancreas (1–3). This tumor is likely to grow into the duct lumen forming a polypoid mass, commonly consisting of tall columnar cells with low-grade atypia and occasionally producing a large amount of mucin, but is unlikely to infiltrate into the pancreatic parenchyma. The potential for metastasis and invasion is generally lower than those of the common type of invasive ductal adenocarcinoma (IDC) of the pancreas (4–6) and the 5-year survival rate after resection of non-invasive (*in situ*) intraductal papillary-mucinous carcinoma (IPMC) has been reported to be >80% (7). Even in invasive IPMC, Yamao et al. reported that the 5-year survival rate was as good as 44% (8) and this figure is higher than that of IDC.

There have been a few recent reports of the development of IDC in the remnant pancreas after partial pancreatectomy for IPMN (9–12). Although the question was raised of how IDC developed after resection of IPMN, there has been no definitive conclusion because the previous analyses were based on conventional histopathology alone (Table 1). However, it

For reprints and all correspondence: Osamu Ishikawa, Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1–3–3 Nakamichi, Higashinari-ku, Osaka 537-8511, Japan

Table 1. Reported cases of invasive ductal adenocarcinoma in the remnant pancreas after resection of IPMC or IPMN

No.	Reference	Age (years)/gender	First tumor		Cut margin	Interval	Second tumor	
			Histology	Location			Histology	Location
1	9	59/M	IPMN	Pt	Negative	1Y9M	IDC (por)	Pb
2	10	63/M	IPMC	Pb	Negative*	5Y8M	IDC (well)	Ph
3	11	74/M	IPMN	Pb	MCH†	2Y6M	IDC (mod)	Ph
4	12	66/M	IPMN	Ph	Negative‡	4Y	IDC (mod)	P
5	Present case	76/F	IPMC	Ph	MCH§	8Y9M	IDC (por)	Pb

IPMC, intraductal papillary-mucinous carcinoma; IPMN, intraductal papillary-mucinous neoplasm; IDC, invasive ductal adenocarcinoma; Pt, pancreatic tail; Pb, pancreatic body; Ph, pancreatic head; MCH, mucous cell hyperplasia; por, poorly differentiated; well, well differentiated; mod, moderately differentiated. *No neoplastic lesions. †Papillary hyperplasia of the metaplastic mucous epithelium with moderate atypia. ‡No continuity between two lesions. §Mucous cell hyperplasia with mild–moderate dysplastic change. All of the previous authors (Nos 1–4) considered that IDC was the second primary tumor because of the different histological features between the two sporadic tumors, non-neoplastic duct epithelium at the cut margin or a long interval between the two events.

would become more reliable if molecular genetic analysis were to be added. In addition to the two sporadic tumors, their surrounding duct epithelium should also be examined, because they commonly have hyperplastic and/or dysplastic changes, which have been considered as precursor lesions by Brat et al. (13). If so, we should be more careful in making an intraoperative histodiagnosis for the cut margin of the pancreas.

The incidence of K-*ras* gene mutation at codon 12 was reported to be 80–95% in IDC (14,15) and around 50% in IPMC (16). This event is generally considered to occur at an earlier phase of pancreatic carcinogenesis than p53 overexpression (17) and Yanagisawa et al. reported that the incidence of K-*ras* mutation was around 60% even in hyperplastic mucous cells obtained from patients with chronic pancreatitis (18). If the DNA sequences at codon 12 are compared among the various lesions from the same patient, more definitive conclusions may be drawn regarding their pathogenesis. Recently, we also experienced a similar case of IDC 9 years after curative resection of IPMC and the DNA sequence of the K-*ras* gene was determined for the two sporadic cancers and their neighbouring 'non-cancerous' duct epithelium.

CASE REPORT

Clinical Course

A 76-year-old woman was referred to our hospital with back pain in December 1988. Except for slight elevation of serum levels of amylase (223 U/l; normal range: 40–160 U/l) and carbohydrate antigen 19-9 (CA19-9, 90 U/ml; normal range: 0–36 U/ml), all other laboratory data were within the normal ranges. Computed tomography (CT) revealed dilation (10 mm in diameter) of the main pancreatic duct (Fig. 1). ERP revealed a filling defect (7 mm in diameter) in the main duct of the pancreatic head. With a preoperative diagnosis of IPMN of the pancreatic head, pancreaticoduodenectomy was performed in June 1989. The cut end of the pancreas was judged to be negative for cancer based on intraoperative histological examina-

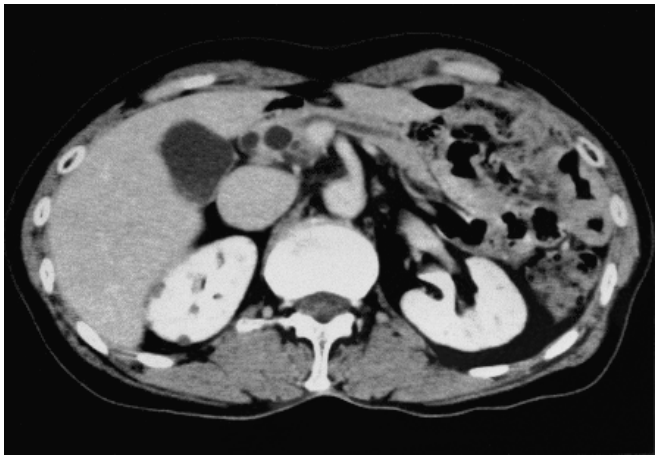

Figure 1. Computed tomography in 1988 revealed dilation (10 mm in diameter) of the main pancreatic duct.

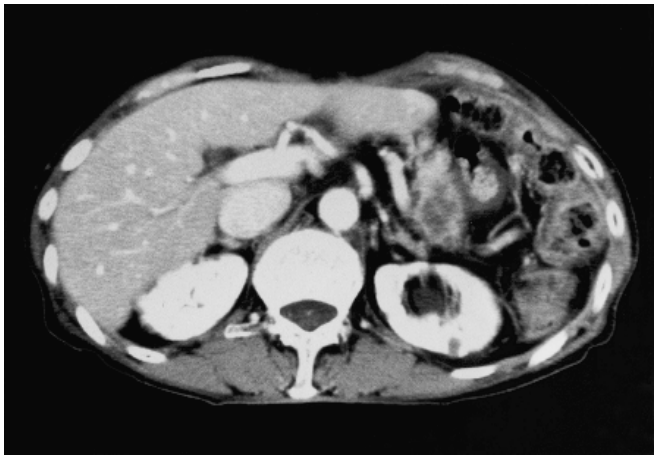

Figure 2. Computed tomography in 1998 revealed a solid mass (3 cm in diameter) close to the anastomotic site of pancreatojejunostomy. The gastric wall and the retroperitoneal spaces were also suspected to be involved with the tumor.

tion of frozen sections and it was anastomosed to the jejunum. The postoperative course had been uneventful for about 9 years postoperatively. However, in March 1998, she experienced pain in the left upper quadrant of the abdomen. Her serum CA19-9 level was again elevated to 826 U/ml and the carcinoembryonic antigen (CEA) level was increased to 9.8 ng/ml (normal range: <5.0 ng/ml). Both CT and magnetic resonance imaging (MRI) revealed a solid mass (3 cm in diameter) close to the anastomotic site of pancreatojejunostomy (Fig. 2). The gastric wall and the retroperitoneal spaces were also suspected to be involved with the tumor and we made a diagnosis of advanced cancer of the pancreatic body. She did not consent to invasive examinations or surgical operation and died owing to the subsequent development of pleuritis and peritonitis carcinomatosis in January 1999. At autopsy, a grayish-white, hard, solid mass (4.5 cm in diameter) was seen in the pancreatic body, involving the surrounding tissues (stomach, retroperitoneal tissues and colon). The main pancreatic duct (1.5 cm in length) intercalated between the anastomotic site and the cranial end of the solid tumor appeared intact with a smooth inner surface. Both pleural and abdominal cavities contained massive bloody fluid and many small nodules, suggesting cancer seeding.

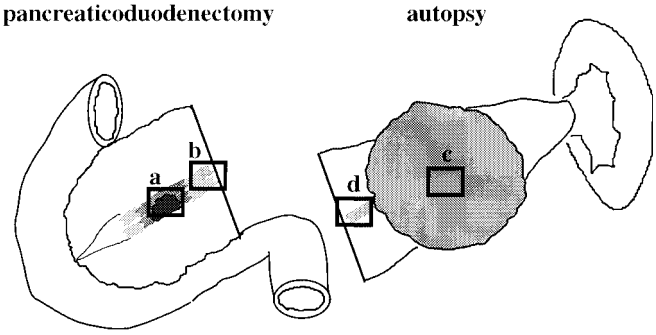

Figure 3. Schema showing the pancreatic head obtained by pancreaticoduodenectomy and the pancreatic body obtained by autopsy. (a) Intraductal polypoid tumor of the pancreatic head (Fig. 4a). (b) Main pancreatic duct around the polypoid tumor of the pancreatic head (Fig. 4b). (c) Solid mass of the remnant pancreatic body (Fig. 4c). (d) Main pancreatic duct intercalated between the solid tumor and the anastomotic site of the remnant pancreatic body (Fig. 4d).

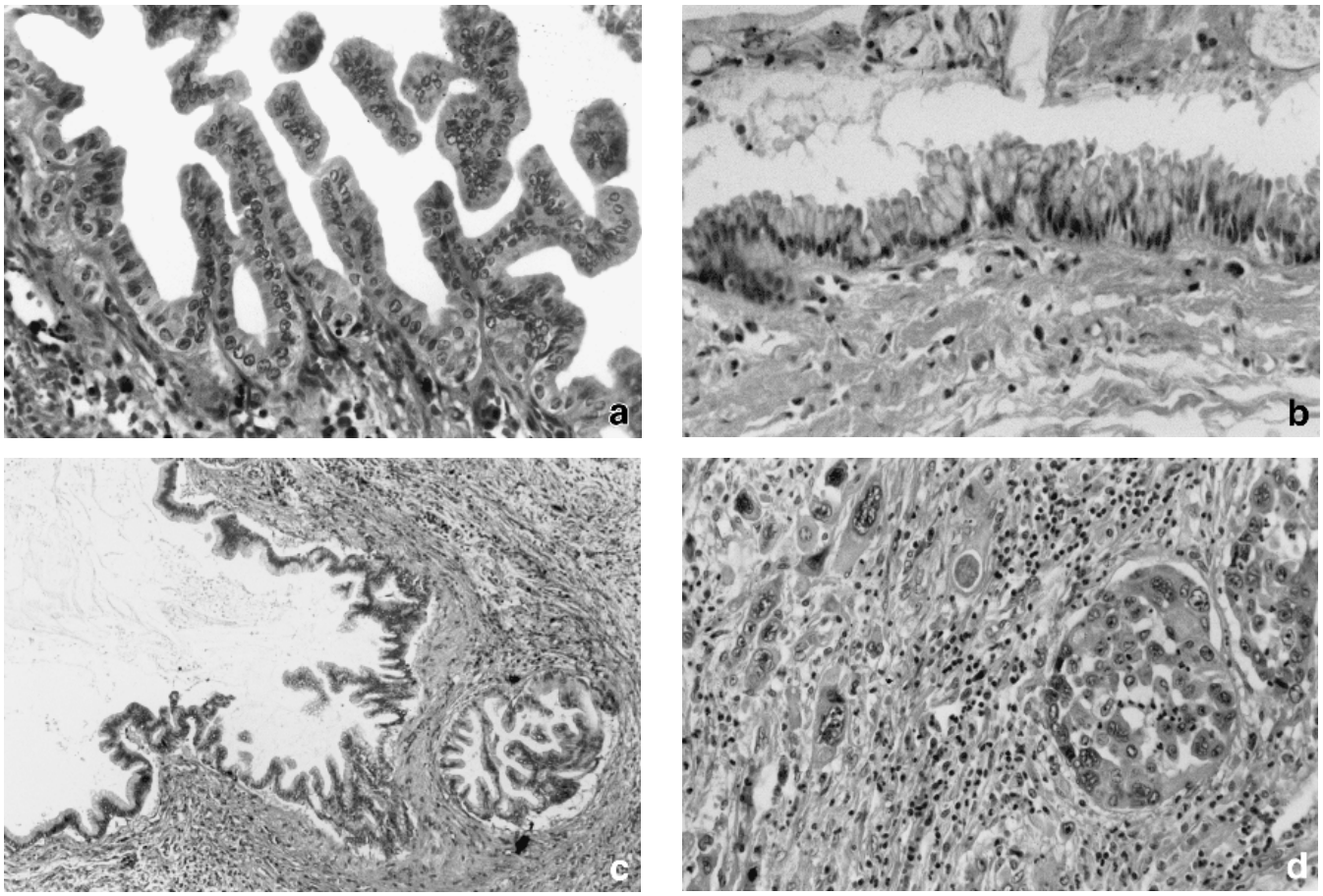

Figure 4. Histological appearances of four lesions (H&E). (a) Non-invasive intraductal papillary-mucinous carcinoma of the pancreatic head (100×) (Fig. 3a). The epithelium shows irregular papillary proliferation with severe atypia. (b) Mucous cell hyperplasia around the intraductal papillary-mucinous neoplasm of the pancreatic head (100×) (Fig. 3b). Tall columnar epithelial cells containing mucin shows mild dysplastic change. (c) Invasive ductal adenocarcinoma (moderately differentiated tubular adenocarcinoma) of the remnant pancreatic body (66×) (Fig.3c). (d) Mucous cell hyperplasia with mild dysplastic change between the solid tumor and the anastomotic site of the remnant pancreatic body (20×) (Fig. 3d). The epithelium shows papillary proliferation consisted of tall columnar cells containing mucin.

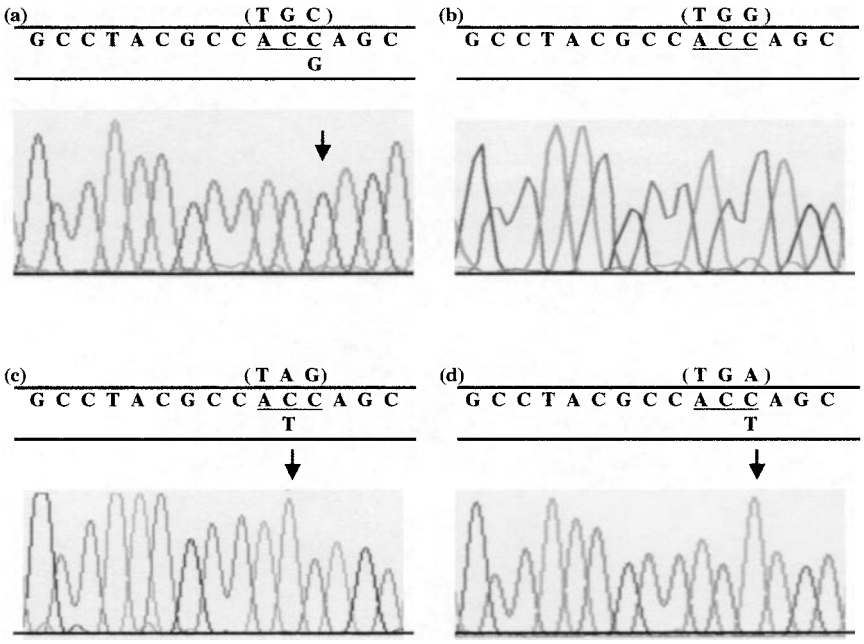

Figure 5. Results of DNA sequence for K-*ras* gene. All sequences show complement DNA of K-*ras* gene code from codon 11 (GCT) to codon 15 (GGC). (a) K-*ras* mutation (CGT) detected in intraductal papillary-mucinous carcinoma of the pancreatic head (Figs 3a, 4a). (b) No mutation (GGT) was detected in the dysplastic duct epithelium at the stump of the pancreatic head (Figs 3b, 4b). (c) K-*ras* mutation (GAT) detected in invasive ductal adenocarcinoma of the remnant pancreas (Figs 3c, 4c). (d) K-*ras* mutation (AGT) detected in mucous cell hyperplasia with mild dysplastic change at the stump of the remnant pancreas (Figs 3d, 4d).

Histopathological Analysis of Specimens Obtained by Surgery and Autopsy (Figs 3 and 4)

Specimens of the polypoid tumor (Fig. 3a) and the main pancreatic duct around the polypoid tumor (Fig. 3b) of the pancreatic head were obtained by pancreaticoduodenectomy. Specimens of the solid mass (Fig. 3c) and the main pancreatic duct intercalated between the solid tumor and the anastomotic site (Fig. 3d) of the remnant pancreatic body were obtained by autopsy. Histologically, the polypoid tumor of the pancreatic head consisted of tall columnar cells that formed papillary proliferation into the duct lumen and produced a large amount of mucin, compatible with IPMN. There was a small focus of *in situ* carcinoma (IPMC), with high ratios of nucleus to cytoplasm and loss of nuclear polarity (Fig. 4a). No cancer extension was seen in the lymph nodes, vessels or perineural spaces. However, the tumor cells were gradually transitional to the surrounding duct epithelium, which also consisted of tall columnar cells with a wide variety of nuclear sizes and nuclear polarity. They also produced mucin and were diagnosed as mucous cell hyperplasia with slight–moderate dysplastic change (Fig. 4b). The solid mass of the pancreatic body was diagnosed as IDC (moderately differentiated adenocarcinoma) with marked invasion beyond the confines of the pancreas (Fig. 4c). The cancer cells formed tubular structures producing a smaller amount of mucin than the polypoid tumor of the pancreatic head. The main pancreatic duct intercalated between the anastomotic site and the tumor of the pancreatic body consisted of tall columnar cells with mild dysplastic changes. They were also diagnosed as mucous cell hyperplasia (Fig. 4d).

Immunohistochemistry and K-*ras* Point Mutation

Immunohistochemical analysis was performed using the avidin–biotin–peroxidase complex method. The IPMC of the pancreatic head was negative for MUC-1 apomucin (Fujirebio Diagnostics, mouse IgG, 100×) and MUC-2 apomucin (Novocastra, mouse IgG, 400×). On the other hand, IDC of the pancreatic body was positive for MUC-1 apomucin.

For genetic diagnosis of K-*ras* gene mutation, the lesions (Figs 3 and 4a–d) were microdissected from unstained sections of paraffin-embedded specimens under microscopy using fine needles, after confirming the identification of the lesions with hematoxylin–eosin staining of adjacent sections. DNA was extracted from microdissected sections and mutation at codon 12 of the K-*ras* gene was examined by direct sequencing after polymerase chain reaction amplification, as described previously (19). Point mutation at codon 12 of the K-*ras* gene was detected in three of four lesions and their changes differed from each other: GGT (glycine) to CGT (arginine) in IPMC (a); no mutation in the dysplastic duct epithelium at the stump of the pancreatic head (b); GGT to GAT (aspartic acid) in IDC of the remnant pancreas (c); and GGT to AGT (serine) in mucous cell hyperplasia with mild dysplastic change at the stump of the remnant pancreas (d) (Fig. 5a–d).

DISCUSSION

When a second cancer is detected in the remnant organ after resection of the first tumor with curative intent, it is occasionally difficult to determine whether it has originated from recur-

rence of the first cancer or is a second newly developed primary cancer. In addition, the role of non-cancerous but precancerous lesions around the primary cancer should also be considered in its pathogenesis. Such considerations become more important as the first cancer is characterized by better prognosis. There have been only a few previous reports of the development of IDC in the remnant pancreas after resection of pancreatic IPMN (9–12) (Table 1). All of these studies concluded that IDC might have originated independently from the first cancer (IPMN), because there are marked differences in histological features between IDC and IPMC: growth pattern, cell atypism, mucin-producing ability, quality of mucin (immunohistochemistry), metastatic potential, etc. However, both of these cancers are of duct cell origin and show a high incidence of K-*ras* point mutation (14–16). In addition, it is not unusual for the advancing points of invasive IPMC to show histological features similar to those of typical IDC, i.e. ductal or ductular structures (tubular adenocarcinoma) (20,21). Hence it is possible that the residual IPMC might transform to IDC during the process of cancer recurrence. Likewise, in the present case, there were many hyperplastic mucous cells located in the intercalated pancreatic duct between the two cancers. Although they appeared to be non-cancerous by conventional histological analysis, they might have had malignant potential to differentiate towards at least one of the two cancers (IDC and/or IPMC). Cubilla and Fitzgerald compared the incidence of mucous cell hyperplasia between cancer-bearing and cancer-free pancreas and the former showed a slightly higher incidence than the latter (22). Yanagisawa et al. also reported that mucous cells had K-*ras* mutation at an incidence of 60% (18). More recently, Brockie et al. (23) and Brat et al. (13) followed patients in whom atypical ductal hyperplasia or carcinoma *in situ* had been seen at the cut margin of the pancreas to detect subsequent IDC in the remnant pancreas.

As described above, K-*ras* gene mutation is a far earlier event in the process of pancreatic carcinogenesis than the development of microscopic alterations including cell atypism, p53 overexpression or DPC4 inactivation (17,24,25). Although most mutations occur at codon 12, there are varieties of mutated sequences among the pancreatic cancers and atypical hyperplasias. Hruban et al. reported that 90% of the K-*ras* mutations at codon 12 in pancreatic cancer were of three types: GAT, GTT and CGT (26). Two other types of mutation, TGT and AGT, were also common in hyperplastic (mucous) cells with or without cell atypism (27). Our results agreed with these observations: CGT in IPMC; GAT in IDC; AGT or no mutation in hyperplastic duct epithelium. In addition, the four foci (a–d) in our case showed different K-*ras* gene sequences from each other and we considered that the cell origins of IDC and IPMN or IPMC might have differed from each other. Likewise, no precursor lesion toward IDC or IPMC was identified in their surrounding duct epithelium even though they had suffered from hyperplastic and/or dysplastic changes in histology. In the future, molecular genetic analysis should be performed in a larger number of similar cases to facilitate the elucidation of histogenetic relationships.

Acknowledgment

This study was supported in part by a grant from the Foundation for Promotion of Cancer Research.

References

1. Sugiyama M, Atomi Y. Intraductal papillary mucinous tumors of the pancreas. Imaging studies and treatment strategies. *Ann Surg* 1998;228:685–91.
2. Uehara H, Nakaizumi A, Iishi H, Tatsuta M, Kitamura T, Okuda S, et al. Cytologic examination of pancreatic juice for differential diagnosis of benign and malignant mucin-producing tumors of the pancreas. *Cancer* 1994;74:826–33.
3. Shyr YM, Su CH, Tsay SH, Lui WY. Mucin-producing neoplasms of the pancreas. Intraductal papillary and mucinous cystic neoplasms. *Ann Sug* 1996;223:141–6.
4. Morohoshi T, Kanda M, Asanuma K, Kloppel G. Intraductal papillary neoplasms of the pancreas. A clinicopathologic study of six patients. *Cancer* 1989;64:1329–35.
5. Rivera JA, Castillo CF, Pins M, Compton CC, Lewandrowski KB, Rattner DW, et al. Pancreatic mucinous ductal ectasia and intraductal papillary neoplasms. *Ann Surg* 1997;225:637–46.
6. Nakagori T, Kenmochi T, Kaimura O, Tokoro Y, Asano T. Intraductal papillary mucinous tumors of the pancreas. *Am J Surg* 1999;178:344–7.
7. Kimura W, Sasahira N, Yoshikawa T, Muto T, Makuuchi M. Ductectatic type of mucin producing tumors of the pancreas. New concept of pancreatic neoplasia. *Hepatogastroenterology* 1996;43:692–709.
8. Yamao K, Ohashi K, Nakamura T, Suzuki T, Shimizu Y, Nakamura Y, et al. The prognosis of intraductal papillary mucinous tumors of the pancreas. *Hepatogastroenterology* 2000;47:1129–34.
9. Ono H, Hayakawa T, Kondo T, Shibata T, Kitagawa M, Sakai Y, et al. A case report of pancreatic cancer after the operation of mucous producing pancreatic tumor. *Suizou* 1989;4:66–71 (in Japanese).
10. Seki M, Hori M, Ueno H, Ohta H, Murakami Y, Ohashi K, et al. A case of metachronous multiple cancers of the pancreas. *Suizou* 1993;8:132–9 (in Japanese).
11. Wakabayashi T, Kawaura Y. An autopsy case of invasive ductal carcinoma of the pancreas developing after resection of a mucin-producing pancreatic tumor. *Suizou* 1999;14:49–55 (in Japanese).
12. Yamaguchi K, Ogawa Y, Takamatsu Y, Ohtsuka T, Nagai E, Nakano K, et al. Diagnostic clues for pancreatic cancer. *Tan to Sui* 2001;22:725–9 (in Japanese).
13. Brat DJ, Lillemoe KD, Yeo CJ, Warfield PB, Hruban RH. Progression of pancreatic intraductal neoplasias to infiltrating adenocarcinoma of the pancreas. *Am J Surg Pathol* 1998;22:163–9.
14. Almoguera C, Shibata D, Forrester K, Martin J, Armheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell* 1988;53:549–55.
15. Tada M, Omata M, Ohto M. Clinical application of *ras* gene mutation for diagnosis of pancreatic adenocarcinoma. *Gastroenterology* 1991;102:233–8.
16. Z'graggen K, Rivera JA, Compton CC, Pins M, Werner J, Castillo CF, et al. Prevalence of activating K-*ras* mutations in the evolutionary stages of neoplasia in intraductal papillary mucinous tumors of the pancreas. *Ann Surg* 1997;226:491–500.
17. Jimenez RE, Warshaw AL, Z'graggen K, Hartwig W, Taylor DZ, Compton CC, et al. Sequential accumulation of K-*ras* mutations and p53 overexpression of pancreatic mucinous cystic neoplasms to malignancy. *Ann Surg* 1999;230:501–11.
18. Yanagisawa A, Ohtake K, Ohashi K, Hori M, Kitagawa T, Sugano H, et al. Frequent c-Ki-*ras* oncogene activation in mucous cell hyperplasias of pancreas suffering from chronic inflammation. *Cancer Res* 1993;53:953–6.
19. Kinoshita M, Katsuragi K, Shin S, Aono T. Amplification and point-mutation alterations of oncogenes in patients with cervical cancer-associated human papillomavirus. *Biomed Res* 1993;14:403–10.
20. Yonezawa S, Taira M, Osako M, Kubo M, Tanaka S, Sakoda K, et al. MUC-1 mucin expression in invasive areas of intraductal papillary mucinous tumors of the pancreas. *Pathol Int* 1998;48:319–22.
21. Yanagisawa A. Intraductal papillary adenocarcinoma of the pancreas. An analysis of invasive carcinomas. *Tan to Sui* 1999;20:9–16 (in Japanese).

22. Cubilla AL, Fitzgerald PJ. Tumors of the exocrine pancreas. In: Atlas of Tumor Pathology, 2nd Series, Fascicle 19. Washington, DC: Armed Forced Institute of Pathology 1984;71–89.
23. Brockie E, Anand A, Albores-Saavedra J. Progression of atypical ductal hyperplasia/carcinoma *in situ* of the pancreas to invasive adenocarcinoma. *Ann Diagn Pathol* 1998;2:286–92.
24. Heinmoller E, Dietmaier W, Zirngibl H, Heinmoller P, Scaringe W, Jauch KW, et al. Molecular analysis of microdissected tumors and preneoplastic intraductal lesions in pancreatic carcinoma. *Am J Pathol* 2000;157:83–92.
25. Lacobuzio-Donahue CA, Klimstra DS, Adsay NV, Wilentz RE, Argani P, Sohn TA, et al. Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas. *Am J Pathol* 2000;157:755–61.
26. Hruban RH, van Mansfeld AD, Offerhaus GJ, van Weering DH, Allison DC, Goodman SN, et al. K-*ras* oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol* 1993;143:545–54.
27. Tada M, Ohashi M, Shiratori Y, Okudaira T, Komatsu Y, Kawabe T, et al. Analysis of K-*ras* gene mutation in hyperplastic duct cells of the pancreas without pancreatic disease. *Gastroenterology* 1996;110:227–31.