Evaluation of Selected Phage Clones for Binding to Human Cancer Cells

The cells were grown to confluency in 6-well plates, acclimated to 4°C for 30 min and washed briefly in PBS containing 1% BSA two times. Then, each selected phage clone diluted in 1 ml of DMEM containing 1% BSA at a concentration of 5×10^7–10^9 pfu was added to each well at 4°C. After 1 h of incubation with gentle agitation, medium containing unbound phages was discarded, and the cells were washed four times in PBS containing 1% BSA. Then, the phage binding to cells was evaluated as described in 'Selection of Cell-Targeting Peptides'. In addition, the ability of selected phage clones to bind to other human cancer cell lines (Hep3B, HepG2, AZ521, DLD-1 and Saos2) was determined in 6-well plates as described above.

Immunofluorescence Analysis of Binding of Selected Phage Clones to Huh-7 Cells in vitro

Huh-7 cells were grown to confluence on an 8-well chamber slide in DMEM containing 10% FBS. The cells were washed with PBS containing 1% BSA, and incubated for 1 h at 4°C in DMEM (containing 1% BSA) containing 2×10^{11} pfu of either a selected phage clone or a control phage. A phage clone displaying no oligopeptide insert (insertless) was used as a negative control. The medium was discarded and the cells were washed four times with PBS containing 1% BSA, fixed with methanol/acetone (–20°C), and blocked with 10% goat serum for 20 min at room temperature. An anti-M13 monoclonal antibody diluted 1:600 in 2% goat serum was added and incubated for 1 h at room temperature. The cells were washed in PBS three times, and then incubated with FITC-conjugated goat anti-mouse immunoglobulin for 1 h at room temperature. The cells were washed in PBS three times, 4′-6-diamino-2-phenylindole was used for nuclear counterstain, and visualized using an Olympus BX60 fluorescence microscope (Olympus, Tokyo, Japan).

The motif Thr-Thr-Pro-Arg-Asp-Ala-Thr (TTPRDAY) was selected as the most promising consensus sequence binding to HCC and studied in more detail.

Competitive Inhibition of the Synthesized Peptide on Phage Accumulation in vitro

To confirm the capacity of the synthesized peptide (TTPRDAY) to bind to HCC, its inhibitory effects on phage accumulation were examined. Huh-7 cells were pre-incubated with the TTPRDAY peptide (synthesized by Sigma Genosys Japan, Ishikari, Japan) or control peptide at 1 or 10 μM for 30 min at 4°C, and then 5×10^8 pfu of the selected phage diluted in 1 ml DMEM containing 1% BSA were added. The phages were allowed to bind to the cells for 1 h at 4°C with gentle agitation. Medium containing unbound phages was discarded, and the cells were then washed four times for 5 min each time in PBS containing 1% BSA, before the cell-associated phages were recovered by lysing the cells in 1 ml/well of 30 mM Tris-HCl (pH 8.0) containing 10 mM EDTA on ice for 1 h. The number of phages recovered was determined by titering multiple dilutions of the eluted phages as described above. The same experiment was repeated using insertless phages and TTPRDAY peptide.

Effect of the TTPRDAY Peptide on Cell Viability

Huh-7 cells were plated in 96-well plates at 5×10^3 cells/well and incubated at 37°C in DMEM containing 10% FCS in either the presence or absence of the TTPRDAY peptide at 0.1, 1 or 10 μM. After 24, 48, 72 and 96 h, the viability of the Huh-7 cells was assessed using the MTS assay, as described previously [9]. Media were replaced with 120 μl of FCS-free DMEM containing 20 μl of CellTiter®96 AQueous One solution reagent (Promega, Madison, Wisc., USA), and the culture plates were incubated at 37°C for 2 h. Next, 100 μl of the medium were transferred to a new 96-well plate and the quantity of the formazan product present was determined by measuring the absorbance at 490 nm using a microplate autoreader (Molecular Devices, Sunnyvale, Calif., USA).

Mutagenicity Study

The Ames test [11, 12] was carried out using histidine-deficient (*his*$^-$) *Salmonella typhimurium* tester strains, TA100 and TA98. The tester strains 100 are responsive to base-pair substitutions, whereas the 98 detect deletions or additions of base pairs (frameshifts). Strains TA100 and TA98 were cultured at 37°C in the presence of different concentrations (0.1, 1, 10, 100 and 1,000 μM) of the selected peptide and revertant *his*$^+$ colonies were counted after a 48-hour incubation period. The presence of revertant colonies after plating on histidine-poor growth media indicates the presence of a mutagen. Each experimental condition was run with duplicate samples. Additionally, the Ames test was performed in the absence or presence of a S9 fraction (mix of metabolizing enzymes from rat liver) to allow not only detection of a direct mutagenic effect, but also of an indirect mutagenic effect brought about by possible metabolites of the applied compound.

Binding of the Selected Phage Clone to Surgically Resected HCC Specimens

Samples of tumor and non-cancerous liver tissue were obtained at the time of hepatectomy from patients with operable HCC. Samples were weighed and homogenized using a motor-driven Teflon-glass homogenizer. The homogenized samples of both the tumor and non-cancerous liver tissue were each divided into two portions, one being used to assess the binding activity of the selected phage clone and the other to assess that of the control phage. Either the selected or the control phage was added to the homogenates of tumor and non-cancerous liver tissue at 5×10^{10} pfu per 100 mg tissue, and allowed to bind to the samples for 20 min at 37°C with agitation (150 rpm). The samples were washed with PBS twice, and then 2 ml of 0.2 M glycine-HCl were added as a general buffer for nonspecific disruption of binding interactions for 3 min, and fluids were neutralized with 300 μl of 1 M Tris-HCl. After washing with PBS, 5 ml of PBS containing 0.5% Tween 20 were added to the cells. Recovery was quantified by titering multiple dilutions of the homogenates, as described above. The experiment was performed on 6 patients with HCC, and in each case, the results were presented as a ratio relative to the recovery of the insertless phage from the non-cancerous liver tissue. In addition, the binding activity of the selected phage clone on surgically resected specimens was evaluated by immunofluorescence analysis. Sample from the tumor tissue was mounted in OCT compound and frozen, and sectioned at 6 μm in a cryostat at –20°C. After washing with PBS twice, the sectioned sample was incubated with 5×10^{10} pfu of the selected phage clone or control phage (insertless) for 30 min. The sample was then washed four times in PBS. This was followed by addition of 100 μl 0.2 M glycine-HCl (pH 2.2) as a general buffer for nonspecific disruption of binding interac-

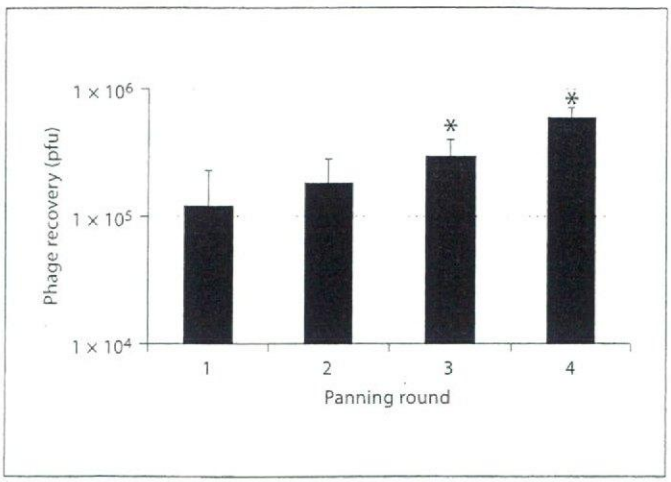

Fig. 1. Phage recoveries from Huh-7 cells in each round of biopanning. Four consecutive rounds of biopanning were performed on the human HCC cell line Huh-7. The phage recovery from each round increased with the number of biopanning passages, being approximately 6-fold higher in the final round than in the first. * $p < 0.05$ compared to the recovery in round 1.

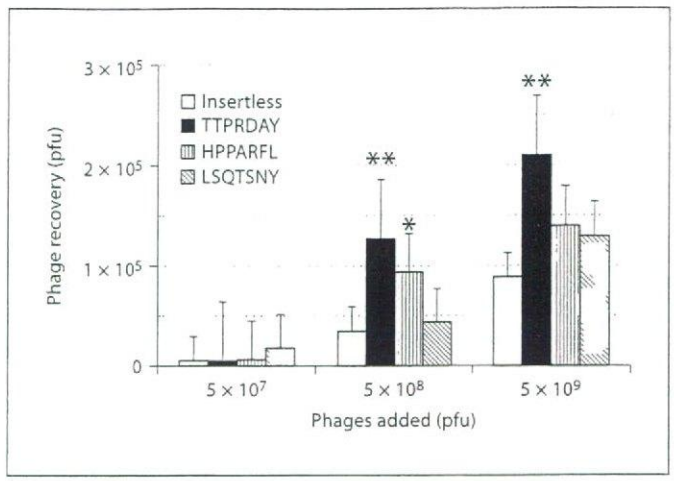

Fig. 2. Assessment of binding activities of selected phage clones expressing TTPRDAY, HPPARFL and LSQTSNY on Huh-7 cells in vitro. Huh-7 cells were cultured in 6-well plates and incubated with phage clones expressing TTPRDAY, HPPARFL or LSQTSNY or control phage (insertless) at a concentration of 5×10^7–10^9 pfu. Cells were then scraped off and phage recoveries were titered by plaque infection assay. The phage clone expressing TTPRDAY showed the highest binding activity at plural concentrations of added phage. ** $p < 0.01$ and * $p < 0.05$ compared to the recovery of insertless phage.

tions for 5 min, and fluids were neutralized with 15 μl 1 M Tris-HCl (pH 9.1). Then, the sample was washed with PBS and immunostained as described in 'Immunofluorescence Analysis of Binding of Selected Phage Clones to Huh-7 Cells in vitro'.

Statistics

Results are presented as the mean and standard deviation of data from three independent experiments, with significance of differences evaluated using Student's t test. In the experiment, to examine the binding of phages to surgically resected human tissue specimens, the results are presented as the mean and standard deviation of the data from six patients.

Approval from the Shinshu University Ethics Committee was obtained before the study, and written informed consent was obtained from all patients for the use of their tissue specimens.

Results

Isolation of the Specific Peptide Binding to Huh-7 Cells

Four consecutive rounds of in vitro biopanning were performed on human HCC. The phage recovery from each round increased with the number of biopanning passages, indicating selection of phage binding to HCC cells (fig. 1).

After each round of biopanning, individual phage plaques were picked up. Their DNA was isolated and sequenced, and the corresponding amino acid sequences of the inserts were deduced. After the first and second rounds of biopanning, the HCC-derived sequences displayed no distinguishable homology (data not shown). However, the HCC-derived sequences from the third and fourth rounds displayed some consensus motifs, and these were selected as candidate peptides that could bind to HCC. After the third and fourth rounds of biopanning, 24 phage plaques were picked up from each replicate, and their DNA was sequenced. We then compared the relative frequencies of every tripeptide motif in each replicate. The motif frequencies were calculated as the prevalence of each motif-containing peptide divided by the total number of isolated peptides. The Thr-Thr-Pro-Arg-Asp-Ala-Tyr (TTPRDAY) motif was the most frequently encountered (5.6%), followed by His-Pro-Pro-Ala-Arg-Phe-Leu (HPPARFL; 4.2%) and Leu-Ser-Gln-Thr-Ser-Asn-Thr (LSQTSNY; 2.8%).

To determine which motif was the best binding peptide, the binding activities of selected phage clones expressing the candidate oligopeptides were assessed in vitro as described above. The phage clone expressing the TTPRDAY motif showed the highest binding activity at plural concentrations of added phage clones (fig. 2), and therefore this motif was selected as the most promising for binding to HCC.

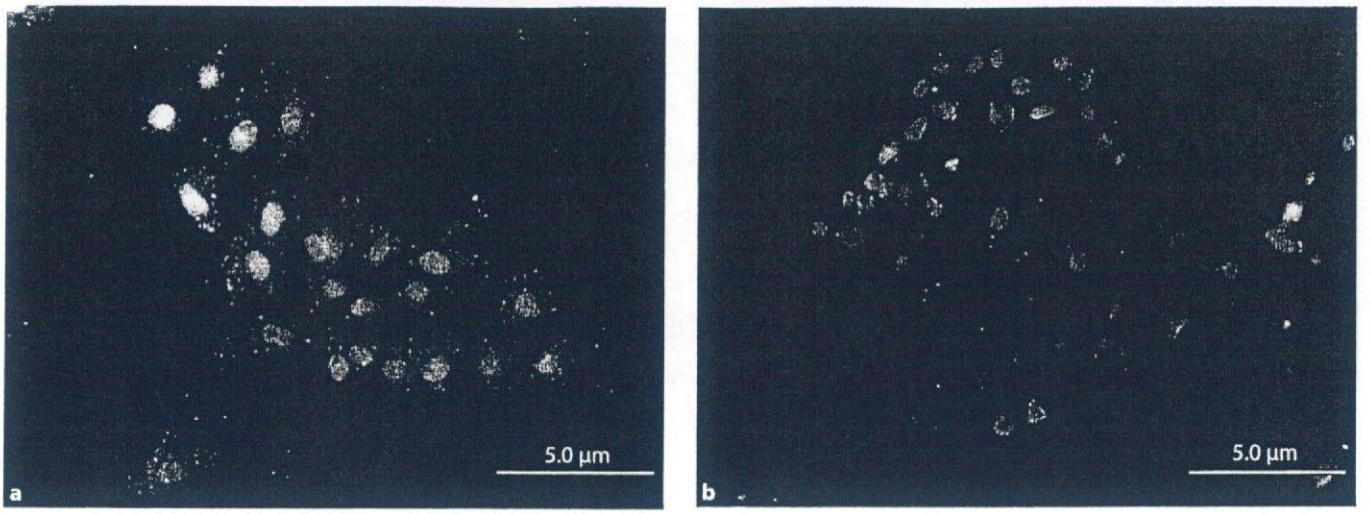

Fig. 3. Immunolocalization of phages on Huh-7 cells. Cells were incubated with phage clones expressing TTPRDAY (**a**) or with control phage (insertless, **b**). Phage-incubated cells were fixed and permeabilized. Phages were visualized by fluorescence using mouse anti-M13 phage antibody followed by an FITC-conjugated goat anti-mouse antibody. 4′-6-Diamino-2-phenylindole was used for nuclear counterstain. ×400.

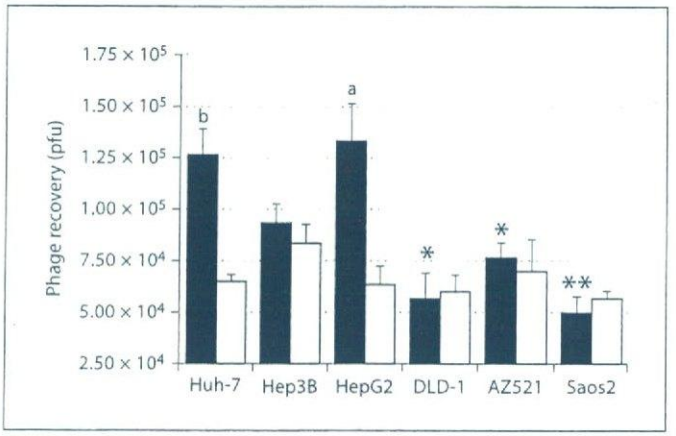

Fig. 4. Evaluation of binding of phage clones expressing TTPRDAY to cultures of Hep3B, HepG2, DLD-1, AZ521 and Saos2 cells in comparison with Huh-7 cells. Each cell line was cultured in 6-well plates, and incubated with phage clones expressing TTPRDAY or control phage (insertless). Cells were scraped off, and phage recoveries from each cell line were titered by plaque infection assay. Black bar = Recovery of TTPRDAY phage; white bar = recovery of insertless phage; ** $p < 0.01$ and * $p < 0.05$ compared to the recovery of TTPRDAY phage from Huh-7 cells; [b] $p < 0.01$ and [a] $p < 0.05$ compared to the recovery of insertless phage from the same cell line.

Heptapeptides containing the consensus motif were analyzed using BLAST (National Center for Biotechnology Information) to search for similarity to known human peptides. Interestingly, TTPRDAY showed 6/7 homology with colonic and hepatic tumor-overexpressed gene protein (ch-TOGp, TTPRDxY).

Immunofluorescence Analysis of Phage Clone Binding to Huh-7 Cells

The ability of the phage clone expressing TTPRDAY to bind to Huh-7 cells was assessed using immunofluorescence (fig. 3). The TTPRDAY phage showed apparently stronger binding activity to Huh-7 than the control phage.

Binding of TTPRDAY-Conjugated Phage Clone to Human Cancer Cell Lines

The binding of the phage clone expressing the TTPRDAY motif to confluent cultures of Hep3B, HepG2, DLD-1, AZ521 and Saos2 cells was evaluated in comparison with its binding to Huh-7 cells (fig. 4). The recoveries of the TTPRDAY phage from HepG2 and Hep3B cells were similar to that from Huh-7. The recoveries of the phage from non-HCC cell lines (DLD-1, AZ521 and Saos2) were significantly lower than that from Huh-7. In addition, the recovery of TTPRDAY phage from Huh-7 and HepG2 was significantly higher than that of the insertless phage

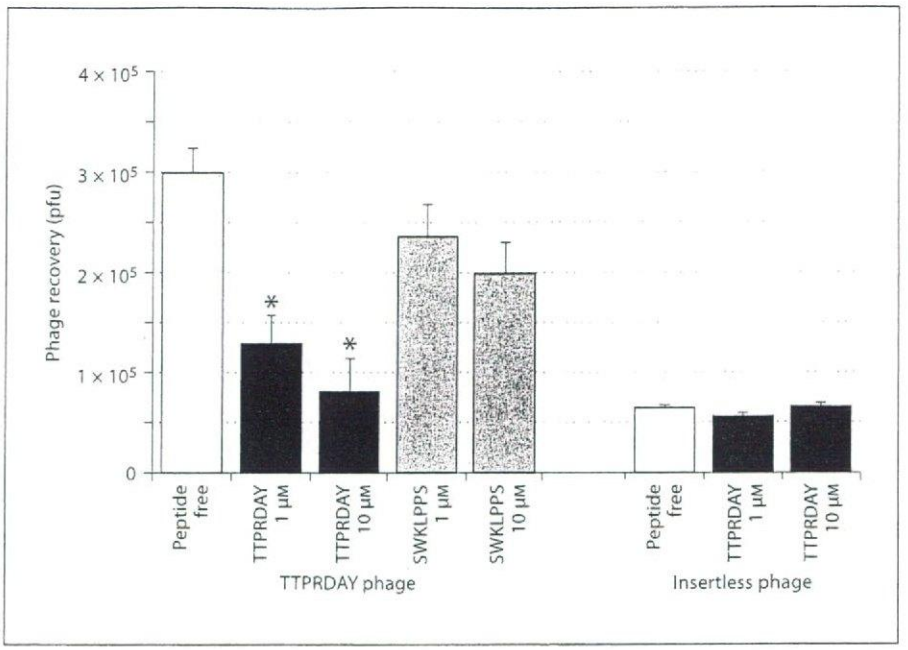

Fig. 5. Competitive inhibition of the synthesized peptides on phage accumulation. Huh-7 cells were cultured on a 6-well plate and pre-incubated with the synthesized TTPRDAY peptide or control peptide (SWKLPPS) at 1 and 10 μM before incubation with phage expressing TTPRDAY. Cells were scraped off and phage recoveries were titered. The same experiment was repeated using insertless phage and TTPRDAY peptide. * $p < 0.01$ compared to the recovery of peptide-free condition.

from the same cell lines. On the other hand, there was no significant difference between the recoveries of TTPRDAY phage and the insertless phage from the other cell lines.

Competitive Inhibition of the Synthesized Peptides on Phage Accumulation in vitro

To confirm the capacity of the synthesized peptides to bind to HCC, cells were pre-incubated with 1 or 10 μM TTPRDAY peptide or control peptide before addition of 5×10^8 pfu of the selected phage. The inhibitory effects of the synthesized peptides on phage accumulation were examined by titering the phages bound to cancer cells. It was found that pre-incubation of cells with the TTPRDAY peptide caused 73% inhibition of the binding activity of the TTPRDAY phage to HCC (fig. 5), while this inhibitory effect of TTPRDAY peptide was not observed when the experiment was performed with insertless phage.

Effect of the Selected Peptide on Cell Viability and Mutagenicity

The possibility that the TTPRDAY peptide might influence cancer cell growth (promotion or inhibition) was evaluated using the MTS assay. The presence of the TTPRDAY peptide had no discernible effect on cell growth (fig. 6).

In addition, the mutagenic potential of TTPRDAY peptide was evaluated using the Ames test. Figure 7 shows

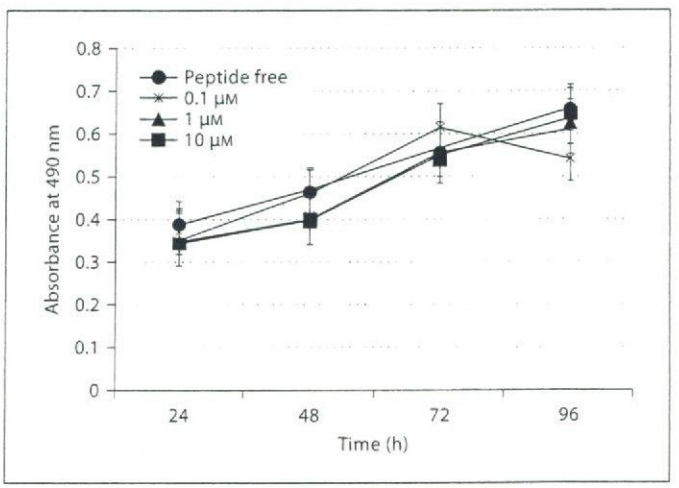

Fig. 6. Effect of the TTPRDAY peptide on cell viability. Huh-7 cells were plated at a density of 5×10^3 per well in a 96-well plate and incubated in DMEM containing 10% FCS, in either the presence or absence of the TTPRDAY peptide at 0.1, 1 or 10 μM. After 24, 48, 72 and 96 h, the viability of the Huh-7 cells was assessed using the MTS assay. Presence or absence of the TTPRDAY peptide made no significant difference to cell growth.

the number of revertant colonies obtained with different concentrations of TTPRDAY peptide: positive control: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide [S9mix(–)], and 2-aminoanthracene [S9mix(+)]. The incubation of

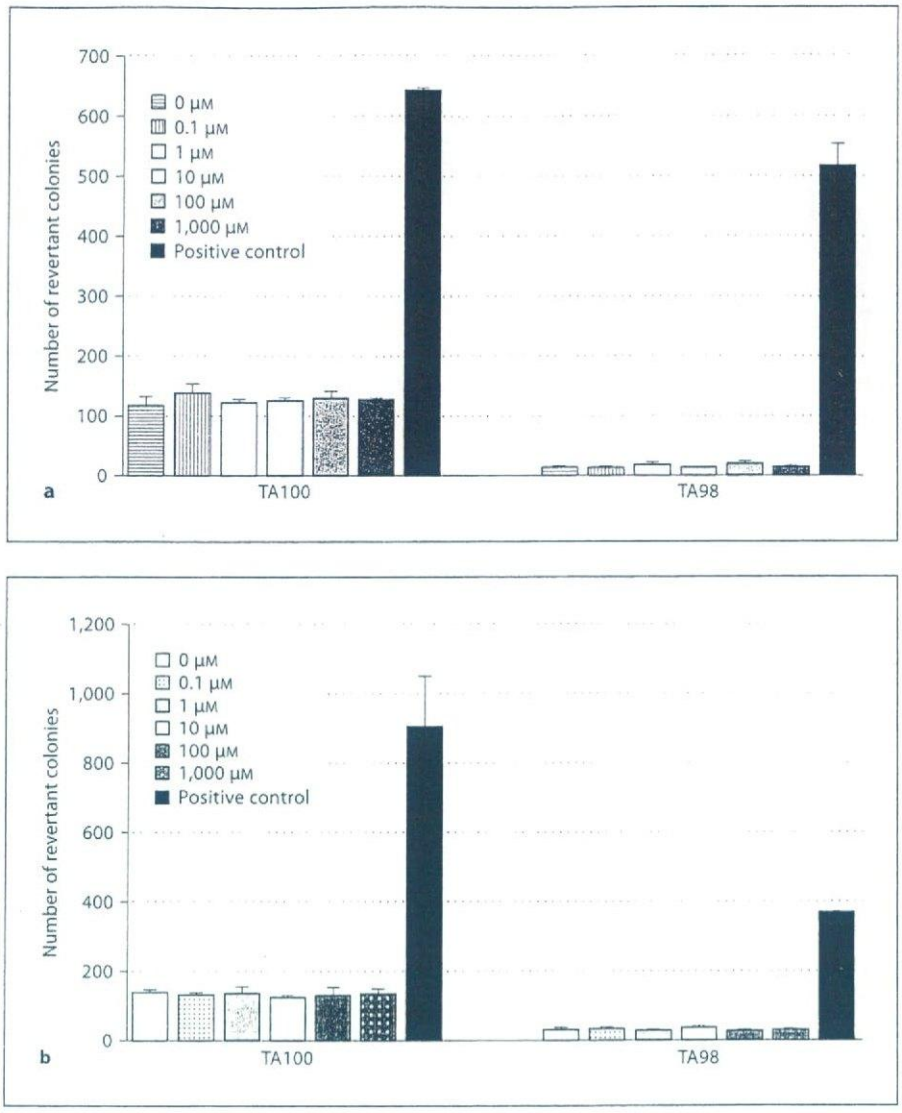

Fig. 7. Evaluation of mutagenicity of TTPRDAY peptide. The Ames test was performed using *S. typhimurium* tester strains, TA100 and TA98, to evaluate the mutagenic potential of TTPRDAY peptide. TA100 and TA98 were cultured in the presence of different concentrations of TTPRDAY peptide (0.1, 1, 10, 100 and 1,000 μM) in the absence (**a**) or presence (**b**) of a S9 fraction isolated from rat hepatocytes. After a 48-hour incubation period, revertant *his*$^+$ colonies were counted. Any concentrations of TTPRDAY peptide cause no increase in the number of revertant colonies.

TTPRDAY peptide with liver enzymes allowed evaluation of the pro-mutagenic potential of TTPRDAY peptide. As seen in figure 7, TTPRDAY peptide did not cause any increase in the number of revertant colonies in the absence (fig. 7a) or presence (fig. 7b) of S9 fraction compared to the control group. The result of this study suggests that TTPRDAY peptide has no mutagenic or pro-mutagenic potential.

Evaluation of Binding of the Selected Phage Clone to Surgically Resected Human Tissue Specimens

Samples of tumor and non-cancerous liver tissue were collected at the time of hepatectomy from 6 patients with operable HCC. The median age of the patients was 70 years (range 59–73 years), and all 6 patients were male. The histology of the tumor was well-differentiated HCC in 4 patients and moderately differentiated HCC in 2. Five patients had liver cirrhosis or chronic hepatitis (hepatitis B virus-related hepatitis in 1; hepatitis C virus-related cirrhosis in 1; hepatitis virus-unrelated cirrhosis or hepatitis in 3, including 1 case of alcoholic hepatitis). TTPRDAY phage showed significantly higher binding to tumor tissue than the control phage (fig. 8a) and also significantly higher accumulation in tumor tissue than in the corresponding non-cancerous liver tissue (fig. 8b). Immunofluorescence analysis was used to characterize the distribution of phage clones expressing the TTPRDAY peptide in the tumor from a patient who had under-

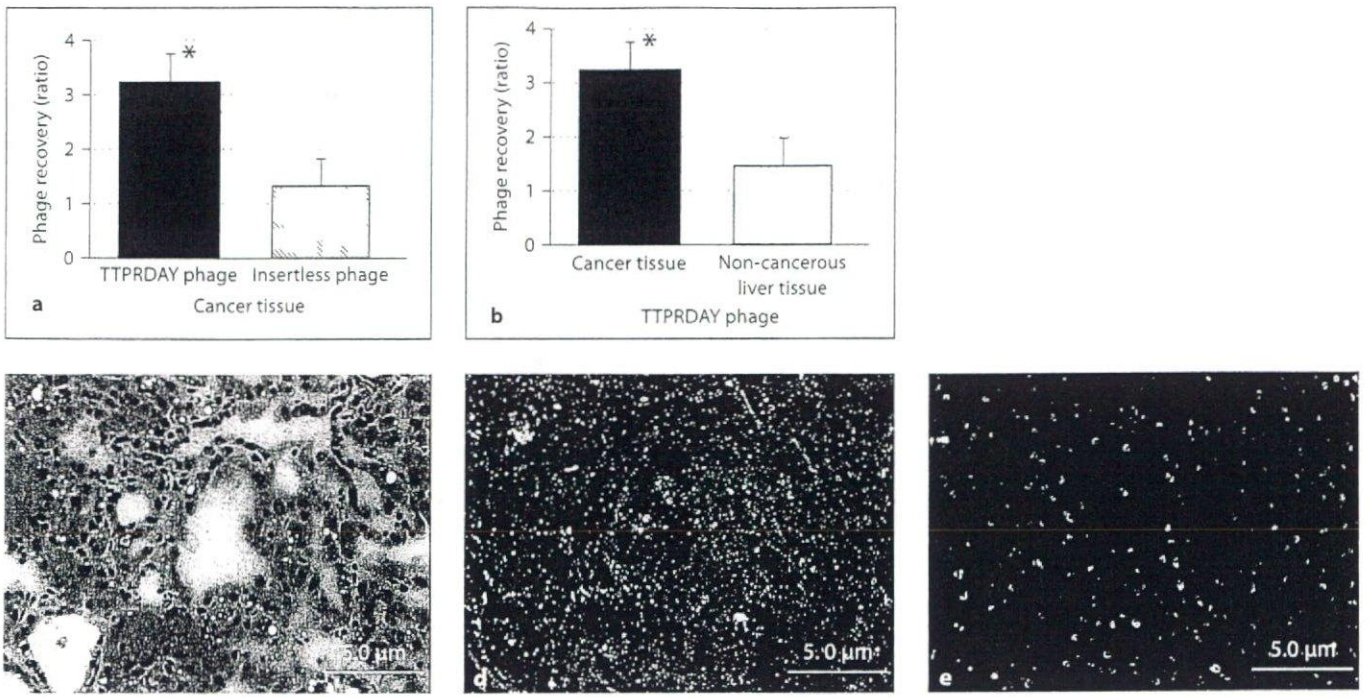

Fig. 8. Evaluation of TTPRDAY phage binding to surgically resected HCC tissue. Samples of cancer and non-cancerous liver tissue were obtained at the time of hepatectomy from 6 patients with HCC. The samples were homogenized and incubated with TTPRDAY phage or control phage (insertless) for 20 min. Phage binding activities were evaluated by plaque infection assay. Recovery of each phage was expressed as a ratio relative to that of the control phage from background liver tissues. In addition, binding of TTPRDAY phage to surgically resected HCC tissue was assessed by immunofluorescence analysis. Sample of cancer tissue was incubated with TTPRDAY phage or insertless phage for 30 min. Phages were visualized by fluorescence using mouse anti-M13 phage antibody followed by an FITC-conjugated goat anti-mouse antibody. 4′-6-Diamino-2-phenylindole was used for nuclear counterstain. **a** Comparison of binding to cancer tissue between TTPRDAY phage and insertless phage. **b** Comparison of TTPRDAY phage binding between cancer tissue and non-cancerous liver tissue. * $p < 0.05$ compared to the recovery of insertless phage or non-cancerous liver tissue. **c** HE staining of surgically resected HCC. **d** Immunostaining for TTPRDAY phage. **e** Immunostaining for insertless phage HCC. ×400.

gone hepatectomy for HCC. The results revealed that TTPRDAY phage particles bound to HCC cells, but the insertless phage showed only low signals (fig. 8d, e).

Discussion

In the present study, we used a phage display library to identify peptide sequences capable of binding to HCC cells. After four rounds of selection, the consensus sequence TTPRDAY was identified and examined in more detail. TTPRDAY showed apparent binding to receptors that are most commonly and specifically upregulated in HCC, since TTPRDAY bound to three different types of HCC cell lines, whereas it showed lower binding to non-HCC cells (fig. 4). In addition, TTPRDAY bound to HCC derived from actual specimens resected from human patients, whereas it showed lower binding to non-cancerous liver tissue from the same patient (fig. 8).

A search for similarity to known human peptides revealed that TTPRDAY has 6/7 homology (TTPRDxY) with colonic and hepatic tumor-overexpressed gene protein (ch-TOGp), which has been reported by Charrasse et al. [13] to be a new human cDNA overexpressed in human HCCs and colonic adenocarcinomas. Strong expression of the ch-TOG gene was also found in the colonic Caco-2 cell line at the proliferative stage, whereas the transcript became undetectable when the cells were in a quiescent enterocyte-like differentiated state [13]. ch-TOGp is reported to be structurally homologous to a high-molecular-weight microtubule-associated protein in *Xenopus* oocytes (XMAP215) [14], but in humans the precise function of ch-TOGp is still unknown [15]. In the present study, TTPRDAY was shown to bind efficiently

to HCC cells but less so to colon cancer cells (fig. 4), despite the fact that ch-TOGp is reportedly related to both HCC and colon cancer. The significance of the homology between TTPRDAY and ch-TOGp is currently unclear, and warrants further investigation.

For the treatment of HCC, tumor-specific targeting is necessary, since HCC has a tendency to develop intrahepatic metastases and multicentric occurrence. Furthermore, an important requirement is that drugs directed against HCC should have no effect on the non-cancerous liver parenchyma, because HCC patients usually have deteriorated liver function. For this reason, research aimed at finding gene therapy vectors that could be targeted to HCC cells using specific monoclonal antibodies is being actively conducted [16]. Because monoclonal antibodies such as AF-20 [17] and Hep27 [18] bind to the antigen uniformly expressed in HCC-derived cell lines and human tumors, including those with distant metastasis, it is likely that they would have high specificity and efficiency as HCC-targeting gene transfer vectors. In addition, single-chain antibody fragments may have potential application for tumor targeting [19]. In fact, Bing et al. [19] have reported HCC-specific single-chain antibody fragments. However, these are relatively large proteins and have some pharmacological limitations, notably a short plasma half-life, unwanted interactions with serum components and a high cost of manufacture. In contrast, TTPRDAY is a simple peptide with excellent stability and a low manufacturing cost. Furthermore, although it consists of only seven amino-acid residues, TTPRDAY is expected to work sufficiently well as a targeting ligand, since peptides containing three amino-acid residues, such as RGD, have been reported to provide the minimal framework for structural formation and protein-protein interactions [20]. In fact, the competitive inhibition of TTPRDAY-presenting phage binding to HCC by the synthesized TTPRDAY peptide implies that the peptide itself has HCC-binding activity.

This study confirmed the ability of TTPRDAY to bind to HCC using surgical specimens derived from patients with operable HCC. Such confirmation is important, because sometimes a peptide that works well in vitro does not work in actual patients. TTPRDAY phage bound to non-cancerous liver tissue approximately 1.4 times more than control phage (fig. 8b), although the difference was not statistically significant. Five of 6 patients contributing to this study had liver cirrhosis or chronic hepatitis, therefore the non-cancerous liver tissue in these cases was not completely normal, and there was a possibility that they had already included micro-dysplasia lesions in the liver. This may be one reason why TTPRDAY phage accumulates in non-cancerous liver tissue slightly higher than control phage. Nevertheless, TTPRDAY phage bound to HCC significantly more than surrounding non-cancerous liver tissue (fig. 8b), and this difference between HCC and non-cancerous tissue could be important for the application of TTPRDAY to HCC-targeted therapy.

In addition, TTPRDAY showed no significant ability to mediate cell proliferation in vitro after binding to HCC cells. This is an important consideration when applying a novel peptide to actual cancer treatment, since it is undesirable to administer potent mitogens to cancer patients. The TTPRDAY peptide has potential therapeutic applications to well-established treatments such as transarterial chemoembolization or systemic chemotherapy for HCC patients. Furthermore, it could be used as a targeting molecule to direct imaging agents to primary HCC and its metastases by using well-developed strategies for chelation to radiolabels. Finally, a peptide-presenting phage display library is a powerful method for identifying ligands that can bind to specific targets [21]. Our findings suggest that the TTPRDAY peptide has considerable potential as a therapeutic and diagnostic reagent for HCC.

Acknowledgements

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Impact of Tumor Spread to the Cystic Duct on the Prognosis of Patients with Gallbladder Carcinoma

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Abstract

Introduction: The importance of gallbladder carcinoma spread to the cystic duct has not yet been described. Although the cystic duct is contiguous with the gallbladder, it is located in the hepatoduodenal ligament and differs in structure from the gallbladder. The incidence and prognostic significance of cancer spread to the cystic duct in patients with gallbladder cancer is unclear.

Methods: Surgical specimens from 42 patients who underwent resection for advanced gallbladder carcinoma were examined retrospectively.

Results: Altogether, 13 (31%) of the patients had cancer spread to the cystic duct. The incidences of perineural invasion, lymph node metastasis, and venous invasion were significantly higher in these patients than in the other 29 patients without cancer spread to the cystic duct ($P = 0.027$, 0.034, and 0.034, respectively). The 3- and 5-year survival rates of these 13 patients were significantly lower than those of the other 29 patients (15.4% vs. 51.0% and 7.7% vs. 46.6%, respectively, $P < 0.0001$ each). Multivariate analysis using the Cox proportional hazard model identified positive cancer spread to the cystic duct and depth of invasion (beyond serosa) as significant independent indicators of a poor prognosis.

Conclusions: Cancer spread to the cystic duct is an indicator of poor prognosis in patients with gallbladder carcinoma. This may be due to the high incidence of concomitant perineural invasion and lymph node metastasis associated with cancer spread.

Although radical cholecystectomy and even more extensive surgery have improved the surgical outcome of patients with gallbladder carcinoma,[1,2] the value of aggressive treatment for advanced cancer is still a controversial issue because of the degree of clinical variation, from a small polypoid lesion to a huge mass involving contiguous structures.[3–5] A uniform system for classifying the mode of cancer spread may help individualize surgical treatment.[3,6] Invasion into the interstitial tissue of the hepatoduodenal ligament is a cause for the poor prognosis of advanced gallbladder cancer.[7] Because the interstitial tissues of the hepatoduodenal ligament are composed of loose fibrous tissue, lymphatic ducts, and nerve fibers, invasion of cancer into this area may progress to the head of the pancreas, the paraaortic fatty tissue, Glisson's sheath in the liver, the hilar bile duct, or any combination of these sites.[5] Hepatoduodenal ligament invasion has been described as an independent factor of tumor spread known as "Binf" (bile duct infiltration) in the general rules for surgical and pathologic studies on cancer of the biliary tract in Japan.[8] Some authors have reported that cancer infiltration of the extrahepatic bile duct is an indicator of poor prognosis factor in patients with gallbladder carcinoma.[5,7,9,10] On

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Table 1.
Surgical procedure in 42 patients with advanced gallbladder carcinoma

Procedure	GBC with CD spread	GBC without CD spread	Total
CX	2	7	9
CX with BDR	2	9	11
HX with BDR	4	8	12
HX without BDR	1	1	2
PD	0	1	1
HPD	4	3	7
Total no. of patients	13	29	42

CX: cholecystectomy; BDR: bile duct resection with lymph node dissection; HX: hepatectomy; PD: pancreatoduodenectomy; HPD: hepatopancreatoduodenectomy.

the other hand, even though the cystic duct is classified as part of the gallbladder in the general rules, no reports have addressed the significance of cancer spread to the cystic duct. Because the cystic duct differs in structure from the gallbladder and is located in the hepatoduodenal ligament, we hypothesize that it is contiguous with the gallbladder, and that tumor spread to the cystic duct can be considered involvement of an organ independent from (not part of) the gallbladder.

Although a few studies have attempted to classify the mode of gallbladder carcinoma spread in the advanced stage,[3,6] the prognostic impact of gallbladder carcinoma spread to the cystic duct remains unclear. The aim of this study was to elucidate the incidence and prognostic significance of gallbladder carcinoma spread to the cystic duct.

PATIENTS AND METHODS

Patients

Between March 1990 and September 2004, a total of 61 patients with gallbladder carcinoma were treated at the Department of Surgery, Shinshu University Hospital. Seven patients with pT1 disease according to the UICC classification[11] were excluded from the study. Also excluded were 12 patients believed to have primary cystic duct carcinoma diagnosed on the basis of an examination of the resected specimen showing that the center of the tumor mass corresponded to the location of the cystic duct.[12] The remaining 42 patients with gallbladder carcinoma that had invaded the subserosal layer or farther (pT2, n = 16; pT3 and pT4, n = 26) were enrolled into the

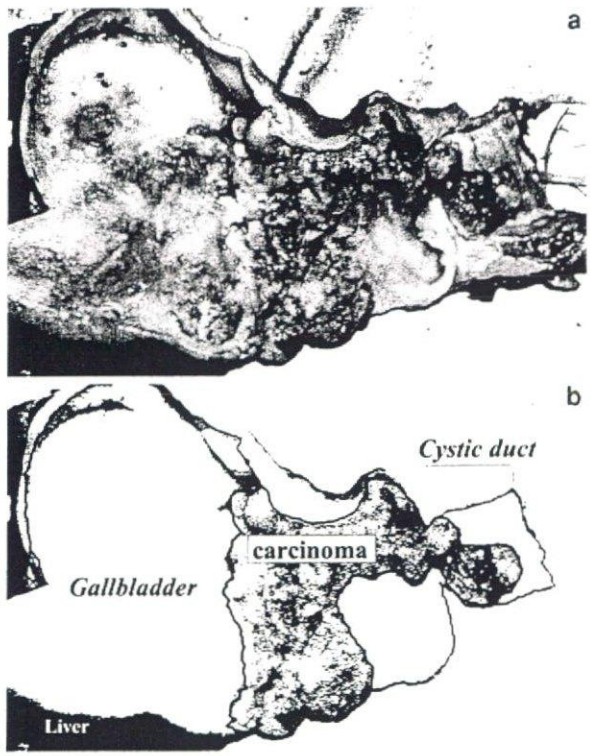

Figure 1. a. Clinical photograph of gallbladder carcinoma spread to the cystic duct. Resected specimen shows gallbladder carcinoma with tumor spread to the cystic duct. **b** Scheme of the specimen.

study. There were 20 men and 22 women with a median age of 68 years (range 27–4 years). The youngest patient with advanced gallbladder carcinoma in the present study also had anomalous pancreaticobiliary ductal junction without bile duct dilatation.[13,14]

The operative procedures used in these 42 patients are described in Table 1. The surgically resected tissue was routinely fixed in 10% formalin, embedded in paraffin, cut serially into 5 μm thick slices, and prepared in the usual manner with hematoxylin and eosin staining. Final confirmation of the spread of gallbladder carcinoma to the cystic duct was made by histologic examination.

Statistical Analysis

Statistical analysis was performed using Fisher's exact probability test. Univariate analysis to identify variables significantly related to survival was done using the Kaplan-Meier method, and differences in survival were evaluated by the log-rank test. Multivariate analysis was then performed to assess the significance of factors identified by univariate analysis using Cox's proportional hazard model. Statistical analysis was carried out using StatView

Table 2.
Clinical futures in patients with advanced gallbladder carcinoma

Variable	GBC with CD spread	GBC without CD spread	*P*
Age (years), mean and range	65.6 ± 14.4 (27–79)	66.2 ± 10.6 (40–84)	0.7791
Sex (male:female)	6:7	14:15	0.9017
Presence of disease			
Sympotomatic disease	12 (92.3%)	22 (75.9%)	0.2192
Incidental disease	1 (7.7%)	9 (31.0%)	0.1055
Cholelithiasis	4 (36.4%)	12 (41.4%)	0.7794
Jaundice	3 (23.1%)	3 (10.3%)	0.2870
ERCP performed	9 (69.2%)	20 (69.0%)	0.9867
Findings of ERCP			
Filling of cystic duct	1 (11.1%)	16 (80%)	< 0.0001
Filling of gall bladder	1 (11.1%)	13 (65%)	0.0032
Type of surgery:R0	10 (76.9%)	23 (79.3%)	0.8657

GBC: gallbladder carcinoma, CD: cystic duct, ERCP: endoscopic retrograde cholangiopancreatography.

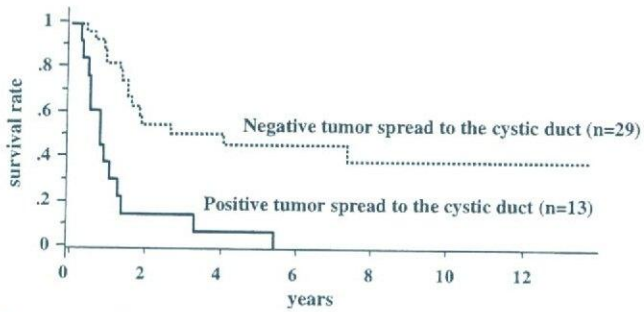

Figure 2. Survival of patients with advanced gallbladder carcinoma stratified according to tumor spread to the cystic duct. The solid line indicates the survival of patients positive for tumor spread to the cystic duct (3-year and 5-year survival rates were 15.4% and 7.7%, respectively). The dotted line indicates survival of patients negative for tumor spread to the cystic duct (3-year and 5-year survival rates were 51% and 46.6%, respectively). Survival was significantly longer in negative patients than in positive patients ($P < 0.0001$).

5.0J software (SAS Institute, Cary, NC, USA). Differences at $P < 0.05$ were considered significant.

RESULTS

Of the 42 patients with advanced gallbladder carcinoma, 13 (31%) had cancer spread to the cystic duct (Fig. 1). There were no significant differences in age, sex, rate of symptomatic disease, incidental carcinoma, presence of cholelithiasis, or presence of jaundice between patients with cancer spread to the cystic duct and patients without spread (Table 2). Of the 42 patients, 29 underwent preoperative endoscopic retrograde cholangiopancreatography (ERCP). The incidence of filling of the cystic duct and gallbladder during ERCP was significantly lower in patients with cancer spread to the cystic duct than in those without spread (Table 2). The 3- and 5-year survival rates of these 13 patients were significantly lower than those of the 29 patients without cancer spread to the cystic duct (15.4% vs. 51.1% and 7.7% vs. 46.6%, respectively; $P < 0.0001$ each) (Fig. 2).

The relations between cancer spread to the cystic duct and other histologic factors are shown in Table 3. The incidences of perineural invasion, lymph node metastasis, and venous invasion were significantly higher in the patients with gallbladder cancer spread to the cystic duct than in the patients without cancer spread ($P = 0.027$, 0.034, and 0.034, respectively). Patients with cancer spread to the cystic duct also had a higher incidence of mural invasion beyond the serosal layer than those without spread (76.9% vs. 55.2%) but not to a significant degree ($P = 0.180$). Also, the incidence of cancer spread to the cystic duct was not significantly related to that of bile duct infiltration (53.8% vs. 24.1%, $P = 0.059$) or pTMN staging ($P = 0.1033$) (Table 3). The incidence of lymph node metastasis in the hepatoduodenal ligament (around the bile duct, hepatic artery, and portal vein) and around the common hepatic artery were higher in patients with cancer spread to the cystic duct than in patients without cancer spread (Table 4).

In the 42 patients with gallbladder carcinoma, 17 clinicopathologic variables were analyzed as possible prognostic factors: age (>68 years or not), sex, symptomatic disease, incidental gallbladder carcinoma, the presence of cholelithiasis, the presence of jaundice, the type of surgery (R0 or R1), histologic type, depth of invasion, positive lymphatic invasion, positive venous invasion, positive perineural invasion, lymph node metastasis, bile duct infiltration, hepatic parenchymal infiltration, AJCC

Table 3.
Histopathologic features in 42 patients with advanced gallbladder carcinoma

Variable	GBC with CD spread (n = 13)	GBC without CD spread (n = 29)	*P*
Histological type			0.420
Papillary and well differentiated adenocarcinoma	3 (23.1%)	16 (55.2%)	
Poorly and moderately differentiated adenocarcinoma	8 (61.5%)	9 (31.0%)	
Other	2 (15.4%)	4 (13.8%)	
Depth of invasion			0.180
Subserosa	3 (23.1%)	13 (44.8%)	
Beyond serosa	10 (76.9%)	16 (55.2%)	
Positive lymphatic invasion	13 (100%)	21 (72.4%)	0.065
Positive venous invasion	12 (92.3%)	19 (65.5%)	0.034
Positive perineural invasion	11 (84.6%)	13 (44.8%)	0.027
Presence of metastatic lymph node	8 (61.5%)	9 (31.0%)	0.034
Presence of bile duct infiltration	7 (53.8%)	7 (24.1%)	0.059
Presence of hepatic infiltration	10 (76.9%)	20 (70.0%)	0.598
TNM stage			0.1033
IB+IIA	2 (15.4%)	12 (41.3%)	
IIB	4 (30.8%)	5 (17.2%)	
III	7 (53.8%)	12 (41.4%)	

Table 4.
Frequency of lymph node involvement in 42 patients with advanced gallbladder carcinoma

Site (site no.)	Frequency (%)			*P*
	GBC (n = 42)	GBC with CD spread (n = 13)	GBC without CD spread (n = 29)	
Cystic LN (12c)	27.8	22.2	33.3	0.632
Pericholedochal LN (12b)	22.2	22.2	22.2	0.379
LN around the proper hepatic artery (12a)	11.1	22.2	0.0	0.029
LN around the portal vein (12p)	27.8	44.4	11.1	0.011
Posterior pancreaticoduodenal LN (13)	66.7	33.3	66.6	0.692
LN around the common hepatic artery (8)	27.8	44.4	11.1	0.002

LN: lymph nodes.
Site number is based on general rules of the Japanese Society of Biliary Surgery.[8]

staging, and cancer spread to the cystic duct. Univariate analysis showed that incidental gallbladder carcinoma, the presence of cholelithiasis , histologic type, depth of invasion, venous invasion, perineural invasion, extrahepatic bile duct invasion, lymph node metastasis, AJCC staging, and cancer spread to the cystic duct as significant prognostic factors (P = 0.040, 0.043, 0.029, 0.036, 0.014 0.004, 0.004, 0.027, and 0.0001, respectively) (Table 5). Multivariate analysis by the Cox proportional hazard model identified positive cancer spread to the cystic duct and depth of invasion (beyond the serosa) as significant independent indicators of a poor prognosis (Table 6).

DISCUSSION

We investigated the role of cancer spread to the cystic duct from gallbladder carcinoma, which is a precondition for infiltration into the bile duct and has an impact on prognosis. A few previous authors have attempted to classify gallbladder carcinoma spread to the hepatoduodenal ligament.[3,6,15] Kondo *et al.* proposed that the "hepatic hilum type," which they defined as tumor infiltration of the hepatic hilum, had a poor prognosis.[6] Miyazaki *et al.* reported that the "biliary type" of spread also had a poor prognosis.[3] However, they did not describe any details of histologic findings or the microscopic pattern of

Table 5.
Univariate analysis of clinical and histopathologic variables

Variable	No.	Survival rate (%) 3 Years	5 Years	P
Age (Years)				
< 69	24	44	39	
≥ 69	18	34	25	
Sex				0.6251
Female	22	35	29	
Male	20	47	41	
Symptomatic disease				0.8243
Yes	33	37	33	
No	9	38	38	
Incidental gallbladder carcinoma				0.0402
Yes	10	76	51	
No (not incidental)	32	26	22	
Cholelithiasis				0.0429
Absent	26	48	38	
Present	16	31	31	
Jaundice				0.1325
Absent	36	44	37	
Present	6	17	17	
Type of surgey				0.098
R0	33	49	41	
R1	9	11	11	
Histologic type				0.0493
Papillary and well differentiated	19	56	50	
Moderately and poorly differtiated	17	21	14	
Other	6	40	40	
Depth of invasion				0.0286
Subserosa	16	74	65	
Beyond serosa	26	23	19	
Lymphatic invasion				0.1314
Absent	8	86	86	
Present	34	33	27	
Venous invasion				0.0358
Absent	11	89	89	
Present	31	28	25	
Perineural invasion				0.0139
Absent	18	64	64	
Present	24	23	14	
Lymph node involvement				0.0036
Absent	24	61	55	
Present	18	21	14	
Bile duct infiltration				0.0035
Absent	28	58	49	
Present	14	7	7	
Hepatic infiltration				0.2848
Absent	12	66	53	
Present	30	31	22	
Cancer spread to the cystic duct				< 0.0001
Absent	29	51	47	
Present	13	15	8	
AJCC staging				0.0271
I	11	81	81	
II	12	30	15	
III	19	26	21	

infiltration into the hepatoduodenal ligament. Shimizu *et al.* reported that infiltration of the hepatoduodenal ligament was evident histologically in 30 (60%) of 50 patients with gallbladder carcinoma. All patients with obstructive jaundice before surgery had hepatoduodenal ligament invasion and cancer cells in the extrahepatic bile duct wall itself, and invasion was seen in 24 of 44 patients without preoperative obstructive jaundice.[15] So far, however, the association between infiltration into the hepatoduodenal ligament and the frequency of lymphatic invasion, venous invasion, or perineural invasion has not been addressed.

In the present study, we showed that cancer spread to the cystic duct was a significant indicator of poor prognosis in patients with advanced gallbladder carcinoma; moreover, in patients with such cancer spread, the incidences of lymph node metastasis in the hepatoduodenal ligament and around the common hepatic artery were higher than in patients without cancer spread to the cystic duct. However, the incidence of cancer spread to the cystic duct was not related to the depth of cancer invasion or bile duct infiltration. Cancer spread to the cystic duct was observed in patients without obvious involvement of the bile duct or mural invasion beyond the serosa. These findings suggest that cancer spread to the cystic duct is a preliminary step of cancer extension to the hepatoduodenal ligament, even in patients with a preoperative diagnosis of locally advanced gallbladder carcinoma. In the present study, the incidence of filling of the cystic duct and the gallbladder during preoperative ERCP were significantly lower in patients with cancer spread to the cystic duct than in those without. This finding could be helpful for diagnosing cancer spread to the cystic duct preoperatively.

The neural network in the hepatoduodenal ligament is highly complex and has not been well described, although it contains rich autonomic nerve networks, especially around the hepatic artery and portal vein (Fig. 3).[16,17] Perineural invasion has been identified by several authors as an important risk factor in patients with hilar and extrahepatic bile duct carcinoma.[18–20] Yamaguchi *et al.* reported that perineural invasion showed no association with either lymphatic or vascular invasion in gallbladder carcinoma but had a close association with extrahepatic bile duct invasion. Once gallbladder carcinoma invades the extrahepatic bile duct through Calot's triangle, the tumor is likely to encroach on nerve tissue and extend along the perineural space.[21] How, then, should cancer spread to the cystic duct be handled? In the present series of patients with gallbladder cancer, the incidence of perineural invasion was significantly higher in patients with cancer spread to the cystic duct than in those without

Table 6.
Results of Cox multivariate regression analysis

Variable	Parameter estimate	Wald chi-squared	*P*	Hazard ratio	95% Confidence interval
Depath of invasion (subscrosa vs. beyond serosa)	1.645	5.771	0.0163	5.184	1.333–19.838
Cancer spread to the cystic duct (yes vs. no.)	2.260	15.545	< 0.0001	9.578	3.117–29.461

such spread. In view of the report of Kaneoka *et al.*,[9] which indicated that once the periductal and intraductal neural network has been infiltrated by cancer spread to both the hepatic hilum and the peripancreatic tissue is likely, there is a potential risk of infiltration into the hepatoduodenal ligament when cancer has spread to the cystic duct, and thus such cancer spread should be handled in the same way as extension of gallbladder carcinoma to the bile duct.

On the other hand, lymph node metastasis in patients with gallbladder carcinoma is a strong indicator of poor prognosis.[22–24] The pattern of lymph node metastasis from gallbladder carcinoma and lymph flow from the gallbladder has been studied by many authors.[2,22,23,25,26] There are three lymphatic pathways: the cholecystoretropancreatic pathway is the main route, the cholecystoceliac pathway is a second route, and the cholecystomesenteric pathway is a third route[26] (Fig. 4). The cystic and pericholedocal lymph nodes are key stations for cancer spread toward more distant lymph nodes.[27] In our study, the incidence of lymph node involvement around the proper hepatic artery, lymph nodes around the portal vein, and lymph nodes around the common hepatic artery was significantly higher in patients with positive tumor spread to the cystic duct. This finding suggests that tumor spread to the cystic duct is associated with a high incidence of lymph node involvement through the "cholecystoceliac" pathway (Fig. 4).

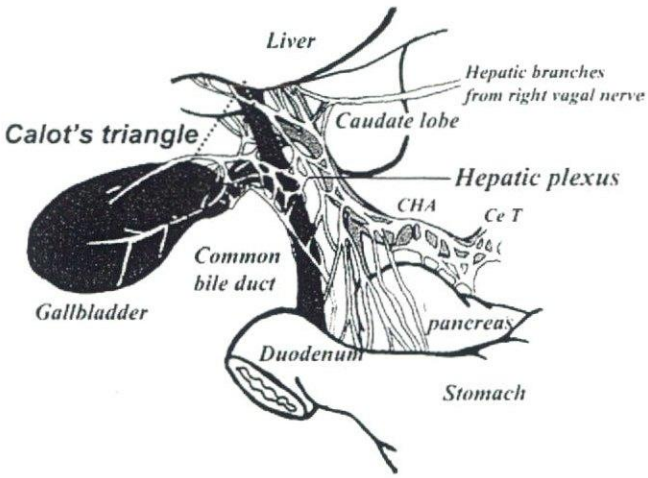

Figure 3. Neural network in the hepatoduodenal ligament. The dotted line's triangle expresses Calot's triangle. CHA: common hepatic artery; Ce T: celiac trunk.

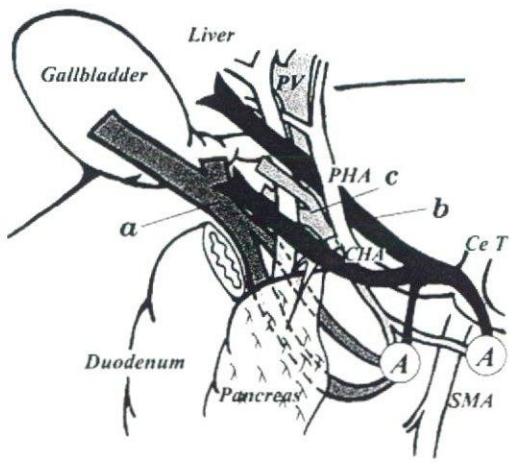

Figure 4. Lymphatic drainage routes of the gallbladder. **a**, Cholecystoretropancreatic pathway, which desends in the right side of the hepatoduodenal ligamant to the posterior surface of the head of the pancreas; **b**, cholecystoceliac pathway, which runs in the left side of the hepatoduodenal ligament and reaches the common hepatic artery and the celiac trunk; **c**, cholecystomesenteric pathway, which runs along the anterior surface of the portal vein and connects with the root of the superior mesenteric artery. CHA: common hepatic artery; PHA: proper hepatic artery; SMA: superior mesenteric artery; PV: portal vein; Ce T: celiac trunk; A: abdominoaortic nodes.

CONCLUSIONS

Although the number of patients was limited, the present study revealed that tumor spread to the cystic duct has a negative impact on the prognosis of patients with advanced gallbladder carcinoma. Perineural invasion and lymph node metastasis toward the cholecystoceliac pathway are characteristic of patients showing spread of advanced gallbladder carcinoma to the cystic duct. It is acceptable to perform extrahepatic bile duct resection for advanced gallbladder carcinoma with bile duct involvement. However, the validity of extrahepatic

bile duct resection as part of radical surgery for advanced gallbladder carcinoma is still controversial when there is no apparent extrahepatic bile duct involvement. The present study indicates the significance of extrahepatic bile duct resection as part of radical surgery for patients with preoperatively suspected (e.g., by ERCP) cancer spread to the cystic duct even if direct invasion to the common bile duct is not apparent. Further studies should be undertaken to clarify whether extensive nerve and lymph node dissection (i.e., extra bile duct resection) provides a survival benefit for patients with spread of gallbladder carcinoma to the cystic duct.

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症例報告

大腸癌肝転移にラジオ波焼灼療法を施行後，肝切除を施行した1例

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大腸癌肝転移に対し他院で行われたラジオ波焼灼（radio frequency ablation；以下，RFA）後に，当科で肝切除術を施行した1例について報告する．症例は50歳の男性で，多発性肝転移の一部の腫瘍に対しRFAが行われ，残りの未治療の腫瘍に対する加療目的で当科に紹介となった．RFA後の腹部CTでは，焼灼部は腫瘍より広範囲に造影欠損が認められ，焼灼により治療しえたと判断されたが，肝切除術により得られた標本の病理組織学的検査では焼灼部に腫瘍の遺残を認めた．大腸癌肝転移に対する治療は肝切除が第1選択であり，RFAの有効性はいまだ議論の多いところである．RFA後の局所再発や，穿刺に伴う播種により根治性を損なうとの報告もあり，大腸癌肝転移の治療としてRFAの適応は慎重に検討すべきである．

はじめに

肝細胞癌は併存する肝機能障害や多発中心性発癌の背景も加わって，小型の肝細胞癌の局所治療としてラジオ波焼灼療法（radio frequency ablation；以下，RFA）が導入されている．しかし，RFA後の腹膜播種，局所再発などが報告されており大きな課題である．大腸癌肝転移に対するRFAの治療効果を組織学的に多数症例で検討した報告は，まだ少ない．一方，大腸癌肝転移症例の多くの肝機能は正常で，長期予後を得るためには切除可能な症例では肝切除が第1選択であることは論をまたない．我々は他院で大腸癌肝転移に対しRFAを施行したのち，当科で焼灼部を含む肝切除術となった症例の病理学的診断を検討することができた．その結果から，切除可能な大腸癌肝転移に対するRFAの適応について文献的考察を加え報告する．

症　例

症例：50歳，男性

既往歴：特記すべきことなし．

現病歴：前医で盲腸癌，同時性肝転移（S8）に対し平成15年1月下旬に回盲部切除術および肝S8部分切除術を施行．同年4月上旬の腹部MRIで6か所（S2，S3，S5，S6，S7に2か所）に最大径1.8cmの多発性肝転移を認め，4月中旬にS2（1.8cm），S5（1.0cm），S6（1.5cm）の3個の腫瘍に対して前医でRFAが施行された（Fig. 1）．RFAはRadionics社のcool-tip needleを用いて行われた（Table 1）．残った肝転移巣の治療のため，肝動注ポート挿入を目的として平成15年5月上旬に当科に紹介となった．

入院時現症：身長164.5cm，体重64kg，上腹部に逆L字型の手術創を認めた．他に特記すべき異常所見なし．

入院時検査所見：CEAの軽度上昇を認めた他には異常所見なし．

RFAの約1か月後となる5月上旬に当院で腹部Angio CTを施行した．

腹部Angio CT：S2，S5，S6の3か所にlow densityに描出される焼灼部を認めた．S2の焼灼部は一部が不均一に造影され，腫瘍の遺残の可能性が示唆された．S5，S6の焼灼部は腫瘍よりも広い範囲でlow densityに描出された（Fig. 2）．

焼灼部に腫瘍の遺残している可能性も否定できず，未治療の転移巣も併せて外科的に切除可能と

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Fig. 1　Abdominal MRI showed a high intensity tumor on T2-weighted imaging in S2, S5 and S6 of the liver.

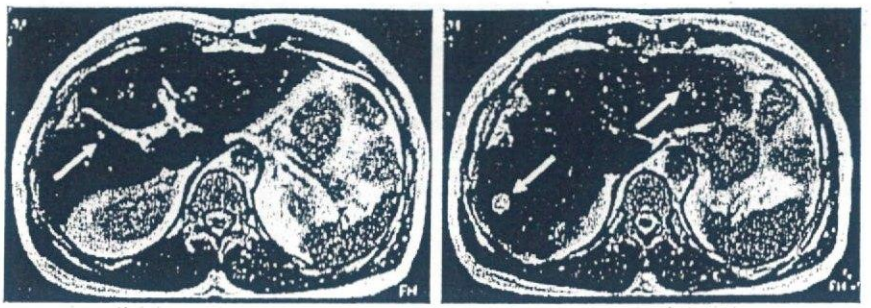


Table 1　RFA techniques

	Output (w)	Ablation time	Maximum central temperature	Impedance
Tumor 1	40-50-60-60-50-50-40	9min.	75℃	90Ω
Tumor 2	40-50-60-60-50-50-40	9min.	68℃	90Ω
Tumor 3	40-50-50-40	9min.	65℃	85Ω

Each ablation was performed with monopolar cooled needle electrodes with an active 2cm-long distal tip. It spent 30 seconds to ablate inserted sites. There was no record that tumor 1 ～ 3 corresponded to each one.

判断し5月中旬に肝S2，S3，S5，S6，S7部分切除術を施行した．

摘出標本：焼灼部は，それぞれ白色から茶褐色の弾性硬な腫瘤として触知された．写真はS6のものである（Fig. 3）．

病理組織学的検査所見：S2，S5，S6のいずれの焼灼部にも，腫瘍・正常肝の壊死組織を背景として，腫瘍の遺残を認めた．写真はS6のものである（Fig. 4）．

術後経過：術後は順調に経過し，平成15年7月上旬に退院した．平成16年1月にCTで傍大動脈領域へのリンパ節転移を認めた．塩酸イリノテカンなどを用いた全身化学療法を行うも著効せず，平成17年2月に死亡した．肝再発は認めなかった．

考　察

医学中央雑誌刊行会の医中誌Webから「肝臓腫瘍」「再発」「肝転移」をキーワードとする1983年～2006年までの論文および米国National Library of MedicineのPub Medからキーワードを「metastatic colorectal cancer，radio frequency ablation」とし，1990年～2006年までの論文を検索した．

大腸癌は肝転移を来しやすく，初診時に，およそ20%前後の患者がすでに肝転移を有し[1]，また大腸癌治癒切除後の肝転移率としては結腸癌で9.2～14.5%，直腸癌で11.6～18.2%と報告されている[2]．現在のところ，大腸癌肝転移については肝切除が最も有効な治療法で，肝切除後の5年生存率は25～40%と良好な成績が報告されている[3)～6)]．当科でもImamuraら[7]が報告するように，切除可能な肺転移以外の肝外転移巣がなければ，両葉多発例や同時性肝転移の症例でも腫瘍数にかかわらず肝切除の適応とし，また残肝容積が不十分と予想される症例には術前に切除予定葉に門脈塞栓術を行った後に肝切除を行う方針をとっており，このような方針で行った当科における肝切除後の5年生存率は42%である．なお，当科では肝切除後にadjuvant chemotherapyは行っていないが，術後に集学的治療を行う施設もみられ，今後の検討が必要と思われる．他施設においても同様に，肝切除を積極的に施行している報告を散見し，その適応は拡大傾向にあると思われる[8)9)]．

肝細胞癌に対してRFAは低侵襲な治療として

Fig. 2 Abdominal CT showed a hypodense area in S2, S5 and S6 of the liver after RFA. The hypodense area in S2 was enhanced heterogeneously. (arrow-A)

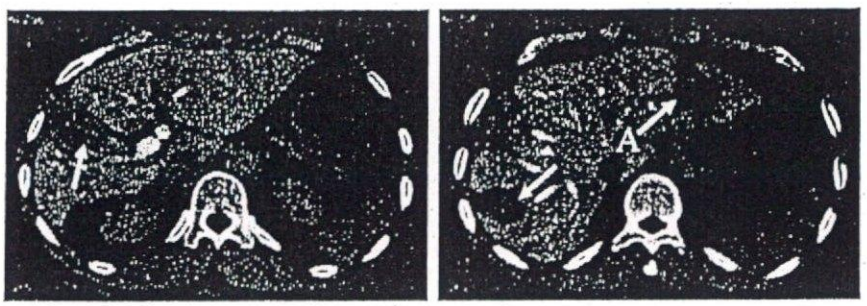
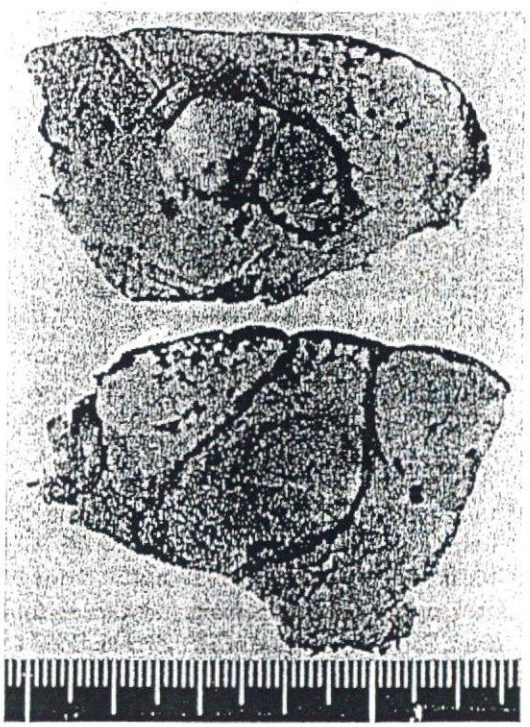


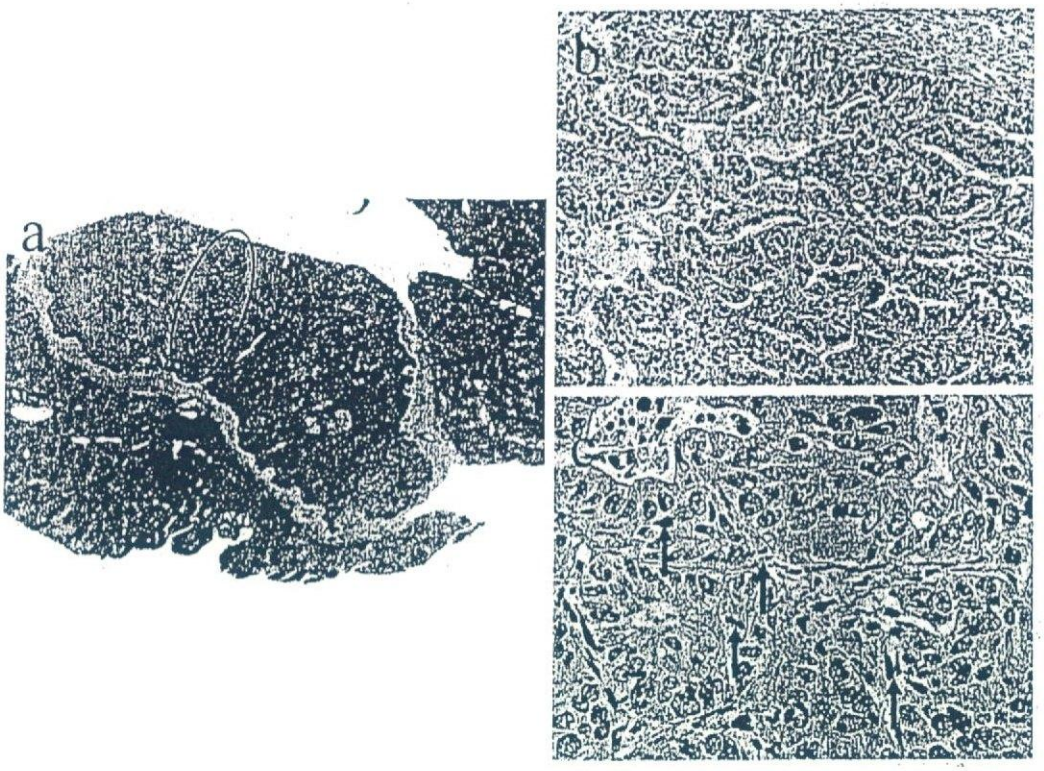
Fig. 3 Resected specimen showed whitish tumor.



導入されつつあり，径3cm未満の肝細胞癌に対しては75～90％の完全壊死が期待できると報告されている[10)~12)]．しかし，転移性肝癌に対するRFAの長期成績についてまとまった報告はなく，治療効果や治療の確実性，安全性については議論の多いところである．De Baere[13)]らは100個の転移性肝癌にRFAを行い，術後1年間の局所制御率は90％であったと，その有効性を報告している．一方，RFA後の局所再発に関する報告も散見される．大川ら[14)]は大腸癌を中心とした転移性肝癌16例に対し，マイクロ波やラジオ波による経皮的穿刺熱凝固療法を施行し，肝細胞癌では局所再発率は14.8％であったが，転移性肝癌では35.3％と有意に高率であったと報告しており，この差は転移性肝癌では腫瘍の硬さや生物学的な悪性度に起因するのではないかと考察している．RFAの技術的な問題や経過観察の間隔などにより，その成績は左右され複数の施設間での成績を単純には比較できないが，Parikhら[15)]の報告によると転移性肝癌のRFA後の局所再発率は8～34％とされている．また，Hoffmanら[16)]は肝細胞癌4例と大腸癌肝転移3例についてRFA後に肝切除を行い，焼灼部の病理組織学的な評価をしており，少なくとも1cmのマージンをもってRFAを施行したが，全例において焼灼部に腫瘍の遺残を確認したと報告している．

別府ら[17)]はRFAが肝切除と同等に大腸癌肝転移にも有効であるとする一方，肝細胞癌に比べ腫瘍に被膜がないため熱が腫瘍内にとどまりにくく，焼灼部のマージンを十分に確保しないと治療部位再発を高率に認めるとして，RFAの適応を肝切除不能例や切除のリスクの高い症例に限定している．症例もS5，S6の焼灼部に造影効果を認めず，術前には完全壊死と判断したが，病理組織学的には腫瘍の遺残を認めた．大腸癌肝転移はCT上，造影効果の低い腫瘤であり，RFA後の壊死の判定が困難であることも，局所再発率を上げる一因であると考える．今後，RFAを肝転移症例に対する治療として確立するのであれば，正確な腫瘍の壊死判定方法と確実な腫瘍の壊死が得られる

Fig. 4　Microscopic findings showed that tumor cells in the surrounding necrotized tissue (a) (b). Mitotic tumor cells were observed (arrows) (c).



RFAの条件について検討し，治療の標準化をはかることが必要と考える．

現在のところ，切除可能な大腸癌肝転移は肝切除が根治可能な唯一の治療法である．肝切除端に腫瘍が露出しないことが予後因子であるということは多くの施設から報告されている．したがって，根治を目指すのであれば，手術でもRFAでも腫瘍を完全に取り除くことが重要と考える．腫瘍の局所遺残や穿刺に伴う播種などの可能性を考慮し，切除可能症例に対するRFAの適応を慎重に検討すべきである．

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A Case of Hepatic Resection for Colorectal Hepatic Metastasis after Radio Frequency Ablation

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We report a case of hepatic resection for hepatic metastasis from colorectal cancer after radio frequency ablation (RFA). A 50-year-old man had multiple liver metastasis from colorectal cancer, and was admitted for complete liver tumor removal after partial RFA elsewhere. CT after RFA showed that each RFA lesion was hypovascular areas, which was larger than the size of the corresponding metastatic tumor, indicating that ablation made each tumor complete necrosis. However, after hepatic resection including RFA ablated lesions, postoperative histopathological findings showed viable tumor cells in ablated lesions in all resected specimens. Hepatic resection is widely accepted as the treatment of choice in colorectal hepatic metastasis, but the definite efficacy of RFA has yet to be established. Serious post-RFA complications such as local recurrence and tumor seeding have been reported, indicating that RFA should be carefully considered before being attempted in treating colorectal hepatic metastasis.

Key words : radio frequency ablation, colorectal hepatic metastasis, hepatic resection
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