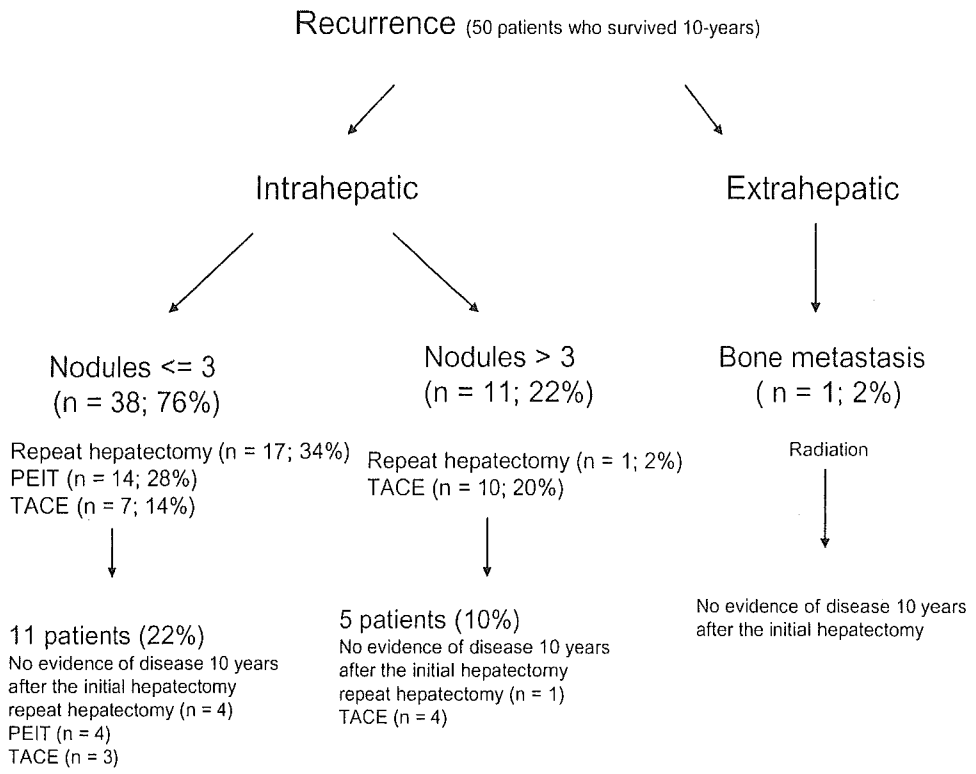

FIGURE 1. The disease recurrence status and therapeutic modalities for treatment of recurrence in the 50 10-year survivors in whom disease recurrence was detected. None of the patients had recurrent tumors exceeding 3.0 cm in greatest dimension or macroscopic evidence of portal or hepatic vein venous tumor involvement. PEIT: percutaneous ethanol injection; TACE: transarterial chemoembolization.

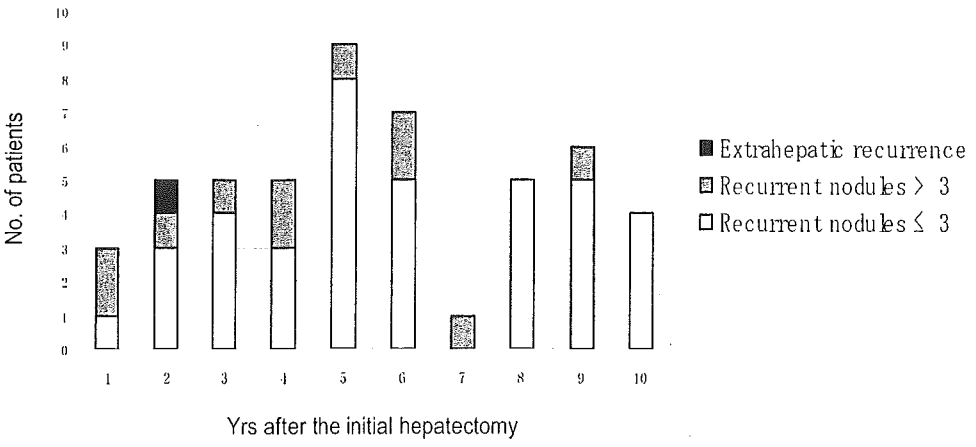

FIGURE 2. Time course of intrahepatic disease recurrence according to the number of recurrent tumor nodules and the development of extrahepatic disease recurrence.

rence.[19,20] Such an initial tumor spread at the time of surgery also strongly influences the overall 10-year-survival, as shown in the current study. A patient age of 56 years or younger was found to be an independent favorable factor. Younger patient age may be associated with better liver functional reserve, hepatitis B virus infection, and nonhepatitis C virus infection. HCC is generally predominant among the elderly and older patients might have higher rates of disease recurrence after removal of the initial hepatic tumor. The value of ICGR15 was not only found to be an appropriate determinant of the extent of resection (segmentectomy, lobectomy, or partial resection), but also one of the most significant factors for long-term survival. It is well known that the value of ICGR15 is well correlated with noncancerous liver parenchyma (normal liver, chronic hepatitis, or cirrhosis), which was reported to be a significant prognostic factor for disease-free survival,[3–6,7–9] but some patients with cirrhosis who have a normal ICGR15 value might undergo resection with a wider surgical margin and achieve long-term survival.

A wider surgical margin and systematic resection also were found to be statistically significant factors on the univariate analysis, but not on the multivariate analysis. The clinical significance of a wider clearance

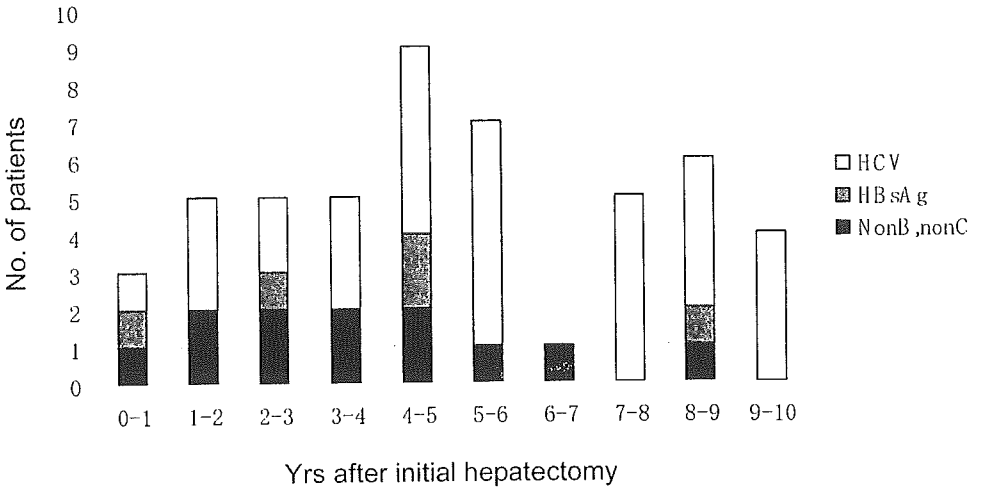

FIGURE 3. Time course of intrahepatic disease recurrence according to the hepatitis virus marker status. HCV: hepatitis virus C antibody positive; HbsAg: hepatitis virus B surface antigen positive; NonB, nonC: hepatitis virus C antibody negative and hepatitis virus B antigen negative.

of noncancerous liver parenchyma, including an appropriate and wide surgical margin and systematic resection, has been considered to be controversial.[21,22] It is difficult to clarify the effectiveness of segmentectomy or lobectomy compared with partial resection using a prospective randomized trial because of the limitation of the functional reserve of the hepatic remnant. Segmentectomy or lobectomy might be recommended as initial treatment for younger patients with good hepatic function and a solitary hepatic nodule because such patients have a chance of achieving long-term survival and wider surgical resections could minimize the chance of microscopic residual tumors or occult metastases.

Manageable disease recurrence also may be expected to improve survival. This speculation is reasonable because 38 of the 50 patients with recurrent HCC (76%) among the 10-year survivors had no more than 3 nodules and these measured at most only 3 cm in greatest dimension, without any macroscopic evidence of tumor thrombus. Tumor recurrence should be principally managed with repeat hepatectomy, which has been established as the most effective treatment modality for recurrent HCC, whenever it is possible.[10-12] Recent studies have demonstrated that percutaneous local ablative therapy provides good local control of small HCC tumors, and might be comparable to surgical resection with regard to long-term outcome.[23] Further investigation of which treatment modalities, including repeat hepatectomy, radiofrequency ablation (RFA), or PEIT, could effectively contribute to the achievement of long-term survival might be necessary. Conversely, HCC patients with underlying cirrhosis, multiple tumors, and/or major venous invasion have little chance of surviving for longer than 10 years because disease recurrence might be unmanageable despite careful postoperative follow-up and appropriate postoperative management. Postoperative adjuvant therapy should be strongly recommended in this group of patients before a clinical manifestation of disease recurrence, because surgery might be potentially noncurative.[19,24] Preoperative TACE was not found to have a significant impact on long-term survival in the current study, but prospective randomized trials are necessary to determine the efficacy of these treatments.

Favorable hepatitis virus marker status, such as hepatitis virus B surface antigen positivity and negative hepatitis C virus antibody status, has previously been reported to be a potentially good predictor of long-term survival.[8,9] The current study did not find viral infection status to be an independent factor influencing the 10-year survival, although hepatitis C virus infection was determined to be an independent risk factor for recurrence among the 10-year survivors. Fifty patients survived for longer than 10 years, although they were found to have tumor recurrence, and the mean time to disease recurrence time in the patients with hepatitis C virus was longer than that in the other patients. The second peak of disease recurrence was observed at 9 years after surgery in patients with positive hepatitis C virus status. These findings suggest multicentric carcinogenesis may play an important role in tumor recurrence in hepatitis C virus-positive, long-term survivors. Recent reports[25,26] have emphasized that interferon therapy might enhance patient survival in selected patients after tumor ablation, and that the oral administration of an acyclic retinoid may prevent the development of second primary tumors. Such treatments should be considered in younger surgical candidates with no underlying cirrhosis, with a solitary HCC tumor, and no vascular

invasion or intrahepatic metastases as determined by pathologic examination.

The distinction between recurrences of latent residual tumors and multicentric carcinogenesis is extremely difficult because clonal discrimination cannot be applied in clinical practice.[6,27] Kumada et al.[2] assessed patterns of disease recurrence in patients with HCC tumors measuring < 2 cm, and multicentric disease recurrence was observed in 29 patients (50.9%). Such a small HCC may appear to be too small to be compared with those occurring in patients who usually undergo surgery. Shimada et al.[12] suggested that the majority of recurrent tumors developing after the initial hepatectomy are caused not by multicentric occurrence but by metastatic recurrence, based on a histologic analysis. In the current analysis, we considered that latent residual tumors (intrahepatic metastases at the time of initial hepatectomy) also may also play a role in disease recurrence, because intrahepatic metastasis was found to be an independent risk factor for disease recurrence among the 10-year survivors as well as in hepatitis C virus-positive survivors. Although the peak of disease recurrence is usually observed at 1.5–2 years after surgery,[5,19] it was found in the current study to occur after 5 years among the 10 year-survivors. Late disease recurrence has been generally observed in other cancers as well, such as renal carcinoma, colorectal carcinoma, and breast carcinoma, and these tumors occasionally demonstrated relatively favorable biologic behaviors.

Wider systematic resection (lobectomy and/or segmentectomy) and/or wider surgical margins should be considered within the limitation of the hepatic functional reserve in younger HCC patients with no underlying cirrhosis because the presence of portal vein invasion and/or microscopic intrahepatic metastases is difficult to determine before surgery. Moreover, if these patients are also positive for the hepatitis C virus, and the absence of portal venous invasion and/or intrahepatic metastases is confirmed by pathologic examination, they may have a good chance of surviving for longer than 10 years and are good candidates for the preventive treatment of multicentric carcinogenesis.

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Increased DNA methyltransferase 1 (DNMT1) protein expression in precancerous conditions and ductal carcinomas of the pancreas

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Aberrant DNA methylation has been shown to play an important role during multistage carcinogenesis in various human organs. The aim of the present study was to evaluate the significance of DNA methyltransferase 1 (DNMT1) protein expression during pancreatic carcinogenesis. Immunohistochemical analysis of DNMT1 in 48 peripheral pancreatic duct epithelia showing no remarkable histological findings without an inflammatory background (DE), 54 peripheral pancreatic duct epithelia with an inflammatory background (DEI), 188 pancreatic intraepithelial neoplasias (PanIN), and 220 areas of invasive ductal carcinoma from surgical specimens resected from 100 patients, was carried out. The average incidence of DNMT1 immunoreactivity increased progressively from DE to DEI ($P = 0.003$), from DE and DEI to PanIN ($P < 0.0001$), among PanIN with different grades of dysplasia (from PanIN I to PanIN II, $P = 0.0012$), from PanIN to invasive ductal carcinomas ($P < 0.0001$) and among invasive ductal carcinomas with different grades of histological differentiation (from well or moderately to poorly differentiated adenocarcinomas, $P < 0.0001$). High-level DNMT1 protein expression in invasive ductal carcinomas was correlated significantly with an advanced t category ($P = 0.0224$) and an advanced stage ($P = 0.0294$). Moreover, patients with invasive ductal carcinomas showing high-level DNMT1 protein expression had a poorer outcome ($P = 0.0469$). These data suggest that increased DNMT1 protein expression participates in multistage pancreatic carcinogenesis from the precancerous stage to malignant progression of ductal carcinomas and may be a biological predictor of poor prognosis. (*Cancer Sci* 2005; 96: 403–408)

Pancreatic cancer is a devastating disease with a very poor prognosis, with a 5-year survival rate of < 3%, and is the fourth or fifth largest cause of cancer-related death worldwide.[1,2] Because ductal carcinomas frequently emerge in pancreases damaged by chronic pancreatitis,[3] at least a proportion of peripheral pancreatic duct epithelia with an inflammatory background may be at the precancerous stage, even though they may show no remarkable histological findings. Recently, Hruban *et al.*[4] suggested a new nomenclature and classification system for pancreatic intraepithelial neoplasia (PanIN) as a precancerous lesion, and proposed a model of progression from PanIN to ductal carcinoma.[5] Elucidation of genetic and epigenetic alterations in such precancerous conditions and ductal carcinomas showing various clinicopathological features would contribute to a better understanding of the molecular basis of multistage pancreatic carcinogenesis.

DNA methylation plays an important role in transcriptional regulation, chromatin remodeling and genomic stability.[6] Overall DNA hypomethylation and regional DNA hypermethylation are commonly observed in various tumors, including pancreatic cancers.[7,8] Furthermore, accumulating evidence suggests that aberrant DNA methylation is involved even in the early and precancerous stages of human carcinogenesis.[9–15]

DNA methyltransferase 1 (DNMT1) is the major human DNMT[16] and increased levels of its mRNA and protein expression have been reported in several human precancerous conditions and cancers.[17–22] We have reported previously that DNMT1 protein overexpression precedes an increase in the proliferating cell nuclear antigen labeling index in precancerous conditions of the urinary bladder,[21] and is significantly correlated with poorer differentiation of liver[19] and stomach[22] cancers and a poor prognosis in patients with liver cancer.[19] However, to our knowledge, there are no reported data on the expression of DNMT1 at both the mRNA and protein levels in pancreatic cancers. In this study we carried out an immunohistochemical analysis of DNMT1 expression in a large series of precancerous conditions and ductal carcinomas of the pancreas to evaluate its significance in multistage pancreatic carcinogenesis.

Materials and Methods

Patients and samples. A total of 48 peripheral pancreatic duct epithelia showing no remarkable histological findings without an inflammatory background (DE), 54 peripheral pancreatic duct epithelia with an inflammatory background (DEI, such ducts were surrounded by infiltrating lymphocytes) and 188 pancreatic intraepithelial neoplasias (PanIN; 50 PanIN IA, 126 PanIN IB and 12 PanIN II) were obtained from surgical specimens resected from 100 patients at the National Cancer Center Hospital, Tokyo, between 1997 and 2002. Invasive ductal carcinomas from this cohort frequently showed histological heterogeneity (e.g. well, moderately or poorly differentiated adenocarcinoma components were simultaneously observed even in tissue sections from any single patient). Then 220 areas of invasive ductal carcinoma (58 well-differentiated adenocarcinomas [WD], 114 moderately differentiated adenocarcinomas [MD], and 48 poorly differentiated adenocarcinomas [PD]) were examined from these 100 patients. The patients comprised 56 men and 44 women with a mean age ± SD of 62.26 ± 10.17 years (range, 33–83 years). Histopathological evaluation of the PanIN and cancers was carried out by three pathologists (Dun-Fa Peng, Yae Kanai, and Nobuyoshi Hiraoka), according to previously published criteria.[4,23] The study was approved by the Ethics Committee of the National Cancer Center, Tokyo.

Immunohistochemistry. Three-micrometer-thick sections of formalin-fixed, paraffin-embedded tissue specimens were deparaffinized and dehydrated. After antigen retrieval by heating

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in an autoclave for 10 min at 120°C, the sections were incubated with 2% normal swine serum to block any non-specific reaction. Then the sections were incubated with a goat antihuman polyclonal antibody against DNMT1 (N16, dilution 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight, followed by incubation with a biotinylated secondary antibody (antigoat IgG, 1:200; Vector Laboratories, Burlingame, CA) and Vectastain Elite ABC reagent (Vector Laboratories) at room temperature for 30 min each. We had previously confirmed the specificity of the goat antihuman DNMT1 polyclonal antibody by western blotting analysis: an immunoreactive band of approximately 193.5 kDa, corresponding to the molecular mass of DNMT1, was detected in human cancer cells, but no non-specific bands were detected with this antibody.[19] The sections were then treated with 3,3′-diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin. Tissue specimens of stomach cancers, in which we had detected positive immunoreactivity for DNMT1 in our previous study,[22] were used as a positive control at each staining. As described previously,[21,24] lymphocytes on the same slide were used as an internal positive control for DNMT1 immunoreactivity.

For each sample ($n = 510$), at least 500 cells were randomly counted. If the lesion was small with less than 500 cells, all the cells were counted. In all samples, DNMT1 immunoreactivity was detected only in the nucleus and never in the cytoplasm and cell membrane. The incidence of DNMT1 immunoreactivity in each sample was expressed as a percentage of all the cells counted. For each patient ($n = 100$), the level of DNMT1 protein expression in the cancer was considered to be low if less than 20% of cancer cells showed DNMT1 immunoreactivity, and high if 20% or more of the cancer cells were positive for DNMT1 after a thorough evaluation of two or three representative tissue sections that frequently showed histological heterogeneity and simultaneously contained well, moderately or poorly differentiated adenocarcinoma components.

Statistics. Comparisons of the incidence of DNMT1 immunoreactivity between or among sample groups (DE, DEI, PanIN, WD, MD, and PD) were analyzed by the Mann–Whitney U or Kruskal–Wallis test. Correlations between the level of DNMT1 protein expression and clinicopathological parameters were analyzed by the χ^2 test. Survival curves were calculated with the Kaplan–Meier method and the significance was analyzed by log–rank test. For all tests, $P < 0.05$ was considered to be the level of significance.

Results

Incidence of DNMT1 immunoreactivity in precancerous conditions and ductal carcinomas of the pancreas. Figures 1 and 2 show examples of immunohistochemical staining for DNMT1 in DE, DEI, PanIN and invasive ductal carcinomas of the pancreas. Figure 3 summarizes the results of immunohistochemistry for DNMT1. There was a significant difference in the average incidence of DNMT1 immunoreactivity between DE and DEI ($P = 0.0003$, Figs 1a,b and 3), and between ductal epithelia showing no remarkable histological findings (DE and DEI) and

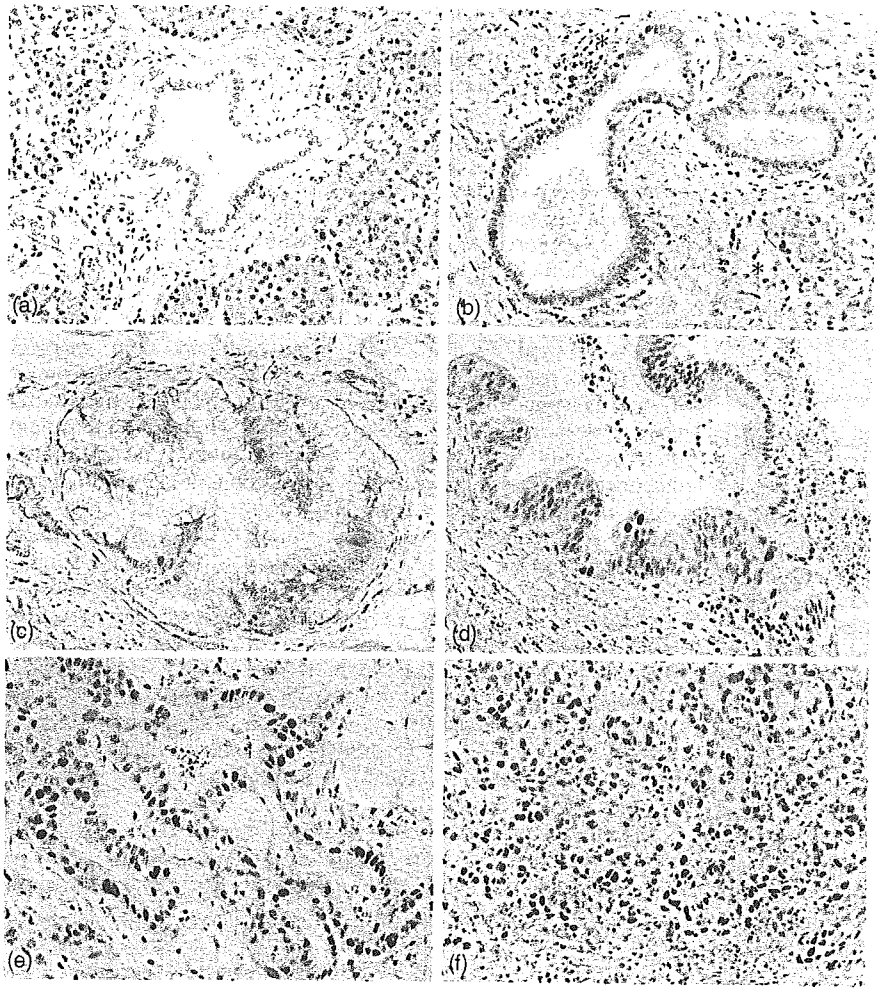

Fig. 1. Immunohistochemical examination for DNA methyltransferase 1 (DNMT1) in tissue specimens. No detectable DNMT1 immunoreactivity was observed in peripheral pancreatic duct epithelia showing no remarkable histological findings without an inflammatory background (a). However, scattered DNMT1-positive cells were observed in peripheral duct epithelia with an inflammatory background accompanied by pancreatic acinar atrophy (b). In panel (b), infiltrating lymphocytes as internal controls show positive immunoreactivity for DNMT1 (*). The incidence of DNMT1 immunoreactivity increased progressively from pancreatic intraepithelial neoplasia (PanIN) IB (c), PanIN II (d), moderately differentiated adenocarcinoma (e), to poorly differentiated adenocarcinoma (f). ×200

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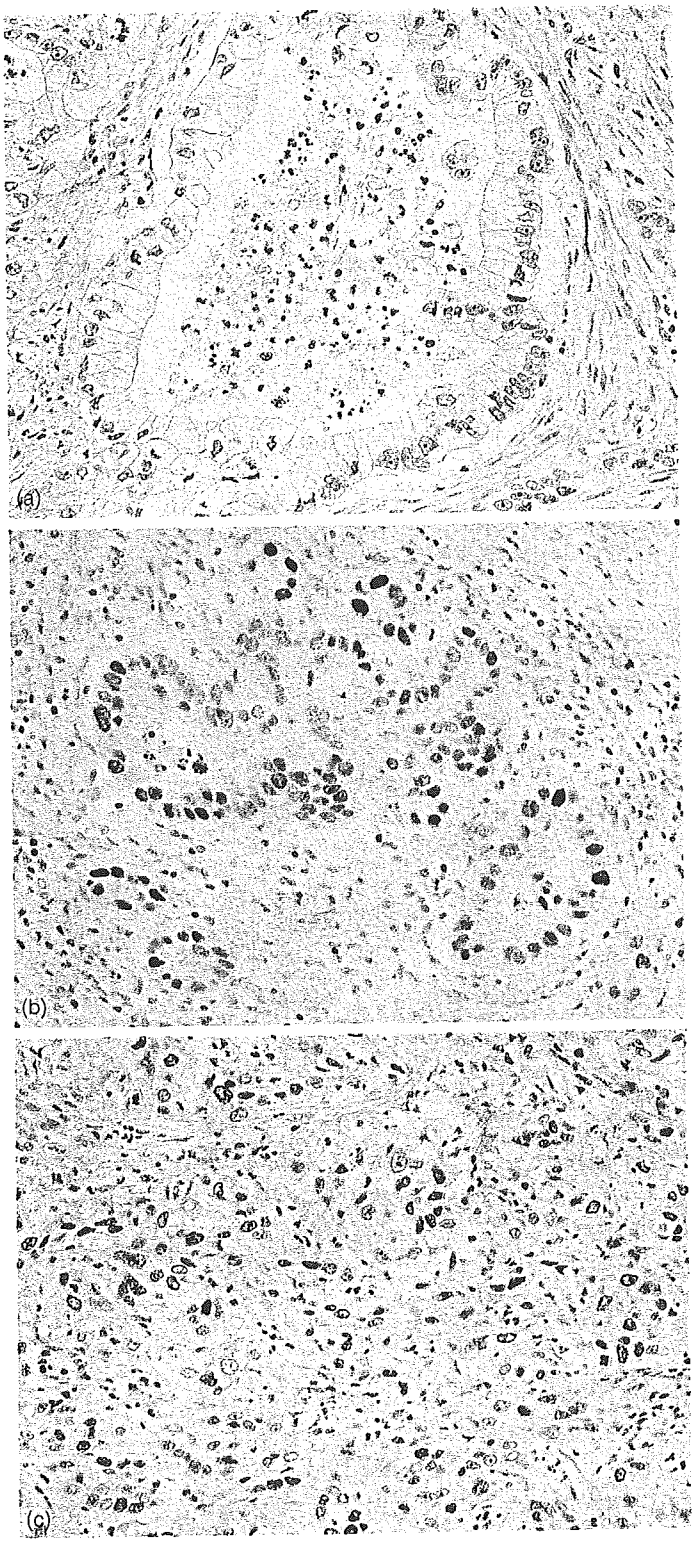

Fig. 2. Heterogeneity of DNA methyltransferase 1 (DNMT1) protein expression among components showing different grades of histological differentiation in a representative cancer from a single patient. Moderately (b) and poorly (c) differentiated adenocarcinoma components showed a higher incidence of DNMT1 immunoreactivity than the well-differentiated adenocarcinoma component (a).

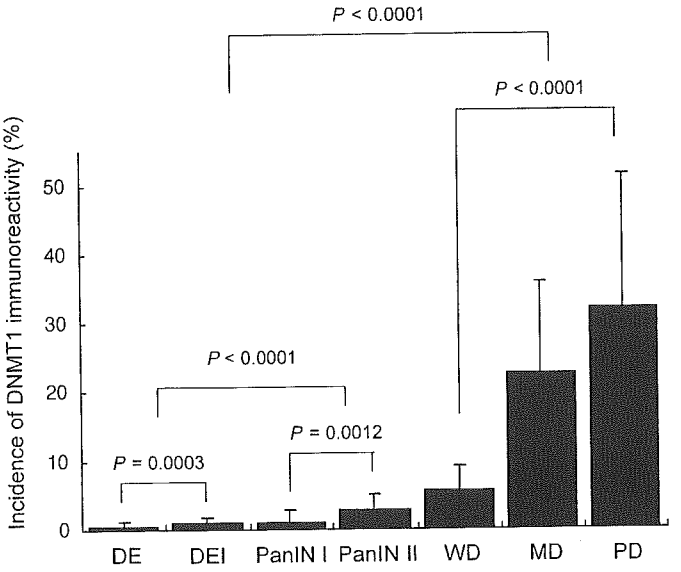

Fig. 3. A summary of the incidence of DNA methyltransferase 1 (DNMT1) immunoreactivity in precancerous conditions and ductal carcinomas of the pancreas. Immunohistochemical examination was performed. For each sample (n = 510) at least 500 cells were randomly counted. The incidence of DNMT1 immunoreactivity in each sample was expressed as a percentage of all the cells counted. A progressive increase in the incidence of DNMT1 immunoreactivity was observed during multistage pancreatic carcinogenesis. The error bars represent standard deviation. DE, peripheral pancreatic duct epithelia showing no remarkable histological findings without an inflammatory background; DEI, peripheral pancreatic duct epithelia with an inflammatory background; PanIN, pancreatic intraepithelial neoplasia; WD, well-differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated adenocarcinoma.

PanIN (PanIN I and PanIN II) ($P < 0.0001$, Fig. 3). A significant difference between PanIN I and PanIN II was detected ($P = 0.0012$, Figs 1c,d and 3), although no PanIN III lesion was ever identified in the non-cancerous pancreatic tissue from the examined tissue sections of all 100 patients. Moreover, there was a significant difference between all PanIN (PanIN I and PanIN II) and all invasive ductal carcinomas (WD, MD and PD) ($P < 0.0001$, Fig. 3), and a progressive increase in the incidence of DNMT1 immunoreactivity was observed among WD, MD and PD ($P < 0.0001$, Figs 1e,f and 3). With respect to the histological heterogeneity observed even in the lesion from a single patient, the incidence of DNMT1 immunoreactivity showed a tendency to increase progressively from its well, moderately to poorly differentiated adenocarcinoma components (Fig. 2).

Correlations between DNMT1 protein expression level and clinicopathological parameters in invasive ductal carcinomas of the pancreas. Correlations between the level of DNMT1 protein expression (low-level or high-level) and clinicopathological parameters[23] in invasive ductal carcinomas from all 100 patients (n = 100) are summarized in Table 1. High-level DNMT1 protein expression in invasive ductal carcinomas was significantly correlated with an advanced t category defined on the basis of the extent of cancer invasion to the anterior pancreatic capsule, retroperitoneal tissue, bile duct, duodenal wall, portal venous system and arterial system[23] and an advanced stage ($P = 0.0224$ and $P = 0.0294$, respectively, Table 1). No significant correlation was observed between the level of DNMT1 protein expression and patient age, sex, and other clinicopathological parameters (Table 1).

Correlation between DNMT1 protein expression level and prognosis of pancreatic cancer patients. We examined the prognostic impact of DNMT1 protein expression in 74 patients who underwent curative resection by partial pancreatoduodenectomy or distal pancreatectomy. The overall survival curve of the 74

Table 1. Correlation between DNMT1 protein expression level and clinicopathological parameters

Clinicopathological parameters*	DNMT1 expression		*P*-value
	Low-level	High-level	
t Category			0.0224
t1	5	0	
t2	22	11	
t3	29	33	
Lymph node involvement			0.0633
negative	13	4	
positive	42	41	
Staging			0.0294
I + II	5	0	
III	16	8	
IVa	21	28	
IVb	14	8	
Invasion pattern			0.2859
α	0	0	
β	30	27	
γ	26	15	
Lymphatic involvement			0.4972
0	4	2	
1	21	14	
2	28	22	
3	3	6	
Venous involvement			0.2197
0	7	1	
1	14	13	
2	30	23	
3	5	7	
Intrapancreatic nerve involvement			0.2907
0	3	2	
1	18	12	
2	21	24	
3	13	5	
Sex			0.2335
Male	29	28	
Female	27	16	
Age (mean ± SD)	62.63 ± 10.13	61.80 ± 10.33	0.6878

DNMT1, DNA methyltransferase 1. *Evaluation of clinicopathological parameters was carried out according to reference (23).

patients with high- and low-level DNMT1 protein expression was calculated by the Kaplan–Meier method (Fig. 4). A significant difference was observed between the groups with high and low DNMT1 protein expression (P = 0.0469, log rank test).

Discussion

We believe that this is the first report to describe the expression of DNMT1 protein in precancerous conditions and ductal cancers of the pancreas. Initially, the incidence of DNMT1 immunoreactivity increased in DEI compared to DE. Inflammatory cell infiltration around the peripheral pancreatic duct was frequently associated with pancreatic acinar atrophy, suggesting that persistent inflammation had long been present in such areas. Although ductal carcinomas are not always preceded by chronic pancreatitis, and the persistent inflammation observed in a part of our cohort may have been induced secondarily by obstruction of the pancreatic ducts as a result of tumor growth, it is well known that chronic pancreatitis significantly increases the risk of developing pancreatic cancer.[3] Therefore, at least a proportion of DEI may be at the precancerous stage even though they may show no remarkable histological findings. Our data suggest that increased expression of DNMT1 protein may participate even in the very early stage of multistage pancreatic carcinogenesis. It has been reported that some pancreatic ducts showing no remarkable histological findings

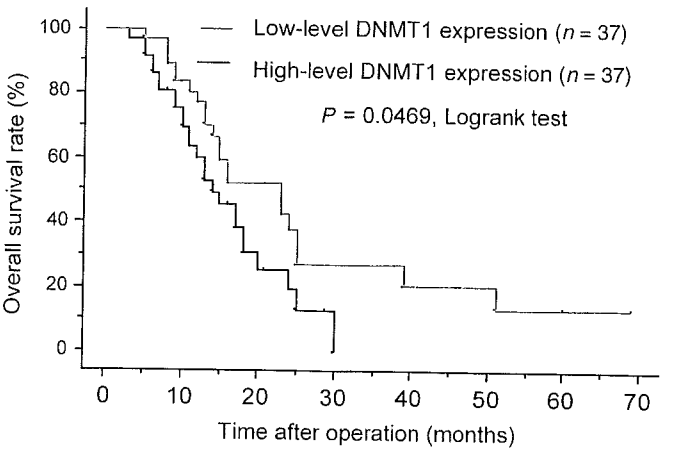

Fig. 4. Kaplan–Meier survival curve. Immunohistochemical examination was performed. For each patient who underwent curative resection by partial pancreatoduodenectomy or distal pancreatectomy (n = 74), the level of DNA methyltransferase 1 (DNMT1) protein expression in the cancer was considered to be low if less than 20% of the cancer cells showed DNMT1 immunoreactivity, and high if 20% or more of the cancer cells were positive for DNMT1 after a thorough evaluation of two or three representative tissue sections. The patients with a high level of DNMT1 protein expression (n = 37) had a poorer prognosis than those with a low level (n = 37) (P = 0.0469, log rank test).

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have K-ras mutation[25] and loss of heterozygosity on some loci.[26] In combination with such genetic aberrations, increased DNMT1 protein expression could play a role in triggering the process of tumorigenesis in ductal epithelia with an inflammatory background. Further studies are needed to understand the molecular mechanisms by which an inflammatory microenvironment affects the level of DNMT1 expression in ductal epithelia.

The incidence of DNMT1 immunoreactivity increased progressively in PanIN compared to DE and DEI and among PanIN that were accompanied by increasing grades of dysplasia, and was further increased in invasive ductal carcinomas, clearly indicating a continuous association of DNMT1 protein overexpression with the progression of multistage pancreatic carcinogenesis. In stomach and colorectal cancers, we have reported that DNMT1 overexpression results in a CpG island methylator phenotype,[20,22] which is defined as frequent DNA hypermethylation on CpG islands that are not methylated in normal tissues.[27] DNA hypermethylation on CpG islands of several cancer-related genes has been reported in PanIN and ductal cancers of the pancreas.[28] By analogy with the findings for stomach and colorectal cancers, DNMT1 protein overexpression may actually result in DNA hypermethylation on such CpG islands of cancer-related genes during pancreatic carcinogenesis. As DNMT1 shows a preference for hemimethylated rather than unmethylated substrates *in vitro*,[29] and targets replication foci by binding to proliferating cell nuclear antigen,[30] it has been considered to be a maintenance form of DNMT that copies methylation patterns after DNA replication. However, in human cancers, unknown factors may potently target DNMT1 to unmethylated substrate DNA, such as CpG islands of specific genes. Moreover, some workers have proposed that DNMT1 possesses both maintenance and *de novo* DNA methylation activity *in vivo*, regardless of its *in vitro* substrate preference.[31] Therefore, it is feasible that DNMT1 protein overexpression actually results in *de novo* and regional DNA hypermethylation during carcinogenesis.

In the present study, we noted that the incidence of DNMT1 immunoreactivity increased abruptly from WD to MD and was further increased in PD (Fig. 3). Even in the same patient, PD components showed a much higher incidence of DNMT1 immunoreactivity than WD or MD components (Fig. 2). Accordingly, we detected a significant correlation between high-level DNMT1 protein expression and an advanced t category and advanced stage at diagnosis defined by the Japan Pancreatic Society.[23] However, high-level DNMT1 protein expression did not show a significant correlation with the clinicopathological parameters defined by the UICC classification.[32] This discrepancy may be attributable to the fact that the classification we used[23] reflects in detail the extent of the tumor by using more parameters than the UICC classification.[32] Our data suggest that DNMT1 protein overexpression was associated with aggressiveness of pancreatic cancers. We then analyzed the overall survival curve by the Kaplan–Meier method (Fig. 4). Almost half of the patients died within the first year after surgery, irrespective of their DNMT1 protein expression level. This appears to reflect the devastating nature of pancreatic cancer. However, a significant difference was observed between the patient groups with a high- and a low-level of DNMT1 protein expression: those with high-level DNMT1 protein expression had a worse prognosis from approximately the second year after surgery. Immunohistochemical examination of DNMT1 in cytological, biopsy or surgically resected specimens may be clinically useful for predicting the outcome of patients with pancreatic cancer.

Although DNMT1 is the major DNMT in humans, the activity of DNMT3a and DNMT3b has also been confirmed.[33] DNA methylation patterns are considered to be established through the cooperation of the three DNMT. Further immunohistochemical studies on the cooperative action of DNMT1 with other DNMT in tissue specimens may provide further understanding of the background factors determining the DNA methylation pattern in pancreatic cancers. DNMT may become a molecular target for preventive and therapeutic strategies against multistage pancreatic carcinogenesis.

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Safety and Effectiveness of Left Hepatic Trisegmentectomy for Hilar Cholangiocarcinoma

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Abstract. Left hepatic trisegmentectomy has been performed for huge malignant tumors, but it is rarely applied in patients with hilar cholangiocarcinoma. Twelve consecutive patients (7 men and 5 women; mean age, 64 years) underwent left hepatic trisegmentectomy in our institution between January 2000 and December 2003. The preoperative management and postoperative outcomes of this surgical procedure were presented and retrospectively analyzed. Preoperative biliary drainage and portal vein embolization were performed in 6 patients (50%) and 9 patients (75%), respectively. The preoperative estimated volume ratio of the posterior segment /the whole liver was 44.8 ± 7.0% (34.3–54.3), the plasma retention rate of indocyanine green at 15 minutes was 8.6 ± 2.2% (4.7–13.7), and the serum total bilirubin level before surgery was 1.0 ± 0.4 mg/dl (0.4–1.7). The serum total bilirubin level on the first postoperative day was 3.3 ± 0.4 mg/dl (1.4–6.2). There was no hospital death or postoperative hepatic failure. The incidence of positive resectional margin was 25%. With biliary decompression and preoperative portal embolization techniques, left hepatic trisegmentectomy was a safe and curative resectional option for hilar cholangiocarcinoma.

Radical surgery including extended right or left hemihepatectomy has been recognized as a standard treatment option of cholangiocarcinoma involving the hepatic hilus [1–4]. Use of biliary decompression and preoperative portal vein embolization (PE) techniques has permitted the adoption of more aggressive surgical resections in this field [5–8].

Left hepatic trisegmentectomy, which is one of the most extensive surgical procedures among the various types of hepatectomy, is usually performed for huge malignant hepatic tumors in adult or pediatric patients [9–12], but it is sporadically applied in patients with advanced carcinoma involving the hepatic hilus because of fear of postoperative hepatic insufficiency due to removal of approximately of 65%–70% of the liver. Clinical details and follow-ups have been little described in the literature [13].

Left hepatic trisegmentectomy can achieve the widest possible clearance of bile duct tumor spreading, when tumors chiefly located in the left hepatic duct are extending to the bifurcation of the anterior and posterior branch of the right hepatic duct with no cancer involvement of right hepatic artery. We described the result of the surgical procedures and clinical data of 12 patients with hilar cholangiocarcinoma who underwent left hepatic trisegmentectomy including removal of the caudate lobe, resection of the extrahepatic bile duct and dissection of the regional lymph nodes, subsequent to biliary drainage and /or PE.

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Patients and Methods

From January 2000 to December 2003, 82 patients with hilar cholangiocarcinoma underwent major liver resections at the National Cancer Center Central Hospital, Tokyo. Of these, 12 patients (12/82; 14.6%) underwent left trisegmentectomy. The hilar cholangiocarcinoma consisted of two type of tumors: (1) hilar bile duct cancer in 7 patients (7/66; 10.6%) and (2) mass-forming cholangiocellular carcinoma involving the hepatic hilus in 5 patients (7/16; 31.3%). Mass-forming cholangiocellular carcinoma was defined as tumor involvement mainly in the hepatic parenchyma. The patients included 7 men and 5 women, ranging in age from 50 to 82 years (mean age: 64 years).

Preoperative Evaluation and Management

Ultrasonography (US), thin-slice computed tomography (CT) with 3-mm collimation and 3 mm/s table movement with a bolus injection of contrast medium [14], angiography, and magnetic resonance cholangiography were carried out to obtain detailed information about local extension of the disease, including the assessment of the proximal and distal end of the hepatic duct. In patients with obstructive jaundice, biliary decompression was established principally by means of percutaneous transhepatic biliary drainage (PTBD) from the posterior inferior branch of the hepatic duct, and cholangiography was performed to assess the proximal tumor extension. Left hepatic trisegmentectomy was indicated by the following conditions: the dominant site of bile duct carcinoma involvement was the left hepatic duct, extending close to the bifurcation of the anterior and posterior branch of the right hepatic duct (Fig. 1A), i.e., Bismuth-Corlette (B-C) type IIIb, IV [15, 16]. Another indication was mass-forming cholangiocellular carcinoma centrally located in the medial segment and/or the left hepatic lobe close to the root of the right anterior portal pedicle (Fig. 1B). Removal of the caudate lobe and

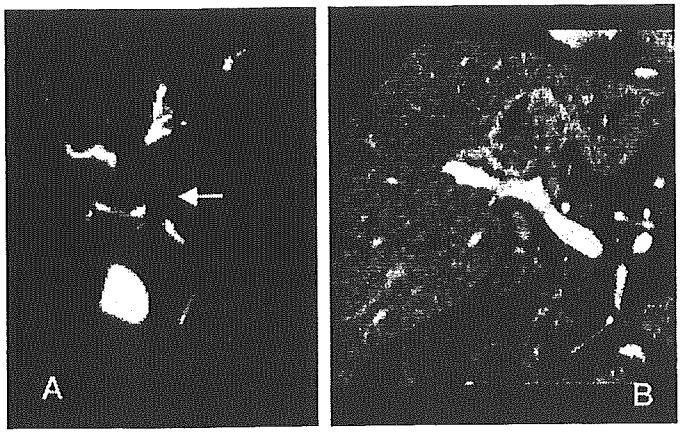

Fig. 1. *A.* Magnetic resonance cholangiography revealed the dominant site of bile duct carcinoma involvement of the left hepatic duct, extending close to the bifurcation of the anterior duct. The posterior hepatic branch (arrow) was separated from the common bile duct (Case 9). *B.* Dynamic computed tomography revealed cholangiocellular carcinoma centrally located close to the root of the right anterior pedicle. The right hepatic duct, which runs along the upper edge of the hilar plate, could not be preserved (Case 10).

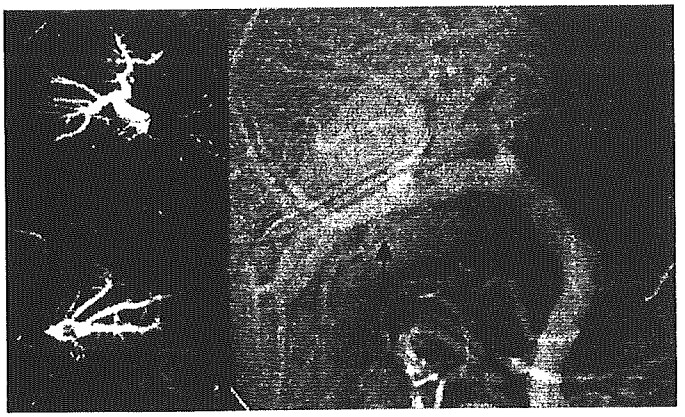

Fig. 2. The anterior branch (*A*) and left portal branch (*B*) of the portal vein were embolized in a percutaneous fashion. After portal embolization, only the posterior branch was observed in a portal phase (*C*) in Case 11.

resection of the extrahepatic duct with left hepatic trisegmentectomy are essential for achieving complete clearance of the tumor with an adequate surgical margin, because the right hepatic duct runs along the upper edge of the right hilar plate. To decrease the risk of postoperative hepatic insufficiency, preoperative PE of the left lobe and the anterior branch (Fig. 2) was performed in patients whose resectional liver parenchymal volume exceeded 60% [4, 17, 18]. Portal Vein embolization was performed when the serum total bilirubin level decreased to below 5.0 mg/dl in patients with obstructive jaundice. The definitive surgery was scheduled 3–4 weeks after PE once liver hypertrophy had been confirmed by successive CT scan and the serum total bilirubin level had decreased to less than 2 mg/dl.

Surgical Procedure

Our standard operative procedure of left hepatic trisegmentectomy was combined with removal of the caudate lobe, resection of

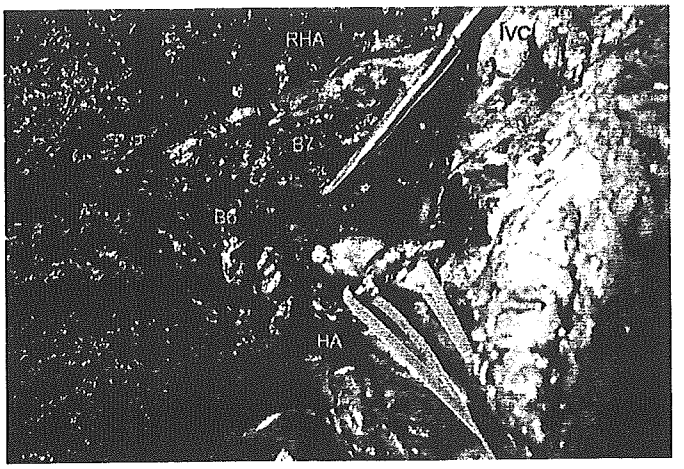

Fig. 3. The surgical field after left hepatic trisegmentectomy. There were two orifices of the posterior bile duct branch. B6: the posterior inferior branch; B7: the posterior superior branch; HA: hepatic artery; PV: portal vein; IVC: inferior vena cava; RHV: right hepatic vein.

the extrahepatic duct, and dissection of the regional lymph nodes. The common bile duct was divided in the pancreas as distally as possible to obtain a negative surgical margin. The duct, lymph nodes, and connective tissue in the hepatoduodenal ligament, posterior to the upper portion of the pancreatic head and around the common hepatic artery, were dissected in an en bloc fashion, leaving only the portal vein and the hepatic artery. The preparation of the hilar structure was identical to that previously described for extended hemihepatectomy [4]. The whole liver was mobilized from the retroperitoneum and the inferior vena cava (IVC) by ligating and dissecting the short hepatic veins caudocranially. On the left side, after retracting up the lateral segment and dividing the ligamentum venosum at the junction with the left hepatic vein, the trunk of the left and middle hepatic veins was dissected. The right hepatic vein was secured after division of the vena cava ligament. The liver was completely separated from the IVC except for the right hepatic vein. The demarcation line between the anterior and posterior sections, and the outer confines of the caudate process and the paracaval portion against the posterior section, were marked by electrocautery. Liver transection was performed, exposing the full length of the right hepatic vein, using the Pringle maneuver (hepatic inflow occlusion time, 15 minutes, and reperfusion time, 5 minutes). A Pean clamp was used to crush the liver tissue without damaging the vessels and ducts. A pediatric Kelly forceps was then passed through these vessels and ducts, which were subsequently ligated on the side of the remaining parenchyma and divided. Finally, the posterior hepatic duct was dissected (Fig. 3). A Roux-en-Y loop was prepared in the usual fashion and hepaticojejunostomy was performed in an end-to-side fashion with interrupted suture of 5-0 polydioxanone.

Clinical features, operative results, changes in serum biochemistry data, and postoperative complication were examined. The percent volume of the posterior segment was calculated before PE and hepatectomy. The resected specimen was examined macroscopically, and the total weight of the resected specimen was measured. During the histological examination, surgeons assisted pathologists in identifying resectional margins for preparation of sections of a fixed specimens. All values were expressed

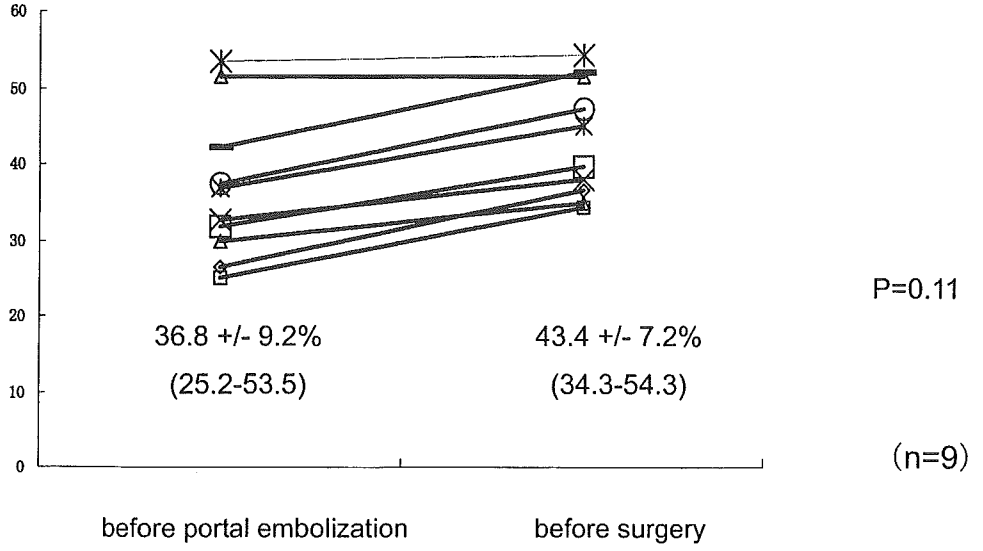

Fig. 4. Change in estimated liver volume ratio of the posterior segment/the whole liver, measured using CT volumetry.

as the mean ± S.D. Statistical significance was determined using student's *t*-test. A p value <0.05 was significant. After discharge, the patient was followed regularly for tumor recurrence by tumor markers, US, and CT.

Results

Six patients (60%) had jaundice and all the patients underwent PTBD, preoperatively. The total bilirubin level before PTBD and before surgery was 11.1 ± 7.4 mg/dl (1.4–24) and 1.2 ± 0.5 mg/dl (0.5–1.7), respectively (p = 0.03). The median duration between PTBD and surgery was 50 days (33–95). Portal Vein embolization was performed in 9 (75%) patients. Four patients underwent transiliac portal vein embolization and another 5 patients the percutaneous transhepatic approach [17]. Three of these had obstruction of the left portal vein due to tumor invasion. In these patients, only the anterior portal branch was embolized. The estimated volume ratio of the posterior segment/the whole liver just before PE and before surgery was 36.8% ± 9.2% (25.2–53.5) and 43.4% ± 7.2% (34.3–54.3), respectively (p = 0.11; Fig. 4). In 3 patients, the estimated volume ratio exceeded 40%. These patients underwent PE because the serum total bilirubin level was not normalized for long intervals and they had persistent jaundice after PTBD or had repeated attacks of cholangitis [18]. The median time between PE and hepatectomy was 22 days (range: 19 to 62 days). There were no complications after PE. Three patients did not undergo PE because the estimated volume ratio just before hepatectomy was 42.6%, 45.0%, and 54.0%, respectively. Two patients had no jaundice and one jaundicized patient recovered promptly a week after PTBD.

Finally, the estimated volume ratio of the posterior segment before surgery in all patients was 44.8% ± 7.0% (34.3–54.3), ICGR15 was 8.6% ± 2.2% (4.7–13.7), and the preoperative total bilirubin level was 1.0 ± 0.4 mg/dl (0.4–1.7). Operative blood loss was 2259 ± 1203 ml (755–5913) and operative time was 662 ± 156 min (420–865). Concomitant procedures included resection and reconstruction of the portal vein in 1 patient, and inferior vena cava in 1. There were no intraoperative complications. There was one stump of the posterior hepatic duct in 9 patients, and two in 3 patients. The former 9 patients were diagnosed as having a cancer-free surgical margin by intraoperative frozen sections. One of the latter 3 patients was diagnosed as having a negative surgical margin after an additional resection, but the other 2 patients finally had positive surgical margins resulting from intraluminal tumor spread.

Table 1. Changes in serum biochemistry data after the operations

	Before surgery	POD1	POD7	POD14
Total bilirubin (mg/dl)	1.0 ± 0.4	3.3 ± 1.4*	2.1 ± 0.9**	1.7 ± 0.9***
GOT(U/l)	44 ± 17	733 ± 604†	56 ± 82	50 ± 67
GPT(U/l)	59 ± 32	648 ± 633‡	118 ± 128	61 ± 68
				n = 12

The postoperative levels of serum total bilirubin, glutamic oxaloacetic transaminases (GOT), and glutamic pyruvic transaminases (GPT) increased most on the first postoperative day, but gradually decreased and returned to the preoperative values within 2 weeks (Table 1). The maximum serum total bilirubin level was less than 6.2 mg/dl, and there were no cases of hepatic failure.

Postoperative and pathological parameters are summarized in Table 2. Postoperative bleeding related to minor pancreatic juice leakage after lymph node dissection of the posterior to upper portion of the pancreas head developed in Case 1, and reoperation was performed. Because of abscess formation near the hepatic transection plane, one patient (Case 9) underwent ultrasound-guided percutaneous drainage without relaparolotomy. Two patients had bilioenteric anastomotic leakage, and 3 had minor bile leakage from the transectional hepatic parenchyma without additional surgical and interventional treatment. There were no operative or hospital deaths. Microscopic examination revealed that the cut end of the proximal bile duct was positive for cancer cells in Cases 3 and 8. The dissection margin in Case 10, a patient who underwent combined resection of the IVC, was positive. Nine patients (75%) were judged to have a cancer free-margin. Metastases to regional lymph nodes were observed in 7 patients (58%).

Table 2. Pathological and postoperative parameters.

No/sex/age	Diagnosis	Total weight of resected specimen	BW	EW	Lymph node metastases	Postoperative complication	Prognosis
1/M/66	HBD	675	N	N	(+)	Postoperative bleeding	Dead 29 months with recurrence (liver, lymph nodes)
2/F/82	CCC	840	N	N	(−)	Bile leakage	Dead 32 months with recurrence (peritoneum)
3/M/62	HBD	503	N	N	(−)	Bile leakage	Alive 48 months without recurrence
4/F/50	CCC	690	N	N	(+)	None	Dead 25 months with recurrence (liver)
5/M/60	CCC	951	N	N	(+)	None	Alive 37 months with recurrence (lymph nodes)
6/M/54	HBD	620	P	N	(-)	None	Alive 34 months without recurrence
7/M/70	CCC	731	N	N	(-)	Bile leakage	Alive 21 months without recurrence
8/F/71	HBD	643	P	N	(+)	Bile leakage	Alive 19 months without recurrence
9/M/50	CCC	390	N	N	(+)	Abscess	Alive 18 months without recurrence
10/F/69	CCC	468	N	P	(+)	Bile leakage	Alive 12 months without recurrence
11/F/73	CCC	517	N	N	(+)	None	Alive 6 months without recurrence
12/M/67	HBD	655	N	N	(−)	None	Alive 5 months without recurrence

HBD: hilar bile duct carcinoma; CCC: cholangiocellular carcinoma involving the hepatic hilus; BW: proximal and distal hepatic/bile duct cut end; EW: dissected surgical margin; P: definitive invasion of the cut end; N: cancer-free margin.

Median follow-up was 23 months (range: 5–48 months). Four patients were diagnosed as having recurrence during follow-up, for a recurrence rate of 33.3%. Up to the end of April 2004, 3 patients have died from recurrence. No anastomotic local recurrence had been recognized in these three patients.

Discussion

Left hepatic trisegmentectomy is one of the most extensive surgical procedures among the various types of hepatectomy. This procedure has been indicated for huge hepatic malignancy, which occupies a large part of the normal or slightly impaired liver parenchyma, and postoperative hepatic insufficiency has rarely been encountered.

The procedure involves removal of approximately 60%–70% of the liver in patients for hilar cholangiocarcinoma, whose hepatic function is sometimes impaired after long-term biliary obstruction and pre-existing cholangitis. In this series, proper preoperative management including biliary decompression and portal embolization led to a reduction in the risk of postoperative hepatic insufficiency and resulted in no mortality. Just before the operation, the mean volume ratio of the posterior segment was estimated at 44.5%, which seemed to be a safe level considering the preoperative ICGR15 level (8.6% ± 2.2%, normal range: <10%) [19]. However, the peak value of the serum total bilirubin level on postoperative day was unusually high compared with that after conventional hepatectomy [19, 20]. There might be a possibility of prolongation of the elevation of serum total bilirubin for a long time and subsequent hepatic failure without the preoperative management.

Serious postoperative complications are another cause of hepatic failure. The incidence of surgery-related complications in our series was 58.3% (7/12). The reported morbidity rate after hepatectomy for hilar bile duct cancer is 36.9%–43.8% [4, 5, and 8], which seems to be high compared with that reported after hepatectomy without hepatico-enteric anastomosis and wide lymph node dissection [19]. Biliary decompression and portal embolization appears to be effective for increasing the safety of such extensive liver resection when an unexpected episode such as postoperative sepsis or bleeding occurs. GOT and GPT levels were increased remarkably on postoperative day 1, in spite of a systematic hepatectomy with small ischemic hepatic remnant. This was probably due to the extensive mobilization of the whole liver, to hepatic vein congestion that resulted from lifting up the remnant liver during hepatic transaction, or to repeated intermittent hepatic ischemia using the Pringle maneuver [19–21]. The transient elevation of GOT/GPT was not related to postoperative liver insufficiency, but careful mobilization of the liver and a quick hepatic transection should be considered when the volume of the hepatic remnant is limited.

Hilar bile duct cancer, which has a longitudinal extent along the bile duct, essentially needs combined wide resection of intra- and extrahepatic bile ducts. The incidence of the positive resectional margin rate was lower in this study, compared with that previously reported, 31.6%–44.2% [4, 5]. On the other hand, mass-forming cholangiocellular carcinoma involving the hepatic hilus has fewer incidences of longitudinal ductal spreading, but requires additional extrahepatic bile duct resection to obtain a negative surgical margin. The surgical margin is one of the most important prognostic factors in the surgical treatment of cholangiocellular carcinoma [22]. The extent of hepatic resection was not a significant prognostic factor, but it was chiefly related to obtaining a cancer-free margin.

The oncological value of dissection of regional lymph nodes is controversial, but it seems to be manageable and effective when lymph node metastases regionally limited [4]. As pathological examination has revealed a lymph node positive rate of 51.7%, candidates for trisegmentectomy seem to be in a more advanced of state of disease compared with those for other types of major hepatectomy [1–5].

Long-term follow-up has not been accomplished, but 9 patients survived for over 12 months and 3 patients died from distant recurrence after 25 months. Until the end of April 2004, there has been no local recurrence, which is the main direct cause of death due to uncontrollable cholangitis. Obtaining a clear resectional margin in patients with hilar cholangiocarcinoma is the only chance for cure, and it provides good quality of life.

In conclusion, once reliable preoperative management and evaluation were prepared, our operative procedure consisting of left hepatic trisegmentectomy, removal of the caudate lobe, resection of the extrahepatic bile duct, and dissection of regional lymph nodes, is a safe and curative treatment option for this disease.

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Surgical Results for Hepatocellular Carcinoma With Bile Duct Invasion: A Clinicopathologic Comparison Between Macroscopic and Microscopic Tumor Thrombus

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Background: The aim of this study was to evaluate the prognostic factors and long-term results after surgery in patients with hepatocellular carcinoma (HCC) with bile duct invasion.

Methods: The records of 38 HCC patients with microscopic (tumor thrombus was found in more than the second order branch of the biliary tree; n = 19) and macroscopic (tumor thrombus was found in no more than the second order branch of the biliary tree; n = 19) bile duct invasion were reviewed in this study. Survival rates were calculated with regard to 18 clinicopathological factors. A log-rank analysis was performed to identify which factors predict the prognosis. The relationships between the degree of bile duct invasion and 17 clinicopathologic factors were also compared.

Results: The overall 1-, 3-, and 5-year survival rates were 79%, 45%, and 33%, respectively. The indicators of a favorable prognosis included no intrahepatic metastases, curative surgical resection, and macroscopic bile duct invasion.

Conclusion: We found a favorable long-term postoperative result for HCC patients with macroscopic bile duct invasion. Even if HCC tumor thrombus is recognized in the major branches of bile duct, extensive and curative surgical treatment should be recommended when hepatic functional reserve is satisfactory without intrahepatic metastases.

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KEY WORDS: **hepatocellular carcinoma; bile duct tumor thrombus; surgical resection**

INTRODUCTION

Hepatocellular carcinoma (HCC) usually spreads intrahepatically via portal vein branches, and the incidence of portal vein invasion was 34%–40% in surgical resected series [1,2]. When tumor thrombi extended to the major portal vein, the prognosis is extremely poor compared with that of microscopic portal vein invasion [3,4]. On the other hand, the rate of intrabiliary growth of HCC is rare, ranging from 2.3% to 13% in surgical and autopsy cases [5,6]. Surgical resection for HCC with obstructive jaundice had been rarely indicated due to poor hepatic functional reserve caused by both underlying liver cirrhosis and cholestasis [7]. Advanced improvement in preoperative management has improved the safety of hepatectomy for patients with obstructive jaundice and there were no significant differences in survival between patients with bile duct thrombi and those without tumor thrombi in the recent reports [8,9].

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However, a long-term result for hepatectomy in patients with bile duct invasion, in special reference to the tumor thrombi extension, has not been well evaluated.

In this study, we retrospectively analyzed the clinicopathological factors in 38 patients with bile duct invasion to examine whether the degree of bile duct invasion influences the prognosis of patients undergoing surgical resection.

PATIENTS AND METHODS

Between January 1990 and December 2002, 1,168 patients underwent hepatic resections for HCC, at the National Cancer Center Hospital, Tokyo, Japan. All the resected specimens were cut into serial 5 mm thick slices and fixed in 10% formalin. After macroscopic examination, slice with greatest dimensions and other slices containing areas of possible bile duct invasion as well as microscopic metastases and portal vein invasion were trimmed for paraffin blocks and into 5 μm microscopic sections, which were then stained with hematoxylin and eosin. The presence of bile duct tumor thrombi was investigated as a part of routine histopathologic examination in all patients [10–13]. Bile duct invasion in the intrahepatic or extrahepatic bile duct was classified into two categories according to the following criteria: microscopic bile duct invasion, which represents that the tumor thrombus can be seen in more than second branch of the biliary tree (excluding the second order branch), and macroscopic bile duct invasion, which represents that tumor thrombus was found in no more than the second order branch, i.e., in the common bile duct, the right or left main hepatic duct, or the second order branch of the intrahepatic bile duct. Thirty eight patients (3.4%) developed bile duct tumor thrombi. There were 32 men and 6 women, and the mean age of them was 62 years (range: 27–83). Nineteen patients (1.7%) with macroscopic bile duct invasion and 19 patients (1.7%) with microscopic invasion were enrolled in this study. Preoperative examination included ultrasonography (US), thin-slice computed tomography (CT) with a bolus injection of contrast medium, angiography, and if necessary, magnetic resonance cholangiography. In six patients (15%) with obstructive jaundice, biliary decompression was performed using percutaneous transhepatic biliary drainage and cholangiography was obtained to assess the tumor extension. Surgery was performed when serum total bilirubin level decreased to less than 2.0 mg/dl. The maximum limit of resectional liver-volume was evaluated based on the indocyanine green retention rate at 15 min (ICG15R) [14,15]. Every cut end of the bile duct was submitted for frozen-section examination. If the tissue was positive for cancer cells, an additional resection and/or combined resection of extra hepatic bile duct to secure negative margin was carried out. Curative surgery was defined when all gross lesions were removed with tumor free-margins. Microscopic examination confirmed the final diagnosis of surgical margin and the presence of direct bile duct wall invasion. Clinical and operative features in patients with macroscopic bile duct invasion were investigated. Eighteen clinicopathologic factors were analyzed with reference to their prognostic significance in all the patients. All significant prognostic factors in univariate analysis were assessed by multivariate analysis. The relationships between the degree of bile duct invasion and the 17 clinicopathological factors were also compared. After discharge from the hospital, all 38 patients were closely followed at our outpatient clinic every 3 months by measurement of the serum alpha-fetoprotein levels, US, and CT. When tumors recurred, percutaneous ethanol injection therapy (PEI), tumor ablation therapy (RFA), transcatheter chemoembolization arterial chemotherapy (TACE), or repeated hepatectomy was performed.

Values are expressed as mean ± SD. Any statistical difference among the groups was analyzed with the unpaired *t*-test or Chi-square test. Survival estimates were calculated by the Kaplan–Meier method. Univariate comparison of the survival curves was made with the log-rank test. Association were considered significant if $P \leq 0.05$. Multivariate regression analysis was performed using the Cox proportional hazards model and variables associated $P < 0.10$ were entered into the final model adopted [16].

RESULTS

Clinical features in patients with macroscopic bile duct invasion were shown in Table I. The mean serum total bilirubin level and ICGR15 were 5.0 ± 7.3 mg/dl (0.3–25.0) and 12.0% ± 7.8% (4.3–31.5), respectively. There was no postoperative liver failure and hospital mortality. Among 19 patients with macroscopic bile duct invasion, 7 patients (37%) had tumor thrombi in the common bile duct (CBD), 5 patients (26%) had in the right hepatic duct, 3 patients (16%) had in the left hepatic duct, and 4 patients (21%) had in the second order branch of the intrahepatic bile duct. The patient (Case 6) had jaundice due to the migration of tumor thrombi in the CBD, which spontaneously disappeared before operation. Direct invasion of the bile duct wall was recognized in three patients (16%) with tumor thrombi in located in the CBD. Bile duct resection and tumor thrombectomy were preformed in five patients (26%) and three patients (16%), respectively.

Overall 1-, 3-, and 5- year survival rate were 79%, 45%, and 33%, respectively. The median duration of survival time was 31 months, ranging from 3 to

TABLE I. Clinical Features of Patients With Macroscopic Bile Duct Invasion

No.	M/F	Age	ICGR15 (%)	Thr extension of bile duct invasion	Operative procedure	Bile duct resection	Tumor thrombectomy
1	M	52	7.0	CBD	Left hepatectomy	No	Yes
2	M	45	31.5	Left hepatic duct	Left hepatectomy	No	No
3	M	60	7.0	The second branch	Left hepatectomy	No	No
4	M	55	22.8	The second branch	Lateral segmentectomy, S8LR[b]	No	No
5	M	41	5.8	Left hepatic duct	Left hepatectomy	Yes	No
6	M	59	21.4	CBD[a]	Right hepatectomy	Yes	No
7	M	62	18.5	CBD[a]	Left hepatectomy	Yes	No
8	M	43	9.6	CBD	Central bisegmentectomy	Yes	No
9	M	49	10.0	The second branch	Medial segmentectomy	No	No
10	M	68	9.6	Right hepatic duct	Right hepatectomy	No	No
11	M	68	4.3	CBD	Anterior segmentectomy	No	Yes
12	M	76	7.9	Left hepatic duct	Left hepatectomy	No	No
13	M	69	11.5	Right hepatic duct	Right hepatectomy	No	No
14	M	58	16.9	Right hepatic duct	Right hepatectomy	No	No
15	M	57	5.8	Right hepatic duct	Right hepatectomy	No	No
16	M	64	21.7	CBD	Medial segmentectomy	Yes	No
17	M	55	6.3	CBD[a]	Left hepatectomy	No	Yes
18	M	83	6.6	The second branch	Medial segmentectomy	No	No
19	M	72	4.6	Right hepatic duct	Right hepatectomy	No	No

ICG15R, the indocyanine green retention rate at 15 min.
[a]CBD, direct invasion to the bile duct wall.
[b]S8LR, segment 8 limited resection.

144 months. Disease-free 1-, 3-, and 5-year survival rates were 45%, 29%, and 16%, respectively. After operation, 26 patients died, 3 are alive with recurrence, and 9 are alive without recurrence. Nine patients have survived for more than 5 years. The liver was the first site of recurrence in all patients. Both intrahepatic and extrahepatic recurrence was recognized 11 patients. The involved extrahepatic organs were lung (six patients), lung and lymph node (one), bone (one), lymph node (one), lymph node and peritoneum (one), and peritoneum (one), who underwent tumor thrombectomy (Case 1).

Eighteen clinicopathologic factors were investigated with reference to their prognostic significance. Factors such as presence of preoperative treatment, the high value of serum alpha-protein (>1,000 mg/dl), presence of jaundice, or positive hepatitis C virus antibody did not influence for survival. The results of the log-lank test are shown in Table II. The indicators of a favorable prognosis included no intrahepatic metastases ($P = 0.0208$), curative surgical resection ($P < 0.0001$), and macroscopic bile duct invasion ($P = 0.0463$). Figure 1 showed cumulative survival curve of patients after resection with macroscopic and microscopic bile duct invasion. Five year survival rate in patients with macroscopic bile duct invasion was 50% and six patients survived more than 5 years after operation. Hepatitis B virus antigen positive tended to have a better prognosis, these difference were not statically significant ($P = 0.0509$). The impaired liver function did not influence the mean duration of survival.

When the significant prognostic factors in univariate analysis were assessed by multivariate analysis, the following factors were found to be independently associated with a favorable prognosis: curative surgical resection, no intrahepatic metastases, and macroscopic bile duct invasion with hazard ratio (95% confidence interval) of 6.099 (2.361–15.755), 2.896 (1.213–6.918), and 0.270 (0.108–0.674), respectively (Table III).

The relationships between the degree of bile duct invasion and the 17 clinicopathological factors were compared. Age (<60), positive hepatitis C virus antibody, positive hepatitis B virus antigen, presence of preoperative treatment, and the high value of serum alpha-protein (>1,000 mg/dl) were not associated with the degree of the bile duct invasion. Table IV shows the results of relationship between the extension of bile duct invasion and 12 clinicopathologic factors. Macroscopic bile duct invasion was strongly associated with sex, symptoms, ICGR15, surgical procedure, and bile duct resection by univariate analysis. There was no significant difference between microscopic bile duct invasion and portal vein invasion ($P = 0.073$).

DISCUSSION

Surgical treatment provides an only cure of chance when tumor grows via intrabiliary. A few reports concerning long-term survivors after hepatectomy in patients with HCC and tumor thrombi described 5-year survival

TABLE II. Possible Clinical and Pathological Risk Factors for Survival

Factor	No. of patients	Proportion surviving (%)			P^c
		1 year	3 years	5 years	
Overall	38	79	45	33	
Age (years)					0.8153
<60	19	89	41	34	
≥60	19	74	45	39	
Sex					0.2044
M	32	78	47	40	
F	6	83	0	0	
Hepatitis B virus antigen					0.0509
Positive	8	88	73	73	
Negative	30	80	36	28	
ICGR15 (%)[a]					0.1637
>15	21	71	46	46	
≤15	17	88	40	26	
Numbers					0.9420
Solitary	19	95	39	39	
Multiple	19	63	46	33	
Size (cm)					0.4833
>6	20	85	41	29	
≤6	18	72	47	47	
Portal vein involvement					0.3180
Positive	27	77	39	34	
Negative	11	82	52	42	
Intrahepatic metastases					0.0208
Positive	19	68	20	13	
Negative	19	89	65	59	
Histologic differentiation					0.5309
Well/mod	21	76	55	44	
Poor	11	81	37	37	
Combined[b]	6	83	17	17	
Nontumorous lesions					0.2311
Cirrhosis	10	80	50	30	
No cirrhosis	28	78	39	39	
Operative procedure					0.9730
Limited resection	12	83	43	32	
Segmentsctomy, lobectomy	26	77	42	38	
Bile duct resection					0.7538
Yes	8	75	38	38	
No	30	79	45	36	
Surgical curability					0.0000
Yes	30	93	51	47	
No	8	25	13	0	
Extension of bile duct invasion					0.0463
Microscopic	19	73	28	21	
Macroscopic	19	84	56	50	

[a]ICG15R, the indocyanine green retention rate at 15 min.
[b]Combined means mixed hepatocellular carcinoma and cholangiocarcinoma.
[c]Log rank test.

rate of around 28%–39% [7–9]. In our series, the duration of survival in patients with HCC with macroscopic bile duct invasion has been 41 months, ranging from 8 to 136 months. Five year survival rate was 50% and six patients survived more than 5 years after operation. The present favorable results, which was relatively better than that of others, may be because surgery could be performed at an earlier stage before macroscopic bile duct invasion did not completely obstruct the common bile duct in 12 patients (63%).

It is generally accepted that HCC with tumor thrombi have a shorter survival after diagnosis than do other HCC patients [5,6]. This is the reason HCC with tumor thrombi is frequently accompanied with portal vein invasion and underlying liver cirrhosis [5,8,9]. However, in this study, macroscopic bile duct invasion was an independent favo-

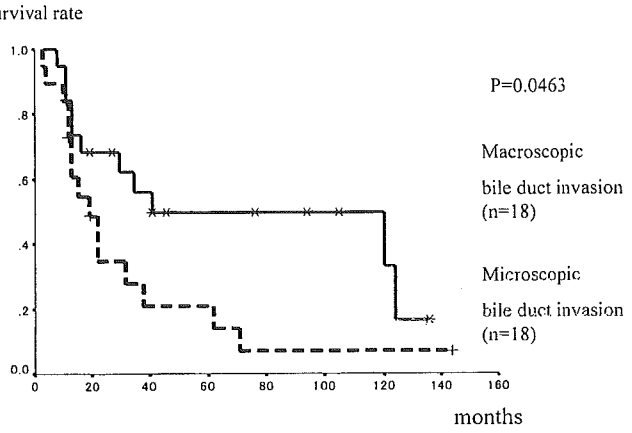

Fig. 1. Cumulative survival curve of patients after resection with macroscopic and microscopic bile duct invasion. There was significant difference in the survival between two groups ($P = 0.0463$).

rable prognostic factor and the degree of bile duct tumor invasion was not related to the incidence of portal vein involvement and intrahepatic metastases. As the difference between patients with macroscopic and microscopic bile duct invasion was the preoperative ICG15R value, wider extent of liver resections (segmentectomy or lobectomy) could be achieved in patients with macroscopic bile duct invasion. Liver cirrhosis was microscopically confirmed in only 3 patients (16%) among 19 patients with macroscopic bile duct invasion. Previously HCC with biliary tumor thrombi was frequently associated with cirrhotic liver in autopsy cases, but Kojiro et al. [5] described intraductal growth could occur in a relatively early phase of the disease because progressive jaundice was the initial sign in one third of these cases, and ductal invasion was seen in a patient with minute HCC. Shiomi et al. [9] recently reported bile duct tumor thrombi were likely to originate in noncirrhotic liver in their surgical cases. Patients in HCC with the bile duct invasion which extended to the larger branch of the biliary tree compared with microscopic bile duct invasion, had infrequency coexistence of portal vein invasion and better functional hepatic reserve. This might contribute a favorable long-term outcome.

The mechanism of macroscopic bile duct invasion has not been well evaluated. Chen et al. [17] described bile duct tumor thrombi as ruptured HCC into the common bile duct. Such a penetration into the intrahepatic bile duct system seemed to be easy to occur in rather soft liver than cirrhotic liver. In our study, the preoperative ICG 15R value was better in patients with further extension of bile duct invasion. Even if tumor thrombi extended to the larger branch, it sometimes spontaneously changed necrosis and behaved like a migration stone with a seldom implantation to the bile duct wall [8,9,17]. Recent report revealed bile duct tumor thrombi in HCC patients might have lower aggressive potential and be less important as a prognostic factor compared with portal vein involvement which represents potential tumor spreading to the whole liver [18]. Yamamoto et al. reported macroscopic intrabiliary invasion of colorectal liver metastases which was observed in 18 patients (12%) with normal liver, was a paradoxical favorable prognostic factor indicating indolent tumor characteristics and favorable post-resection outcome [12,19]. However, a different pathogenesis between microscopic and macroscopic bile duct invasion could not be explored in their study.

The frequent intrahepatic recurrence of HCC after hepatic resection results from intrahepatic metastasis and multicentric occurrence in the remnant liver. Percutaneous ethanol injection, ablation therapy, or transarterial chemoembolization, are well established treatments for recurrent HCC after surgery. However, such non-surgical therapy after bile duct resection has sometimes caused a severe cholangitis or liver abscess [20,21]. Therefore, bile duct resection and bilioenteric anastomosis should not be performed in patients with HCC and direct removal of tumor thrombus has been strongly recommended [8,9,17]. In our series, four patients underwent bile duct resection because direct tumor invasion of the bile duct wall was recognized intraoperatively. On the other hand, tumor thrombectomy through choledocotomy might be a major cause of peritoneum dissemination [22]. One patient with peritoneal recurrence, who underwent tumor thrombectomy, had simultaneously intrahepatic multiple recurrence. Considering the incidence of peritoneal recurrence and the simultaneous intrahepatic and extrahepatic recurrence, this procedure did not seem to be contraindicated because postoperative intrahepatic recurrence frequently occurs in patients with HCC. There is no evidence combined resection of bile duct

TABLE III. Results of Cox Multivariate Regression Analysis

Variables	B	SD	*P*	Hazard ratio	95% CI
Surgical curativity (yes vs. no)	1.808	0.484	0	6.099	2.361–15.755
Intrahepatic metastases (no vs. yes)	1.063	0.444	0.017	2.896	1.213–6.918
BDTT extension (micro vs. macro)	−1.310	0.467	0.005	0.27	0.108–0.674