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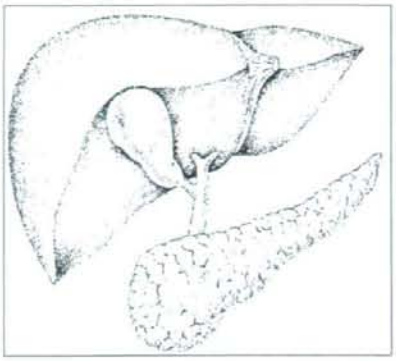

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Journal of HBP Surgery

Adjuvant treatments for resectable pancreatic cancer

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Abstract
Pancreatic cancer remains one of the most challenging malignancies to treat successfully. The majority of patients present with unresectable advanced-stage cancer, and only 20% of patients can undergo resection. Even if surgical resection is performed, the recurrence rate is high and the survival rate after surgery is poor. Therefore, effective adjuvant therapy is needed to improve the prognosis of patients with pancreatic cancer. Until now, no universally accepted standard adjuvant therapy for this disease has been available: chemoradiotherapy followed by chemotherapy is considered the optimal therapy in the United States, while chemotherapy alone is the current standard in Europe. However, recent randomized controlled trials (RTOG [Radiation Therapy Oncology Group] 9704; CONKO [Charité Onkologie]-001; and a Japanese study) have suggested a benefit of adjuvant chemotherapy with gemcitabine for patients with resectable pancreatic cancer. This article will review the clinical trials of adjuvant therapy for this disease, including the results of recent trials.

Key words Pancreatic cancer · Adjuvant therapy · Chemoradiotherapy · Chemotherapy

Introduction

Surgical resection offers the only opportunity for cure in patients with pancreatic cancer; therefore, detection at an early stage, especially International Union Against Cancer (UICC) stage I, is essential to increase long-term survival. However, no valid method of screening has been established for this disease, and only 20% of all pancreatic cancers are detected at the resectable stage. In addition, because the recurrence rate after surgery is high, the 5-year survival rate in patients with resectable pancreatic cancer is 20% or less. Because surgical resection alone has limitations, the development of nonsurgical treatment as adjuvant therapy is important. Recently, various attempts at adjuvant therapy have been reported for this disease. This review focuses on the outcomes of clinical trials, including randomized controlled trials (RCTs), of adjuvant therapy for resectable pancreatic cancer.

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Controversies associated with adjuvant therapy for pancreatic cancer

For breast cancer and colorectal cancer, the survival benefits of adjuvant therapy in resectable cases have been shown in large-scale RCTs, and a standard adjuvant therapy has been established on a global scale. In regard to pancreatic cancer, the survival benefits of chemotherapy using gemcitabine (GEM) for unresectable advanced cases have been evaluated internationally. However, as far as adjuvant therapy for resectable pancreatic cancer is concerned, no globally accepted standard therapy has yet been established. Major factors underlying this situation are: (1) difficulty in conducting large-scale RCTs because the number of resectable pancreatic cancer cases is not large enough; and (2) lack of consensus about the significance of adjuvant chemoradiotherapy between United States and European physicians. According to the results of clinical studies including RCTs carried out in the United States and Europe, most United States physicians now support the validity of adjuvant chemoradiotherapy, while most in Europe have a negative view of adjuvant chemoradiotherapy and a positive view of adjuvant chemotherapy. It is therefore difficult to establish an adjuvant therapy which can serve as a global standard, and it is desirable that global cooperative studies be carried out to reach a consensus regarding the validity of adjuvant therapy.

Adjuvant chemoradiotherapy

Table 1 summarizes the results of RCTs reported to date on adjuvant chemoradiotherapy for resectable pancreatic cancer. The GITSG (Gastrointestinal Tumor Study Group)[1] and RTOG (Radiation Therapy Oncology Group) 9704[2] trials were carried out in the United States, and the EORTC (European Organization for Research and Treatment of Cancer)[3] and ESPAC (European Study Group For Pancreatic Cancer)-1[4] trials were carried out in Europe.

Although GITSG is an RCT that was carried out more than 20 years ago,[1] the results of this study still have major impacts even at present. In this RCT, the adjuvant chemoradiotherapy group was compared with an observation group, yielding a significantly longer survival period in the former, with the median survival time (MST) being 20 months vs 11 months ($P = 0.035$). The regimen evaluated in GITSG was a combination of split-course radiotherapy (20 Gy × 2) and bolus 5-fluorouracil (5-FU) injection, followed by maintenance chemotherapy using bolus 5-FU injection. This RCT has been criticized for the very small scale of the study (only 43 subjects were analyzed because of difficulties in case enrollment) and the poor results in the observation group despite curative resection being carried out in these patients. However, because no RCT results that invalidate the results of the GITSG have been reported from the United States, many clinicians in the United States still consider chemoradiotherapy as a standard adjuvant therapy for resectable pancreatic cancer.

In Europe, an RCT was carried out by EORTC as a follow-up to the GITSG.[3] The regimen evaluated in the EORTC trial resembled that employed in GITSG, except that 5-FU was administered by continuous intravenous infusions during irradiation and no maintenance chemotherapy was used. The EORTC trial involved 114 subjects (larger than the number of GITSG subjects) and demonstrated a tendency for slightly better outcomes in the chemoradiotherapy group as compared to the observation group, although the difference was not statistically significant (MST, 17.1 months vs 12.6 months; $P = 0.099$). Following the report of the EORTC trial results, European clinicians began to question the efficacy of postoperative chemoradiotherapy for resectable pancreatic cancer, but some commented that, in view of the slightly better outcomes in the chemoradiotherapy group, a significant difference in outcome would have been obtained if a larger number of subjects had been studied. In response to the criticism that the scales of the studies were too small to draw any valid conclusions from the GITSG and EORTC trials, a larger-scale RCT was planned by ESPAC in the 1990s (ESPAC-1).[4] In this RCT, not only the significance of chemoradiotherapy but also that of chemotherapy was assessed using the 2 × 2 factorial design. ESPAC-1 adopted the GITSG regimen for chemoradiotherapy and a combined 5-FU + leucovorin (LV) regimen for chemotherapy. The final analysis of the data from 289 subjects revealed a survival-prolonging effect of chemotherapy, but chemoradiotherapy exerted no significant efficacy as compared to the group who did not receive chemoradiotherapy, with the outcome being less favorable in the chemoradiotherapy group than in the no-chemoradiotherapy group (MST, 15.9 months vs 17.9 months; $P = 0.053$). Although some problems have been raised regarding this study (e.g., quality control for radiotherapy, low compliance with the instructions on the assigned therapy, and problems with the analytical method), many European clinicians now view postoperative chemoradiotherapy negatively.

In the United States, there was a long break in reports on RCTs of adjuvant therapy for resectable pancreatic cancer. Recently, the RTOG presented the results of a

Table 1. Randomized controlled trials of adjuvant chemoradiotherapy for resectable pancreatic cancer

Author	Year of publication	Treatment	Number of patients	MST (months)	2-Year survival rate	*P* value (log-rank test)
Kalser and Ellenberg[1] (GITSG)	1985	5-FURT → 5-FU	21	20	42%	0.035
		Observation	22	11	15%	
Klinkenbijl et al.[3] (EORTC)	1999	5-FURT	60	17.1	37%	0.099
		Observation	54	12.6	23%	
Neoptolemos et al.[4] (ESPAC-1)	2004	5-FURT	145	15.9	29%	0.053
		No 5-FURT	144	17.9	41%	
Regine et al.[2] (RTOG 9704)	2006	All patients				
		GEM → 5-FURT → GEM	221	18.8	NA	0.15
		5-FU → 5-FURT → 5-FU	221	16.9	NA	
		Pancreas head only				
		GEM → 5-FURT → GEM	187	20.6	NA	0.033
		5-FU → 5-FURT → 5-FU	194	16.9	NA	

5-FURT, chemoradiotherapy using 5-fluorouracil; 5-FU, 5-fluorouracil; GEM, gemcitabine; MST, median survival time; NA, not available

large-scale RCT involving the analysis of the data from 442 subjects.[7] This RCT was designed to identify an optimal chemotherapy to be added to chemoradiotherapy, rather than to evaluate the validity of chemoradiotherapy. Chemoradiotherapy using 5-FU was administered to both groups, and 5-FU was compared with GEM as the agent used for chemotherapy to be added to chemoradiotherapy. When the data from the entire population were analyzed, no significant difference in the survival period was noted between the 5-FU group and the GEM group, but the GEM group had significantly better outcomes when the analysis was confined to cases of pancreatic head cancer (MST, 20.6 months vs 16.9 months; $P = 0.033$). There is an open question as to the meaning of the significant difference demonstrated by the analysis of pancreatic head cancer alone. At present, however, chemoradiotherapy using 5-FU is often combined with chemotherapy using GEM in the United States.

As a new attempt at chemoradiotherapy, combinations of GEM and radiotherapy have been actively studied since the latter half of the 1990s. Because this combined therapy was shown to induce relatively intense adverse reactions, modifications of the regimen have been called for, e.g., reducing the GEM dose level and/or radiation dose or narrowing the irradiated field. Blackstock et al.[5] conducted a phase II study, using a regimen combining twice weekly GEM treatment (40 mg/m^2) with 50.4 Gy radiotherapy, and reported an MST of 18.3 months. At present, a large-scale RCT by EORTC is underway, comparing GEM followed by chemoradiotherapy using GEM vs GEM alone (EORTC 40013). As another noteworthy chemotherapeutic approach, we can cite a regimen involving the combined use of three drugs (5-FU, cisplatin, and interferon-alpha) reported by the Mason Medical Center. The investigators at this facility applied this therapy in 43 patients who had undergone surgical resection, and reported a very favorable outcome (5-year survival rate of 55%).[6] At present, a multicenter phase II study (ACOSOG [American College of Surgeons Oncology Group]-Z05031) is underway in the United States to assess the reproducibility of this study. In Germany, an RCT (CapRI [adjuvant ChemoRadioImmunotherapy of pancreatic carcinoma] trial) is now underway, comparing chemoradiotherapy using a combination of these three drugs with the chemotherapeutic regimen used in the ESPAC-1 trial (5-FU + LV).[7]

In recent years, active efforts have been made to develop adjuvant therapy combining radiotherapy with new treatment modalities such as molecular-targeted drugs and vaccine therapy. To date, the efficacies of these new therapies remain to be clarified.

Adjuvant chemotherapy

Table 2 summarizes the results of RCTs reported to date concerning adjuvant chemotherapy for resectable pancreatic cancer. As stated above, adjuvant chemoradiotherapy began to be used as a standard therapy in the 1980s in the United States. For this reason, evaluation of adjuvant chemotherapy is difficult in the United States. Evaluation of adjuvant chemotherapy has thus been carried out primarily in Europe and Japan.

5-FU had been used as a major drug for adjuvant chemotherapy since before GEM began to be used for pancreatic cancer in the latter half of the 1990s. Several combined therapy regimens involving 5-FU had been attempted during that period. The earliest attempt was the RCT reported in 1993 by Bakkevold et al.[8] from Norway. In that study, postoperative AMF therapy

Table 2. Randomized controlled trials of adjuvant chemotherapy for resectable pancreatic cancer

Author	Year of publication	Treatment	Number of patients	MST (months)	2-Year survival rate	*P* value (log-rank test)
Bakkevold et al.[8] (Norway)	1993	ADR + MMC + 5-FU	30[a]	23	43%	0.10
		Observation	31[a]	11	32%	
Takada et al.[9] (Japan)	2002	5-FU + MMC	81	NA	NA	NS
		Observation	77	NA	NA	
Neoptolemos et al.[4] (ESPAC-1)	2004	5-FU + LV	147	20.1	40%	0.009
		No 5-FU + LV	142	15.5	30%	
Kosuge et al.[10] (Japan)	2006	5-FU + cisplatin	45	12.5	NA	0.94
		Observation	44	15.8	NA	
Oettle et al.[12] (CONKO-001)	2007	GEM	179	22.1	47.5%	0.06
		Observation	175	20.2	42%	
Kosuge et al.[15] (Japan)	2007	GEM	58	22.3	48.3%	0.29
		Observation	60	18.4	39.8%	

ADR, adriamycin; MMC, mitomycin C; 5-FU, 5-fluorouracil; LV, leucovorin; GEM, gemcitabine; MST, median survival time; NA, not available; NS, not significant

[a] Including ampulla of Vater cancer

(adriamycin + mitomycin C + 5-FU) was compared with observation, involving 61 patients with surgically resected pancreatic cancer, including ampulla of Vater cancer. The authors reported that the MST was longer in the chemotherapy group (23 months) than in the observation group (11 months), although analysis of the overall survival period revealed no significant intergroup difference.[8] In Japan, Takada et al.[9] compared combined 5-FU + mitomycin C therapy with observation, and Kosuge et al.[10] compared combined 5-FU + cisplatin therapy with observation, but neither of these studies revealed a significant intergroup difference in survival periods.

In contrast to these studies, the ESPAC-1 trial[4] revealed the usefulness of adjuvant chemotherapy involving 5-FU. When the adjuvant chemotherapy (5-FU + LV) was analyzed using a 2×2 factorial design in that study, the survival time was significantly longer in the adjuvant chemotherapy group than in the group without adjuvant chemotherapy (MST, 20.1 months vs 15.5 months; $P = 0.009$).[4] A metaanalysis was conducted on the results of RCTs reported before ESPAC-1 (GITSG,[1] EORTC,[3] Bakkevold et al.,[8] Takada et al.,[9] ESPAC-1[4]).[11] The analysis revealed that chemotherapy involving 5-FU reduced the risk of death significantly (hazard ratio, 0.75; 95% confidence interval, 0.64–0.90; $P = 0.001$). The ESPAC-1[4] findings, which revealed the survival benefit of adjuvant chemotherapy in a large-scale RCT, now have major impacts, and there is a prevailing view in Europe that chemotherapy should be used as a standard adjuvant therapy for resectable pancreatic cancer.

Next to 5-FU, GEM has been actively studied in the adjuvant setting. German investigators, including Oettle et al.,[12] compared a GEM therapy group with an observation group after surgical resection of pancreatic cancer (CONKO [Charité Onkologie]-001). The results of their study were presented at an American Society of Clinical Oncology (ASCO) 2005 meeting. In the CONKO-001 study, GEM was administered for six courses by the routine dosing method. The data from 354 patients in total from the two groups were analyzed. The disease-free survival (DFS), which served as a primary endpoint of the study, was significantly longer in the GEM group than in the observation group (median DFS, 13.4 months vs 6.9 months; $P < 0.001$). In the analysis of overall survival, the survival period tended to be longer in the GEM group than in the observation group, although this difference was not statistically significant (MST, 22.1 months vs 20.2 months; $P = 0.06$).

An RCT of GEM vs observation has also been conducted in Japan, and the results were reported at an European Cancer Conference (ECCO) 14 meeting.[13] In that study, data from 118 subjects were analyzed, and the GEM group received three treatment courses (shorter than the period in the CONKO-001 study). The DFS was significantly longer in the GEM group than in the observation group (median DFS, 11.4 months vs 5.0 months; $P = 0.01$). In the analysis of overall survival, the GEM group tended to show more favorable results than the observation group, but the difference was not significant (MST, 22.3 months vs 18.4 months; $P = 0.29$). Most of the adverse reactions of GEM observed in that study were temporary, and severe adverse reactions were rare. The results of GEM therapy in Japan were quite akin to those of the CONKO-001 study. This high reproducibility suggests the effectiveness of adjuvant chemotherapy using GEM.

In Europe, a large-scale RCT (ESPAC-3) involving comparisons among three groups (observation, 5-FU + LV, and GEM) is now underway. In Japan, active efforts are currently being made to develop novel adjuvant chemotherapy using S-1, following the report of favorable outcomes of S-1 therapy for advanced pancreatic cancer.[14]

Conclusions

Although no adjuvant chemotherapy that serves as a global standard for pancreatic cancer has yet been established, RCTs of this type of therapy have been actively performed in recent years, yielding increasing evidence of the benefits of such therapy.

In Japan, GEM has increasingly been accepted as the treatment of choice for patients after the surgical resection of pancreatic cancer, based on the CONKO-001 trial[12] and the results of a Japanese RCT.[13] Because the prognosis of patients with pancreatic cancer is still poor, advances based on research into adjuvant therapy are desired. When considering the adoption of adjuvant therapies in clinical cases, it is essential to adequately inform individual patients of the fact that no universally accepted standard adjuvant therapy has yet been established for pancreatic cancer, in addition providing an explanation of adverse reactions. Then, if the patient agrees to undergo adjuvant therapy, the treatment should be carried out carefully, paying close attention to adverse reactions.

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HOW TO DO IT

Hepatobiliary resection with inferior vena cava resection and reconstruction using an autologous patch graft for intrahepatic cholangiocarcinoma

Tsuyoshi Sano · Kazuaki Shimada · Satoshi Nara · Minoru Esaki · Yoshihiro Sakamoto · Tomoo Kosuge

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Abstract
Background In patients with advanced cholangiocarcinoma involving the inferior vena cava (IVC), an extended hepatobiliary resection with combined resection and reconstruction of the IVC is often prerequisite to obtain a clear resection margin.
Materials and methods We present our approach to repair of approximately half of a cross-sectional wall defect of the IVC using an autologous external iliac venous patch graft during extended hepatobiliary resection with a total hepatic vascular exclusion technique. The harvested external iliac vein graft was incised longitudinally and trimmed to fit the IVC defect. After multiple stay sutures, a continuous running suture using 4–0 prolene was made.
Results Two patients who underwent this complex surgery survive 20 and 27 months after surgery, respectively. Morbidity of transient edema of the ipsilateral lower leg potentially related to graft harvesting was noted in one patient after surgery.
Conclusions The external iliac vein patch graft for IVC resection and reconstruction during hepatobiliary resection is technically simple, produces no stenosis or caliber change in the reconstructed IVC, and is applicable for at least half or less of a cross-sectional defect of the IVC wall to be reconstructed.

Keywords Inferior vena cava · Hepatectomy · Hepatobiliary resection · Patch graft

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Introduction

There have been several reports describing hepatectomy with inferior vena cava (IVC) resection and reconstruction for various diseases such as hepatocellular carcinoma, metastatic colorectal carcinoma, or leiomyosarcoma of the IVC. [1–6] Intrahepatic cholangiocellular carcinoma (CCC) originating from or involving the caudate lobe occasionally necessitates an extensive hepatobiliary resection including concomitant IVC resection and reconstruction to achieve negative surgical margins. In the setting of IVC resection and reconstruction, the total hepatic vascular exclusion technique (THVE) [7–10] is sometimes useful to reduce the blood loss during the procedure, although the indication or necessity of active veno-veno bypass between portal system and systemic circulation remains controversial. Actually, the hemodynamic state during short-term clamping of the IVC may be maintainable by rapid fluid replacement, and wedge resection and subsequent direct longitudinal suture of the IVC would not require THVE. However, such time-consuming methods as total replacement or repairing the wide defect of the IVC wall may necessitate vascular grafting under THVE with or without active veno-veno bypass between the portal system and systemic circulation.

This report highlights CCC treated by an extended hepatobiliary resection with IVC resection after patch repair using an autologous external iliac vein graft.

Case 1: Left trisectionectomy

A 72-year-old woman presented with epigastralgia. Computed tomography (CT) depicted a mass with heterogeneous enhancement measuring 6×4.5 cm in the caudate lobe of the liver (Fig. 1a–d). CT revealed vascular invasion of the left (LHV) and middle hepatic vein (MHV), and IVC. cavography showed a marked narrowing part of the IVC and well-developed intrahepatic venous anastomoses between the inferior (IRHV) or middle right hepatic (MRHV) and superior right hepatic vein (SRHV; Fig. 1e). The extrahepatic collateral pathway was not opacified. After left trisectional portal vein embolization (PVE), [11, 12] a laparotomy was conducted.

Upper median with right transverse oblique thoracoabdominal incision (inverted T-shape incision) through the ninth intercostal space was done to assure an adequate operative view. At first, regional lymphadenectomy including the retropancreatic and hepatoduodenal ligament nodes was performed, and skeletonization of the hepatic hilum progressed. After division of the left portal vein, left hepatic artery, and anterior branch of the right hepatic artery, a demarcation line appeared corresponding to the right portal fissure. The whole liver was mobilized, and the SRHV and the common trunk of the MHV and LHV were encircled.

A left lower paramedian incision was made, and the left external iliac vein was harvested as an autologous vein graft through the extraperitoneal approach. The blood access route both from the stump of the left portal vein and the right femoral vein to the left subclavian vein was obtained before liver parenchymal transection. Liver transection along the demarcation line on the right portal fissure was started only under intermittent hepatic pedicle occlusion. Increased bleeding from the transection plane was documented, and THVE was started with active veno-veno bypass using an active centrifugal force pump (Biopump; Bio-Medicus, MN, USA). [10] The SRHV was exposed on the raw surface of the liver, and the IRHV and MRHV were clamped with vascular forceps in the liver parenchyma. Then, the common trunk of LHV and MHV was divided and closed, and the retrohepatic IVC was clamped just below the confluence of the common trunk of LHV and MHV. Finally, the ventral wall of the IVC was concomitantly resected with a cancer-free margin, and a left hepatic trisectionectomy with caudate lobe resection was completed (Fig. 2a).

The defect of the IVC wall was half of the vessel cross section and oval in shape. An external iliac vein graft was longitudinally incised and trimmed to fit the IVC defect. A total of four stay sutures were placed, and reconstruction was completed with running sutures of 4–0 polypropylene (Figs. 2b, 3a). Finally, a hepaticojejunostomy was done using a Roux-en Y jejunal limb. Total operation time was 863 min, pedicle occlusion time was 130 min, and THVE time was 75 min. Intraoperative blood loss was 2,775 g. The maximum serum total bilirubin level was 5.7 mg/dl on the seventh postoperative day.

Histologically, the tumor involvement extended to the adventitia of the IVC wall, and all surgical margins were negative for cancer (Fig. 3b). The patient was discharged on the 26th postoperative day and is alive 27 months after the operation without any sign of tumor recurrence.

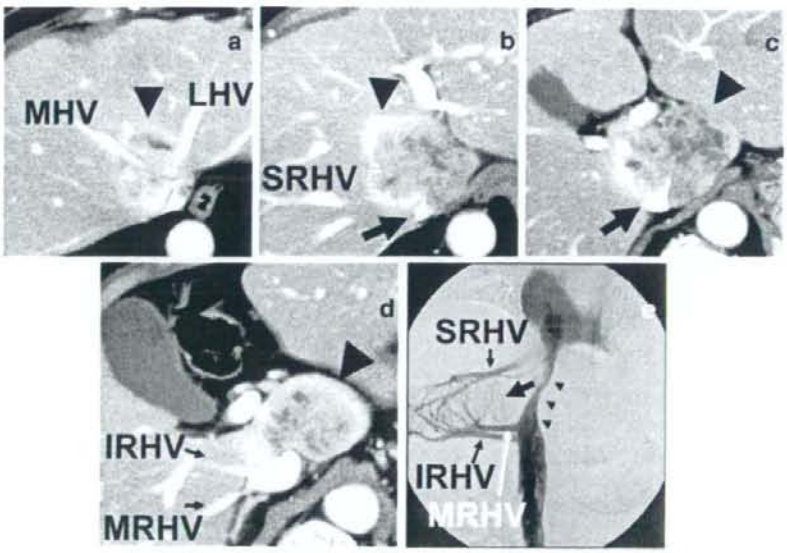

Fig. 1 Computed tomography depicts an ill-defined tumor showing irregular enhancement (*arrowheads*) (**a**–**d**). LHV and MHV are involved by the tumor (**a**). IVC is narrowed (**b**) and deformed (**c**), suggesting tumor involvement. Cavography shows narrowing of IVC (*arrowheads*) and well-developed intrahepatic anastomoses between SRHV and MRHV or IRHV (*arrows*) (**e**). *LHV* left hepatic vein, *MHV* middle hepatic vein, *IVC* inferior vena cava, *SRHV* superior right hepatic vein, *MRHV* middle right hepatic vein, *IRHV* inferior right hepatic vein

Fig. 2 **a** Intraoperative photograph just after left hepatic trisectionectomy, total caudate lobectomy, and combined IVC resection. Half cross-sectional defect (*white arrows*) of the IVC. IRHV and MRHV are clamped with vascular forceps (*black arrows*). **b** Intraoperative photograph just after reconstruction of IVC using an autologous left external iliac vein graft patch. *IVC* inferior vena cava, *IRHV* inferior right hepatic vein, *MRHV* middle right hepatic vein

Case 2: Right trisectionectomy

A 64-year-old man was referred to our institute for surgical treatment of an irregular-shaped mass 7×6 cm in size located in the caudate lobe on CT. After right PVE, a laparotomy was conducted. A paraaortic lymph node dissection was performed due to the suspicion of metastatic cancer, and the nodes were confirmed to be negative for cancer metastasis by intraoperative frozen section. During skeletonization dissection of the hepatoduodenal ligament, a portal vein sleeve resection and direct end-to-end anastomosis before liver resection were performed due to tumor invasion of the portal bifurcation. In this case, no intrahepatic collateral venous pathway was detected, so a short THVE was used to facilitate IVC repair using a right external iliac vein graft similar to case 1 (Fig. 4). The liver transection progressed along the falciform ligament (Fig. 5a).

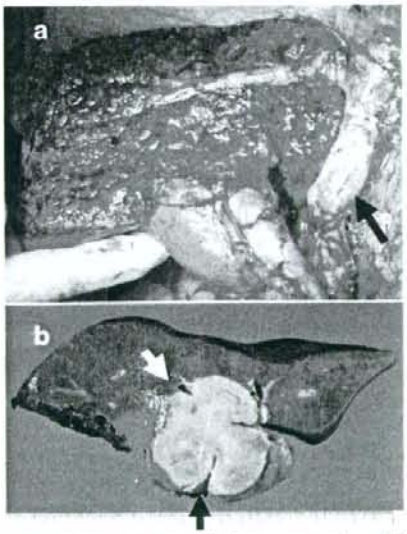

Fig. 3 **a** Blood flow was restored after reconstruction of IVC. No caliber change is seen on the reconstructed IVC (*arrow*). **b** Cut surface of resected specimen shows a round-shaped tumor involving the IVC (*black arrow*) and middle hepatic vein (*white arrow*). *IVC* Inferior vena cava

Total operation time was 780 min, pedicle occlusion time was 60 min, and IVC clamp time was 41 min. Intraoperative blood loss was 2,110 g, and no red blood cell transfusion was required. The maximum serum total bilirubin level was 3.4 mg/dl on the seventh postoperative day. Postoperatively, intractable ascites possibly due to lymphorrhea after paraaortic lymph nodes dissection were encountered along with right leg edema due to harvesting the right external iliac vein. Those morbidities could be managed conservatively.

The resected specimen showed cancer involvement beyond the IVC wall and exposure into the intraluminal space (Fig. 5b). The patient was discharged on the 62nd postoperative day and is alive with lung metastasis 20 months after the resectional surgery.

Discussion

Development of the intrahepatic collateral circulation suggested the possible necessity of an active veno-veno bypass during liver transection with the THVE technique. [10] In this condition, liver parenchymal transection may injure the intrahepatic collateral circulation and lead to massive bleeding from the liver transection plane under simple portal pedicle occlusion. Thus, if we can detect this unusual finding preoperatively, the THVE technique would be essential, and preparation of an active veno-veno bypass might also be advisable. Although intermittent THVE is a potential countermeasure for this situation, it runs the risk of considerable bleeding from the transection plane during the release of inflow and outflow occlusion.

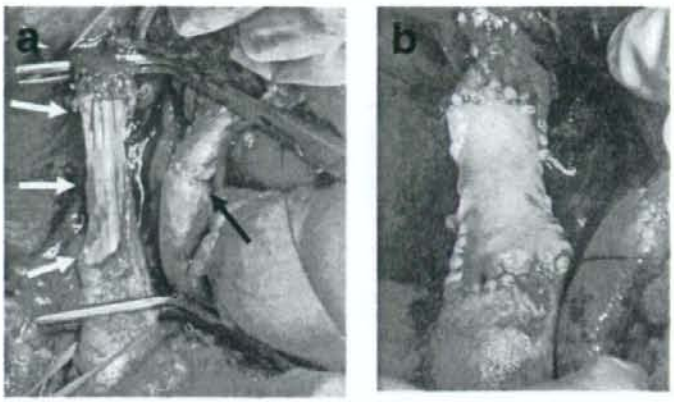

Fig. 4 **a** Intraoperative view after right trisectionectomy, total caudate lobectomy, and combined resection of IVC shows wall defect of half or more of the vessel cross-section (*white arrows*). Portal vein resection and reconstruction had already been completed (*black arrow*). **b** The right external iliac vein was longitudinally incised, trimmed, and fixed with stay sutures. *IVC* Inferior vena cava

Actually, the risk of infectious complications such as intraabdominal abscess after hepatectomy without bilioenterosotmy might be low. Thus, some hepatic surgeons concluded that routine prophylactic abdominal drainage after hepatectomy is not required. However, their reports described hepatectomy without bilioenterostomy for such liver tumors as hepatocellular carcinoma or metastatic liver tumor. [13–15] Using a polytetrafluoroethylene graft [16] during hepatectomy without bilioenterostomy might be beneficial for a risk of morbidity related to harvesting the graft. Although major hepatobiliary resection for perihilar cholangiocarcinoma is becoming more common and safer, [17] anastomotic insufficiency rates of hepaticojejunostomy more than 5% have been reported. [18, 19] This observation suggests that an intraabdominal septic complication after hepatobiliary resection is more frequent than hepatectomy without bilioenterostomy. The morbidity relating to the harvesting of the external iliac vein graft in our case is transient and not life-threatening, and thereafter, no anticoagulant therapy was required. On the other hand, a prosthetic graft must fit for a case producing a long segmental defect of IVC to be reconstructed; an autologous vein graft is too short or small for the size or length of the defect. Segmental resection and reconstruction of IVC using autologous vein graft potentially causes a caliber change of the reconstructed IVC that is a risk for thrombosis. [20] A graft to repair an IVC defect must be selected in terms of the risk and benefits.

Our strategy in cases of hepatobiliary resection potentially requiring IVC resection and reconstruction is as follows: Direct suture of the IVC wall is the first choice if possible, followed by repair with an autologous vein patch graft, and finally, an interposition graft for segmental repair of the resected IVC. With an interposition graft, an autologous vein graft is desirable, and an artificial graft is the final solution. Candidates for an autologous vein graft in IVC repair include the left renal vein, [21] external iliac vein, and external jugular vein. In case 1, at first, we attempted to harvest the left renal vein as a graft. However, marked distention of the left renal vein was encountered just after clamping the confluence of the left renal vein. This may suggest left renal congestion potentially relating to left renal dysfunction. Thus, we did not harvest the left renal vein. Both iliac and jugular vein graft necessarily involved an additional wound for harvesting, and jugular vein harvesting is also associated with a cosmetic disad-

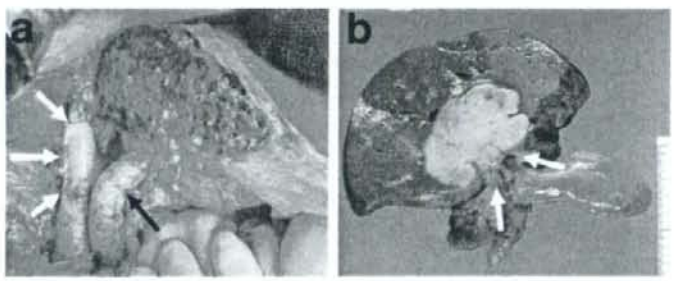

Fig. 5 **a** Intraoperative photograph after right trisectionectomy, total caudate lobectomy, portal vein resection and reconstruction (*black arrow*). IVC resection and reconstruction (*white arrows*) developed no caliber change of reconstructed IVC. **b** Cut surface of resected specimen shows a round-shaped tumor involving the IVC (*white arrow*). *IVC* inferior vena cava

vantage in terms of a neck wound. On the other hand, external iliac vein grafting might produce transient edema of the ipsilateral lower extremity as documented in case 2.

The hepatic venous involvement by the tumor is a factor greatly influencing selection of the appropriate type of hepatectomy. In this setting in which either a right or left hepatic trisectionectomy can be selected to achieve an R0 resection, there are two concerns: the technical feasibility and safety of the operation. A right trisectionectomy usually produces smaller transection surface than a left trisectionectomy and is technically a simple procedure. Moreover, concomitant portal vein resection and reconstruction before liver transection are more easily achieved in a right-side hepatectomy. On the other hand, considering the safety of the operation, the future remnant liver volume that is calculated by CT volumetry is critical in the selection of the type of hepatectomy. Preoperative PVE is one of the crucial options to facilitate the design or to select a wider range of extended hepatobiliary resection procedures.

In conclusion, the external iliac vein patch graft for IVC resection and reconstruction during the hepatobiliary resection is technically simple and produces no stenosis or caliber change in the reconstructed IVC. This method is applicable for at least a cross-sectional IVC wall defect of half or less to be reconstructed and is feasible by general hepatobiliary surgeons without any special liver transplant technique.

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Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma

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Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and human epidermal growth factor receptor 2 (HER2) have been considered as potential therapeutic targets in cholangiocarcinoma, but no studies have yet clarified the clinicopathological or prognostic significance of these molecules. Immunohistochemical expression of these molecules was assessed retrospectively in 236 cases of cholangiocarcinoma, as well as associations between the expression of these molecules and clinicopathological factors or clinical outcome. The proportions of positive cases for EGFR, VEGF, and HER2 overexpression were 27.4, 53.8, and 0.9% in intrahepatic cholangiocarcinoma (IHCC), and 19.2, 59.2, and 8.5% in extrahepatic cholangiocarcinoma (EHCC), respectively. Clinicopathologically, EGFR overexpression was associated with macroscopic type ($P = 0.0120$), lymph node metastasis ($P = 0.0006$), tumour stage ($P = 0.0424$), lymphatic vessel invasion ($P = 0.0371$), and perineural invasion ($P = 0.0459$) in EHCC, and VEGF overexpression with intrahepatic metastasis ($P = 0.0224$) in IHCC. Multivariate analysis showed that EGFR expression was a significant prognostic factor (hazard ratio (HR), 2.67; 95% confidence interval (CI), 1.52–4.69; $P = 0.0006$) and also a risk factor for tumour recurrence (HR, 1.89; 95% CI, 1.05–3.39, $P = 0.0335$) in IHCC. These results suggest that EGFR expression is associated with tumour progression and VEGF expression may be involved in haematogenic metastasis in cholangiocarcinoma.
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Keywords: cholangiocarcinoma; epidermal growth factor receptor; vascular endothelial growth factor; human epidermal growth factor receptor 2; immunohistochemistry; prognosis

Cholangiocarcinoma arises from the ductal epithelium of the bile duct tree and is classified anatomically into intrahepatic cholangiocarcinoma (IHCC) and extrahepatic cholangiocarcinoma (EHCC). The incidence and mortality rates of cholangiocarcinoma, especially those of IHCC, are increasing worldwide (Khan *et al*, 2005). Complete resection is the only way to cure the disease at present. Moreover, because cholangiocarcinoma is difficult to diagnose at an early stage and extends diffusely, most patients have unresectable disease at clinical presentation, and prognosis is very poor (5-year survival is 0–40% even in resected cases) (Khan *et al*, 2005; Sirica, 2005). Therefore, novel effective therapeutic strategies are urgently required to improve the prognosis. Among potential therapeutic targets, several studies have revealed overexpression of epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor 2 (HER2) protein, amplification, and mutation of these genes (Ito *et al*, 2001; Aishima *et al*, 2002; Ukita *et al*, 2002; Altimari *et al*, 2003; Gwak *et al*, 2005; Nakazawa *et al*, 2005; Leone *et al*, 2006) as well as overexpression of vascular endothelial growth factor (VEGF) protein (Hida *et al*, 1999; Tang *et al*, 2006) in cholangiocarcinoma.

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Epidermal growth factor receptor and HER2 are members of the ErbB receptor tyrosine kinase family. Binding of ligands, such as epidermal growth factor and transforming growth factor alpha (TGFα), to their extracellular ligand-binding domain initiates intracellular signalling cascades, leading to progression, proliferation, migration, and survival of cancer cells (Olayioye *et al*, 2000; Yarden and Sliwkowski, 2001). Vascular endothelial growth factor plays a key role in tumour-associated neo-angiogenesis, which contributes to providing a tumour with oxygen, nutrition, and a route for metastasis. It binds to VEGFR (vascular endothelial growth factor receptor), and leads to survival, proliferation, and migration of endothelial cell (Tabernero, 2007). Expression of these molecules has been reported to have prognostic significance in several cancers (Gusterson *et al*, 1992; Han *et al*, 2001; Nicholson *et al*, 2001; Des Guetz *et al*, 2006; Mohammed *et al*, 2007). Recently, agents targeted at these molecules have been used clinically, such as trastuzumab in breast cancer (Gonzalez Angulo *et al*, 2006), gefitinib, and erlotinib in non-small cell lung cancer, and bevacizumab in colorectal cancer (Tabernero, 2007). In cholangiocarcinoma, a phase II study of erlotinib (Philip *et al*, 2006) and some case reports of combined chemotherapy including cetuximab (Sprinzl *et al*, 2006; Huang *et al*, 2007) have been reported.

However, no previous studies have clarified associations between the expression of these molecules and clinicopathological

factors or prognosis in patients with cholangiocarcinoma. To elucidate the biological significance and potential of these molecules as therapeutic targets, we investigated EGFR/VEGF/HER2 expression and attempted to elucidate their associations with various clinical features as well as patient survival in 236 cases of cholangiocarcinomas.

MATERIALS AND METHODS

Patients

A total of 236 patients with cholangiocarcinoma (male 160; female 76) who had undergone tumour resection and been diagnosed histologically as having adenocarcinoma of the bile duct at the National Cancer Center Hospital, Tokyo, between January 1991 and August 2004, were enrolled in the present study. Median patient age and follow-up period were 65 years and 875 days, and median tumour sizes of IHCC and EHCC were 4.8 and 3.0 cm, respectively. Detailed characteristics of patient with IHCC and EHCC are presented in Tables 1 and 2. All patients were followed for more than 100 days. Follow-up examination was performed using computed tomography, abdominal ultrasonography, and measurement of the serum carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) levels every 3-6 months. Recurrence was diagnosed by clinical, radiological, or pathological methods, but mainly by radiological evaluation including computed tomography and ultrasonography. Clinical and pathological profiles were obtained from the database of hepatobiliary tumours based on the medical records of the patients. This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan, and written informed consent was obtained from all patients.

All cases were anatomically classified into two groups: IHCC and EHCC. Tumours arising from the bilateral hepatic duct or distal common bile duct were classified as EHCC. The numbers of IHCC and EHCC cases were 106 and 130, respectively.

Histological assessment

Tumour staging and histological classification were assessed according to *TNM Classification of Malignant Tumours* (Sobin and Wittekind, 2002) defined by the International Union Against Cancer (UICC) and the *World Health Organization Histological Classification of Tumours* (Hamilton and Altonen, 2000). Macroscopic types of IHCC were defined with reference to *General Rules for the Clinical and Pathological Study of Primary Liver Cancer* (Liver Cancer Study Group of Japan, 2003): (1) the mass-forming type (MF), which develops an apparent tumour in the liver; (2) the periductal infiltrating type (PI), which spreads along the bile duct; (3) the intraductal growth type (IG), which is confined within the bile duct, and divided into two groups: the mass-forming group (MF and MF mixed with PI or IG) and the non-mass forming group (PI and/or IG). Macroscopic types of EHCC were divided into polypoid type and non-polypoid type (including nodular, scirrhous constricting, and infiltrating types). Other clinicopathological factors were categorised into groups that are presented in Table 1 (IHCC) and Table 2 (EHCC). Because the classifications and clinicopathological factors used in IHCC and EHCC are different, statistical analyses were performed separately.

Immunohistochemistry

Immunohistochemistry (IHC) for EGFR, VEGF, and HER2 was performed using a polymer-based method (Envision™ + Dual Link System-HRP (Dako, DK-2600 Glostrup, Denmark)). Sources and dilutions of primary antibodies were as follows: anti-EGFR (mouse monoclonal, clone 31G7; Zymed, South San Francisco, CA, USA; 1:100), anti-VEGF (rabbit polyclonal; Zymed; 1:50), and anti-HER2 (rabbit polyclonal; Dako; 1:300).

Formalin-fixed, paraffin-embedded serial tissue sections (4 μm) were placed on silane-coated slides for IHC. Sections cut through the maximum tumour diameter were selected for IHC evaluation. The sections were deparaffinised and rehydrated in xylene and grade-diluted ethanol (50-100%), and submerged for 20 min in 0.3% hydrogen peroxide with absolute methanol to block endogenous peroxidase activity. Antigen retrieval for EGFR, VEGF, and HER2 was carried out by adding Digest-all™3 pepsin solution (Zymed) at 37°C for 10 min for EGFR, near boiling in 0.01 M citrate buffer (pH 6.0) for 15 min for VEGF, and heating in 0.01 M citrate buffer at 121°C for 10 min by pressure cooker for HER2. After protein blocking, the sections were incubated with each primary antibody at room temperature for 1 h, followed by incubation with

Table 1 Characteristics of the IHCC patients

Factors	Categories	Population
Age	<65 years old	54 (50.9%)
	≥65 years old	52 (49.1%)
Gender	Male	64 (60.4%)
	Female	42 (39.6%)
Tumour size	≤5.0 cm	55 (55.6%)
	>5.0 cm	44 (44.4%)
Macroscopic type	Non-mass forming	17 (16.0%)
	Mass forming	89 (84.0%)
Invasion of portal vein	Negative	23 (21.9%)
	Positive	82 (78.1%)
Invasion of hepatic vein	Negative	56 (54.9%)
	Positive	46 (45.1%)
Intrahepatic metastasis	Negative	75 (70.8%)
	Positive	31 (29.2%)
Lymph node metastasis	Negative	62 (58.5%)
	Positive	44 (41.5%)
UICC pT	1+2	71 (68.3%)
	3+4	33 (31.7%)
UICC stage	1+2	45 (42.5%)
	3A+3B+3C	61 (57.5%)
Histological classification	Well	22 (20.8%)
	Mod	79 (74.5%)
	Por	5 (4.7%)
Lymphatic vessel invasion	Negative	20 (18.9%)
	Positive	86 (81.1%)
Venous invasion	Negative	19 (17.9%)
	Positive	87 (82.1%)
Perineural invasion	Negative	29 (27.4%)
	Positive	77 (72.6%)
Hepatic surgical margin	Negative	89 (84.0%)
	Positive	17 (16.0%)
Bile duct margin	Negative	91 (85.8%)
	Positive	15 (14.2%)

Well = well differentiated adenocarcinoma; Mod = moderately differentiated adenocarcinoma; Por = poorly differentiated adenocarcinoma. In some factors, data were not available for all cases.

Table 2 Characteristics of the EHCC patients

Factors	Categories	Population
Age	<65 years old	60 (46.2%)
	≥65 years old	70 (53.8%)
Gender	Male	96 (73.8%)
	Female	34 (26.2%)
Tumour size	≤3.0 cm	72 (56.3%)
	>3.0 cm	56 (43.7%)
Macroscopic type	Polypoid	21 (16.8%)
	Non-polypoid	104 (83.2%)
Depth of tumour invasion	Within FM	13 (10.0%)
	Beyond FM	117 (90.0%)
Invasion of portal vein	Negative	97 (74.6%)
	Positive	33 (25.4%)
Invasion of hepatic artery	Negative	127 (97.7%)
	Positive	3 (2.3%)
Lymph node metastasis	Negative	71 (54.6%)
	Positive	59 (45.4%)
UICC pT	1+2	49 (37.7%)
	3+4	81 (62.3%)
UICC stage	1A+1B	37 (28.5%)
	2A+2B+C	93 (71.5%)
Histological classification	Pap	20 (15.4%)
	Well	31 (23.8%)
	Mod	62 (47.7%)
	Por	17 (13.1%)
Lymphatic vessel invasion	Negative	16 (12.3%)
	Positive	114 (87.7%)
Venous invasion	Negative	19 (14.6%)
	Positive	111 (85.4%)
Perineural invasion	Negative	23 (17.7%)
	Positive	107 (82.3%)
Dissected periductal structures margin	Negative	109 (83.8%)
	Positive	21 (16.2%)
Bile duct margin	Negative	92 (70.8%)
	Positive	38 (29.2%)
Invasion to other organ	Negative	53 (40.8%)
	Positive	77 (59.2%)

FM = fibromuscular layer; Pap = papillary adenocarcinoma; Well = well differentiated adenocarcinoma; Mod = moderately differentiated adenocarcinoma; Por = poorly differentiated adenocarcinoma. In some factors, data were not available for all cases.

Envision+ Dual Link reagent at room temperature for 30 min, and visualised using 3,3′-diaminobenzidine tetrahydrochloride as a chromogen. Finally, the sections were counterstained with haematoxylin. Sections were gently rinsed in phosphate-buffered saline between the incubation steps.

Evaluation of immunohistochemistry

All sections were evaluated by DY, HO, and TS without the knowledge of any clinical or pathological information, and cases for which consensus could not be reached were discussed to decide the evaluation. Based on the Herceptest™ (Dako) criteria, intensities of both EGFR and HER2 were defined as follows: 0, no membrane staining or membrane staining in ⩽10% cancer cells; 1+, faint and partial membrane staining in >10% cancer cells; 2+, moderate and complete membrane staining in >10% cancer cells; 3+, strong and complete membrane staining in >10% cancer cells. Intensities of VEGF were defined as follows: 0, no cytoplasmic staining or cytoplasmic staining in ⩽30% cancer cells; 1+, faint cytoplasmic staining, equivalent to the intensity of normal bile duct epithelium within the same section, in >30% cancer cells; 2+, moderate cytoplasmic staining in >30% cancer cells; 3+, strong cytoplasmic staining in >30% cancer cells. For cases showing mixed intensity, the predominant intensity was selected as the final IHC score. A final IHC score of 2+ or 3+ was defined as positive for expression of each protein.

Statistical analysis

Associations between results of IHC and clinicopathological factors were assessed by χ^2 test. Cumulative survival rates and survival curves were calculated by the Kaplan–Meier method, and log-rank test was performed for the comparison of survival curves. Cox's proportional hazard model was performed to estimate hazard ratio (HR) and 95% confidence interval (CI) of each outcome (death and recurrence). Multivariate analyses were performed using the factors identified to be risk factors for each outcome by univariate analyses, without UICC pT and UICC Stage, which are composed of other factors. All P-values reported are two-sided, and significance level was set at $P<0.05$. All statistical analyses were performed with the Statview 5.0 statistical software package (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Expression of EGFR, VEGF, and HER2 protein in cholangiocarcinoma

Representative cases of positive staining for each protein are shown in Figure 1 (A, EGFR; B, HER2; C, VEGF). Epidermal growth factor receptor, VEGF, and HER2 were expressed in 29 (27.4), 57 (53.8), and 1 (0.9%) of the 106 IHCCs, respectively, and in 25 (19.2), 77 (59.2), and 11 (8.5%) of the 130 EHCCs, respectively. Microscopically, EGFR was mostly overexpressed in the moderately and/or poorly differentiated component, which is characterised by infiltration (52 of 54 EGFR-positive cases, Figure 1D), whereas only two cases showed EGFR overexpression in the well-differentiated component. In contrast, HER2 was preferentially expressed in the well-differentiated component. In 6 of 12 HER2-positive cases, HER2 was expressed only in well-differentiated component (Figure 1E), and 5 progressive cases showed positive HER2 staining in both the well and moderately and/or poorly differentiated components and 1 case only in moderately differentiated component. There was no association between VEGF expression and histological features.

Associations between EGFR, VEGF, and HER2 expression and clinocopathological factors

Statistical analyses of HER2 were performed only in EHCC cases because of the small number of HER2-positive cases in IHCC. In IHCC, VEGF expression was significantly associated with intrahepatic metastasis ($P=0.0224$). There was no significant association between EGFR expression and any clinicopathological factors.

In EHCC, EGFR expression was significantly associated with macroscopic type (0% in the polypoid type, 24.0% in the non-polypoid type; $P=0.0120$), lymph node metastasis ($P=0.0006$), UICC Stage ($P=0.0424$), lymphatic vessels invasion ($P=0.0371$), and perineural invasion ($P=0.0459$). Human epidermal growth factor receptor 2 expression was significantly associated with

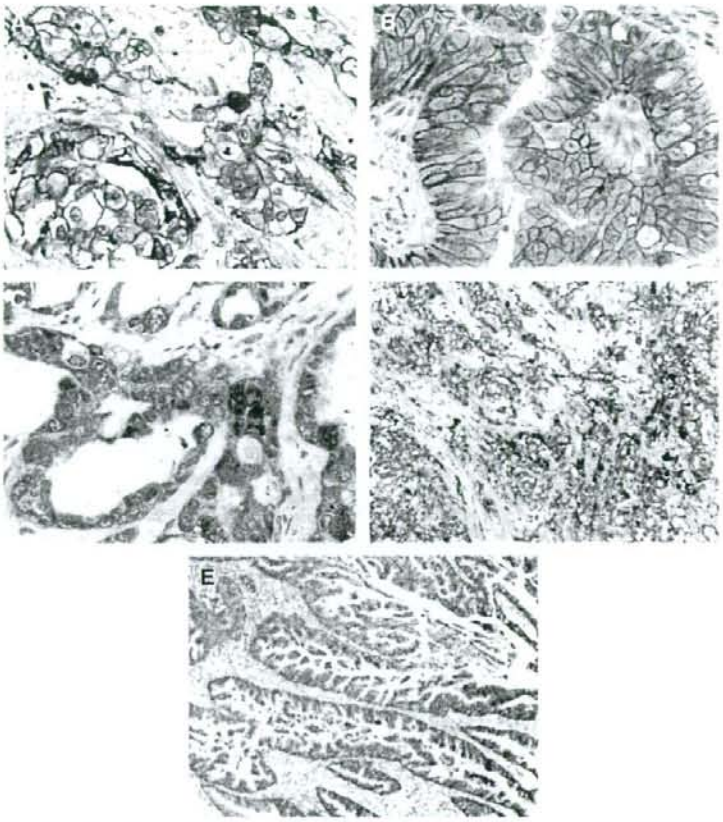

Figure 1 Representative immunohistochemical staining of (**A**) EGFR, (**B**) HER2, and (**C**) VEGF in cholangiocarcinoma (×400 magnification). (**D**) Epidermal growth factor receptor tends to be expressed in the poorly differentiated component (×100 magnification). (**E**) Human epidermal growth factor receptor 2 is preferentially expressed in more differentiated areas such as the glandular or papillary component (×100 magnification).

macroscopic type (23.8% in the polypoid type, 5.8% in the non-polypoid type; $P=0.0078$), histological classification (25% in papillary adenocarcinoma, 9.7% in well differentiated adenocarcinoma, 3.2% in moderately differentiated adenocarcinoma, 5.9% in poorly differentiated adenocarcinoma; $P=0.0237$), and invasion to other organs (3.9% in invasive cases, 15.1% in non-invasive cases; $P=0.0242$). VEGF expression was not significantly associated with any factors in EHCC.

Detailed results of associations between EGFR/VEGF/HER2 expression and clinicopathological factors are shown in Supplementary information 1 (IHCC) and Supplementary information 2 (EHCC).

Univariate and multivariate analyses regarding overall survival and tumour recurrence in cholangiocarcinoma

The number of dead and the median survival time were 70 cases and 724 days in IHCCs, and 76 cases and 1197 days in EHCCs, respectively. The number of recurrence and the median recurrence time were 64 cases and 522 days in IHCCs, and 78 cases and 960 days in EHCCs, respectively.

Overall 5-year cumulative survival for patients with IHCC and EHCC was 33.0 and 41.6%, respectively, and no significant difference was identified between the groups ($P=0.0599$). The survival curves stratified by EGFR expression status are shown as Figure 2. Five-year survival for patients with EGFR-positive and EGFR-negative tumours was 17.7 and 47.1% for IHCC, and 26.4 and 45.6% for EHCC, respectively. There was a significant difference between EGFR-positive and -negative cases for both IHCC ($P=0.0008$) and EHCC ($P=0.0204$).

The results of multivariate analyses following univariate analyses regarding overall survival and tumour recurrence are shown in Table 3 (IHCC) and Table 4 (EHCC).

In IHCC, 13 factors including EGFR expression were identified as significantly prognostic by univariate analysis. Multivariate analysis revealed that EGFR expression was an independent prognostic factor (HR, 2.67; 95% CI, 1.52–4.69; $P=0.0006$), along with mass-forming macroscopic group (HR, 2.96; 95% CI, 1.06–8.31; $P=0.0390$), intrahepatic metastasis (HR, 2.91; 95% CI, 1.60–5.29; $P=0.0005$), and lymph node metastasis (HR, 1.96; 95% CI, 1.04–3.69; $P=0.0375$). In EHCC, 14 factors including EGFR expression were identified as significantly prognostic by univariate analysis. Multivariate analysis revealed that lymph node metastasis (HR, 2.03; 95% CI, 1.16–3.55; $P=0.0133$) and a histological classification of moderately differentiated adenocarcinoma (HR for papillary adenocarcinoma, 4.23; 95% CI, 1.08–16.50; $P=0.0380$) and poorly differentiated adenocarcinoma (HR for papillary adenocarcinoma, 13.22; 95% CI, 3.10–56.45; $P=0.0005$) were significant prognostic factors.

Multivariate analysis following univariate analysis for risk factors of tumour recurrence revealed that EGFR expression in IHCC was a significant risk factor of tumour recurrence (HR, 1.89;

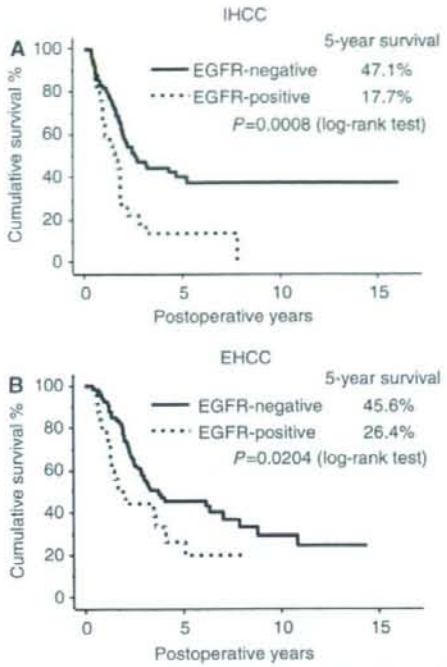

Figure 2 Survival curves stratified by EGFR expression in (**A**) IHCC and (**B**) EHCC (Kaplan–Meier method). The outcome of EGFR-positive cases was significantly worse than that of EGFR-negative cases in both IHCC ($P=0.0008$) and EHCC ($P=0.0204$) (by log-rank test).

95% CI, 1.05–3.39; $P=0.0335$), along with intrahepatic metastasis (HR, 2.36; 95% CI, 1.31–4.25; $P=0.0044$), lymph node metastasis (HR, 2.24; 95% CI, 1.19–4.22; $P=0.0126$), and venous invasion (HR, 6.74; 95% CI, 1.31–34.73; $P=0.0225$), whereas, in EHCC, lymph node metastasis (HR, 1.75; 95% CI, 1.03–2.98; $P=0.0394$) and dissected periductal structures margin (HR, 1.81; 95% CI, 1.03–3.16; $P=0.0383$) were independent risk factors of tumour recurrence, but EGFR expression was not associated with tumour recurrence even in univariate analysis.

Table 3 Multivariate analyses regarding overall survival and tumour recurrence in IHCC (Cox's proportional hazard model)

	Overall survival			Tumour recurrence		
	HR	95% CI	P-value	HR	95% CI	P-value
Macroscopic type						
Non-mass forming	1.00			1.00		
Mass forming	2.96	1.06–8.31	0.0390	3.06	1.00–9.40	0.0505
Invasion of portal vein						
Negative	1.00			1.00		
Positive	0.67	0.30–1.47	0.31	1.01	0.43–2.41	0.98
Invasion of hepatic vein						
Negative	1.00			1.00		
Positive	1.19	0.66–2.12	0.57	1.17	0.65–2.14	0.60
Intrahepatic metastasis						
Negative	1.00			1.00		
Positive	2.91	1.60–5.29	0.0005	2.36	1.31–4.25	0.0044
Lymph node metastasis						
Negative	1.00			1.00		
Positive	1.96	1.04–3.69	0.0375	2.24	1.19–4.22	0.0126
Histological classification						
Well differentiated	1.00			1.00		
Moderately differentiated	1.24	0.56–2.75	0.60	0.65	0.28–1.53	0.32
Poorly differentiated	2.09	0.58–7.49	0.26	1.35	0.32–5.72	0.69
Lymphatic vessel invasion						
Negative	1.00			1.00		
Positive	3.31	0.80–13.65	0.0982	1.37	0.41–4.56	0.61
Venous invasion						
Negative	1.00			1.00		
Positive	4.07	0.97–17.09	0.0551	6.74	1.31–34.73	0.0225
Perineural invasion						
Negative	1.00			—		
Positive	0.60	0.26–1.36	0.22	—	—	—
Bile duct margin						
Negative	1.00			—		
Positive	1.84	0.91–3.73	0.0923	—	—	—
EGFR expression						
Negative	1.00			1.00		
Positive	2.67	1.52–4.69	0.0006	1.89	1.05–3.39	0.0335

Abbreviations: CI = confidence interval; HR = hazard ratio.

DISCUSSION

This study, analysing EGFR/VEGF/HER2 expression in the largest cohort of cholangiocarcinoma reported so far, showed for the first time that EGFR expression in IHCC is significantly associated with poor prognosis. In addition, our study confirmed previously reported prognostic factors in cholangiocarcinoma, such as macroscopic type, intrahepatic metastasis, lymph node metastasis, and histological classification (Yamamoto *et al*, 1998; Ohtsuka *et al*, 2002; Morimoto *et al*, 2003; DeOliveira *et al*, 2007). Expression of EGFR or HER2 is known to be a prognostic factor in some cancers (Gusterson *et al*, 1992; Nicholson *et al*, 2001), but no previous study has clarified the influence of these molecules on prognosis in cholangiocarcinoma (Ito *et al*, 2001; Altimari *et al*, 2003; Nakazawa *et al*, 2005), probably because cholangiocarcinoma is a relatively rare cancer and collection of a large cohort is difficult. Indeed, most previous studies were performed on the basis of only 50 cases at most. Although it is unclear why EGFR expression in IHCC is an independent prognostic factor, it may be associated with frequent relapse of cancer because EGFR expression is also a risk factor for tumour recurrence.

In contrast to IHCC, EGFR expression was not an independent prognostic factor in EHCC, but was associated with clinical features that may represent tumour progression and invasion, such as lymph node metastasis and apparent stromal invasion in EHCC. Because cancer tissue tends to be heterogeneous, histological diagnosis is generally decided on the basis of the degree of differentiation that predominates. In order to elucidate the biological significance of each protein, we microscopically examined positive cases in detail and compared their expression with histological components, and found that EGFR tended to be expressed in the poorly differentiated component, which is characterised by infiltration in both IHCC and EHCC. Similar results have been reported in bladder cancer (Neal *et al*, 1985), oesophageal adenocarcinoma (Wilkinson *et al*, 2004), and IHCC (Ito *et al*, 2001), although the studies were based on small cohorts. These findings indicate that EGFR expression may be a relatively late event in the development of cholangiocarcinoma and

Table 4 Multivariate analyses regarding overall survival and tumour recurrence in EHCC (Cox's proportional hazard model)

	Overall survival			Tumour recurrence		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Tumour size						
⩽3.0 cm	1.00			—		
>3.0 cm	1.29	0.71–2.35	0.41	—	—	—
Macroscopic type						
Polypoid	1.00			—		
Non-polypoid	0.44	0.16–1.26	0.13	—	—	—
Depth of tumour invasion						
Within FM	1.00			1.00		
Beyond FM	1.26	0.19–8.60	0.81	1.16	0.24–5.57	0.85
Invasion of portal vein						
Negative	1.00			1.00		
Positive	1.48	0.81–2.69	0.20	1.59	0.92–2.75	0.94
Lymph node metastasis						
Negative	1.00			1.00		
Positive	2.03	1.16–3.55	0.0133	1.75	1.03–2.98	0.0394
Histological classification						
Papillary	1.00			1.00		
Well differentiated	3.40	0.85–13.66	0.0849	0.91	0.33–2.51	0.85
Moderately differentiated	4.23	1.08–16.50	0.0380	1.19	0.47–3.02	0.72
Poorly differentiated	13.22	3.10–56.45	0.0005	2.80	0.99–7.87	0.0516
Lymphatic vessel invasion						
Negative	1.00			1.00		
Positive	1.78	0.29–11.10	0.54	2.36	0.45–12.37	0.31
Venous invasion						
Negative	1.00			1.00		
Positive	3.93	0.81–19.12	0.0898,	1.89	0.52–6.92	0.34
Perineural invasion						
Negative	1.00			1.00		
Positive	1.94	0.58–6.53	0.29	0.98	0.38–2.51	0.97
Dissected periductal structures margin						
Negative	1.00			1.00		
Positive	1.20	0.67–2.17	0.54	1.81	1.03–3.16	0.0383
Invasion to other organ						
Negative	1.00			1.00		
Positive	1.02	0.53–1.94	0.96	0.94	0.53–1.69	0.84
EGFR expression						
Negative	1.00			—		
Positive	1.04	0.55–1.96	0.90	—	—	—

HR = hazard ratio; CI = confidence interval; FM = fibromuscular layer.

associated with invasion and progression. Because it has been previously reported that poor differentiation is associated with unfavourable outcome in other cancers (Sohn *et al*, 2000; Hassan *et al*, 2005), the association between EGFR expression and poor differentiation may also be a reason that EGFR expression is a prognostic factor.

Though the prognostic factors were different between IHCC and EHCC, it may be due to the difference of anatomical character, which extrahepatic bile duct is near from other organs and is not surrounded by liver parenchyma in contrast to intrahepatic bile duct. The intrahepatic epithelium is distinct from the extrahepatic epithelium in terms of development and differentiation (Shiojiri, 1997), and the risk factors, pathogenesis, and clinical features of IHCC and EHCC are different (Strom *et al*, 1985; Nakeeb *et al*, 1996; Shaib *et al*, 2007). Although no previous studies have elucidated EGFR function in normal bile duct epithelium, EGFR overexpression might play distinct roles in IHCC and EHCC.

Vascular endothelial growth factor expression was detected frequently, being evident in about 60% of our study cases, which is consistent with previous studies (31.4–75.6%) (Hida *et al*, 1999; Tang *et al*, 2006). Our study revealed that VEGF expression was significantly associated with intrahepatic metastasis in IHCC. Vascular endothelial growth factor is a key molecule in angiogenic pathway. Angiogenesis is an essential component in the process of metastasis, and this has been partly confirmed by studies showing that microvessel density (MVD) is associated with metastasis and a poorer outcome in a range of cancers (Weidner *et al*, 1991; Zetter, 1998). It has also been reported that high MVD is an independent prognostic factor in node-negative IHCC (Shirabe *et al*, 2004) and is associated with VEGF expression in IHCC (Tang *et al*, 2006), although no study has clarified the involvement of angiogenesis in the process of metastasis in cholangiocarcinoma. Our result suggests that VEGF plays an important role in the process of cholangiocarcinoma metastasis by promoting angiogenesis.

Human epidermal growth factor receptor 2 was expressed in only 11 of 130 EHCC cases (8.5%) and in one of 106 IHCC cases (0.9%). The proportion of HER2-positive cases reported previously has varied from 4.2 to 81.8% (Ito *et al*, 2001; Aishima *et al*, 2002; Ukita *et al*, 2002; Altimari *et al*, 2003; Nakazawa *et al*, 2005), and the discrepancy may be due to differences in staining procedure or tumour location. In contrast to EGFR expression, HER2 expression was associated with more favourable clinical features, such as a polypoid macroscopic type and absence of other organ involvement. The proportion of HER2-positive cases in papillary adenocarcinoma was higher than in other histological types, consistent with some previous reports claiming that HER2 expression in cholangiocarcinoma is associated with an early disease stage (Endo *et al*, 2002; Nakazawa *et al*, 2005). Microscopically, HER2 is preferentially expressed in well differentiated component, and it is also expressed in dedifferentiated components (moderately and/or poorly differentiated components) in progressive cases. This indicates that HER2 overexpression is maintained from an early stage of carcinogenesis in cases that are HER2-positive.

Recently, the efficacy of molecular targeting therapy for various molecules including EGFR/VEGF/HER2 has been proved clinically in a wide range of cancers. Epidermal growth factor receptor inhibitor has been reported to be effective in a cholangiocarcinoma cell line (Yoon *et al*, 2004), and a phase II study of erlotinib, an EGFR inhibitor, in patients with advanced biliary cancer has been reported. In this study, the progression-free rate at 6 months as a primary end point was 17% (7/42) despite the fact that disease condition was severe, and the disease control rate was 50% (20/42) (Philip *et al*, 2006). This study suggested the clinical applicability of the EGFR inhibitor to cholangiocarcinoma. Several clinical trials demonstrating the efficacy of VEGF inhibition for other cancers have been reported (Hurwitz *et al*, 2004; Sandler *et al*, 2006), and VEGF upregulation in tumour cells is considered to be a mechanism of resistance to EGFR inhibitors (Viloria Petit *et al*, 2001). Therefore, dual inhibition of both EGFR and VEGF may exert a synergistic effect.

In summary, we have shown that EGFR and VEGF expression is relatively common in cholangiocarcinoma. Moreover, in IHCC, EGFR expression is an independent prognostic factor and VEGF expression is associated with intrahepatic metastasis. In EHCC, EGFR expression is associated with clinical factors involved in tumour progression and invasion. Our results suggest the validity and significance of molecular targeting agents for EGFR and/or VEGF pathway, and that further preclinical and clinical studies are warranted for improving the clinical outcome of cholangiocarcinoma.

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Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc)

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