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How I Do It

# Extensive hilar bile duct resection using a transhepatic approach for patients with hepatic hilar bile duct diseases

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**KEYWORDS:**
Benign bile duct stricture;
Bile duct resection;
Hilar cholangiocarcinoma;
Transhepatic approach

**Abstract**

**BACKGROUND:** Extensive hilar bile duct resection beyond the second- or third-order intrahepatic biliary radicals is usually required for patients with hilar cholangiocarcinoma as well as those with benign inflammatory stricture. Most hilar cholangiocarcinomas is resected with combined major hepatectomy to obtain free surgical margins. The purpose of this study was to show the surgical procedure and the usefulness of extensive hilar bile duct resection using a transhepatic approach for patients with hilar bile duct diseases.

**METHODS:** Five patients with hepatic hilar bile duct disease and who were unfit for major hepatectomy for several reasons underwent extensive hilar bile duct resection by way of a transhepatic approach. Four of the patients had hilar bile duct cancer, including 1 with mucous-producing bile duct cancer of low-grade malignancy and 1 with a postsurgical benign bile duct stricture.

**RESULTS:** After extensive hilar bile duct resection, bile duct stumps ranged in number from 2 to 7 mm (mean 4). Surgical margins at bile duct stump were free of cancer in all 4 cancer patients. The long-term outcomes were as follows: 3 patients are alive at the time of publication, and 2 patients have died.

**CONCLUSIONS:** A transhepatic approach may be useful when performing extensive hilar bile duct resection bile duct stricture of biliary disease at the hepatic hilus, especially in high-risk patients who are unfit for major hepatectomy as well as in those having benign bile duct stricture and low-grade malignancy.


Most biliary tract cancers involving the hilar bile duct, such as hilar cholangiocarcinoma and gallbladder carcinoma, must be resected by hepatectomy and extrahepatic bile duct resection to obtain cancer-free surgical margins. However, in patients who are unfit for major hepatectomy because of liver dysfunction and general poor condition, combined major hepatectomy with bile duct resection might incur increased surgical risk.[1–3] In contrast, low-grade malignancies, such as mucin-producing bile duct tumors, may sometimes be radically resected without performing combined major hepatectomy, even when there is a tumor in the upper third of the extrahepatic bile duct.[4,5] We applied a transhepatic approach to extensive hilar bile duct resection in 5

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**Table 1** Patient characteristics and operative data

| Patient no/age (y) | Sex | Disease | No. of BDS | Surgical data Time | Blood loss (mL) |
|---|---|---|---|---|---|
| 1/61 | Male | Bile duct cancer | 7 | 7 h 16 min | 390 |
| 2/76 | Male | Benign stricture | 4 | 6 h 38 min | 1,023 |
| 3/76 | Male | Bile duct cancer | 3 | 5 h 32 min | 465 |
| 4/80 | Male | Bile duct cancer | 3 | 7 h 18 min | 680 |
| 5/72 | Male | Bile duct cancer | 5 | 8 h 13 min | 1,400 |

BDS = bile duct stumps.

patients with hilar bile duct disease. This approach may provide a sufficient surgical view and facilitate extensive resection of the hilar bile duct, facilitating reconstruction by bilioenteric anastomosis. The purpose of this study was to show the usefulness of extensive hilar bile duct resection using a transhepatic approach for patients with hilar bile duct diseases.

## Methods

Between October 2001 and December 2006, 5 patients with hepatic hilar bile duct disease underwent extensive hilar bile duct resection using a transhepatic approach. The patients' characteristics are listed in Table 1. There were 4 patients with hilar bile duct cancer, including 1 with mucous-producing bile duct cancer of low-grade malignancy, and 1 with postsurgical benign bile duct stricture. The ages of the 5 patients ranged from 61 to 80 years. Selection criteria for this surgical procedure, used in the 4 patients with bile duct cancer, were that the cancer was localized at the hilar bile duct had not invaded the extramural liver or vessels, such as the portal vein and the hepatic artery, and that curative resection with extensive hilar bile duct resection alone was indicated for treating the cancer based on the evaluation of preoperative imaging findings. Furthermore, the surgical stress of major hepatectomy was deemed to be unacceptably extreme for 3 patients because of advanced age, liver dysfunction, or otherwise general poor condition (Table 2).

## Surgical procedures

Under a subcostal transverse skin incision in the upper abdomen, the right hepatic lobe was mobilized by dissecting the coronary ligament. The gallbladder was mobilized from the gallbladder bed of the liver. The hepatoduodenal ligament was skeletonized, and the portal vein and the hepatic artery were tracked down into the intrahepatic Glissonian sheath until the second-order intrahepatic Glissonian sheath at the right side and the umbilical portion at the left side. The liver parenchyma was transected along the Cantlie line as the interlobar border between the right and the left hepatic lobes after identifying the demarcation line by hemilobar inflow vascular clamping.

By identifying the hepatic vein branch from the right anterior segment (S5 and S8) and the left medial segment (S4), we determined on which side of the middle hepatic vein the transected line would be situated. Preoperative imaging, especially enhanced computed axial tomography (CAT), is important for identification of the branch of the major hepatic vein before applying the transhepatic approach. In addition, intraoperative Doppler ultrasonography could be useful for identifying the branches of the major hepatic vein. If the vein branch from segments 5 or 8, with a confluence into the middle hepatic vein, had a larger caliber than the vein branch from segments 5 or 8 with a confluence into the right hepatic vein, it was judged to be significant drainage vein that should be preserved. Similar judgment was rendered in the vein branch from segment 4 with a confluence into the middle or left hepatic veins. Therefore, in the case of remarkable hepatic vein branches of the right anterior segment with a confluence into the middle hepatic vein, the liver parenchyma was transected along the left side of the middle hepatic vein without ligating these vein branches to avoid congestive injury of the right anterior hepatic segment (Fig. 1). In contrast, in the case of significant hepatic vein branches of the left medial segment with a confluence into the middle hepatic vein, the liver should be transected on the line along the right side of the middle hepatic vein (Fig. 2), or the these veins should be preserved as much as possible by not transecting the liver

**Table 2** Reasons for avoiding major hepatectomy in 5 patients who underwent surgical resection by way of transhepatic approach

| Patient no | Reasons |
|---|---|
| 1 | Localized bile duct tumor of low-grade malignancy |
| 2 | Benign stricture, old age, liver dysfunction (ICG 20%) |
| 3 | Localized bile duct cancer, old age, liver dysfunction (ICG 15%) |
| 4 | Old age, liver dysfunction (ICG 15%) |
| 5 | Localized bile duct cancer, liver dysfunction (ICG 14%) |

ICG = indocyanine green; retention rate at 15 minutes after the intravenous injection of 0.5 mg/kg body weight.

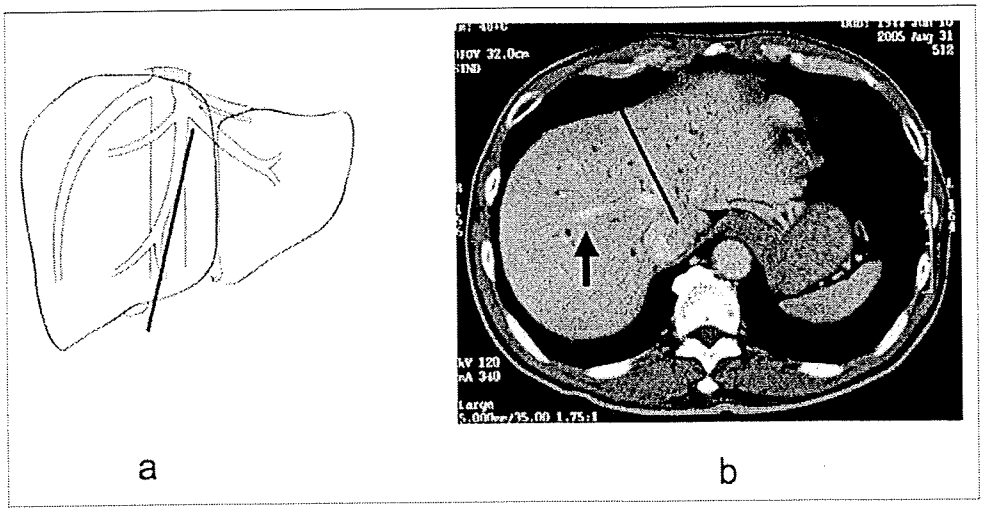


**Figure 1** A large drainage vein from segment 8 pouring into the middle hepatic vein in illustration (1a) and on CAT (1b).

completely on the line along the left side of the middle hepatic vein.

After transecting the liver parenchyma between the right and the left lobes by CUSA under inflow vascular clamp, it is possible to expose the hepatic hilar bile duct extensively without excising any liver volume (Fig. 3). The lower bile duct should be ligated and divided at the level of the intrapancreatic portion as low as possible to obtain a negative margin of the bile duct stump. It is possible to resect the upper bile duct at the level of the second-or third-order branch of the intrahepatic bile duct bilaterally (Fig. 4). Therefore, at the right side of the hepatic transected plane, there will be several bile duct stumps of the anterior and posterior segments. At the left side of hepatic transected plane, there will usually be 2 or 3 bile duct stumps, such as B2 from segment 2, B3 from segment 3 of the lateral segment, and B4 from the medial segment of the liver. After extensive resection of the hilar bile duct and extrahepatic bile duct, bilioenteric anastomosis can easily be performed with a single-layer interrupted suture of 5-0 PDSII (Ethicon) under sufficient surgical view in an end-to-side fashion by Roux-en-Y loop of the jejunum. Biliary stent tubes should routinely be placed in each anastomosis through the retrograde transhepatic route (Fig. 5). However, with bile duct stump diameter <2 mm, a thin biliary stent tube should be placed through the transjejunum route. Hemostasis on the liver transected surface should be carefully performed before the abdomen is closed.

## Results

The surgery data of 5 patients are listed in Table 1. After extensive hilar bile duct resection, bile duct stumps ranged in size from 3 to 7 mm (mean 4.4). Surgical margins at bile

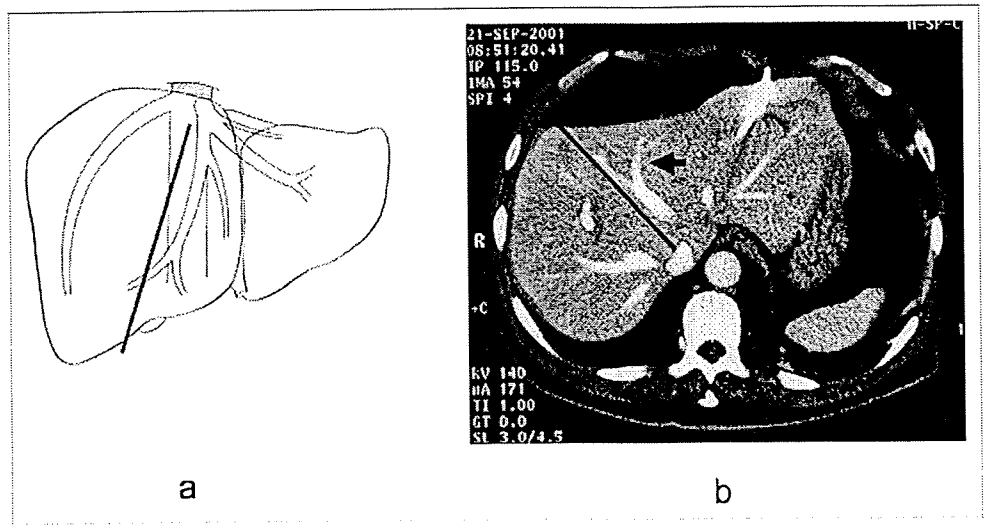


**Figure 2** A large drainage vein from segment 4 pouring into the middle hepatic vein in illustration (2a) and on CAT (2b).

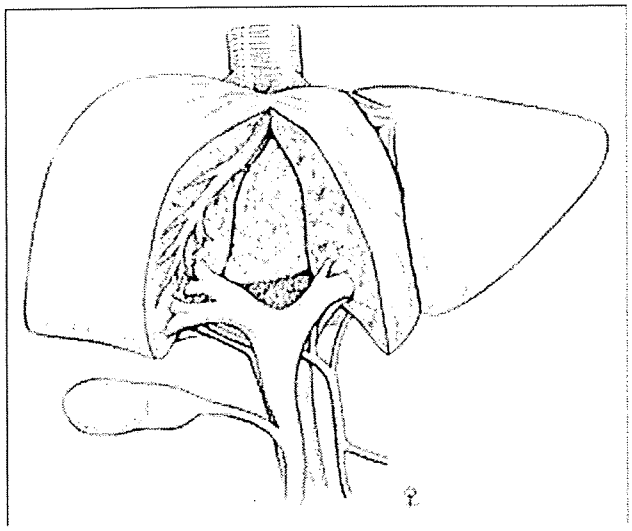

**Figure 3** Sufficient, extensive exposure of the hilar bile duct after liver transection.

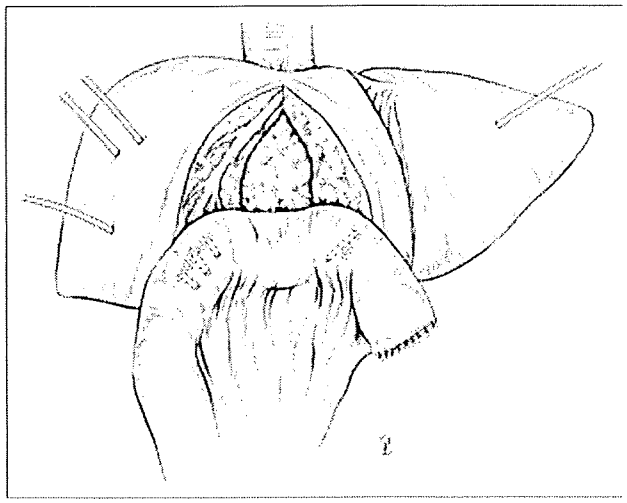

**Figure 5** Bilioenteric anastomosis, with sufficient surgical view, using a Roux-en-Y jejunal loop.

duct stumps were free of cancer in all 4 patients with hilar bile duct cancer. Surgery time ranged from 5 hours 32 minutes to 8 hours 13 minutes (mean 7 hours), and surgical blood loss ranged from 390 to 1400 g (mean 792). There were no surgical deaths, but 1 patient suffered from biliary fistula that required a lengthy hospital stay because of delayed healing. Hospital stays ranged from 28 to 97 days (mean 47). The postsurgical outcomes were as follows: 3 patients (2 with bile duct cancer and 1 with benign bile duct stricture) are alive (during early observation periods of 8 to 38 months) at the time of publication, and 2 cancer patients have died (Table 3).

## Comments

The transhepatic anterior approach was recently reported by Yamamoto et al[6] as useful in surgical resection for patients with liver cancer present in the caudate lobe and also by Chi-Leung et al[7] for resection in patients with hugeliver cancer. They also reported the usefulness of a transhepatic anterior approach in patients with liver cancer, especially in those who had undergone isolated caudate lobectomy for deeply localized liver cancer, and in patients with right hemihepatectomy for huge liver cancers, such as those invading the retroperitonium and diaphragma. Herein we propose the usefulness of a transhepatic approach for patients with bile duct disease at the hepatic hilus, such as benign hilar bile duct stricture, and patients with low-grade malignant or localized hilar cholangiocarcinoma, especially those who are unfit for major liver resection.

This approach can offer a sufficient surgical view to enable clear and extensive visualization of the hilar bile duct, including the second- and third-order branches of the intrahepatic bile duct. This may enable extensive hilar bile duct resection without excision of any volume of liver parenchyma, which may be appropriate for patients with liver dysfunction and for those in whom surgical major hepatectomy is contraindicated. Templeton and Dodd[8] and Waddell[9] previously reported the similar approach of anatomic separation of the

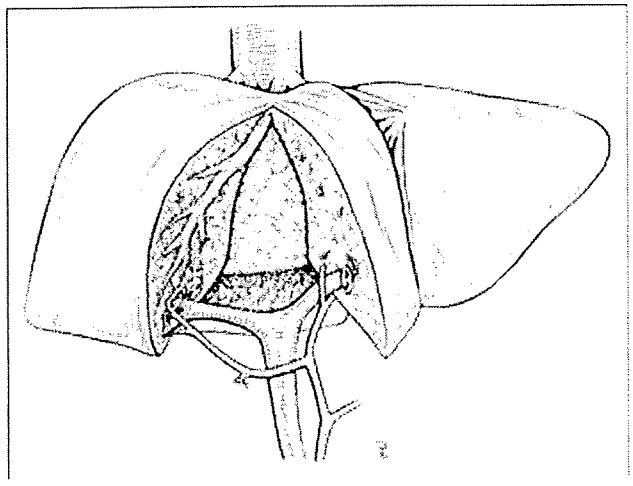

**Figure 4** Numerous intrahepatic bile duct stumps are clearly exposed after extensive hilar bile duct resection.

**Table 3** Outcomes of 5 patients who underwent surgical resection by way of transhepatic approach

| Patient no | Complications | Hospital stay (d) | Long-term outcome |
|---|---|---|---|
| 1 | — | 28 | Alive at 30 months |
| 2 | — | 53 | Alive at 28 months |
| 3 | Biliary fistula, pleural effusion | 97 | Alive at 23 months |
| 4 | — | 28 | Died at 38 months |
| 5 | Wound infection | 29 | Died at 29 months |

right and left lobes of the liver to expose intrahepatic bile ducts in the patients with upper bile duct stricture. However, they did not describe the liver transecting line in detail, especially concerning the point of hepatic venous congestion. We previously reported refined approaches to central hepatectomy for hilar cholangiocarcinoma, such as segment 1 and 4 hepatectomy, as parenchyma-preserving types of hepatectomy[10,11] and have shown their usefulness for patients for whom major hepatectomy would incur high risk. It has been reported in many previous articles that the caudate lobe should be excised in most patients with hilar cholangiocarcinoma because cancer invasion extends into the intrahepatic bile duct branch of segment 1.[12,13] Therefore, extensive hilar bile duct resection must be used only in patients with hilar cholangiocarcinoma in whom cancer invasion seems not to extend into the intrahepatic bile duct branches of segment 1. The correct evaluation of these findings may require meticulous preoperative imaging findings and may sometimes be difficult. In contrast, hilar bile duct tumor of low-grade malignancy may not invade deeply into the intrahepatic bile duct branch of segment 1.[4,5] In our patient (patient no. 1) with mucous-producing bile duct tumor, pathologic findings showed no clear invasion into the intrahepatic bile duct branch of segment 1.

The second advantage of this procedure is that it provides a sufficient surgical view after extensive hilar bile duct resection, thus facilitating recognition. Using the standard procedure, ie, hilar bile duct resection without liver parenchymal transection, it is usually difficult to obtain sufficient favorable surgical view for segment I resection when the surgeon is reconstructing the bilioenteric bypass. We did not encounter failure of bilioenteric anastomosis in any of the 5 patients in our series. In 1 patient (patient no. 3), biliary fistula appeared and persisted for a long time after surgery. However, in this patient, biliary fistula was induced by bile leakage from the transected liver surface, not from leakage of the bilioenteric anastomosis. The broad transected surface of the liver may be a disadvantage of the transhepatic approach. However, surgical blood loss was not such that blood transfusion was required in any patient in our series.

In the transhepatic approach, the liver parenchyma should be transected the length of the boundary between the right and left hepatic lobes, along which the middle hepatic vein runs. Therefore, a decision must be made as to along which side of the middle hepatic vein should the liver parenchyma be transected: the right side or the left side of the wall? When considering which to choose, it is important to recognize the segmental drainage veins from segments 4 and 8 into the middle hepatic vein. If the segmental drainage vein from segment 4 makes a confluence into the left hepatic vein, the transhepatic approach may be followed along the left side of the middle hepatic vein (Fig. 2). The segmental drainage vein from segment 8 is sometimes a major drainage vein from the anterior segment, with a confluence into the middle hepatic vein.[14,15] In such patients, right-sided transection should be avoided as much as possible to lessen the chance of postsurgical congestion and subsequent damage to the anterior segment of the liver (Fig. 1). Therefore, it is important to evaluate the drainage veins of segments 4 and 8 before and after surgery when the transhepatic anterior approach is used. In fact, there was no serious postsurgical liver dysfunction in any of the 5 patients of our series.

In conclusion, the transhepatic approach may be useful when performing extensive hilar bile duct resection in patients with biliary disease at the hepatic hilus, especially in high-risk patients unfit for major hepatectomy, as well as in the patients with benign bile duct strictures and low-grade malignancies.

## References


1. Bernard L. Simon WH, Guy JM. Current and future trends in management and treatment of Klatskin tumour. J Clin Oncol 2002;7:91–102.
2. William RJ. Cholangiocarcinoma of the extrahepatic bile ducts. Semin Surg Oncol 2000;19:156–76.
3. Steven AC. Biliary tract cancer. Surg Oncol;1997:273–307.
4. Shibahara H, Tamada S, Goto M, et al. Pathologic features of mucin-producing bile duct tumors: two histopathologic categories as counterparts of pancreatic intraductal papillary-mucinous neoplasms. Am J Surg Pathol 2004;28:327–38.
5. Oshikiri T, Kashimura N, Katanuma A, et al. Mucin-secreting bile duct adenoma—clinicopathological resemblance to intraductal papillary mucinous tumor of the pancreas. Dig Surg 2002;19:324–7.
6. Yamamoto J, Takayama T, Kosuge T, et al. An isolated caudate lobectomy by the transhepatic approach for hepatocellular carcinoma in cirrhotic liver. Surgery 1992;111:699–702.
7. Chi-Leung L, Sheuntg-Tat F, Chung-Mau L, et al. Anterior approach for major right hepatic resection for large hepatocellular carcinoma. Ann Surg 2000;232:25–31.
8. Templeton JY, Dodd GD. Anatomical separation of the right and left lobes of the liver for intrahepatic anastomosis of the biliary ducts. Ann Surg 1963;157:287–91.
9. Waddell WR. Exposure of intrahepatic bile ducts through interlobar fissure. Surg Gynecol Obstet 1967;124:491–500.
10. Miyazaki M, Ito H, Nakagawa K, et al. Segments I and IV resection as a new approach for hepatic hilar cholangiocarcinoma. Am J Surg 1998;175:229–31.
11. Miyazaki M, Ito H, Nakagawa K, et al. Parenchyma-preserving hepatectomy in the surgical treatment of hilar cholangiocarcinoma. J Am Coll Surg 1999;189:575–83.
12. Mizumoto R, Kawarada Y, Suzuki H, et al. Surgical treatment of hilar carcinoma of the bile duct. Surg Gynecol Obstet 1986;162:153–8.
13. Henri B, Richard N, Tom D. Management strategies in resection for hilar cholangiocarcinoma. Ann Surg 1992;215:31–8.
14. Sheung-Tat F, Chung-Mau L, Chi-Leung L, et al. Safety and necessity of including the middle hepatic vein in the right lobe graft in adult-to-adult live donor liver transplantation. Ann Surg 2003;238:137–48.
15. Sano K, Makuuchi M, Miki K, et al. Evaluation of hepatic venous congestion: proposed indication criteria for hepatic vein reconstruction. Ann Surg 2002;236:241–7.

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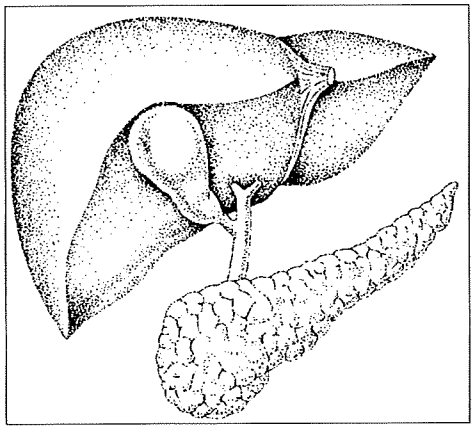

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# Risk factors for biliary tract and ampullary carcinomas and prophylactic surgery for these factors

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**Abstract**
Curative resection is the only treatment for biliary tract cancer that achieves long-term survival. However, patients with advanced biliary tract cancer have only a limited prognosis even after radical surgical resection. Thus, to improve the long-term results, the early detection of biliary tract cancer and subsequent cure seem to be essential. The purpose of this study was to review the literature concerning the risk factors for cancerous and precancerous lesions of the biliary tract, and prophylactic surgery for these factors. It has been reported that pancreaticobiliary maljunction (PBM) with bile duct dilatation is a risk factor for gallbladder cancer and bile duct cancer, while PBM without bile duct dilatation is a risk factor for gallbladder cancer. Thus, in the former group, a prophylactic excision of the common bile duct and gallbladder should be recommended, while in the later group, a prophylactic cholecystectomy without bile duct resection may be the appropriate surgical procedure. It has also been reported that primary sclerosing cholangitis (PSC) is a risk factor for cholangiocarcinoma. Patients with PSC often develop advanced cholangiocarcinoma with a poor prognosis. In patients with PSC, therefore, strict follow-up should be recommended. Adenoma and dysplasia have been regarded as precancerous lesions of gallbladder cancer. A polypoid lesion of the gallbladder that is sessile, has a diameter greater than 10 mm, and /or grows rapidly, is highly likely to be cancerous and should be resected. Although gallstones seem to be closely associated with gallbladder cancer, there is no evidence of a direct causal relationship between gallstones and gallbladder cancer. Thus, a cholecystectomy is not advised for asymptomatic cholecystolithiasis. Controversy remains as to whether adenomyomatosis of the gallbladder and porcelain gallbladder are associated with gallbladder cancer. With respect to ampullary carcinoma, adenoma of the ampulla is considered to be a precancerous lesion. This article discusses the risk factors for cancerous and precancerous lesions of the biliary tract and prophylactic treatment for these factors.

**Key words** Biliary tract neoplasms · Risk factors · Prophylaxis therapy · Gallstones · Pancreaticobiliary maljunction · Precancerous conditions · Gallbladder · Guidelines

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## Introduction

One of the possible causes of biliary tract cancer may be chronic and continuous stimulation of the biliary tract, and cholangitis due to gallstones and the reflux of pancreatic juice into the biliary tract.[1,2] The underlying diseases that potentially cause such a condition include pancreaticobiliary maljunction (PBM),[3] primary sclerosing cholangitis (PSC),[4–7] chronic cholecystitis,[1] gallstones,[1,8] and adenomyomatosis.[9] In such patients, chronic inflammation potentially causes pathological changes of the biliary epithelium resulting in precursors of biliary tract cancer.[1]

Although curative resection is the only treatment for biliary tract cancer that achieves long-term survival, patients with advanced cancer have only a limited prognosis even after radical surgical resection. To improve the long-term results, therefore, the early detection of precancerous and cancerous lesions, and subsequent cure seem to be essential.

In this article, we discuss the predisposing factors for bile duct cancer, gallbladder cancer, and ampullary car-

cinoma, using a format of clinical questions (CQs) and responses. In the responses to the CQs, recommendations for treatment are noted (grades of these recommendations are defined in Table 1[10]). Also, levels of evidence are given (in parentheses) for findings in reference citations (see definitions of levels in Table 2[10]).

**CQ 1 What are the risk factors for biliary tract and ampullary carcinomas?**

***Pancreaticobiliary maljunction (PBM) with bile duct dilatation and primary sclerosing cholangitis (PSC) are risk factors for biliary tract cancer (see side memos 1 and 2). PBM, particularly that without bile duct dilatation, is a risk factor for gallbladder cancer.***

***There are no evident risk factors for ampullary carcinoma.***

The prevalence rate of biliary tract cancer varies in different geographical regions. Chile and Japan have the highest rate in the world, followed by East Asia and India (level V).[1,2] Also, there are age differences in the incidence of biliary tract cancer.[1] It has been reported that the risk factors for biliary tract cancer are, possibly, chronic and continuous stimulation and inflammation of the biliary tract.[1,2] Polypoid lesions of the gallbladder and adenomyomatosis have been regarded as a risk factors for gallbladder cancer. In this section, we review the respective risk factors for bile duct cancer, gallbladder cancer, and ampullary carcinoma.

**Table 1.** Strength of recommendations[10]

| |
|---|
| A, Strongly recommend performing the clinical action |
| B, Recommend performing the clinical action |
| C1, The clinical action may be considered although there is a lack of high-level scientific evidence for its use. May be useful |
| C2, Clinical action not definitively recommended because of insufficient scientific evidence. Evidence insufficient to support or deny usefulness |
| D, Recommend not performing the clinical action |

**Table 2.** Levels of evidence[10]

| | |
|---|---|
| Level I | Systematic review/meta-analysis |
| Level II | One or more randomized clinical trials |
| Level III | Nonrandomized controlled trials |
| Level IV | Analytic epidemiology (cohort studies and case-control studies) |
| Level V | Descriptive study (case reports and case-series studies) |
| Level VI | Opinions of expert panels and individual experts not based on patient's data |

## Risk factors for bile duct cancer

*Pancreaticobiliary maljunction (see CQ 2)*

A retrospective nationwide survey (1990 to 1999) of PBM in Japan revealed that 10.6% of PBM patients with bile duct dilatation were complicated by biliary tract cancer, and 33.6% of these biliary tract cancers were bile duct cancer[3] (level IV). PBM with bile duct dilatation is considered as a risk factor for bile duct cancer.

*Primary sclerosing cholangitis (PSC)*

Patients wit PSC carry an increased risk of bile duct cancer. Five percent to 10% of PSC patients develop bile duct cancer[4–7] (level V). Bile duct cancer associated with PSC is often advanced with a poor prognosis. PSC, therefore, should be recognized as a risk factor for bile duct cancer.

Controversy remains as to whether bile duct cancer is related to chronic inflammation due to gallstones or some gene mutations[8,11,12] (level IV).

## Risk factors for gallbladder cancer

*Pancreaticobiliary maljunction (see CQ 2)*

There have been many studies that reported PBM as a risk factor for gallbladder cancer. A nationwide survey (1990 to 1999) of PBM revealed that the prevalence rate of biliary tract cancer was 10.6% in the group with bile duct dilatation, while the prevalence rate was 37.9% in the group without bile duct dilatation[3] (level IV). With respect to the PBM patients who developed biliary tract cancer, the incidence of gallbladder cancer was 64.9% in those with PBM with bile duct dilatation, whereas the incidence was 93.2% in those with PBM without bile duct dilatation. Therefore, PBM is an evident risk factor for gallbladder cancer. Of note, the frequency of gallstones in patients with PBM associated with gallbladder cancer is low.[13]

*Gallstones and porcelain gallbladder (see CQ 3)*

It has been well established that gallstones are closely associated with gallbladder cancer[1,8] (level V). It has also been reported that a stone size of more than 3 cm,

a family history of gallbladder cancer, and the duration of cholelithiasis are potential risk factors for developing gallbladder cancer.[1,14–16] However, there is no evidence of a direct causal relationship between gallstones and gallbladder cancer. Gracie and Ransohoff[17] followed-up the subsequent history of 123 patients with asymptomatic gallstones for 10 years or longer, and revealed that there was no case of gallbladder cancer reported among that group.

Controversy remains as to whether patients with "porcelain gallbladder" carry a risk of gallbladder cancer. It has been reported that porcelain gallbladder is often complicated by gallbladder carcinoma[18,19] (level IV), while another report suggests that porcelain gallbladder is not associated with gallbladder carcinoma[20] (level IV).

*Adenoma of the gallbladder (Figs. 1 and 2; also see CQ 4)*

There is consensus regarding the existence of two models through which malignant transformation is produced: the adenoma-carcinoma sequence and the dysplasia-carcinoma sequence. Intestinal and gastric metaplasias appear to be the pathway through which epithelial dysplasia is produced.[21] Yamagiwa[22] examined 110 cases of resected gallbladder carcinoma and found dysplasia adjacent to carcinoma in 46 of the 110 cases, and this change was frequently found in lesions at an early stage and in well-differentiated carcinoma (level V).

Kubota et al.[23] reported that in patients with polypoid lesions of the gallbladder, the respective diameters of adenomas and cancers were 6.9 mm (range, 4 to 13 mm) and 25.7 mm (range, 5 to 50 mm). In that study, 75% of the adenomas and 13% of the cancers had a diameter of less than 10 mm. It has also been reported that when a polypoid lesion of the gallbladder is sessile, has a diameter greater than 10 mm, and /or grows rapidly, it is highly likely to be cancerous.[23–25] In such cases, surgical resection should be recommended.

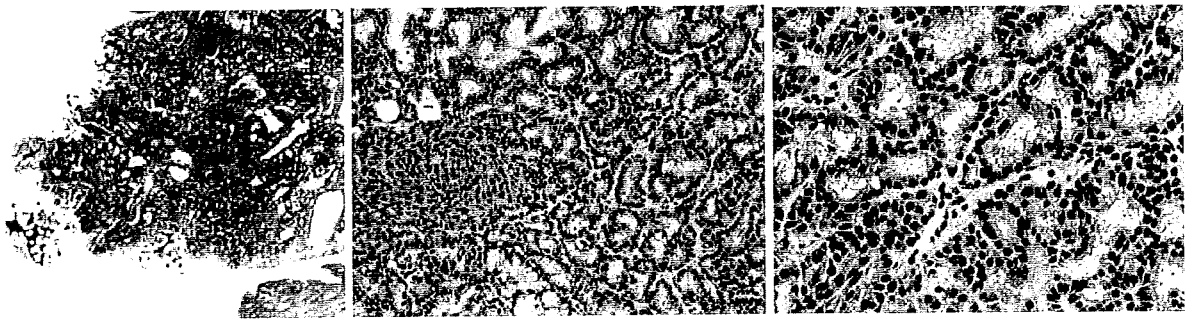
*Adenomyomatosis (Fig. 3; 4)*

Adenomyomatosis has not been considered to have malignant potential.[1] Nabatame et al.[9] studied the relationship between adenomyomatosis and gallbladder cancer by examining 4560 gallbladders (2031 from male patients and 2529 from female patients; age 14 to 94 years) resected for gallbladder cancer, gallstones, or other diseases. In that study, the incidence of gallbladder carcinoma was higher in patients with segmental adenomyomatosis (22/334; 6.6%) than in those without (181/4226; 4.3%; $P = 0.049$). This difference was more marked in patients equal to or older than 60 years of age ($P < 0.001$). However, the magnitude of risk for gallbladder cancer in patients with adenomyomatosis has not been clearly established.[1,26]

**Risk factors for ampullary carcinoma**

Kimura et al.[27] histologically investigated the papilla of Vater in 576 autopsy cases of elderly people and revealed that the incidences of group 3 and 4 epithelia in the common channel were significantly higher than those in the intraduodenal portion of the bile duct, pancreatic duct, or duodenal epithelia. They also investigated

**Fig. 1.** Macroscopic photograph of adenoma of the gallbladder



a b,c

**Fig. 2a–c.** Histological examination of adenoma of the gallbladder. **a** Low magnification; **b** intermediate magnification; **c** high magnification (H&E)

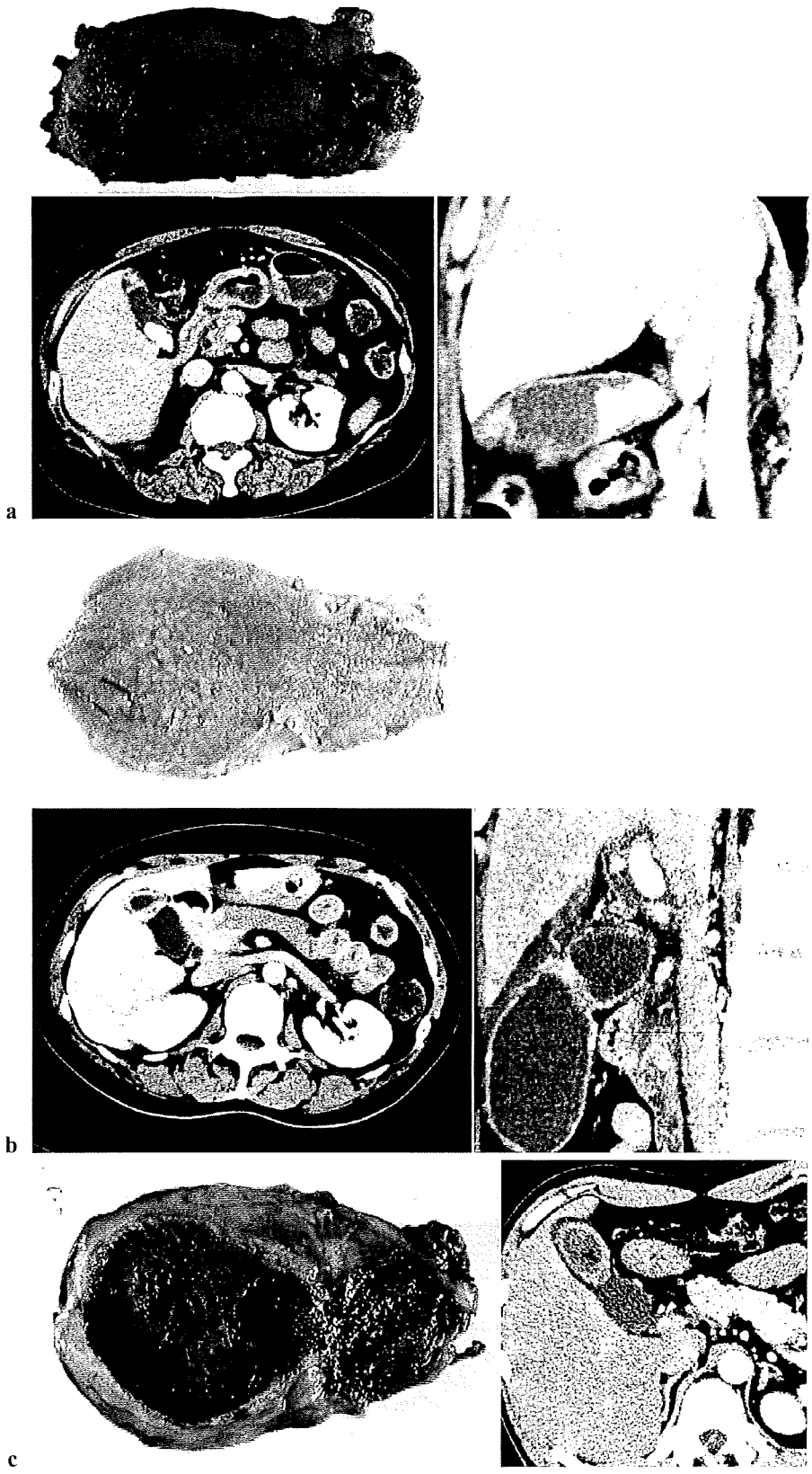


**Fig. 3a–c.** Findings in adenomyomatosis. **a** Fundal type: circumscribed hypertrophy of the fundus of the gallbladder with Rokitansky-Aschoff sinus. Case complicated by cholecystolithiasis. *Upper,* resected specimen; *lower left,* abdominal computed tomography (CT; cross section); *lower right,* abdominal CT (sagittal section). **b** Segmental type: hypertrophy circumscribing the gallbladder with Rokitansky-Aschoff sinus. *Upper,* resected specimen; *lower left,* abdominal computed tomography (CT; cross section); *lower right,* abdominal CT (sagittal section). **c** Diffuse (segmental-diffuse) type: hypertrophy and Rokitansky-Aschoff sinus from the gallbladder body to the fundus. Gallstones in the fundus. *Left,* resected specimen; *right,* abdominal CT

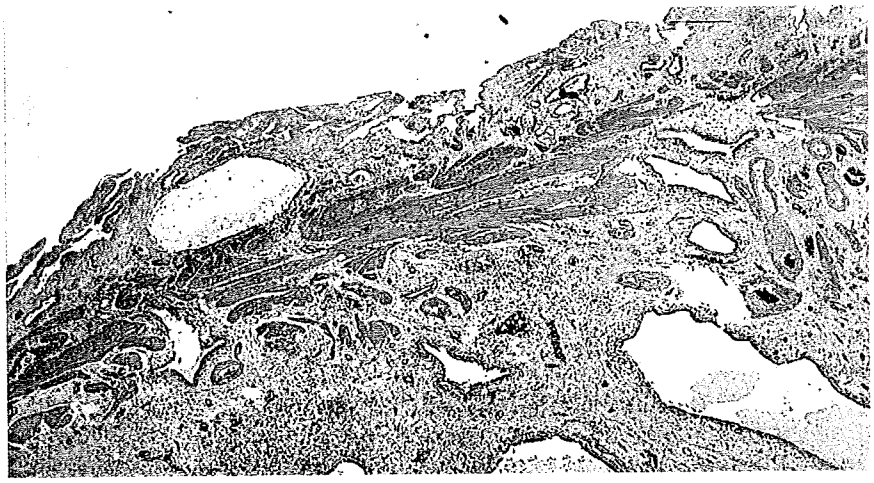


**Fig. 4.** This Loupe image shows growing and dilating Rokitansky-Aschoff sinuses from the muscularis propria to the subserosa, and the growing smooth muscle fiber and collagen fiber surrounding them (H&E)

resected specimens from the patients with carcinoma of the papilla of Vater and found tha the common channel was the most frequent site for the possible origin of carcinoma (level IV). These results suggest that the common channel is the most important site in the pathogenesis of carcinoma of the papilla of Vater. With regard to the incidence of "adenoma" surrounding carcinoma of the papilla of Vater, the values have been reported to range from 82% to 91%[27] (level IV). Therefore, the adenoma-carcinoma sequence is very important in the pathogenesis of carcinoma of the papilla of Vater. In addition, familial adenomatous polyposis (FAP) is notable for the risk of adenoma in the ampulla of Vater.

**Side memo 1**
**Primary sclerosing cholangitis (PSC)**

Definition: recurrent or persistent chronic inflammatory disease of extra- and intrahepatic bile ducts resulting in obliterative fibrosis. No effective treatment has been discovered to date, and liver transplant is required in the terminal stage. Although autoimmune abnormality is suspected, the underlying pathogenesis is still unknown.

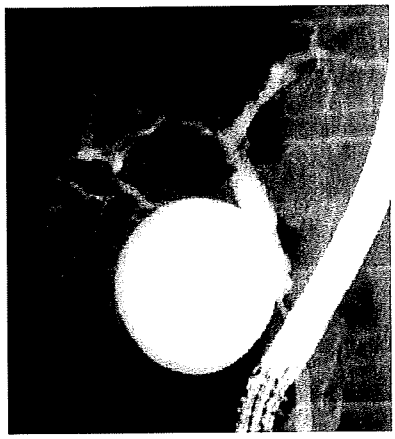


**Fig. 5.** Endoscopic retrograde cholangiopancreatography image of PSC. Multifocal stricturing of intrahepatic and extrahepatic bile ducts, and shaggy appearance of extrahepatic bile ducts

Diagnostic criteria

1. Typical radiological findings
   Typical radiological findings of bile duct are;
   Multifocal stricturing
   — Beaded appearance; short or/and annular strictures interspersed among normal or slightly inflamed ducts. This appearance is thought to reflect the fibrous stricture portions and the normal segments of the bile duct. One-fifth of the cases present band-like strictures that are extremely short, and one-fourth present diverticulum-like outpouchings
   — Pruned-tree appearance; diminished arborization of the intrahepatic ducts
   — Shaggy appearance; diffuse mural irregularities of extrahepatic bile ducts
2. Typical clinical presentation
   Important clinical history: inflammatory bowel disease, bile obstruction
   Blood examination: high alkaline phosphatase (ALP; two or three times upper limit of normal) for 6 months or more
3. Deductive diagnosis
   Exclude secondary sclerotic cholangitis as follows:
   — Infectious cholangitis due to AIDS
   — Malignant neoplasms or similar disease in the bile duct (excluding PSC accompanied by early-stage cholangiocarcinoma)
   — Previous surgery of the biliary tract (excluding cholecystectomy)
   — Bacterial cholangitis accompanying biliary tract stricturing or biliary calculus
   — Ischemic cholangitis due to floxuridine

**Side memo 2**
**Diagnostic criteria of pancreaticobiliary maljunction (PBM; see Fig 6)**

Definition: PBM is a congenital anomaly consisting of a union of the pancreatic and bile ducts located outside the duodenal wall.

Diagnostic criteria: PBM is diagnosed by either radiological or anatomical findings

1) Radiological findings
It is necessary to confirm the lack of sphincter action at the union of the pancreatic and bile ducts. However, because clarification of the lack of sphincter action is often difficult, the following radiological findings of endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTHC), intraoperative cholangiography, or similar methods are used to verify whether there is a long common channel or a complicated confluence of the pancreatic and bile ducts.
2) Anatomical findings
Confirm the abnormal anatomical confluence of the pancreatic and bile ducts outside the duodenal wall or confirm the complicated confluence by surgery, autopsy, or other procedures.

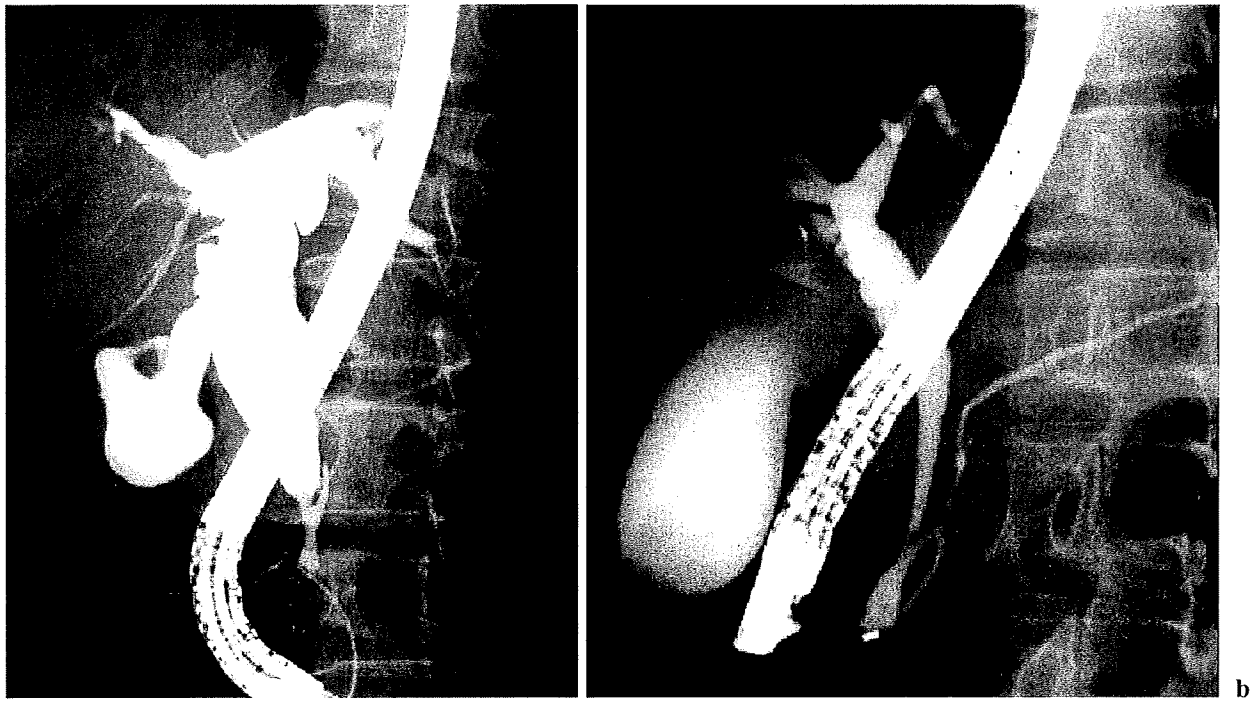



**Fig. 6a,b.** Endoscopic retrograde cholangiopancreatography (ERCP) image of pancreaticobiliary maljunction (PBM): a patient with congenital bile duct dilatation (Kotani IV a type). **a** The pancreatic duct joins the biliary duct. **b** Pancreaticobiliary maljunction without bile duct dilatation (Fig 6a, with permission from Koyanagi K, Aoki T, editors. *Pancreaticobiliary maljunction*. Tokyo: Igaku Tosho Shuppan: 2002. p 25, Fig. 4[29])

**CQ 2 Is prophylactic treatment necessary for pancreaticobiliary maljunction (PBM)?**

***PBM with bile duct dilatation is a risk factor for bile duct and gallbladder cancer. Prophylactic excision of the gallbladder and common bile duct should be recommended for PBM with bile duct dilatation (recommendation grade C1)***

***PBM without bile duct dilatation is a risk factor for gallbladder cancer. A prophylactic cholecystectomy is the appropriate surgical procedure for PBM without bile duct dilatation. (recommendation grade B)***

As mentioned above, a retrospective nationwide survey (1990 to 1999) of PBM revealed that the incidence of biliary tract cancer was 10.6% in the group with bile duct dilatation; of these biliary tract cancers, 64.9% were gallbladder carcinoma and 33.6% were bile duct cancer.[3] Therefore, PBM with bile duct dilatation should be considered as a risk factor for bile duct cancer and gallbladder cancer, and in these patients, prophylac-

and an inflammatory polyp in 1. The respective diameters of the adenomas and cancers were 6.9 mm (range, 4 to 13 mm) and 25.7 mm (range, 5 to 50 mm); 75% of the adenomas and 13% of the cancers had a diameter of less than 10 mm (level IV). Chijiiwa and Tanaka[36] also examined 44 patients who underwent cholecystectomy for polypoid lesions of the gallbladder, and reported that the sex ratio, symptoms, and the presence of gallstones were not significantly different between patients with carcinoma and those with benign polypoid lesions, and that the size (>10 mm), number of polypoid lesions (single), and age (> or =60 years) were significant indicators of carcinoma.

It has been reported that when a polypoid lesion of the gallbladder is sessile, has a diameter greater than 10 mm, and/or grows rapidly, it is highly likely to be cancerous[23–25,36–39] (level IV). In such cases, surgical resection should be considered.

**Side memo 3**
**Polypoid lesions of the gallbladder (see Figs. 7–10)**

Definition: "Polypoid" is the general term for torous lesions protruding into the lumen of the gallbladder, no matter whether they are neoplastic or nonneoplastic. The majority of polypoid lesions are nonneoplastic lesions such as cholesterol polyp, adenomyomatosis, or inflammatory polyp. Benign neoplastic lesions include adenoma and metaplastic polyp, while malignant neoplastic lesions include gallbladder cancer

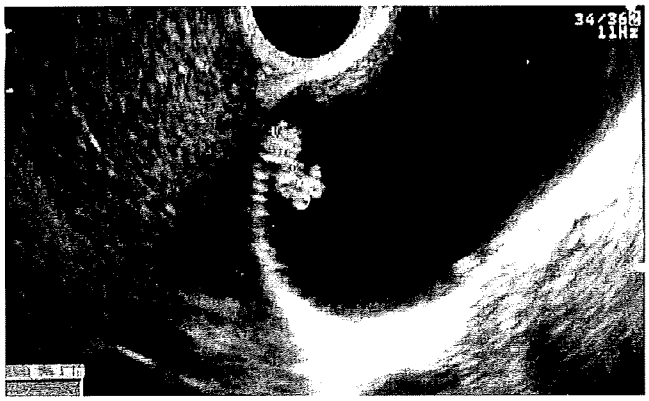


**Fig. 7.** Ultrasound image of a cholesterol polyp: sessile polyp that has a higher echoic signal than the liver

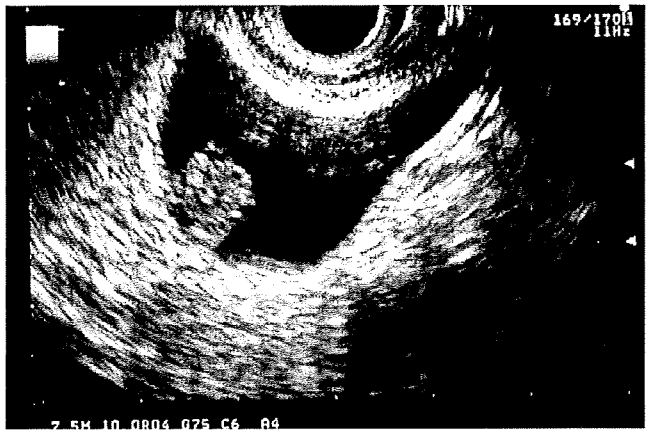


**Fig. 8.** Ultrasound image of adenoma of the gallbladder: this image indicates a semipedunculate polyp with isoechoic signal

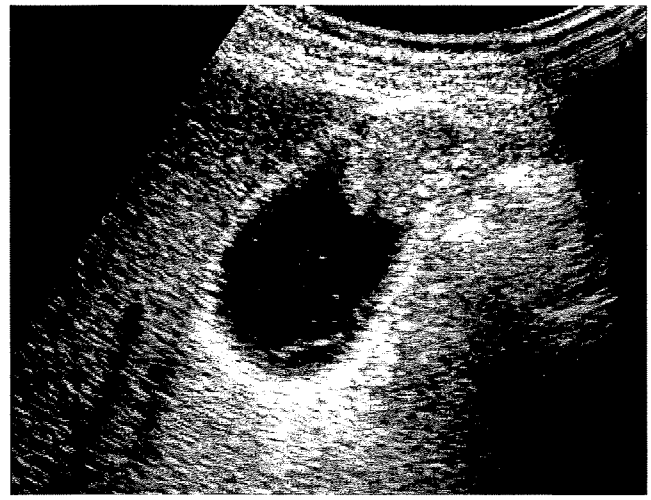


**Fig. 9.** Ultrasound image of early-stage gallbladder carcinoma: a pedunculated protrusion with a relatively high echoic signal. Depth of tumor invasion is limited to the mucosal layer

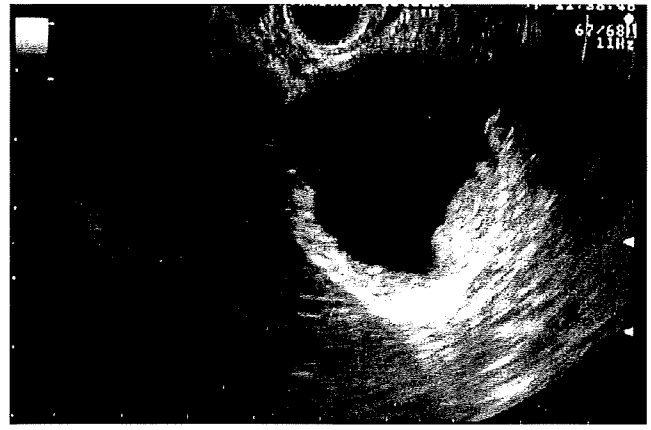


**Fig. 10.** Ultrasound image of gallbladder carcinoma: sessile protruding lesion. Depth of tumor invasion is to the subserosa

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## References

1. Sheth S, Bedford A, Chopra S. Primary gallbladder cancer: recognition of risk factors and the role of prophylactic cholecystectomy. Am J Gastroenterol 2000;95:1402–10.
2. Matsukura N, Yokomuro S, Yamada S, Tajiri T, Sundo T, Hadama T, et al. Association between *Helicobacter bilis* in bile and biliary tract malignancies: *H. bilis* in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract. Jpn J Cancer Res 2002;93:842–7.
3. Tashiro S, Imaizumi T, Ohkawa H, Okada A, Katoh T, Kawaharada Y, et al. Pancreaticobiliary maljunction: retrospective and nationwide survey in Japan. J Hepatobiliary Pancreat Surg 2003;10:345–51.
4. Rosen CB, Nagorney DM, Wiesner RH, Coffey RJ, LaRusso NF. Cholangiocarcinoma complicating primary sclerosing cholangitis. Ann Surg 1991;213:21–5.
5. Rosen CB, Nagorney DM. Cholangiocarcinoma complicating primary sclerosing cholangitis. Semin Liver Dis 1991;11:26–30.
6. Callea F, Sergi C, Fabbretti G, Brisigotti M, Cozzutto C, Medicina D. Precancerous lesions of the biliary tree. J Surg Oncol Suppl 1993;3:131–3.
7. Franco J, Saeian K. Biliary tract inflammatory disorders. primary sclerosing cholangitis and primary biliary cirrhosis. Curr Gastroenterol Rep 1999;1:95–101.
8. Hsing AW, Bai Y, Andreotti G, Rashid A, Deng J, Chen J, et al. Family history of gallstones and the risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. Int J Cancer 2007;121:832–8.
9. Nabatame N, Shirai Y, Nishimura A, Yokoyama N, Wakai T, Hatakeyama K. High risk of gallbladder carcinoma in elderly patients with segmental adenomyomatosis of the gallbladder. J Exp Clin Cancer Res 2004;23:593–8.
10. Takada T, Miyazaki M, Miyakawa S, Tsukada K, Nagino M, Kondo S, et al. Purpose, use, and preparation of clinical practice guidelines for the management of biliary tract and ampullary carcinomas. J Hepatobiliary Pancreat Surg 2008;15:2–6.
11. Su WC, Shiesh SC, Liu HS, Chen CY, Chow NH, Lin XZ. Expression of oncogene products HER2/Neu and Ras and fibrosis-related growth factors bFGF, TGF-beta, and PDGF in bile from biliary malignancies and inflammatory disorders. Dig Dis Sci 2001;46:1387–92.
12. Matsumoto Y, Fujii H, Itakura J, Matsuda M, Yang Y, Nobukawa B, Suda K. Pancreaticobiliary maljunction: pathophysiological and clinical aspects and the impact on biliary carcinogenesis. Langenbecks Arch Surg 2003;388:122–31.
13. Tanaka K, Nishimura A, Yamada K, Ishibe R, Ishizaki N, Yoshimine M, et al. Cancer of the gallbladder associated with anomalous junction of the pancreatobiliary duct system without bile duct dilatation. Br J Surg. 1993;80:622–4.
14. Kumar JR, Tewari M, Rai A, Sinha R, Mohapatra SC, Shukla HS. An objective assessment of demography of gallbladder cancer. J Surg Oncol. 2006;93:610–4.
15. Diehl AK. Gallstone size and the risk of gallbladder cancer. JAMA. 1983;250:2323–6.
16. David FR, William AG. Treatment of gallstones. Ann Intern Med 1993;119:606–19.
17. Gracie WA, Ransohoff DF. The natural history of silent gallstones. N Engl J Med 1982;307:798–800.
18. Stephen AE, Berger DL. Carcinoma in the porcelain gallbladder: a relationship revisited. Surgery 2001;129:699–703.
19. Tazuma S, Kajiyama G. Carcinogenesis of malignant lesions of the gallbladder. The impact of chronic inflammation and gallstones. Langenbecks Arch Surg 2001;386:224–9.
20. Towfigh S, McFadden DW, Cortina GR, Thompson JE, Tompkins RK, Chandler C, et al. Porcelain gallbladder is not associated with gallbladder carcinoma. Am Surg 2001;67:7–10.
21. Roa I, de Aretxabala X, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer. J Surg Oncol 2006;93:615–23.
22. Yamagiwa H. Mucosal dysplasia of gallbladder: isolated and adjacent lesions to carcinoma. Jpn J Cancer Res 1989;80:238–43.
23. Kubota K, Bandai Y, Noie T, Ishizaki Y, Teruya M, Makuuchi M. How should polypoid lesions of the gallbladder be treated in the era of laparoscopic cholecystectomy? Surgery 1995;117:481–7.
24. Ishikawa O, Ohhigashi H, Imaoka S, Nakaizumi A, Kitamura T, Sasaki Y, et al. The difference in malignancy between pedunculated and sessile polypoid lesions of the gallbladder. Am J Gastroenterol 1989;84:1386–90.
25. Sugiyama M, Atomi Y, Yamato T. Endoscopic ultrasonography for differential diagnosis of polypoid gallbladder lesions: analysis in surgical and follow up series. Gut 2000;46:250–4.
26. Aldridge MC, Bismuth H. Gallbladder cancer: the polyp-cancer sequence. Br J Surg 1990;77:363–4.
27. Kimura W, Futakawa N, Zhao B. Neoplastic diseases of the papilla of Vater. J Hepatobiliary Pancreat Surg 2004;11:223–31.
28. Sekido H, Shomada H, Morita T, Matsuo K, Kunihoro O, Miura Y, et al. Indications for surgery and optimal extent of resection of the bile duct: surgical treatment for pancreaticobiliary maljunction. In: Koyanagi K, Aoki T, editors. Pancreaticobiliary maljunction. Tokyo: Igaku Tosho Shuppan; 2002. p. 303–10.
29. Kobayashi S, Asano T, Yamasaki M, Kenmochi T, Saigo K, Ochiai T. Prophylactic excision of the gallbladder and bile duct for patients with pancreaticobiliary maljunction. Arch Surg. 2001;136:759–63.
30. Ohuchida J, Chijiiwa K, Hiyoshi M, Kobayashi K, Konomi H, Tanaka M. Long-term results of treatment for pancreaticobiliary maljunction without bile duct dilatation Arch Surg. 2006;141:1066–70.
31. Masuhara S, Kasuya K, Aoki T, Yoshimatsu A, Tsuchida A, Koyanagi Y. Relation between K-ras codon 12 mutation and p53 protein overexpression in gallbladder cancer and biliary ductal epithelia in patients with pancreaticobiliary maljunction. J Hepatobiliary Pancreat Surg 2000;7:198–205.
32. Ando H, Ito T, Nagaya M, Watanabe Y, Seo T, Kaneko K. Pancreaticobiliary maljunction without choledochal cysts in infants and

children: clinical features and surgical therapy. J Pediatr Surg 1995;30:1658–62.
33. Matsubara T, Zhi LZ, Miura H, Sakurai Y, Imazu H, Imai H, et al. High carcinogenic potential of biliary epithelium in patients with pancreaticobiliary maljunction. In: Koyanagi K, Aoki T, editors. Pancreaticobiliary maljunction. Tokyo: Igaku Tosho Shuppan; 2002. p. 285–94.
34. Maringhini A, Moreau JA, Melton LJ 3rd, Hench VS, Zinsmeister AR, DiMagno EP. Gallstones, gallbladder cancer, and other gastrointestinal malignancies. An epidemiologic study in Rochester, Minnesota. Ann Intern Med 1987;107:30–5.
35. Attili AF, De Santis A, Capri R, Repice AM, Maselli S. The natural history of gallstones: the GREPCO experience. The GREPCO Group. Hepatology 1995;21:655–60.
36. Chijiiwa K, Tanaka M. Polypoid lesion of the gallbladder: indications of carcinoma and outcome after surgery for malignant polypoid lesion. Int Surg 1994;79:106–9.
37. Myers RP, Shaffer EA, Beck PL. Gallbladder polyps: epidemiology, natural history and management. Can J Gastroenterol 2002;16:187–94.
38. Mainprize KS, Gould SWT, Gilbert JM. Surgical management of polypoid lesions of the gallbladder. Br J Surg 2000;87:414–7.
39. Akatsu T, Aiura K, Shimazu M, Ueda M, Wakabayashi G, Tanabe M, et al. Can endoscopic sonography differentiate nonneoplastic from neoplastic gallbladder polyps? Dig Dis Sci 2006;51:416–21.

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# FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer

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Pancreatic cancer has one of the highest mortalities among all malignancies and there is an urgent need for new therapy. This might be achieved by resolving the detailed biological mechanism, and in this study we examined how pancreatic cancer cells develop aggressive properties by focusing on signalling through the fibroblast growth factor (FGF)10 and FGF receptor (FGFR)2, which play important roles in pancreatic organogenesis. Immunostaining of pancreatic cancer tissues showed that FGFR2 was expressed in cancer cells, whereas FGF10 was expressed in stromal cells surrounding the cancer cells. Patients with high FGFR2 expression in cancer cells had a shorter survival time compared to those with low FGFR2 expression. Fibroblast growth factor 10 induced cell migration and invasion of CFPAC-1 and AsPC-1 pancreatic cancer cells through interaction with FGFR2-IIIb, a specific isoform of FGFR2. Fibroblast growth factor 10 also induced expression of mRNA for membrane type 1-matrix metalloproteinase (MT1-MMP) and transforming growth factor (TGF)-$\beta$1, and increased secretion of TGF-$\beta$1 protein from these cell lines. These data indicate that stromal FGF10 induces migration and invasion in pancreatic cancer cells through interaction with FGFR2, resulting in a poor prognosis. This suggests that FGF10/FGFR2 signalling is a promising target for new molecular therapy against pancreatic cancer.
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**Keywords:** pancreatic cancer; fibroblast growth factor 10; fibroblast growth factor receptor 2; cancer stromal cell



Pancreatic cancer has one of the highest mortalities among all malignancies, and is the fourth most common cause of cancer death in the United States and the fifth in Japan (Li *et al*, 2004; Willett *et al*, 2005). Although significant advances are now being made into the management of the disease, the 5-year survival rate still remains poor (Willett *et al*, 2005; Ghaneh *et al*, 2007). Therefore, there is an urgent need for new therapies for pancreatic cancer, based on an improved understanding of the molecular biology of the disease. The high mortality rate of pancreatic cancer is, in part, owing to difficulties of early diagnosis, the high incidence of metastatic disease at the time of diagnosis, and rapid progression of the disease. In addition, although newer adjuvant modalities are greatly increasing the prognosis (Ghaneh *et al*, 2008), most patients who undergo the surgery eventually relapse and die of the disease, even with curative resection (Li *et al*, 2004; Willett *et al*, 2005).

An understanding of the mechanisms underlying the biological aggressiveness of pancreatic cancer may be key for development of new therapies. Therefore, in this study we examined the molecular mechanisms underlying cellular invasion and metastasis of pancreatic cancer cells. Cellular and genetic studies have shown that tumour growth is not determined by malignant cancer cells alone, but also by cells in the tumour stroma. Supply of oxygen and nutrients by endothelial cells of blood vessels are critical for maintenance of the tumour microenvironment, and stromal fibroblasts are the principal source of extracellular matrix, which serves as a scaffold for cancer cells (Kalluri and Zeisberg, 2006). In addition, recent studies have revealed more active roles of stromal cells in tumour initiation and progression through direct interaction with tumour cells. For example, stromal cell-derived factor-1 (SDF-1/CXCL12) released from fibroblasts promotes cancer cell proliferation through a specific receptor, CXCR4, in several types of malignancies, including breast cancer (Orimo *et al*, 2005) and pancreatic cancer (Koshiba *et al*, 2000; Marchesi *et al*, 2004). Immune cells also play important roles in cancer progression (Pollard, 2004); for example, tumour-associated macrophages induced by colony-stimulating factor 1 promote invasiveness of cancer cells (Lin *et al*, 2001). Given this background, we hypothesized that stromal cell–cancer cell interactions have an important role in acquisition of the aggressive character by pancreatic cancer, and we examined signalling molecules that may be associated with this mechanism.

The molecular mechanisms underlying carcinogenesis are often similar to those in organogenesis. Interactions between stromal and parenchymal cells are important during organ development, and signals from stromal cells regulate epithelial cell growth and differentiation in pancreatic development. The classical tissue recombinant study by Golosow and Grostein (1962) showed that growth and morphogenesis of the developing pancreas depend on mesenchymal interactions, and more recently advances in molecular biology have allowed the molecular basis of this interaction to be established. We have shown that signals from endothelial cells and mesenchymal cells surrounding the

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pancreatic bud are crucial for initiation of pancreatic development from endoderm (Yoshitomi and Zaret, 2003; Jacquemin *et al*, 2006). Especially, we found that fibroblast growth factor-10 (FGF10) from mesenchymal cells maintained expression of Ptf1a, a critical transcription factor for initiation of pancreatic development, in pancreatic progenitor cells (Jacquemin *et al*, 2006). Mice deficient in FGF10 or the FGF receptor-2 (FGFR2)/IIIb isoform, the specific receptor for FGF10 (Igarashi *et al*, 1998), show impaired pancreatic development (Bhushan *et al*, 2001; Pulkkinen *et al*, 2003). However, it is unknown if FGF10/FGFR2-IIIb-signalling is associated with carcinogenesis in pancreatic cancer. In this study, we show that FGF10/FGFR2-signalling has an important role in pancreatic cancer progression, and we suggest that these results may lead to a new therapy and a better prognosis for patients with pancreatic cancer.

## MATERIALS AND METHODS

### Patients and tissue samples

Pancreatic cancer tissues were obtained from 76 pancreatic cancer patients who underwent curative surgical resection in the Department of General Surgery, Chiba University Hospital, Chiba, Japan, from June 2001 to April 2006. All patients were diagnosed histologically as primary invasive pancreatic ductal carcinoma. The patient characteristics are summarised in Table 1. The study protocol was approved by the Ethics Committee of our institute and written informed consent was obtained from all patients.

### Immunohistochemistry

Paraffin-embedded tissues were cut into 4 $\mu$m serial sections and deparaffinised. The sections were placed in citrate buffer (10 mmol l$^{-1}$ pH 6.0) with 0.2% Tween 20 and boiled in a microwave oven (two times × 6 min) to retrieve the antigen. They were then rinsed and blocked in 10% $H_2O_2$ solution with methanol for 10 min. Next, the sections were incubated with goat anti-human FGF10 polyclonal antibody (R&D Systems, Minneapolis, MN, USA) at 1 : 20 dilution, mouse anti-human FGFR2 monoclonal antibody (R&D Systems) at 1 : 10 dilution, or rabbit anti-human CD3 monoclonal antibody (ready-to-use without dilution) (Thermo Fisher Scientific Anatomical Pathology, Fremont, CA, USA) overnight at 4°C. They were then rinsed in PBS and incubated for 60 min with a secondary antibody labelled with streptoavidin–biotin–peroxidase for goat polyclonal antibody (DAKO LSAB + ™ System, DAKO, Glostrup, Denmark), or dextran polymer-peroxidase for mouse monoclonal antibody (DAKO EnVision™ System, DAKO). For detection of anti-CD3 antibody, CSA II biotin-free catalysed amplification system with rabbit link (DAKO) was used. The bound complex was visualised using diaminobenzidine liquid chromogen (Dako) and counterstained with haematoxylin. Goat polyclonal IgG (Santa Cruz Biotechnology. Santa Cruz, CA, USA) at an optimal dilution was used as a negative control.

### Cell lines and culture conditions

AsPC-1 cells were maintained in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) and MIA PaCa-2, PANC-1 and CFPAC-1 cells

**Table 1** Characteristics of pancreatic cancer patients in IHC analysis

| | | FGFR2 | | | FGF10 | | |
|---|---|---|---|---|---|---|---|
| | Total | Low | High | *P*-value | Low | High | *P*-value |
| | 76 | 37 | 39 | | 34 | 42 | |
| *Sex* | | | | NS | | | NS |
| M | 44 | 18 | 26 | | 19 | 25 | |
| F | 32 | 19 | 13 | | 15 | 17 | |
| *Age (years)* | | | | NS | | | NS |
| Mean | 65.0 | 65.0 | 64.5 | | 65.0 | 64.5 | |
| ± s.d. | ± 9.2 | ± 9.3 | ± 9.4 | | ± 9.3 | ± 8.8 | |
| Stage | | | | NS | | | NS |
| IA | 2 | 2 | 0 | | 2 | 0 | |
| IB | 2 | 2 | 0 | | 2 | 0 | |
| IIA | 15 | 9 | 6 | | 4 | 11 | |
| IIB | 53 | 22 | 31 | | 24 | 29 | |
| III | 4 | 2 | 2 | | 2 | 2 | |
| *Histology* | | | | NS | | | NS |
| *Tubular adenocarcinoma* | | | | | | | |
| Well. | 7 | 3 | 4 | | 4 | 3 | |
| Mod. | 47 | 26 | 21 | | 19 | 28 | |
| Poor. | 13 | 5 | 8 | | 9 | 4 | |
| *Invasive carcinoma derived from intraductal tumour* | 4 | 2 | 2 | | 0 | 4 | |
| Anaplastic carcinoma | 3 | 1 | 2 | | 2 | 1 | |
| Adenosquamous carcinoma | 2 | 0 | 2 | | 0 | 2 | |
| *Resection status* | | | | | | | |
| Negative | 51 | 26 | 25 | NS | 23 | 29 | NS |
| Positive | 25 | 11 | 14 | | 12 | 13 | |
| *Adjuvant chemotherapy* | | | | | | | |
| − | 21 | 9 | 12 | NS | 9 | 12 | NS |
| + | 55 | 28 | 27 | | 25 | 30 | |

Mod = moderately differentiated; NS = no significant; Poor = poorly differentiated; s.d. = standard deviation; Well = well differentiated. Patient stage was determined according to UICC TNM classification.

were cultured with DMEM medium (Sigma-Aldrich, St Louis, MO, USA). All cell lines were incubated in a humidified atmosphere containing 5% $CO_2$ at 37°C. Each medium contained 10% fetal bovine serum (Invitrogen), 100 U ml$^{-1}$ penicillin and 0.1 mg ml$^{-1}$ streptomycin sulphate (Sigma-Aldrich).

For some experiments, cells were seeded ($1 \times 10^6$ cells in 2 ml medium per well) in 6-well plates, and cultured for 24 h. Then cells were cultured with serum-free medium for another 24 h and changed to new serum-free medium with recombinant human FGF10 (R&D systems) or/and FGFR2-IIIb/IgG Fc Chimera (R&D systems) for indicated time in each experiments. Cells and medium were harvested for further experiments.

Transforming growth factor $\beta$ 1 (TGF-$\beta$1) concentration was measured using Quantikine Human TGF-$\beta$1 immunoassay kit (R&D systems).

### Reverse transcription–PCR

Total RNA was extracted from cultured cells, pancreatic cancer tissues and adjacent normal tissues with an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. cDNA was synthesised from 1 $\mu$g of total RNA with SuperScript™ III First-Strand Synthesis SuperMix for reverse transcription (RT)–PCR (Invitrogen). Polymerase chain reaction was performed with the following primer sets: FGF10, forward 5-ACATTGTGCCTCAGCCTTTC-3, reverse 5-CCCCTTCTTGTT CATGGCTA-3; FGFR2-IIIb, forward 5-TATATAGGGCAGGCAAC CA-3, reverse 5-GCTGAAGTCTGGCTTCTTGG-3; FGFR2-IIIc, forward 5-GTGCTTGGCGGGTAATTCTA-3, reverse 5-GCTGAAGTCT GGCTTCTTGG-3; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5-GTCAGCCGCATCTTCTTTTG-3, reverse 5-TTCACACCCATGACGAACAT-3. The RT–PCR conditions for FGF10, FGFR2-IIIb, FGFR2-IIIc and GAPDH were as follows: 94°C for 2 min, 40 cycles at 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s, with an extension step of 7 min at 72°C at the end of the last cycle.

### Quantitative RT–PCR

Quantitative RT–PCR was performed as described previously (Mitsuhashi *et al*, 2003). Primers for 18 genes related to cancer invasion and motility have been described by Ide *et al* (2006). The mRNA levels of these genes (E-Cadherin, N-Cadherin, Snail, MMP-1, MMP-2, MMP-7, MMP-9, MT1-MMP, TIMP-2, uPA, TGF-$\beta$1, HGF, c-Met, RhoA, CD44, Integrin-$\alpha$4, Integrin-$\beta$4, and VEGF-A) were determined as the absolute number of copies normalised against the GAPDH mRNA copy number (Mitsuhashi *et al*, 2003). These experiments were performed three times independently.

### Cell migration assay

A migration assay was performed in 12-well plates using a Quantitative Cell Migration™ Assay Kit (Chemicon International, Temecula, CA, USA) with an 8.0 $\mu$m pore size collagen-coated chamber membrane. The cells were seeded ($1 \times 10^5$ cells in 0.3 ml of serum-free medium) in the upper chamber and cultured for 24 h for attachment. The medium was then replaced by fresh serum-free medium for another 24 h, before addition of recombinant human FGF10 (100 ng ml$^{-1}$) (R&D Systems) to the lower chamber. In some experiments, recombinant human FGFR2-IIIb/IgG Fc Chimera (500 ng ml$^{-1}$) (R&D Systems) was also added to the lower chamber. The cells were incubated for 12 h and the number of cells that passed through the membrane was counted according to the manufacturer's instructions. All experiments were performed in triplicate and independently at least three times.

### Cell invasion assay

An invasion assay was performed in 24-well plates using a BD Biocoat™ Matrigel™ Invasion Chamber (BD Biosciences, Bedford, MA, USA) with an 8.0 $\mu$m. pore size polyethylene terephthalate (PET) membrane coated with Matrigel. The inserts were rehydrated by adding 0.5 ml of warm culture medium at 37°C for 2 h. The cells were seeded ($5 \times 10^5$ cells in 0.5 ml of serum-free medium) in the upper chamber and cultured as described in the method for the migration assay. The number of seeded cells, culture conditions and other items were also similar to those for the migration assay. The cells were incubated for 24 h and the number of cells that passed through the membrane was counted according to the manufacturer's instructions. All experiments were performed in triplicate and independently at least three times.

### Statistical analysis

Values are expressed as means ± s.d. The distribution of categorical data for FGFR2 immunostaining in tissue samples and for clinicopathological characteristics were assessed by a $\chi^2$ test and a Fisher's exact test. Survival time was calculated using the Kaplan–Meier method and compared by log-rank test. Cell migration and cell invasion data were analysed using a Student's *t*-test and a Mann–Whitney *U*-test. Statistical significance was assumed for $P<0.05$.

## RESULTS

### Expression of FGFR2 in pancreatic cancer cells and FGF10 in cancer stromal cells

To examine the expression pattern of FGFR2 and FGF10 in pancreatic cancer tissues, we performed immunohistochemical staining of 76 tissue samples of invasive pancreatic ductal carcinoma and of normal pancreatic tissues. FGF receptor-2 immunoreactivity was weak to moderate in pancreatic ductal cells in normal tissues (Figure 1A; arrow) and acinar cells (Figure 1A; arrowhead), as well as in islet cells (data not shown), as described previously (Ishiwata *et al*, 2002). On the other hand, immunostaining of FGF10 did not occur in normal pancreatic tissue, as also described previously (Ishiwata *et al*, 2002) (Figure 1B).

In pancreatic cancer tissues, cancer cells expressed FGFR2 at various levels (Figure 1C), but did not express FGF10 (Figure 1D). Pancreatic cancer tissue often contains a few islet cells (Figure 1E and 1F; arrowheads) and we compared the expression levels of FGFR2 in cancer cells and islets. In 39 cases (51.3%), FGFR2 immunoreactivity in cancer cells was stronger than in islets (high expression group, Figure 1E). In the other 37 cases (48.7%), FGFR2 immunoreactivity was not found or was faint in cancer cells, and was weaker than in normal islet cells (low expression group, Figure 1F). There was no correlation between FGFR2 immunoreactivity levels and tumour histological findings (Table 1).

Pancreatic cancer cells did not express FGF10 in any samples (Figure 1D), but scattered cells in stroma surrounding the cancer cells showed strong expression of FGF10 (Figure 1D; arrows). Fibroblast growth factor 10-positive stromal cells in cancer tissues were found in 42 cases (55.3%), and interestingly, were mainly localised close to cancer cells (Figure 1D and 1E). Moreover, of the 42 cancer tissue samples with FGF10-positive stromal cells, 29 (69.0%) showed high FGFR2 expression in cancer cells, and there was a significant correlation between the presence of FGF10-positive stromal cells and high FGFR2 expression in cancer cells ($P=0.013$).

Next we examined which kind of cancer stromal cells expressed FGF10. We stained sequential sections with antibody against FGF10 and stromal cell markers (CD68; macrophage marker, $\alpha$-smooth muscle actin; activated fibroblast marker, CD3; T-cell

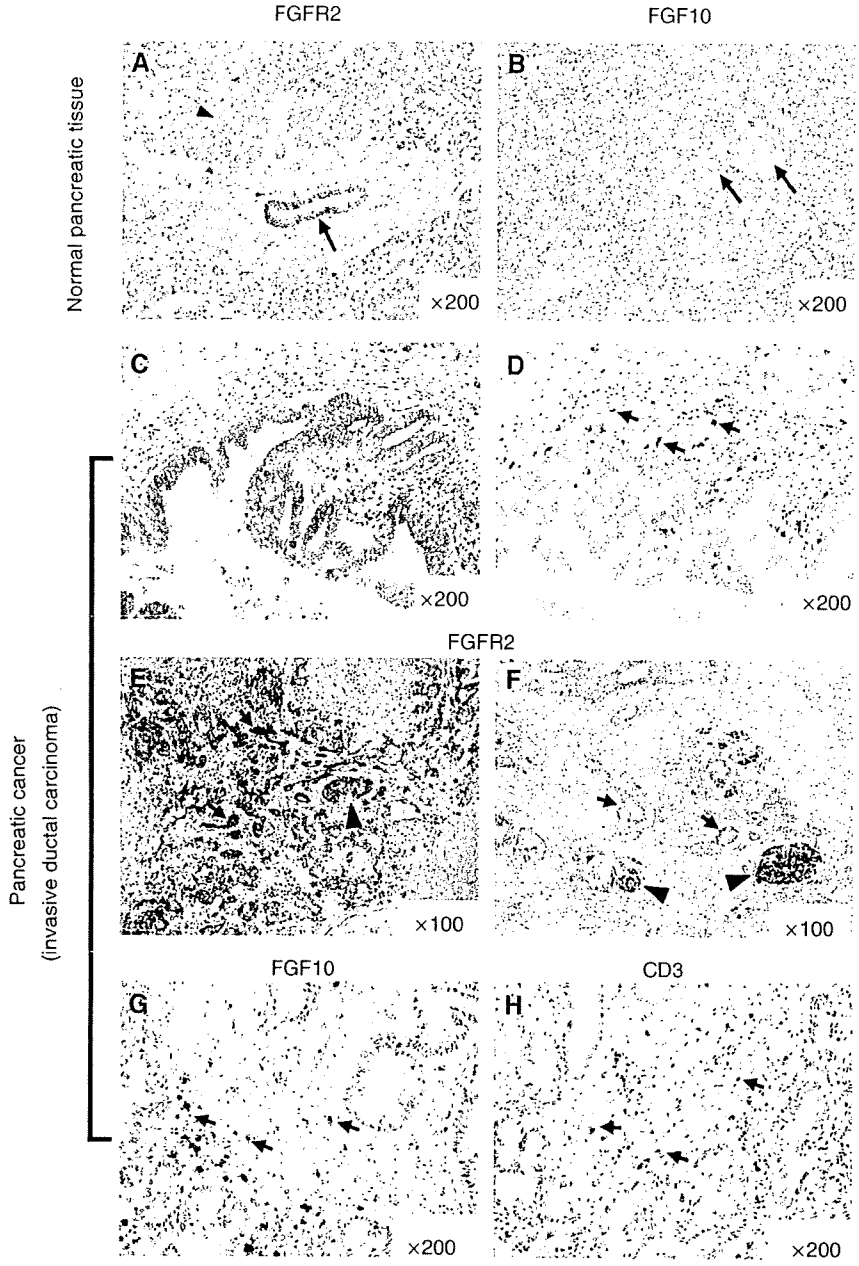


**Figure 1** Expression patterns of FGFR2 and FGF10 in normal pancreas and pancreatic cancer. The magnification is shown in the right bottom corner of each figure. (**A** and **B**) Immunostaining of FGFR2 (**A**) and FGF10 (**B**) in normal pancreas, showing that FGFR2 is expressed weakly in ductal cells (**A**, arrow) and acinar cells (**A**, arrow head), and that no obvious FGF10 staining is found in normal pancreatic tissue, including ductal cells (**B**, arrows). (**C** and **D**) Immunostaining of FGFR2 (**C**) and FGF10 (**D**) in pancreatic cancer tissues, showing that FGFR2 is expressed in cancer cells (**C**), whereas FGF10 is expressed in scattered cells in the stroma surrounding cancer cells (**D**, arrows). (**E** and **F**) Immunostaining of FGFR2 in pancreatic cancer cells. (**E**) Representative result from the FGFR2 high expression group, indicating higher FGFR2 expression in cancer cells (arrows) compared with islets (arrow head). (**F**) Representative result from the FGFR2 low expression group, showing lower FGFR2 expression in cancer cells (arrows) compared with islet (arrow heads). (**G** and **H**) Immunostaining of FGF10 (**G**) and CD3 (**H**), marker for T cell. Fibroblast growth factor 10 and CD3 are both expressed in scattered cells with similar cell shape in the stroma surrounding cancer cells (arrows).

marker). Within them, CD3-positive stromal cells, T-cells, were located similar to FGF10-positive stromal cells and also has similar cell shapes (Figure 1G and 1H; arrows). However, due to the technical difficulties, we could not demonstrate that FGF10-expressing cells were identical with T-cells.

Overall, the results show that FGFR2 is expressed in pancreatic cancer tissue, and that its ligand, FGF10, is expressed in stromal cells.

## FGFR2 expression levels correlates with prognosis of pancreatic cancer patients

Next, we examined whether strong expression of FGFR2 in cancer cells correlated with patient prognosis. The 76 patients were divided into two groups according to the expression level of FGFR2 in cancer cells (the high and low expression groups in Table 1). The expression level of FGFR2 was not correlated with

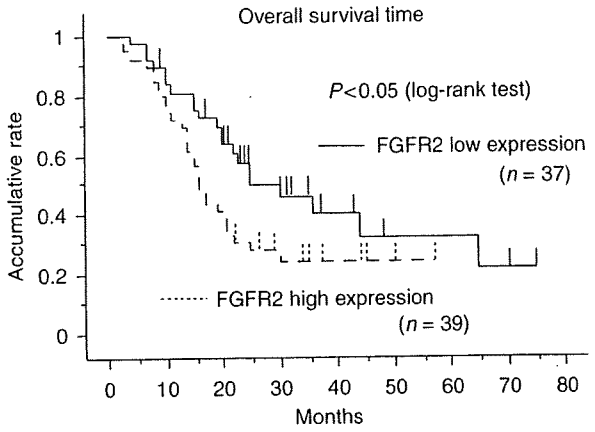


**Figure 2** Kaplan–Meier survival curves for patients with high and low FGFR2 expression. Patients with low FGFR2 expression had a significantly longer overall survival time compared to those with high FGFR2 expression ($P<0.05$ by log-rank test).

clinicopathological factors such as sex, age and pathological stage (Table 1). There were no statistical differences in the resection status of both groups. Also, the ratios of patients who received adjuvant chemotherapy (using gemcitabine) were similar in both groups, indicating that patients in both groups were treated with similar therapeutic approaches after surgery (Table 1). Interestingly, Kaplan–Meier analysis showed that patients with high FGFR2 expression had a significantly shorter overall survival time compared to those with low expression levels (Figure 2) ($P=0.047$ by log-rank test). Moreover, patients with high FGFR2 expression had significantly more nodal invasion, a larger tumour size, and a worse UICC Stage (Table 2; $P=0.0263$, $P=0.0469$, and $P=0.022$, respectively, by $\chi^2$-test). There was no significant correlation between survival time and the presence of FGF10 in stromal cells in cancer tissue (data not shown).

## FGF10/FGFR2-IIIb signalling induces cell migration and invasion

The expression pattern of FGF10 and FGFR2 in cancerous tissue and the poor prognosis of patients with high FGFR2 expression in cancer cells indicate that a stromal cell–epithelial cell interaction through FGF10/FGFR2 signalling might induce the malignant properties of pancreatic cancer. To examine this hypothesis, we analysed the effects of FGF10 on the proliferation, invasion and migration of pancreatic cancer cells. For this purpose, four pancreatic cancer cell lines were used: MIA PaCa-2, PANC-1, CFPAC-1 and AsPC-1 cells.

First, we examined whether these cell lines expressed FGFR2 and FGF10. Reverse transcriptase–PCR analysis showed that all four cell lines did not express FGF10 mRNA, consistent with the results of immunostaining showing FGF10 expression in stromal cells, but not in cancer cells, in pancreatic cancer tissue (Figure 3A). Fibroblast growth factor 10 activity is dependent on its binding to the FGFR2-specific isoform, FGFR2-IIIb (Igarashi *et al*, 1998). Therefore, a primer set for FGFR2-IIIb was designed with a 5 primer for its specific exon in the FGFR2 gene. Reverse transcriptase–PCR analysis with this primer showed that CFPAC-1 and AsPC-1 cells expressed the FGFR2-IIIb isoform, whereas the other two cell lines did not do so (Figure 3A).

To examine cancer cell proliferation, the cells were stimulated with various concentrations of FGF10 ($10-200\,ng\,ml^{-1}$), but no effect of FGF10 was observed in any of the four cell lines (data not shown). Next, we examined whether FGF10 affects migration or invasion of pancreatic cancer cells. Representative results from the cell migration and invasion assays are shown in Figure 3B.

**Table 2** Clinico-pathological features of pancreatic cancer patients in FGFR2-IHC analysis

| | | FGFR2 expression of IHC | | |
|---|---|---|---|---|
| | Total | Low expression | High expression | |
| | 76 | 37 | 39 | *P*-value |
| T (1,2,3/ 4) | 42/34 | 23/14 | 19/20 | NS |
| N (0/ 1,2,3) | 20/56 | 14/23 | 6/33 | 0.0263 |
| M (0/ 1) | 67/9 | 35/2 | 32/7 | NS |
| ly (0/ 1,2,3) | 14/62 | 9/28 | 5/34 | NS |
| v (0/ 1,2,3) | 31/45 | 19/18 | 12/27 | NS |
| ne (0/ 1,2,3) | 11/65 | 7/30 | 4/35 | NS |
| Size (</≥; 30 mm) | 37/39 | 23/14 | 14/25 | 0.022 |
| IA, IB, IIA/IIB, III | 19/57 | 13/24 | 6/33 | 0.0469 |
| Poor/others | 13/63 | 8/29 | 5/34 | NS |

ly = lymphatic invasion; M = distant metastasis; N = nodal metastasis; ne = neural invasion; NS = no significant; Poor = poorly differentiated adenocarcinama; T = tumour depth; v = venous invasion. Patient stage was determined according to UICC TNM classification.

Interestingly, FGF10 stimulated cell migration and invasion of cells that expressed FGFR2-IIIb (CFPAC-1 and AsPC-1), but not of cells without expression of the specific receptor (MIA PaCa-2 and PANC-1) (Figure 3C and 3D). For CFPAC-1 cells, migration was almost doubled (Figure 3C) and invasion was increased by 1.5 times (Figure 3D) following stimulation with FGF10 ($100\,ng\,ml^{-1}$). Similar results were obtained for AsPC-1 cells (Figure 3C and 3D).

To confirm that the effects of FGF10 on pancreatic cells were mediated through FGFR2-IIIb, we used a molecular hybrid including the FGFR2-IIIb extracellular domain and the carboxy-terminal Fc region of human IgG (recombinant human FGFR2-IIIb/IgG Fc Chimera). Such hybrids inhibit signalling through the FGFR2-IIIb receptor by antagonising ligand binding (Jung *et al*, 1999). Stimulated migration of CFPAC-1 cells by $100\,ng\,ml^{-1}$ FGF10 was completely inhibited by addition of FGFR2-IIIb/IgG Fc Chimera ($500\,ng\,ml^{-1}$) (Figure 3E; compare the second and third columns). The effects of the chimera were due to inhibition of signalling through FGFR2-IIIb, rather than to a direct effect of the chimera on the cells, as addition of chimera alone did not affect cell migration (Figure 3E; compare the first and fourth columns). Invasion of CFPAC-1 cells stimulated by FGF10 was also inhibited by FGFR2-IIIb/IgG Fc Chimera (Figure 3F). Overall, these results indicate that FGF10 stimulates migration and invasion of cancer cells through its specific receptor, FGFR2-IIIb.

## Upregulation of MT1-MMP and TGF-$\beta$1 mRNA by FGF10

To examine the molecular mechanisms through which FGF10 signalling induces migration and invasion of pancreatic cancer cells with FGFR2-IIIb expression, we analysed whether FGF10 stimulation induced mRNA expression of 18 genes related to cell mobility in CFPAC-1 cells (see Materials and methods for the 18 genes). CFPAC-1 cells were cultured with recombinant human FGF10 ($100\,ng\,ml^{-1}$) for 12, 24 and 48 h, and then total RNA were extracted and the mRNA levels of the 18 genes were analysed by quantitative RT–PCR. Among these genes, FGF10 upregulated the expression levels of MT1-MMP (Figure 4A) and TGF-$\beta$1 (Figure 4B) mRNAs in a time-dependent manner. In CFPAC-1 cells, the mRNA expression levels for both genes were almost 10 times higher after FGF10 stimulation for 48 h compared with the level before stimulation. The concentration of TGF-$\beta$1 in culture medium was also upregulated in time-dependent manner in CFPAC-1 cells (Figure 4C) and also in AsPC-1 cells (data not shown). The secretion of TGF-$\beta$1 by FGF10 stimulation in CFPAC-1 cells was inhibited by FGFR2-IIIb/IgG chimera (Figure 4D), indicating FGF10-induced TGF-$\beta$1 secretion through FGFR2.