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Journal of HBP Surgery

Advanced gallbladder cancer: Indian "middle path"

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Abstract
Gallbladder cancer (GBC) is common in northern India. The western world has a pessimisctic attitude towards GBC resulting in inadequate management of even early GBC. At the other extreme is the Japanese aggressivism with high mortality but very few actual long-term survivors. The Indian surgeons have adopted a Buddhist "middle path" — aggressive surgical approach for "less advanced" GBC and non-surgical palliative approach for "more advanced" GBC. We rely heavily on staging laparoscopy to detect metastatic deposits on liver, peritoneum and omentum, and upper gastrointestinal endoscopy (UGIE) to detect duodenal infiltration which indicates unresectability as we do not perform pancreaticoduodenectomy for GBC. Our favoured procedure is extended cholecystectomy (EC) which includes a 2 cm nonanatomical wedge of liver in the GB bed and the lymph nodes in hepatoduodenal ligament, behind the duodenum and head of pancreas and along the hepatic artery to the right of celiac axis. EC can achieve R0 resection in patients with T1-T2 and T3 (fundus/body — hepatic bed type) disease. For T3 (neck — hepatic hilum type) and T4 disease major hepatic resection is required. In selected patients with nodally advanced GBC, a non-curative simple cholecystectomy with post-operative chemoradiotherapy may improve survival. GBC is an "Indian disease" and Indian surgeons have to be prepared to accept the "challenge" of GBC.

Key words Gallbladder neoplasms · Biliary tract neoplasms · Cholelithiasis

Gallbladder cancer — an "Indian disease"

Gallbladder cancer (GBC) is the most common biliary tract malignancy worldwide — more common than cholangiocarcinoma, which somehow has received more attention than GBC from surgeons. Incidence rates of GBC are low (around 1 per 100 000 per year) in the west (United States, United Kingdom, Australia, and New Zealand). Areas of high incidence rates are in Central and South America, Central and eastern Europe, and Japan. Recently, the Indian Council of Medical Research[1] has reported that incidence rates for GBC in women in northern India — more than 9 per 100 000 per year — are one of the highest in the world. GBC is the most common malignancy of the gastrointestinal tract in women[1] and the most common cause of malignant surgical obstructive jaundice in northern India.[2] Several northern Indian centers had reported many experiences with GBC in the 1970s and 1980s.[3–6] The All India Institute of Medical Sciences (AIIMS), New Delhi group has highlighted the dismal prognosis in patients with GBC.[7,8] The contributions of the Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow (SGPGIMS) group were recognized when it was invited by the International Gastro-Surgical Club (IGSC) to be the guest editor of a special issue of its journal, *Hepatogastroenterology*, on GBC, in which authors from Chile, France, Japan, the Netherlands, the United Kingdom, and the United States contributed.[9] The Varanasi group recently edited a special issue of the *Journal of Surgical Oncology*,[10] in which the SGPGIMS group highlighted the Indian experience and contribution[11] and the surgical attitudes and approaches to GBC.[12]

We have called GBC an "Indian disease".[13]

Advanced gallbladder cancer

Overall prognosis in patients with GBC is dismally poor, with median survival of less than 6 months and 5-year survival of 5%–10%. This is because most patients, when symptomatic and diagnosed, have an advanced

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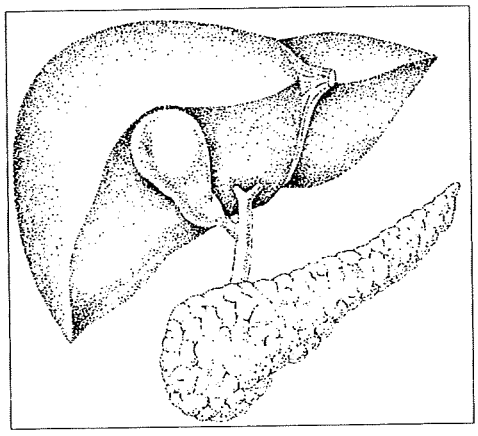

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Recent advance in the treatment of hilar cholangiocarcinoma: hepatectomy with vascular resection

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Abstract
Radical surgical resection has been revealed to be the only hope of cure for the patient with hilar cholangiocarcinoma. Therefore, major efforts have been made to increase the resection rate by surgeons employing combined hepatic resection and vascular resection of the portal vein and the hepatic artery. Especially, the technical feasibility and surgical safety of hepatic resection with combined portal vein resection have recently been reported by several authors. On the other hand, there have been few reports of combined hepatic artery resection in hilar cholangiocarcinoma. There are fears that combined vascular resection with extended hepatectomy for hilar cholangiocarcinoma may lead to high surgical morbidity and mortality. Herein, we describe the results of aggressive surgical approaches in our series, and we also review the outcomes of hepatic resection with combined vascular resection in the previously reported literature.

Key words Hilar cholangiocarcinoma · Portal vein · Hepatic artery · Vascular resection

Introduction

Radical surgical resection has been revealed to be the only hope of cure for the patient with hilar cholangiocarcinoma. Therefore, surgeons have made major efforts to increase the resection rate, with the first reports of these increases being made between the 1980s and 1990s.[1–5] Extended hepatectomy and combined vascular resection have played a major role in the increase of the resection rate for hilar cholangiocarcinoma. However, these extended surgeries had high surgical morbidity and mortality rates, although they led to a slight improvement in the prognosis of the patients.[6–8] To avoid the occurrence of lethal postoperative complications such as liver failure and sepsis, various useful strategies, such as preoperative portal vein embolization[9] and parenchyma-preserving hepatectomy[10–12] have been developed in recent years.

Surgical techniques of hepatectomy with combined vascular resection have also been refined very well recently.[13–15] These techniques have brought about a remarkable decrease in the occurrence of surgical complications, which may improve the outcome of patients with hilar cholangiocarcinoma.[16,17]

Here, we review, from the latest data, the clinical implications of combined vascular resection in the surgical treatment of hilar cholangiocarcinoma.

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Portal vein resection

Surgical techniques

Combined portal vein resection was selected according to the extent of the tumor invasion to the portal vein, as determined by preoperative and intraoperative evaluation. Only when curative surgical resection is to be obtained by combined portal vein resection should the portal vein be resected. Our previous report[10] revealed that curative resection, lymph node involvement, and vascular resection were significant independent prognostic factors after surgical resection in patients with hilar cholangiocarcinoma. Therefore, combined portal vein resection should be indicated for patients with locally advanced hilar cholangiocarcinoma without extensive lymph nodal involvement or distant metastases. Most patients with combined portal vein resection undergo hemihepatectomy with contralateral portal vein reconstruction.[18] The decision on whether to perform combined vascular resection is finally made according to the intraoperative macroscopic findings of

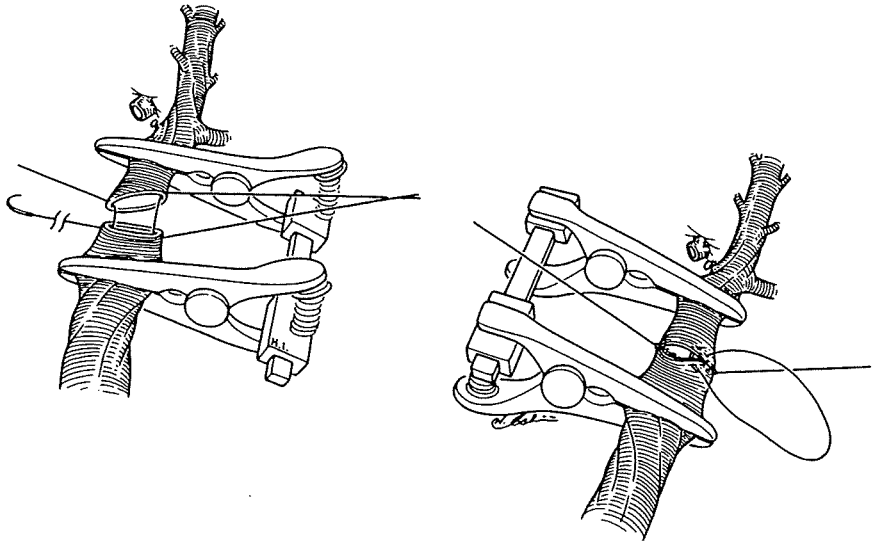

Fig. 1. Portal vein reconstruction done in a continuous fashion with a 6–0 nonabsorbable suture

Table 1. Combined vascular resection in hilar cholangiocarcinoma

Vessels	Number of patients
Portal vein reconstruction	41
Right portal vein	
Left portal vein	
Main portal trunk	
End-to-end	39
Autologous vein graft	2
Hepatic artery reconstruction	9
Left hepatic artery	3
Right hepatic artery	3
No reconstruction	
Right anterior hepatic artery	1
Right hepatic artery	1
Left hepatic artery	1

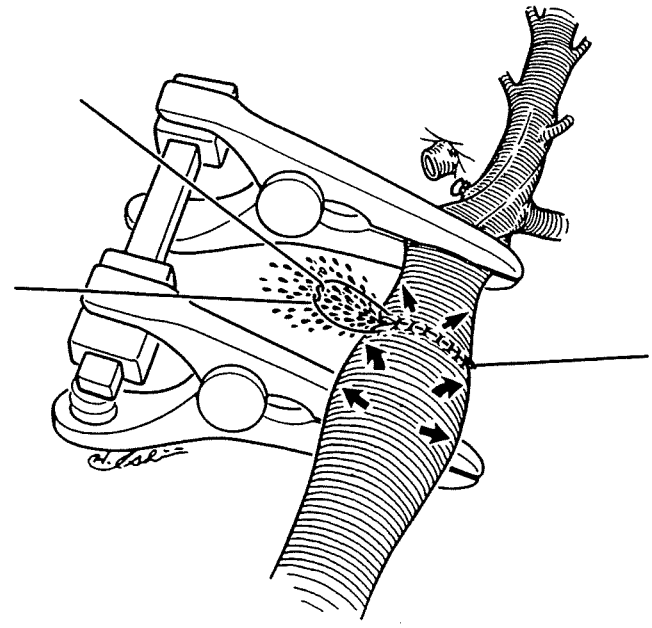

Fig. 2. Final ligature of the continuous suture should be done following expansion of the proximal portal vein (*arrows*) by the release of a proximal vascular clamp to avoid stricture of the anastomosis

tumor invasion to the vessels, together with the preoperative imaging findings. In our series of 41 patients with portal vein resection, the portal vein was reconstructed in an end-to-end fashion in 39 patients (95%), and autologous vein grafts, using the left renal vein,[15] were required in 2 patients (Table 1). Other autologous vein grafts, such as the external and internal iliac veins[16] and the jugular vein,[19] have been reported as suitable grafts for portal vein reconstruction. However, in most portal vein resections, direct end-to-end reconstruction could be performed without using segmental grafts. A continuous suture with a 6-0 nonabsorbable thread should be used for the reconstruction of the portal vein (Fig. 1). The use of growth factors does not seem to be required for the reconstruction if sufficient expansion of the reconstructed portal vein is obtained by the release of a proximal vascular clamp before the final ligature of the continuous suture (Fig. 2).

To avoid stricture of the reconstructed portal vein, the surgeon should carefully reconstruct the portal vein without kinking in the reconstructed portion. All operative procedures included resection of the extrahepatic duct, as well as the gallbladder, and the creation of a bilioenteric anastomosis using a Roux-en-Y jejunal loop. Biliary stent tubes for the bilioenteric anastomosis were placed through a retrograde transhepatic route. The patency of reconstructed portal veins can be evaluated by Doppler ultrasonography and enhanced com-

puted tomography (CT) during short-term and long-term follow-up after surgery.

Surgical results

Combined portal vein resection in hilar cholangiocarcinoma has been shown, by Hemming et al.,[17] Ebata et al.,[13] and in our report[10] to not bring about an increase in surgical morbidity and mortality rates. Hemming et al.[17] reported that 26 patients who underwent portal vein resection had an operative mortality of 4%, which was not different from the 12% mortality in 34 patients who did not undergo portal vein resection. Ebata et al.[13] also reported that surgical mortality was similar in patients who did and did not undergo portal vein resection (9.6% vs 9.3%). In our series, surgical mortality, including hospital deaths after surgery, was 6.8% of all 161 patients resected. However, the mortality rate in the portal vein resection group (n = 34) was 8.8%, which was not significantly different from 4.2% in the non-vascular resection group (n = 118). The long-term prognosis after surgery was affected by the combined vascular resection, as reported by Ebata et al.[13] and by us.[10] In our series, multivariate analysis showed that combined vascular resection of the portal vein was a significantly poor prognostic factor in the surgically resected patients. Survival rates in the non-vascular resection group were 63%, 39%, and 30%, 1, 3, and 5 years after surgical resection. These results were significantly better than those in the combined vascular resection group (survival rates of 42%, 17%, and 14% at 1, 3, and 5 years after surgical resection, respectively). However, the survival rates in the non-resection group were 15% and 0% at 1 and 2 years (Fig. 3). There was a significant difference in survival rates between the portal-vein resection group and the non-resection group

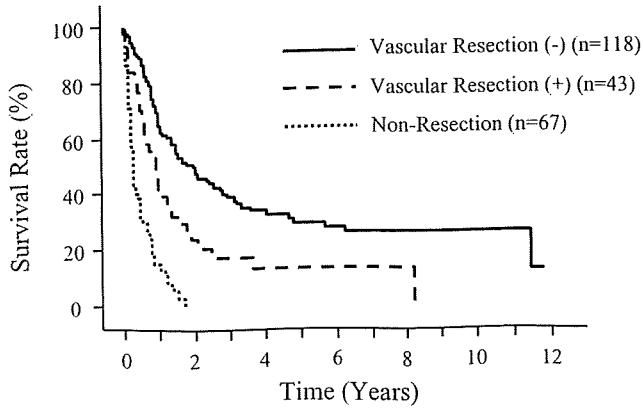

Fig. 3. Survival of patients with hilar cholangiocarcinoma after surgical resection. *Non-resection*, non-vascular resection

($P < 0.001$). In all our patients who underwent curative resection, the survival rates of the portal-vein resection group and the non-vascular resection group were 47%, 31%, 25%, and 72%, 52%, 41% at 1, 3, and 5 years after surgery, respectively. Median survivals in these two groups were 340 and 1157 days, respectively. On the other hand, Neuhaus et al.[20] reported that combined portal vein resection in extended right hepatectomy brought about a beneficial effect on the prognosis after surgery. They addressed the usefulness of prophylactic portal vein resection for the purpose of non-touch isolation from cancer invasion. However, this approach and strategy can be applied only to those patients who undergo extended right hepatectomy, but it cannot be employed for patients who undergo left hepatectomy.

Hepatic artery resection

Surgical technique

The decision to carry out combined hepatic artery resection can be made according to the intraoperative macroscopic findings of tumor invasion to the hepatic artery, together with the preoperative imaging findings, especially thin-slice multidetector CT findings. The hepatic artery can be reconstructed in an end-to-end fashion in most patients, usually associated with the use of the gastroduodenal artery by its division (Fig. 4). In hilar cholangiocarcinoma, the communication of the right and left hepatic arteries through the hilar plate is disrupted by hilar bile duct resection, despite the carrying out of parenchyma preserving hepatectomy such as caudate lobectomy and S4 + S1 resection. Unilateral hepatic arterial flow cannot supply contralateral lobar arterial flow after the amputation of the unilateral hepatic artery, which results in severe liver infarction and abscess, and breakdown of the bilioenteric anastomosis because of a reduction of bile duct arterial blood flow. Therefore, in hilar cholangiocarcinoma, contralateral hepatic arterial reconstruction should be considered to be necessary even with parenchyma preserving hepatectomy after unilateral hepatic artery resection.[21] Of course, after hepatic artery resection in hemihepatectomy, hepatic artery reconstruction should be done to avoid the occurrence of lethal postoperative liver failure. In our experienced cases, most patients with hepatic artery resection had concomitant portal vein resection; in such instances, the blood flow of both the hepatic artery and the portal vein could have been interrupted during resection and reconstruction, which might induce long-term ischemic liver damage. Therefore, to avoid long-term ischemic liver injury during both the resection and reconstruction of the hepatic artery and the portal vein, simultaneous resection and interruption

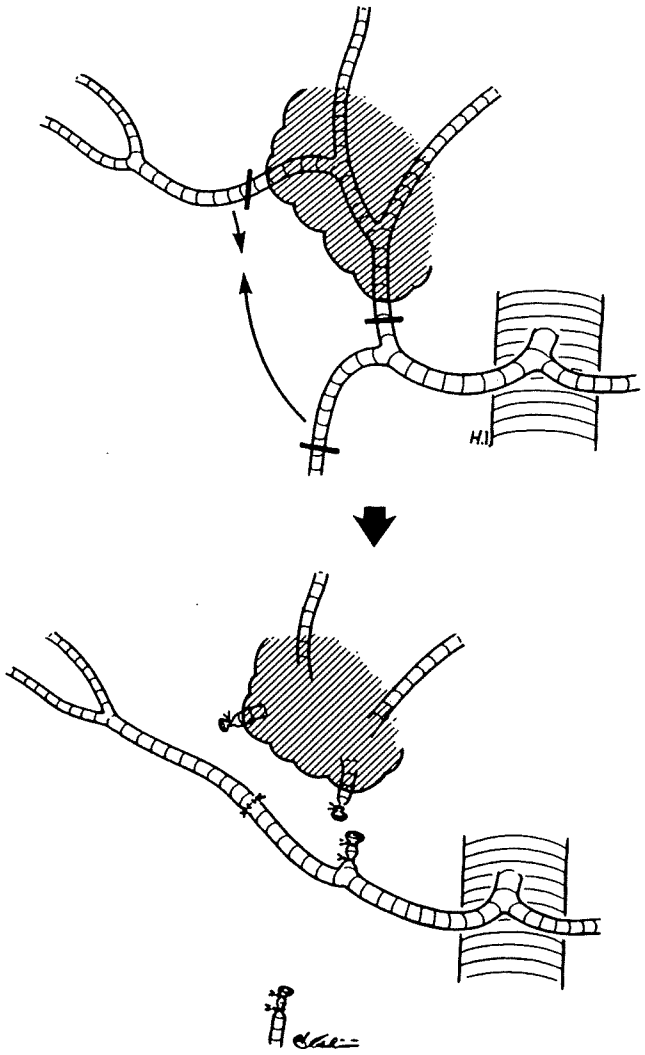

Fig. 4. Reconstruction of the right hepatic artery using the gastroduodenal artery. The *thin arrows* show utilization of divided gastroduodenal artery and the *thick arrow* show after reconstruction

of both vessels should be avoided, as shown in Fig. 5. The portal vein should be resected first and reconstructed prior to hepatic artery resection. Then, immediately after the reconstruction of the portal vein, the hepatic artery can be resected with the surgical specimen. Finally, the hepatic artery should be reconstructed (Fig. 6). These techniques of vascular resection and reconstruction may reduce the ischemic liver damage that could occur during simultaneous resection and reconstruction of both the portal vein and the hepatic artery. Postoperatively, at our institution, systemic prostaglandin E1 is routinely administered for a week, to avoid the occurrence of reconstructed arterial thrombosis, but heparin is not administered. The patency of the reconstructed hepatic artery can be evaluated by Doppler ultrasonography after surgery.

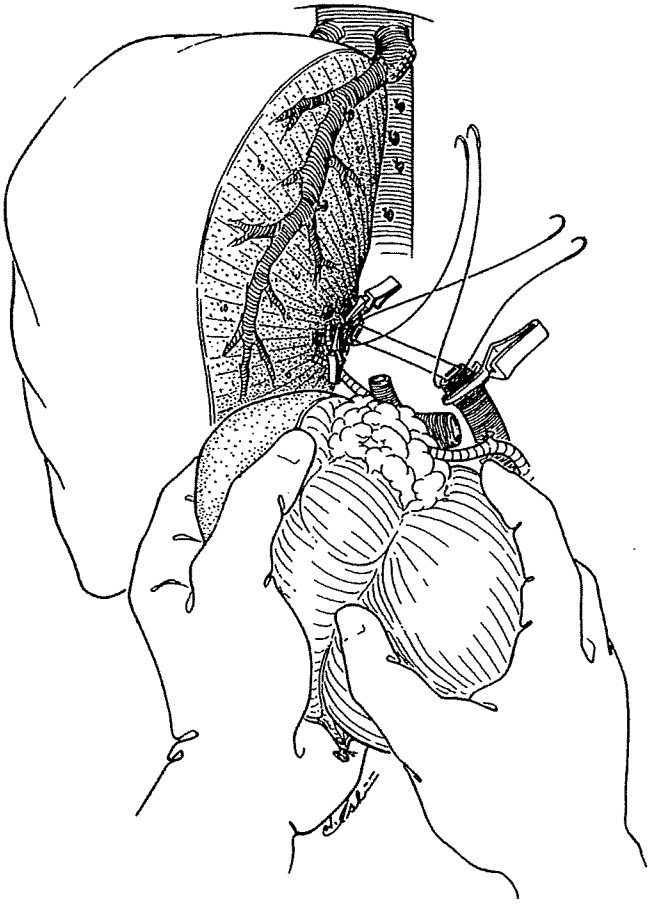

Fig. 5. In combined resection of both the portal vein and the hepatic artery, portal vein reconstruction is done first before combined hepatic arterial resection

Surgical results

There have been a few reports in the literature on the outcome of hepatic artery resection in patients with hilar cholangiocarcinoma (Table 2[6–8,10,14,16,17,22–25]). Most reports of combined hepatic artery resection are of very small series of patients (fewer than 10), and the high surgical morbidity and mortality rates have been reported. Gerhards et al.[6] reported combined hepatic artery resection in 9 patients, but the mortality rate in their series was 56% (5 of 9 patients). Most authors have reported surgical mortality including those patients who underwent portal vein resection, as a combined vascular resection group. In our series, 2 of the 9 patients with hepatic artery resection had late obstruction of the reconstructed hepatic artery. As compared with the surgical mortality of 6.8% in all 161 patients resected in our series, the mortality rate of 33% in the hepatic-artery resection group was significantly higher than that in the non-vascular resection group ($P < 0.001$). The survival rates of hepatic artery resection group in our series were 11%, 0%, and 0%, 1, 3, and 5 years after

Table 2. Hepatic artery resection in hilar cholangiocarcinoma

Year	Author	Total number of patients resected	PV	HA	PV + HA	Mortality
1990	Tsuzuki[22]	25 (4%)	3	0	2	
1997	Klempnauer[23]	151 (9.9%)	37	0	1	17%
1998	Iwatsuki[24]	34 (14.7%)	5	3	1	
1998	Madariaga[25]	28 (14%)	5	2	1	
1999	Miyazaki[10]	93 (10%)	24	2	6	
2000	Gerhards[6]	112 (18%)	3	2	7	50%
2000	Lee[7]	128 (6.3%)	29	0	0	13%
2002	Capussotti[14]	36 (2.8%)	5	1	0	0%
2004	Kondo[16]	40 (0%)	8	8	2	0%
2005	Hemming[8]	39 (9%)	20	0	3	
2006	Hemming[17]	60 (8%)	23	0	3	4%

Percentages in parentheses show surgical mortality in all patients with resection
PV, portal vein resection; HA, hepatic artery resection; PV + HA, resection of both portal vein and hepatic artery; mortality, surgical mortality in patients with combined vascular resection

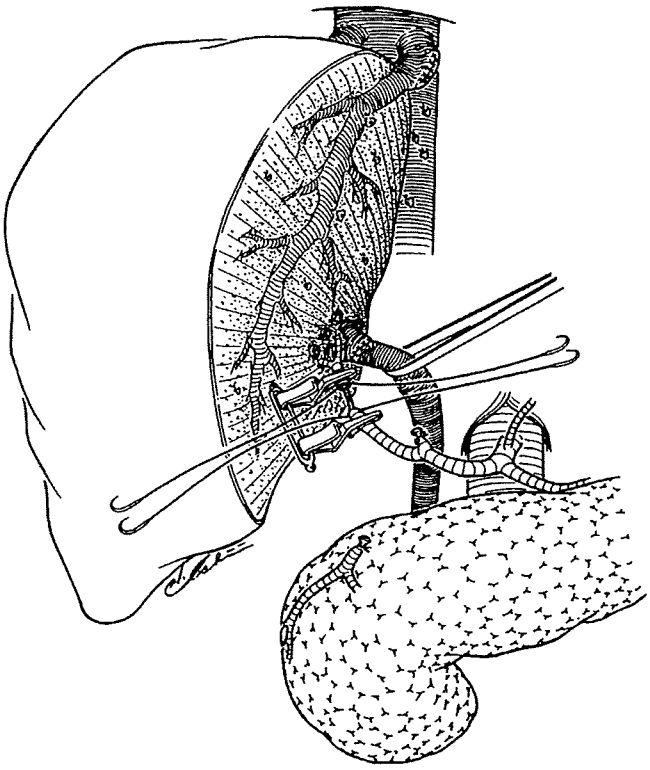

Fig. 6. Finally, in combined resection of both the portal vein and the hepatic artery, the hepatic artery is reconstructed after portal vein reconstruction

surgical resection and the survival rates in the non-resection group were 15% and 0% at 1 and 2 years. There was no significant difference between the hepatic artery resection group and the non-resection group. From this point of view, the performance of hepatic artery resection must be considered only for very strictly selected patients.

Conclusion

Combined vascular resection of the portal vein and hepatic artery is now feasible in the surgical management of hilar cholangiocarcinoma; in particular, portal vein resection and reconstruction may have some beneficial effects on the prognosis without leading to an increase in surgical complication rates. However, combined hepatic artery resection does not seem to improve the outcome of patients with hilar cholangiocarcinoma. Future studies of large series will have to be evaluated to identify the clinical implications of hepatic artery resection in patients with hilar cholangiocarcinoma.

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CCAAT/enhancer binding protein-β promotes the survival of intravascular rat pancreatic tumor cells via antiapoptotic effects

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A transcriptional factor, CCAAT/enhancer binding protein-β (C/EBP-β), regulates a variety of cell functions in normal and neoplastic cells. Although the involvement of C/EBP-β in metastasis has been demonstrated clinicopathologically in several types of human cancer, the mechanism by which it functions during the multistep process of metastasis remains largely unknown. We investigated the role of C/EBP-β in the intravascular step of hematogenous metastasis in a rat pancreatic tumor cell line, AR42J-B13, as this step profoundly affects metastatic efficiency. C/EBP-β-transfected AR42J-B13 (βB13) cells acquired considerable resistance against serum toxicity, which was primarily mediated by apoptosis *in vitro*. Upregulated expression of Bcl-2 and Bcl-xL was seen in βB13 cells. Enhanced early survival of intraportally injected βB13 cells in the BALB/c *nu/nu* male mice liver, detected by the mRNA of a vector-specific gene, was observed. Nick-end labeling analysis of the tumor-injected liver revealed significantly lower rates of apoptosis among intravascular βB13 tumor cells than among empty vector-transfected AR42J-B13 (mB13) cells. Finally, intrasplenically injected βB13 cells established a larger number of colonies in the liver than did the mB13 cells. These findings suggest that C/EBP-β may enhance hematogenous metastasis and its antiapoptotic effects may promote the survival of intravascular tumor cells. (*Cancer Sci* 2007; 98: 1706–1713)

Metastatic inefficiency has been observed in a variety of metastatic experiments in animals.[1] The vast majority of intravascular tumor cells are killed, and only a very small fraction of such cells successfully establish metastasis in a target organ.[1,2] Harmful intravascular factors include inflammatory and immune cells such as natural killer cells,[3] blood flow stress,[4] antibody binding followed by cytotoxicity,[5] and serum constituents such as lipoproteins.[6] In addition, anoikis, a form of apoptosis resulting from the loss of anchorage growth, is thought to be among the important reasons for intravascular tumor cell death.[7] Recent studies have indicated that intravascular growth is crucial in the establishment of organ-destructive metastases of clinical importance; thus, the results of such studies have indicated intravascular tumor cells in the target organ as important therapeutic targets.[8,9] The mechanism by which intravascular tumor cells survive or die remains largely unknown, although the apoptosis of intravascular tumor cells is expected to be crucial for achieving metastatic inefficiency.[10]

CCAAT/enhancer binding protein-β is a transcription factor with three domains: DNA-binding, basic leucine zipper and activation domains.[11] Leaky ribosomal scanning of C/EBP-β mRNA generates isoforms, that is, the activating form (LAP) and the inhibitory form (LIP).[12] LAP contains both activation and basic leucine zipper domains, whereas only the latter is present in LIP. LIP can therefore act as a dominant negative inhibitor of C/EBP function by forming non-functional heterodimers with other members of this protein family.[12]

CCAAT/enhancer binding protein-β regulates the expression of a variety of genes, including the genes involved in the differentiation of adipocytes,[13,14] immune function,[15,16] female reproduction,[17] cell survival,[18] and tumor invasiveness and progression.[19,20] In addition, C/EBP-β is highly expressed in hepatocytes during hepatogenesis and liver regeneration. The maintenance of adult liver cell function by processes such as the synthesis of serum proteins requires C/EBP-β, and therefore C/EBP-β is a member of the liver-enriched transcription factors.[21,22] In this context, it is of interest that C/EBP-β has been reported to lead to the transdifferentiation of a rat pancreatic tumor cell line, AR42J-B13, into a hepatocellular direction.[23] Thus, models using AR42J-B13 cells are useful for understanding the molecular and cellular events that occur during hepatic transdifferentiation.[24] Clinically, adenocarcinomas with hepatic differentiation are highly malignant due to frequent vascular invasion and highly metastatic potency.[25,26] However, it remains unknown whether or not the enhancement of metastatic potency takes place in the hepatic-differentiated AR42J-B13 cells.

Recent studies have suggested that the expression of C/EBP-β in breast cancer cells correlates with the clinicopathology of this disease.[27] Data indicative of the importance of C/EBP-β have also been obtained from studies of renal tumors[19] and colorectal tumors.[28] Another recent study demonstrated that the upregulation of an antiapoptotic protein, Bcl-2, was mediated by C/EBP-β in t(14;18) lymphoma cells.[18] Taken together, these findings led us to hypothesize that C/EBP-β could induce a survival phenotype in intravascular tumor cells, possibly via its antiapoptotic activity. We therefore investigated C/EBP-β-induced tumor cell survival or death with exposure to pure serum, as well as in an intravascular microenvironment *in vivo* using AR42J-B13 cells.

Materials and Methods

Cell culture and transfection of C/EBP-β. A rat pancreatic tumor cell line, AR42J-B13 (kindly provided by Professor Itaru Kojima, Gunma University, Japan), was maintained in DMEM (Sigma Chemical Co., St Louis, MO, USA) supplemented with 10% FBS (Sigma Chemical Co.) under 5% CO_2 at 37°C. The expression vector for the C/EBP-β gene, pcDNA3-C/EBP-β (kindly provided by Professor David Tosh, University of Bath, UK), was transfected into the AR42J-B13 cells using lipofectamine (Invitrogen,

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Abbreviations: AFP, α-fetoprotein; βB13, C/EBP-β-transfected AR42J-B13; C/EBP-β, CCAAT/enhancer binding protein-β; DMEM, Dulbecco's modified Eagle's medium, FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase, LAP, liver-activating protein; LIP, liver inhibitory protein; mB13, mock-transfectec AR42J-B13; mTOR, mammalian target of rapamycin; PBS, phosphate-bufferec saline; PCR, polymerase chain reaction; RT, reverse transcription; SDS, sodiun dodecylsulfate; TBS-T, 100 mM Tris (pH 7.5), 150 mM NaCl and 0.05% Tween-20.

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Carlsbad, CA, USA) according to the manufacturer's protocol. After transfection, we carried out a selective culture using G418 (800 μg/mL) (Invitrogen) to establish a βB13 clone that stably expressed C/EBP-β. The empty vector pcDNA3 was also transfected into the AR42J-B13 cells, and we obtained a clone of a mock transfectant, mB13.

Cell proliferation assay. βB13 and mB13 cells (3×10^4 cells/well) were cultured in 96-well microtiter plates. Two culture media with different concentrations of FBS (10 or 100%) were applied. We added the standard amount of DMEM powder to 100% FBS to create the 100% FBS medium. The number of viable cells was measured based on the absorption of WST-1 (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium; monosodium salt) using Cell Counting Kits (Dojindo, Kumamoto, Japan).

Flow cytometric detection of apoptosis. βB13 and mB13 cells were cultured in medium with either 10 or 100% FBS for 24 h, and flow cytometric detection of early apoptosis in these cells was carried out. Early apoptotic cells were defined as annexin V-positive and propidium iodide-negative cells. FITC-conjugated annexin V and propidium iodide were purchased from BD Biosciences (Heidelberg, Germany).

Western blot analysis. The cells were homogenized in cell lysis buffer with protease inhibitors (PBS with 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5 mM phenylmethyl sulfonyl fluoride, 3% aprotinin, 0.1 mM leupeptin, 0.1 mM pepstatin A and 0.1 mM chymostatin), and the samples were then stored on ice for 2 h. The extracted proteins (50 μg) were mixed with sample buffer (0.5 M Tris-HCl [pH 6.8], 20% SDS, 1% bromophenol blue, 20% glycerol, 10% β-mercaptoethanol), and electrophoresis was carried out on 15% SDS–polyacrylamide gels (SPU-15, ATTO, Tokyo, Japan). The proteins were then transferred to nitrocellulose membranes (Nihon Eido, Tokyo, Japan). After the non-specific binding sites were blocked, the blots were incubated with primary antibodies (1.5 μg/mL) in TBS-T containing 2% non-fat skim milk for 4 h at 4°C. The membranes were washed with three successive solutions of TBS-T containing 2% skim milk at room temperature for 30 min, and were then incubated with HRP-conjugated anti-immunoglobulin (1:2000 dilution) for 2 h at 4°C. The membranes were then washed with three successive TBS-T solutions for 30 min, and the signals were detected by enhanced chemiluminescence using a Hybond ECL protocol (Amersham Pharmacia Biotech, Buckinghamshire, UK). The antibodies used were as follows. Mouse monoclonal anti-C/EBP-β, goat polyclonal anti-AFP, antialbumin, antiamylase and the HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal anti-Bcl-2 antibody was purchased from R&D Systems (Minneapolis, MN, USA). Monoclonal anti-Bcl-xL, anti-Bax and anti-Bad antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Mouse monoclonal antirat β-actin antibody was obtained from Sigma Chemical Co.

Animals. Male BALB/c *nu/nu* mice, 6–8 weeks old, were obtained from Japan SLC (Hamamatsu, Japan). All mice were maintained under specific pathogen-free conditions at the Center for Animal Experimentation, Chiba University Graduate School of Medicine. Regular laboratory food and tap water for drinking were made available *ad libitum*. All animal experiments were carried out under the guidelines of the National Research Council and Chiba University.

Subcutaneous xenografts. Viable βB13 and control mB13 cells (1×10^7/100 μL PBS) were injected subcutaneously into the dorsal surface of nude mice under anesthetization of the animals with diethylether. Mice were killed when the tumor size reached a volume of approximately 1 cm^3. The tumor tissues were used for immunohistochemistry and western blot analysis.

Immunohistochemical staining. Formalin-fixed, paraffin-embedded sections were stained with hematoxylin–eosin, and these sections were also used for immunohistochemical analysis. Immunostaining was carried out using labeled streptavidin–biotin–peroxidase (Dako Cytomation Co., Kyoto, Japan) and microwave antigen retrieval techniques. Mouse monoclonal anti-C/EBP-β (1:100; Santa Cruz Biotechnology) and mouse monoclonal anti-Bcl-2 (1:100; R&D Systems) were used as the primary antibodies. Diaminobenzidine tetrahydrochloride substrate was used to visualize positively stained cells.

Detection of immediate entrapment of intraportally injected B13 cells and nick-end labeling for detection of apoptosis in tissue sections. Male nude mice, 6–8 weeks old, were used. Either βB13 or mB13 cells (1×10^7/100 μL PBS) were injected intraportally using 27G needles under anesthetization of the animals with diethylether. The livers were then removed 2 h after the injection. The livers were cut into two slices, and each slice was formalin fixed and paraffin embedded. Four mice were injected with βB13 cells, and four were injected with mB13 cells. The immediate entrapment of intraportally injected βB13 or mB13 cells was evaluated by counting the number of cells in five random 1×1 cm^2 fields in the histological slides. The nick-end labeling detection of apoptosis in the histological sections was carried out using an Apoptosis *In situ* Detection Kit (Wako, Tokyo, Japan) according to the manufacturer's instructions. Based on the staining pattern, tumor cells within the blood vessels in the liver were determined to be either apoptotic or non-apoptotic.

Detection of viable βB13 or mB13 cells in the mouse liver. An incision was made in the abdominal wall, and the portal vein was exposed under anesthetization of the animals with diethylether. βB13 or mB13 cells (1×10^4/100 μL PBS) were injected into the portal vein with 27G needles. After 24 h, the mice were killed and the livers were removed ($n = 4$ in each group). Total RNA was prepared from the liver tissues. The RT-PCR detection of Neo^r mRNA was then carried out.

RNA isolation and RT-PCR. Total RNA was obtained using an RNAeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed by random priming using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). PCR was carried out in a volume of 25 μL containing 1 μL first-strand cDNA, forward and reverse primer (0.4 μM each), dNTP (0.2 mM), $MgCl_2$ (1.5 mM), PCR buffer and *Taq* polymerase (Amersham Pharmacia Biotech). Samples were amplified through 35 consecutive cycles, or through other numbers of cycles in order to evaluate sample quantity. Each cycle consisted of denaturation at 95°C for 60 s, annealing at 54°C for 30 s, and extension for 60 s, with a final extension for 5 min at 72°C. A 6-μL volume of the PCR mixture was electrophoresed in a 2.0% agarose gel and stained with ethidium bromide. The following PCR primers for β-actin and the vector-derived neomycin resistance gene (Neo^r) were used: for rat or mouse β-actin, 5′-CTC TTT GAT GTC ACG CAC GAT TTC C-3′ and 5′-ATC CTG ACC CTG AAG TAC CCC ATT G-3′, amplifying a 430-bp fragment; and for Neo^r, 5′-GCT TGG GTG GAG AGG CTA TTC GG-3′ and 5′-GCC AGT CCC TTC CCG CTT CAG TG-3′, amplifying a 235-bp fragment.

Blood-borne metastasis to the liver. An incision was made in the left abdominal wall after the animals were anesthetized with diethlyether. Either βB13 or mB13 cells (1×10^6/100 μL PBS) were injected into the spleen. At 6 weeks after the injection, the mice were killed and the livers were removed (number of mice in each group: βB13, 14; mB13, 15). The entire liver from each mouse was cut evenly into three slices, and a tissue section from each slice was stained with hematoxylin–eosin. Microcolonies of tumor cells were counted under a microscope. The sum of the counts from the three slices was considered as the number of micrometastatic colonies in the liver.

Statistical analysis. Statistical analysis of the results was carried out using Student's *t*-test. StatView J-5.0 software (SAS

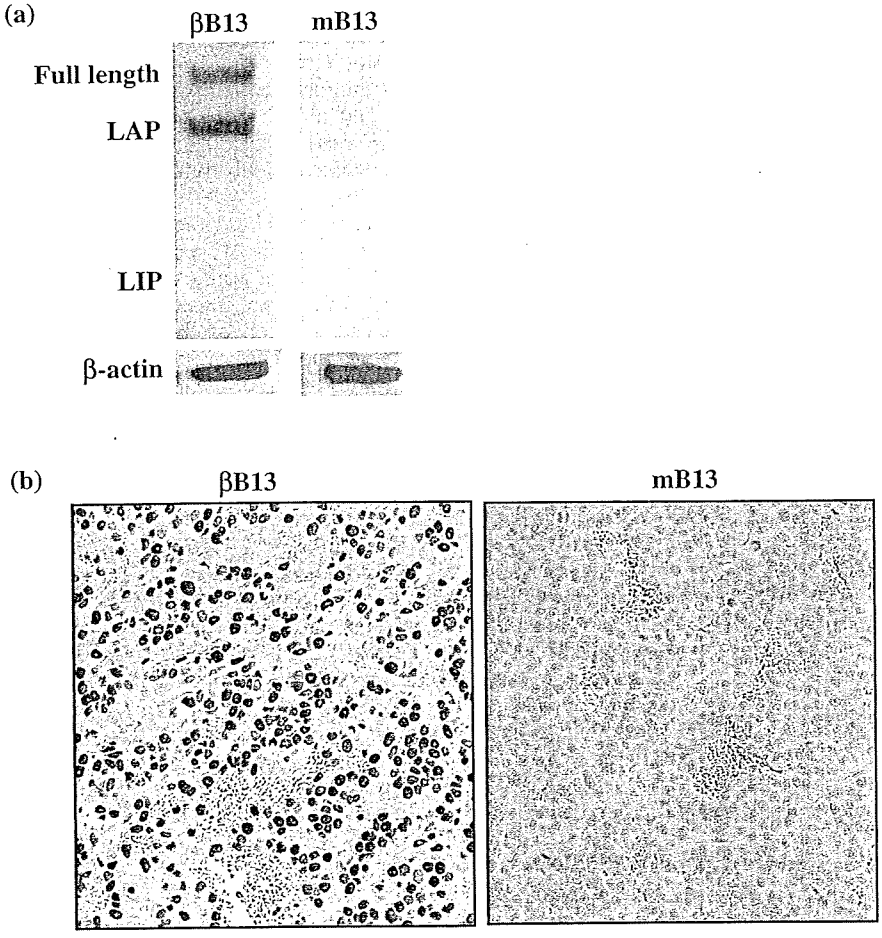

Fig. 1. Transfection of C/EBP-β to rat pancreatic tumor cells. (a) The C/EBP-β-transfected AR42J-B13 (βB13) cells expressed the full-length and activated isoform liver-activating protein (LAP), whereas the inhibitory isoform liver inhibitory protein (LIP) was not detectable by western blot analysis. Negligible C/EBP-β protein was detected in mock-transfected AR42J-B13 (mB13) cells. (b) Xenografted βB13 cells (left) expressed high levels of C/EBP-β, whereas xenografted mB13 cells (right) did not.

Institute, Cary, NC, USA) was used for all statistical analyses. All *P*-values below 0.05 were considered statistically significant.

Results

Generation of βB13 cells and their properties. Either empty (pcDNA3) or C/EBP-β expression vectors (pC/EBP-β) were stably transfected into AR42J-B13 cells, and the clones obtained were tested for C/EBP-β expression by western blot analysis. The C/EBP-β-transfected clone (βB13) was found to produce activating isoforms of C/EBP-β proteins, namely, full-length C/EBP-β and LAP. No LIP was detected. The empty vector-transfected clone (mB13) did not produce any detectable C/EBP-β protein (Fig. 1a). To examine the localization of C/EBP-β in the cellular compartments, immunohistochemical detection was used. Intense nuclear localization was observed in the subcutaneous βB13-cell tumors, but not in the mB13-cell tumors (Fig. 1b).

As it had previously been reported that C/EBP-β-transfected AR42J-B13 cells transdifferentiate toward hepatocytes,[23] the expression of AFP, albumin and amylase was investigated by western blot analysis. AFP expression was induced, and a slight upregulation of albumin protein was also seen, which indicated the presence of C/EBP-β-modified, cell lineage-specific proteins in the AR42J-B13 pancreatic tumor cells. However hepatic transdifferentiation was not complete in the case of the βB13 cells, as indicated by a slight upregulation of exocrine pancreatic cell-specific protein amylase (Fig. 2).

Viability, proliferation and apoptosis in 100% FBS in βB13 cells. Tumor cells are directly, without interposition of the basement membrane, exposed to serum when they enter blood vessels. Here, we evaluated the viability and proliferation of AR42J-B13

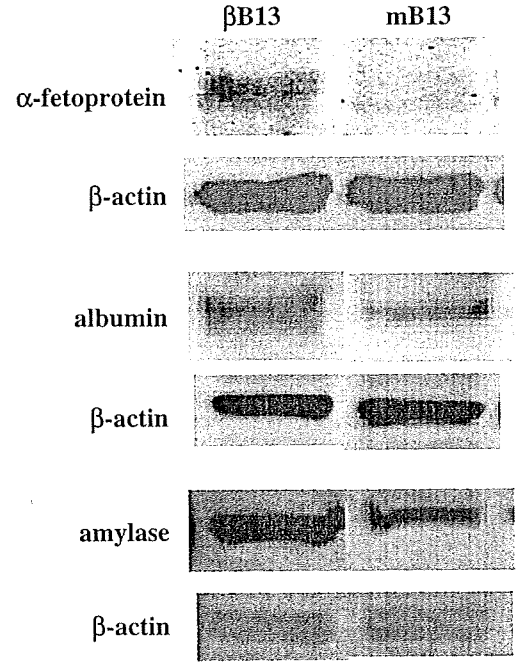

Fig. 2. Regulation of cell type-specific proteins by C/EBP-β. α-Fetoprotein expression was induced in C/EBP-β-transfected AR42J-B13 (βB13) cells. An equivalent upregulation of both albumin and amylase was seen in βB13 cells.

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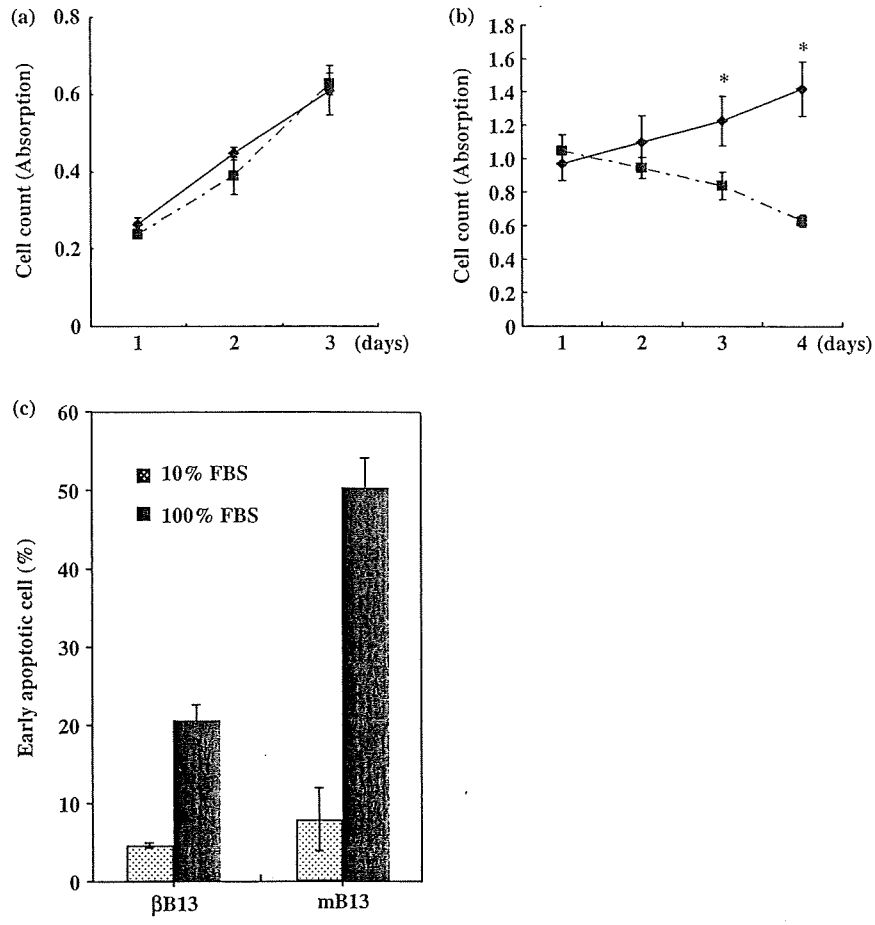

Fig. 3. Anti-apoptotic properties in C/EBP-β-transfected AR42J-B13 (βB13) cells. (a) Proliferation of βB13 (◆) and mB13 (■) cells in ordinary culture medium (10% fetal bovine serum [FBS]). (b) Proliferation of βB13 (◆) and mB13 (■) cells in 100% FBS (*$P < 0.0001$). (c) Early apoptotic cells in 24-h culture in 100% FBS. Early apoptotic cells were defined as the annexin V-positive, propidium iodide-negative cell population, as determined by flow cytometric examination. Early apoptotic cells in 100% FBS were more numerous in βB13 than in mB13 cell cultures.

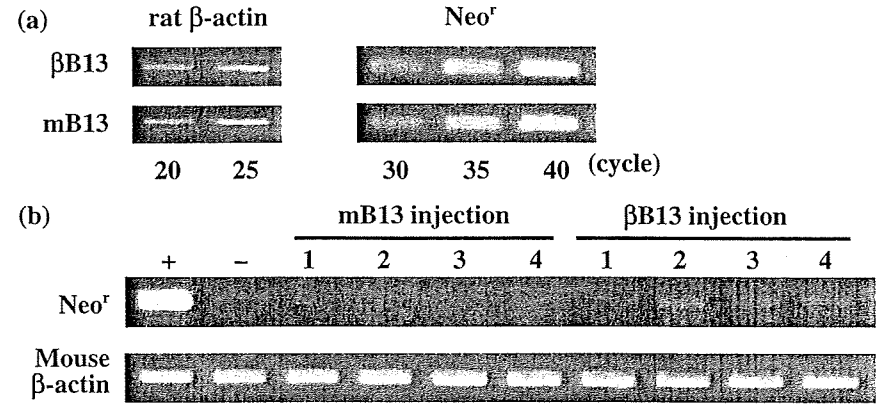

Fig. 4. Early survival of intraportally injected C/EBP-β-transfected AR42J-B13 (βB13) cells in liver tissue. (a) Expression of Neo^r mRNA in βB13 and mB13 cells. Semiquantitative reverse transcription–polymerase chain reaction analysis revealed that Neo^r mRNA expression was almost identical among cultured βB13 and mB13 cells. (b) Early survival of rat tumor cells (B13) 24 h after intraportal injection. Neo^r mRNA expression was detected in three out of four livers in βB13-injected animals, and in none of four livers in mB13-injected animals. +, βB13 cells as a positive control; –, liver tissue from a phosphate-buffered saline-injected mouse as a negative control.

cells in 100% FBS *in vitro*. When incubated in 10% FBS, no differences between βB13 and mB13 cells were observed in terms of either viability or proliferation (Fig. 3a). The βB13 cells were able to proliferate in 100% FBS, whereas no proliferation of mB13 cells was observed in a 100% FBS environment ($P < 0.0001$) (Fig. 3b). Early apoptotic cells, recognized as annexin V-positive and simultaneously propidium iodide-negative cells in 100% FBS, were present in much greater numbers among the mB13 cells than among the βB13 cells, whereas in 10% FBS, apoptosis appeared to occur at a similar rate among mB13 and βB13 cells (Fig. 3c).

Augmented early survival of intraportally inoculated βB13 cells in the liver. As the product of the neomycin-resistance gene *Neo^r* has the potential to be used as a marker of surviving cells in mouse tissues, the detection of Neo^r mRNA was carried out by RT-PCR. Semiquantitative RT-PCR was done in order to verify that Neo^r mRNA was produced in identical amounts by mB13 and βB13 cells. Almost identical amplification of the housekeeper gene rat β-actin was observed in mB13 and βB13 cells both treated for 20 as well as 25 cycles. In addition, the signals for the products amplified for 25 cycles were more intense than those amplified for 20 cycles; these results indicated the exponential stage of amplification and provided support for the notion that the total cDNA amount produced from mB13 and βB13 cells was identical. No difference in Neo^r mRNA expression was observed between mB13 and βB13 cells with respect to cDNA with exponential amplifications of 30, 35 and 40 cycles (Fig. 4a).

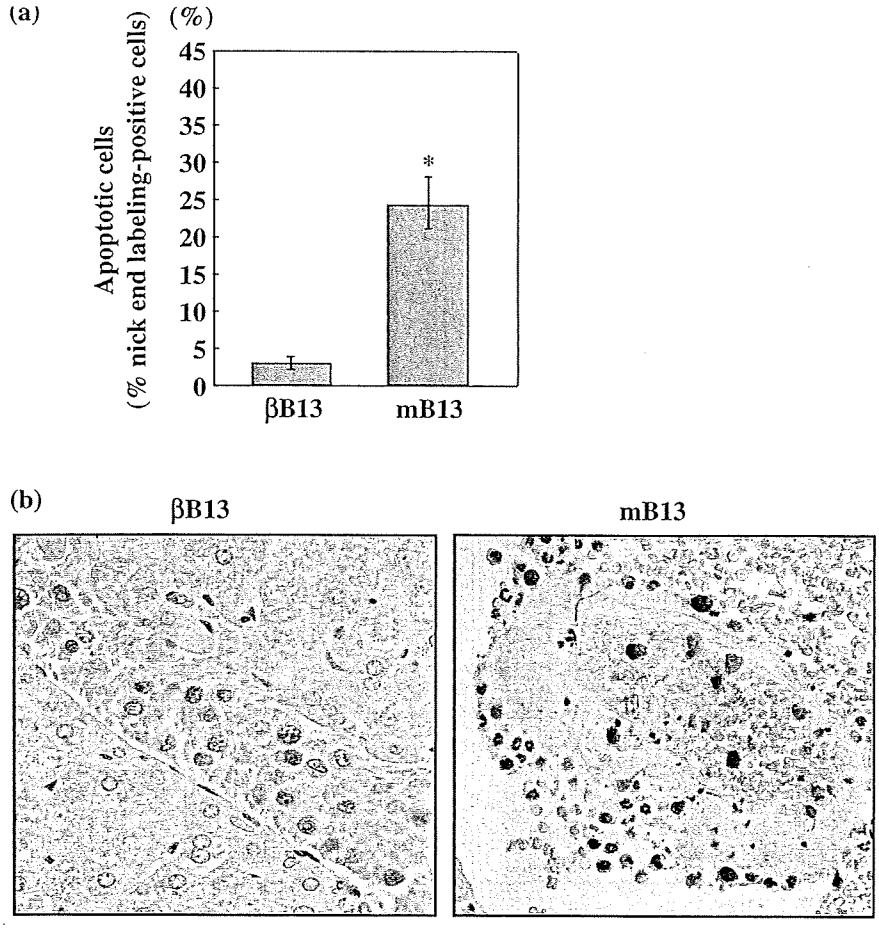

Fig. 5. Apoptosis among C/EBP-β-transfected AR42J-B13 (βB13) and mB13 cells intraportally injected into the mouse liver. Liver tissues were removed from cell-injected mice, and intravascular tumor cells were examined for apoptosis by the nick-end labeling method. (a) Nick-end labeling-positive cells in intravascular βB13 and mB13 cells in the liver (*$P = 0.001$). Cells with fragmented DNA were more numerous among mB13 cells than among βB13 cells. (b) Nick-end labeling-positive cells in intravascular βB13 (left) and mB13 (right) samples.

The results of this preliminary investigation suggest that the amount of amplified Neo^r mRNA product in mouse tissues could serve as a marker of the number of viable (surviving) B13 cells. The amplified products of Neo^r mRNA were detected 24 h after intraportal injection in three out of four mouse liver tissue samples that had been inoculated with βB13 cells, whereas the products of Neo^r mRNA were detected in none of four liver samples previously inoculated with mB13 cells (Fig. 4b). These observations suggest that the βB13 cells acquired an advantage that allowed them to maintain viability in the liver after the intraportal injection of the cells.

Anti-apoptotic property of intraportally inoculated βB13 cells. The amplified products of Neo^r mRNA were only detected in βB13 cell-injected mouse liver tissue samples. However, in this experiment, the results of RT-PCR may have been affected by a difference in the number of entrapped cells. Thus, we microscopically investigated livers in which tumor cells had been intraportally injected. The inoculated B13 cells were located within small branches of the portal veins and sinusoidal vasculature in the liver. The inoculated B13 cells could be distinguished from the intravascular leukocytes based on cellular size and shape, the presence of nuclear pleomorphisms, and the chromatin pattern. The immediate entrapment of intravascularly injected B13 cells was examined as the sum of apparent viable and degenerated B13 cells in the liver; this sum did not differ in the case of either βB13 ($377.6 \pm 253.7/cm^2$) or mB13 cells ($442.2 \pm 424.8/cm^2$).

Next, the nick-end labeling method of detecting fragmented DNA was carried out in order to visualize apoptotic B13 cells. Significantly more mB13 cells than βB13 cells were positive, thus indicating that the βB13 cells were antiapoptotic (Fig. 5) in the vasculature.

Induction of Bcl-2 in AR42J-B13 cells by C/EBP-β. We examined the expression of Bcl-2 and its family members, Bcl-xL, Bax and Bad, because antiapoptotic properties were observed in βB13 cells, both *in vitro* and *in vivo*. Western blot analysis revealed that expression of the antiapoptotic proteins Bcl-2 and Bcl-xL was upregulated in the βB13 cells under normal culture conditions, whereas no remarkable change was observed in expression of the apoptosis-inducing proteins Bax and Bad (Fig. 6a). Immunohistochemical analysis revealed that Bcl-2 was more frequently positive among βB13 cells than among mB13 cells in the liver of tumor-injected mice (Fig. 6b).

Enhanced metastatic properties of β-B13 cells *in vivo*. The number of established microcolonies and the maximal diameter of individual microcolonies were examined 6 weeks after intrasplenic inoculation of βB13 or mB13 cells. Both the number and maximal size of the colonies were greater in the βB13-inoculated livers compared with the mB13-inoculated livers (Fig. 7), thus indicating that C/EBP-β enhanced the blood-borne metastatic properties of AR42J-B13 cells.

Discussion

The formation of microcolonies within blood vessels precedes the establishment of clinically relevant metastatic tumors;[8,9] survival and proliferation in blood vessels increases the opportunity for extravasation and growth into large metastatic nodules. Here, inoculation with βB13 and mB13 cells both exhibited early intrahepatic entrapment 2 h after intraportal cell injection, and early intrahepatic entrapment was observed at the same rate in both groups. One striking observation was that the intravascular βB13 cells were more likely to escape apoptosis than were the control mB13 cells. The upregulation of Bcl-2 and

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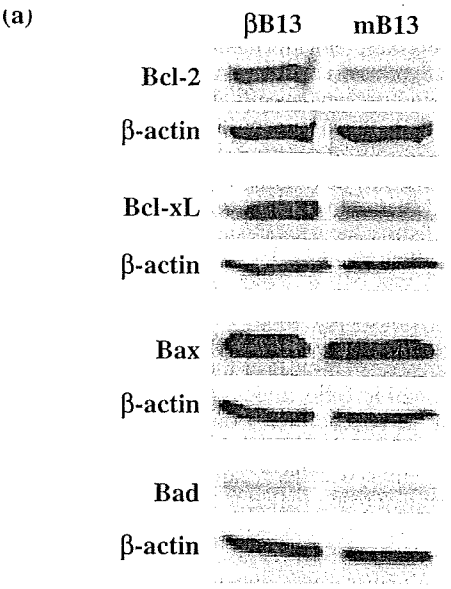

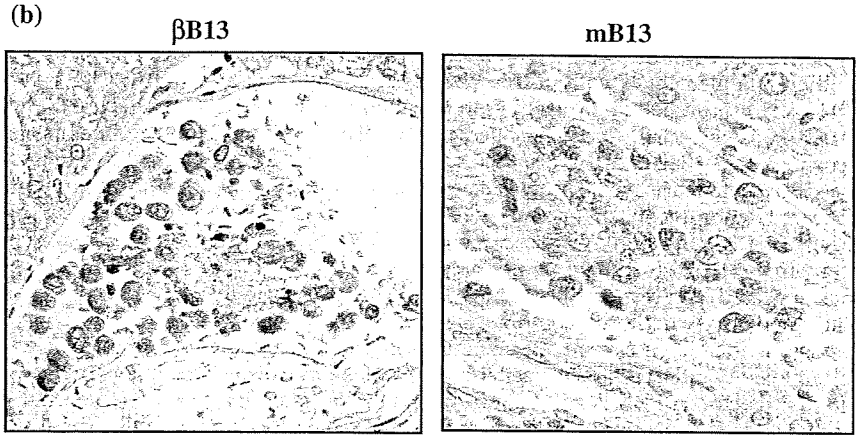

Fig. 6. The expression of Bcl-2 family proteins in C/EBP-β-transfected AR42J-B13 (βB13) cells. (a) Augmented expression of Bcl-2 and Bcl-xL was observed in βB13 cells cultured *in vitro* by western blot analysis. (b) Representative result of the immunohistochemical study of Bcl-2 expression *in vivo*. The intravascular βB13 cells stained positive for Bcl-2.

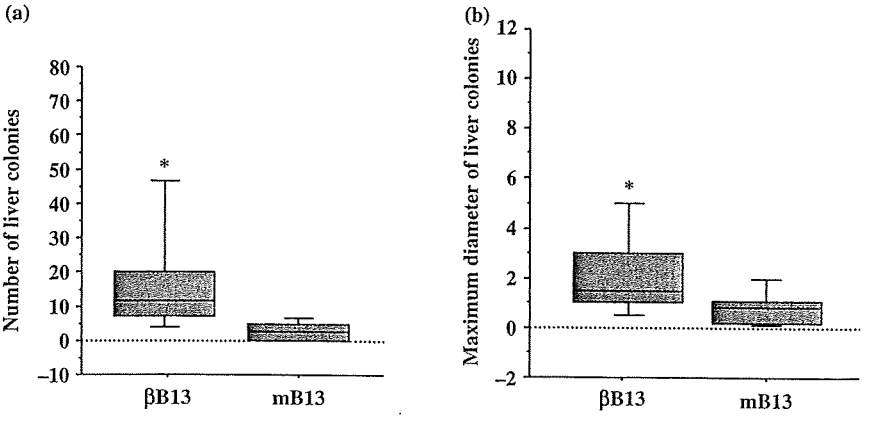

Fig. 7. Metastatic capacity of C/EBP-β-transfected AR42J-B13 (βB13) cells. (a) The number of established colonies detected 6 weeks after an intraportal injection of βB13 or mB13 cells (*$P = 0.0104$). (b) The maximal diameter of individual microcolonies 6 weeks after an intraportal inoculation with βB13 or mB13 cells (*$P < 0.0001$). The boxes show a range of 25–75%. The horizontal bars in each box indicate median values.

Bcl-xL, a key regulator in cell survival, was seen in βB13 cells, as is also seen in t(14;18) lymphoma cells.[18] These observations indicated that βB13 cells acquired the property of early survival (2 h) in the intravascular milieu. The detection of vector-specific mRNA in the liver 24 h after injection also supported the notion of enhanced early intravascular survival via C/EBP-β activity. Additionally, intrasplenically injected βB13 cells were found to establish more numerous colonies in the liver, which indicated an enhancement of the metastatic potential of βB13 cells. These results suggest the possibility that the promotion of metastatic ability was, in this case, at least partially mediated by the antiapoptotic properties of βB13 cells in the intravascular milieu.

However, the mechanisms underlying the enhanced metastatic potency of βB13 cells should be carefully assessed, because it is also possible that other C/EBP-β functions, such as the

upregulation of certain molecules known to be important for cancer metastasis,[29] can also mediate metastatic enhancement. It has been demonstrated that the increased expression of apoptotic inhibitors results in resistance to anoikis among cancer cells in circulation.[30] Thus, it is also possible that the antiapoptotic effects of C/EBP-β could antagonize anoikis, in turn resulting in increased intravascular survival.[30,31] However, no significant difference in proliferation was observed between βB13 and mB13 cells cultured in a non-adherent dish, which suggested that anoikis was not suppressed in βB13 cells (Kishimoto *et al.*, unpublished data).

Many tumor cells were observed in the liver 2 h after cell injection, but mB13 cells could not be detected by RT-PCR analysis 24 h after cell injection. This indicated that most of the mB13 cells were eliminated during the 24 h following cell injection. Previous observations are not in conflict with our results; the majority of cell loss occurred within 24–48 h of the intravascular invasion of transformed rat embryo cells,[10] and during the first 1.5 h to 3 days in an experiment using melanoma cells.[32]

Culture medium supplemented with 10–20% FBS is used optimally for *in vitro* cell expansion, but higher serum concentrations are often inappropriate for cultivating tumor cells. Common epithelial cells do not make direct contact with blood plasma; however, carcinoma cells are exposed to plasma when they enter vessels. In this context, we examined cell proliferation in culture medium supplemented with 100% FBS. It was observed that βB13 cells proliferated in 100% serum culture, whereas the mB13 cells did not, which indicated that C/EBP-β conferred upon the AR42J-B13 cells a tolerance to serum toxicity, even though the species difference should be considered. The observed tolerance appeared to be mediated to some degree by an antiapoptotic mechanism, because serum-induced apoptosis was less frequently seen among βB13 than among mB13 cells *in vitro*. These findings appeared to correspond with the *in vivo* observations in this study, and also with those of a previous study showing enhanced metastasis in a colon cancer cell line that conferred tolerance to serum toxicity induced by dexamethasone treatment.[33] However, the toxic factor in FBS is still unknown, and thus further study will be needed to establish that antiserum toxicity is involved in the mechanism of metastatic efficiency.

The results obtained with the present experimental system appear to suggest that a single transcription factor, C/EBP-β, can simultaneously regulate both a metastatic property and the direction of differentiation in solid tumor cells. It has been shown that C/EBP-β can force AR42J-B13 cells to transdifferentiate in a hepatocellular direction, in a study that revealed the ability of C/EBP-β-expressing AR42J-B13 cells to produce AFP, a protein that serves as a marker of embryonal hepatocytes.[23] Similar AFP expression was reproduced in the present study. In addition, albumin expression was found to be slightly increased. Clinically, aberrant hepatic differentiation is well described (e.g. AFP production associated with highly malignant properties with frequent metastasis); however, the precise mechanism governing the emergence of hepatic differentiation within adenocarcinomas remains unclear. Interestingly, in AFP-producing gastric adenocarcinoma cell lines, C/EBP-β is expressed, and its isoforms are regulated by a predominance of LAP.[34]

The introduced C/EBP-β cDNA generated both full-length and LAP isoforms in AR42J-B13 cells. In addition, a far less inhibitory isoform, LIP, was generated. Several lines of evidence have indicated that C/EBP-β-expressing cells exhibit a unique LAP/LIP ratio, depending on the cell type, thus suggesting that C/EBP-β does not always function in a positive manner when the expression of LIP exceeds negligible levels. The results of the present study suggested that the activating isoforms of C/EBP-β most likely mediate antiapoptosis in intravascular AR42J-B13 cells. Thus, it can be hypothesized that the inhibitory isoform does not necessarily mediate this function, and this lack of mediation most likely results in a tendency toward unaltered or even augmented apoptosis. The LAP/LIP ratio is known to be regulated by several intracellular proteins, including the mTOR signal pathway proteins,[35] RNA-dependent kinase pathway proteins[35] and triplet repeat-binding proteins.[36,37] For example, rapamycin, an inhibitor of mTOR, is known to modulate the LAP/LIP ratio, resulting in a relative LIP increase.[35] These ratio regulators might alter C/EBP-β-induced modulation, thereby exerting an influence on intravascular survival.

To summarize, we will review the three important findings of this study, although the results were obtained from only one established cell line. First, the expression levels of Bcl-2 and Bcl-xL were upregulated in C/EBP-β-introduced AR42J-B13 cells. Next, the viability of these cells was preserved by an escape from apoptosis in the liver vasculature during the early period following the *in vivo* intraportal injection of C/EBP-β-introduced AR42J-B13 cells. Finally, more numerous metastatic colonies were generated in the livers of those mice that had been intrasplenically inoculated with C/EBP-β-introduced AR42J-B13 cells. These results provide support for the hypothesis that the antiapoptotic activity of C/EBP-β promotes the survival of tumor cells in an intravascular microenvironment, a step considered important for the establishment of metastasis.

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Original Paper

Pylorus-preserving Pancreatoduodenectomy: Preoperative Pancreatic Function and Outcome

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ABSTRACT

Background/Aims: To investigate the effects of preoperative pancreatic function on gastric emptying, body weight, and quality of life after pylorus-preserving pancreatoduodenectomy.

Methodology: Thirty-one patients who underwent pylorus-preserving pancreatoduodenectomy were divided into 2 groups according to preoperative pancreatic exocrine and endocrine function (normal *vs.* abnormal). Gastric emptying, body weight, and quality of life were evaluated before surgery, 1-2 months after surgery (short term), and 6-12 months after surgery (long term).

Results: Short-term body weight was significantly decreased in comparison to preoperative body weight regardless of preoperative exocrine and endocrine pancreatic function. Body weight returned to the preoperative level by 12 months after surgery in patients with normal preoperative pancreatic function but not in patients with abnormal pancreatic function. In both groups, gastric emptying was delayed at 1-2 months after surgery and then returned to the preoperative value by 12 months. Short-term quality of life did not differ from preoperative quality of life in either group, but long-term quality of life improved to beyond the preoperative level in both groups.

Conclusions: Preoperative pancreatic function appears to significantly influence long-term body weight after pylorus-preserving pancreatoduodenectomy.

KEY WORDS: Pancreatic function; Gastric emptying; PPPD

ABBREVIATIONS: Pylorus-Preserving Pancreatoduodenectomy (PPPD); Quality Of Life (QOL); Pancreatoduodenectomy (PD); Delayed Gastric Emptying (DGE)

INTRODUCTION

Traverso and Longmire introduced pylorus-preserving pancreatoduodenectomy (PPPD) in 1978 (1), and it is now the standard surgical procedure for treatment of periampullary lesions. It is thought that PPPD prevents long-term complications such as dumping and anorexia by preserving the reservoir function of the stomach and the duodenum-derived intestinal hormones and that, in comparison to standard pancreatoduodenectomy (PD), it improves nutritional status and quality of life (QOL) (2-5). However, complications can occur after PPPD. For example, delayed gastric emptying and impaired pancreatic function can result from the resection, and nutritional status may remain insufficient. Few studies have investigated the relation between pancreatic function and gastric emptying, nutritional status, and QOL over the long term after PPPD even though pancreatic function and gastric emptying are important indicators of postoperative nutritional status and QOL. The aim of this study was to investigate the effects of preoperative pancreatic exocrine and endocrine function on gastric emptying and recovery of body weight over the long term after PPPD.

METHODOLOGY

The present study included 31 Japanese patients who underwent PPPD in the Department of Surgery and Oncology at Kyushu University Hospital January 1994 through December 2001. The group comprised 19 men and 12 women who ranged in age from 46 to 81 years, with a mean age of 63.2 years. PPPD was performed for 19 malignant and 12 benign diseases: ampullary carcinoma, n=9; bile duct carcinoma, n=6; pancreas carcinoma, n=4; intraductal papillary adenoma of the pancreas, n=6; chronic pancreatitis, n=4; serous cystadenoma of the pancreas, n=1; and chronic cholangitis, n=1. All patients were followed up, and cancer recurrence was ruled out for more than 1 year after PPPD.

Of the 31 patients, 27 underwent gastrointestinal reconstruction by the Imanaga method (6) and 4 by the Traverso method (1). The proximal duodenum was transected 2-6cm distal to the pyloric ring. The Imanaga reconstruction procedure has been described previously (7-11): end-to-end duodenojejunostomy, end-to-side pancreatojejunostomy and hepaticojejunostomy, in that order. In Traverso reconstruction, pancreatojejunostomy is performed 5cm from the closed end of the jejunum, this is followed by hepaticojejunostomy 10cm distally and end-to-side duodenojejunostomy 30cm more distally.

Before surgery, the fecal chymotrypsin level (cut-off value: 13.2 U/g) and fasting blood sugar level (cut-

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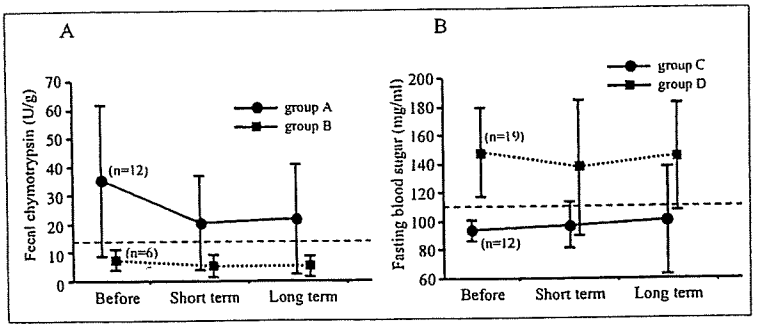

FIGURE 1 Changes in pancreatic **(A)** exocrine (fecal chymotrypsin) and **(B)** endocrine (fasting blood sugar) function.

off value: 110mg/mL) were examined in each patient for evaluation of pancreatic exocrine and endocrine function, respectively. Patients were divided into 2 groups according to preoperative pancreatic exocrine and endocrine function, and they were classified in subgroups of normal and abnormal group: group A, fecal chymotrypsin level was normal, group B, fecal chymotrypsin level was abnormal, group C, fasting blood sugar level was normal, group D, fasting blood sugar level was abnormal. Gastric emptying, body weight, and QOL were determined before surgery, 1-2 months after surgery (short term) and 6-12 months after surgery (long term). Gastric emptying was evaluated by the acetaminophen method as previously reported (7). The indices of gastric emptying were calculated from the area under the serum acetaminophen concentration curve for 90 minutes (AUC 90). Changes in each patient's body weight were calculated by referring to the preoperative level as 100%. QOL was assessed by means of a modified Kurihara questionnaire (12), which we have used previously (11,13). The questionnaire consisted of 23 items divided into 2 categories: physical (questions 1-13) and psychosocial (questions 14-23).

All values are expressed as means ± standard deviation (SD). Statistical analyses were carried out with unpaired *t*-test. A *P* value of less than 0.05 was considered significant.

RESULTS

Pancreatic Exocrine and Endocrine Function

The mean fecal chymotrypsin level in group A was decreased in the short term after surgery, but kept within normal limit in the short and long term. The level in group B with pancreatic exocrine insufficiency did not differ between time points. The mean fasting blood sugar levels in groups C and D did not differ between time points. Thus, normal or abnormal preoperative pancreatic exocrine and endocrine function did not appear to influence postoperative pancreatic function **(Figure 1A, B)**.

Influence of Preoperative Pancreatic Exocrine Function

Short-term gastric emptying was delayed in both groups (group A: before surgery, 550.8±243.9 μg·90min/mL; short term, 412.4±146.3μg·90min/mL, and group B: before surgery, 649.8±294.6μg·90 min/mL; short term, 478.5±348.9μg·90min/mL). Long-term gastric emptying returned to the preoperative state in both groups (group A: long term, 702.2±284.4μg·90min/mL, and group B: long term, 761.7±345.7μg·90min/mL **(Figure 2A)**. Short-term body weight significantly decreased in both groups, while long-term body weight returned to the preoperative value in group A (short term, 90.9±4.3%; long term, 95.1±5.1%) but not in group B (short term, 89.5±5.3%; long term, 90.7±7.1%) **(Figure 2B)**. Short-term QOL was decreased in group B (group A: before surgery, 70.1±16.3%; short term, 71.0±9.5%, and group B: before surgery, 71.3±15.3%; short term, 62.5±10.2%), but long-term QOL increased to greater than the preoperative level in both groups (group A: long term, 84.5±9.6%, and group B: long term, 78.4±8.2%) **(Figure 2C)**.

Influence of Preoperative Pancreatic Endocrine Function

Short-term gastric emptying was delayed in both groups (group C: before surgery, 679.1±267.0μg·90min/mL; short term, 456.1±220.1μg·90min/mL, and group D: before surgery, 596.8±262.2μg·90min/mL; short term, 410.4±150.8μg·90min/mL), and long-term gastric emptying returned to the preoperative level in both groups (group C: long term, 789.4±259.4μg·90min/mL, and group D: long term, 711.3±212.2μg·90min/mL) **(Figure 3A)**. Short-term body weight was decreased significantly in both groups. Whereas long-term body weight significantly recovered and returned to the preoperative value in group C (short term, 90.6±4.4%, long term,

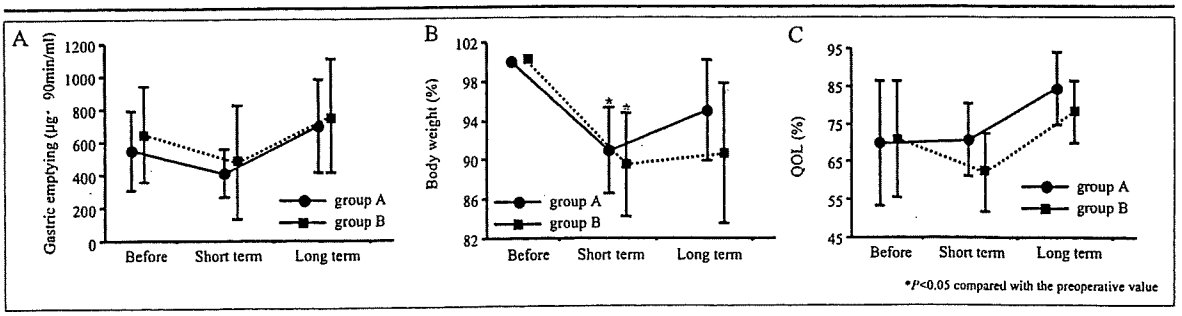

FIGURE 2 Changes in **(A)** gastric emptying (AUC 90), **(B)** body weight, and **(C)** QOL in groups A and B.

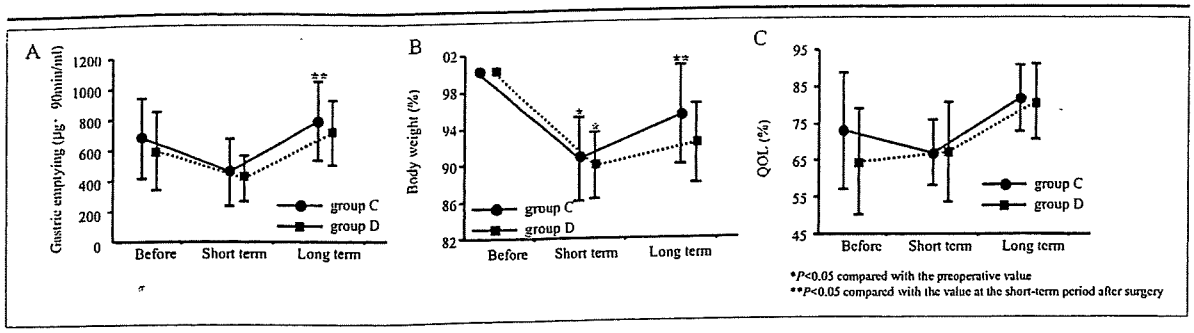

FIGURE 3 Changes in (A) gastric emptying (AUC 90), (B) body weight, and (C) QOL in groups C and D.

95.3±5.4%, $P<0.05$), there were no significant changes in group D (short term, 89.9±3.5%, long term, 92.2±4.2%) **(Figure 3B)**. Short-term QOL decreased in group C (before surgery, 73.0±15.8%, short term, 67.0±8.9%) and was similar in group D (before surgery, 64.5±14.4%, short term, 67.1± 13.5%). However, long-term QOL increased to greater than the preoperative level without a significant difference in both groups (group C: long term, 81.9±9.0%, and group D: long term, 80.9±10.3%) **(Figure 3C)**.

DISCUSSION

Several reports have suggested that body weight is better after PPPD than after standard PD. Kozuschek *et al.* reported that 43% of patients who underwent standard PD reached preoperative body weight after 1 year, whereas 86% of patients who underwent PPPD reached their preoperative weight (2). Braasch *et al.* reported that 28 patients who underwent PPPD reached 93% pre-illness weight and 106% preoperative weight at the time of follow-up (14). Zerbi *et al.* reported that patients who underwent PPPD had reached a mean 92% of the usual pre-illness body weight at 6 months after surgery, showing significantly better recovery of body weight than that of patients after standard PD (15). Yamaguchi *et al.* reported that postoperative loss of more than 3kg body weight was evident in 62% of patients after PPPD, that the maximum body weight loss was seen about 4.2 months after PPPD, and that body weight returned to the preoperative level 4.8 months thereafter (16). In our PPPD patients, long-term body weight was greater than 90% in all groups, and these results resembled those of previous reports. Short-term body weight decreased significantly after PPPD in patients with normal preoperative pancreatic function and in patients with abnormal preoperative pancreatic function. However, long-term body weight returned to the preoperative level in patients with normal preoperative pancreatic function, but not in patients with abnormal preoperative pancreatic function. Recovery of body weight is an important determinant of nutritional status, and our results suggest that the relation between pancreatic function and body weight is also important.

Gastric emptying is also an important determinant of nutritional status. Early delayed gastric emptying (DGE) is one of the most relevant and frequent postoperative complications and has been reported to range between 20% and 50% (17-23). The cause of DGE is not yet clear, and several factors are thought to play a role in DGE. These include gastric dysmotility after PPPD attributed to disruption of the gastroduodenal neural connection (24) and gastric dysrhythmia due to postoperative complications such as anastomotic leakage, intraabdominal abscess, and bleeding (25,26). Murakami *et al.* reported that residual pancreatic fibrosis is the most important cause of DGE after PPPD without complications (27). We previously reported that gastric emptying was delayed but returned to the preoperative level by 6 months after surgery (7). We obtained similar results in the present study, and there was no significant difference in postoperative gastric emptying between patients with normal and abnormal preoperative pancreatic function. Long-term gastric emptying was restored to the preoperative level, whereas recovery of body weight was poor in patients with abnormal preoperative pancreatic function. This suggests that preoperative pancreatic function is more important determinant of postoperative nutritional status.

Short-term QOL was the same or slightly lower than preoperative QOL. However, long-term QOL was high in comparison to preoperative and short-term QOL. It is likely that the QOL score improved with the increases in food intake and nutritional status. It is also possible that patients' anxiety over their disease state was relieved after surgery, positively influencing QOL. The difference in long-term QOL between our patients with normal and abnormal preoperative pancreatic functions suggests on indirect link between preoperative pancreatic function and improvement in QOL.

In conclusion, preoperative pancreatic function influenced the recovery of body weight after PPPD, however, it did not influence the recovery of gastric emptying or QOL.

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