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


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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

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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-

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

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

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.

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 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.
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 μg/ml) and MUC-2 apomucin (Novocastra, mouse IgG, 400 μg/ml). 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 pre-
cancerous 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 con-
cluded 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
usual 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).

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 con-
ventional 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 inci-
dence 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 carcino-
ma 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 muta-
tion 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. Like-
wise, no precursor lesion toward IDC or IPMC was identified
in their surrounding duct epithelium even though they had suf-
fered 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.

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