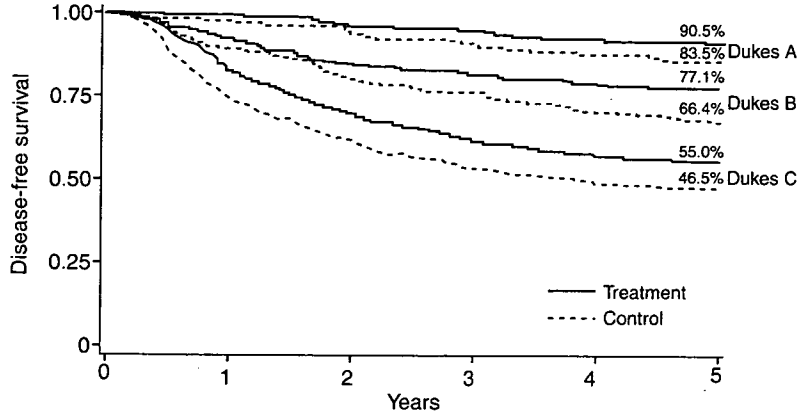

	No. at risk						
Dukes A	Treatment	148	147	143	140	134	132
	Control	128	124	119	115	111	108
Dukes B	Treatment	377	347	315	303	291	282
	Control	316	280	250	237	217	206
Dukes C	Treatment	589	487	403	354	320	298
	Control	532	396	323	277	251	227

Figure 6 Disease-free survival curves by tumour stage and by treatment.

The recent development of O-FPs has therefore opened new perspectives. Oral fluorinated pyrimidines may mimic continuous regimens without its technical inconvenience and deterring patients' quality of life. In patients with advanced colorectal cancer, the efficacy of UFT (typical and most prescribed O-FP) plus oral LV (Carmichael *et al*, 2002; Douillard *et al*, 2002) or of capecitabine alone (Hoff *et al*, 2001; Van Cutsem *et al*, 2001) seems comparable in terms of the efficacy with significantly less significant severe haematologic toxicities and/or stomatitis. The risk of severe hand-foot syndrome is lower in UFT than with capecitabine, but the risk of severe diarrhoea and other gastrointestinal symptoms is higher in UFT and in UFT/oral LV treatment for Western patients.

In Japan, UFT have been administered for many years especially for patients with curatively resected colorectal cancers. For some unknown reason, severe gastrointestinal toxicities are much less frequent in Japanese patients, and patients usually prefer oral chemotherapy especially in an adjuvant setting (Borner *et al*, 2002).

Furthermore, with regard to rectal cancer, it is a difficult objective for a clinical trial to accrue enough patients, compared to colon cancer, and despite the fact that several attempts of determining a standard adjuvant treatment for rectal cancer, almost no clinical trial has succeeded in showing a relevant survival benefit of adjuvant treatment, except one with preoperative radiotherapy (Swedish Rectal Cancer Trial, 1997).

In this context, several Japanese groups conducted randomised clinical trials comparing UFT with surgery alone for curatively resected rectal cancers. Five such trials were identified after a meticulous search, and are included in the present meta-analysis. This meta-analysis was restricted to trials that had been randomised centrally and from which no patient had been excluded for any reason. It represents the largest series of properly randomly assigned patients receiving the single oral adjuvant O-FP agent, that is, UFT, for rectal cancer comparing with patients receiving no therapy after curative tumour resection.

This meta-analysis found a statistically significant benefit of UFT with regard to overall survival (OS) (hazard ratio = 0.82; $P = 0.02$) as well as DFS (hazard ratio = 0.73; $P < 0.0001$), and LRFS (hazard ratio = 0.68; $P = 0.0026$). As can seen by comparing the data in Figures 1 and 4, the data from the NSAS-CC and TAC-CR study show benefits that are, apparently, larger than the others. As shown in Table 1, the dosage and duration of treatment with UFT in the NSAS-CC and TAC-CR trials differed from those in the other three trials; the dose intensity of UFT was higher in the former two trials. Several studies have reported that a high-dose intensity of UFT improves survival in patients given postoperative adjuvant chemotherapy for gastric cancer (Sugimachi *et al*, 1997; Danno *et al*, 2001). The higher dose intensity of UFT in the NSAS-CC and TAC-CR trials may have influenced the outcomes.

Most of the Japanese rectal cancer patients did not receive pre- or postoperative radiotherapy in any of the trials. Although radiotherapy has been considered one of the standard adjuvant treatments in the Western countries, significant survival benefit has not been shown with reproducibility (Wolmark *et al*, 2000; Colorectal Cancer Collaborative Group, 2001). The ostensible advantage of adjuvant radiotherapy is to decrease local recurrence of rectal cancers. As compared with postoperative chemoradiotherapy, preoperative chemoradiotherapy does not improve OS, but inhibits local recurrence and reduces toxicity (Sauer *et al*, 2004). In our study, however, LRFS was also significantly better in the UFT group compared to surgery alone group. As far as our results are concerned, UFT might also be useful in preventing local recurrence in Japanese patients who usually do not receive radiotherapy in an adjuvant setting.

Also, there is still a debate whether adjuvant chemotherapy for early stage rectal cancer is feasible (Buyse and Piedbois, 2001). In terms of numbers needed to treat, these benefits imply that approximately 20 patients need to be treated for one more patient to survive 5 years, and approximately 10 to be treated for one fewer patient to suffer a cancer recurrence within 5 years, regardless of disease stage. Our results show that the therapy is beneficial in Stage II patients not only Stage III patients with nodal involvement (Mamounas *et al*, 1999; Gray *et al*, 2004). As for early stage disease, further investigations are needed to assess potential benefits of treatment because events were infrequent and hazard ratios were small.

Regardless of the disease stage and patient background characteristics, there is a need for further trials involving UFT and new agents that are effective in advanced disease, such as irinotecan, oxaliplatin, and monoclonal antibodies.

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ORIGINAL PAPER

Comparative analysis of intraperitoneal minimal free cancer cells between colorectal and gastric cancer patients using quantitative RT-PCR: possible reason for rare peritoneal recurrence in colorectal cancer

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Abstract Peritoneal recurrence has a much lower incidence in colorectal cancer (CRC) patients than gastric cancer (GC) patients. The aim of this study is to clarify the reason for the rare peritoneal recurrence in CRC as compared with GC. The incidence and the abundance of free tumor cells in the peritoneal lavages from 102 CRC and 126 GC patients who underwent curative surgery were assessed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) with carcinoembryonic antigen (CEA) and cytokeratin 20 (CK20) as genetic markers. Prognostic significance of CEA and CK20 mRNA was also compared between CRC and GC after 2 years of follow-up by Kaplan–Meyer method with overall and peritoneal recurrence-free survival as endpoints. Positivity rate and average values of CEA and CK20 mRNA in peritoneal lavages of CRC patients, which are correlated to the depth of tumor invasion (pT category), were essentially the same as those of GC cases. Overall survival was significantly (marginally) worse in CEA mRNA (CK20 mRNA)-positive CRC patients than negatives like GC. However, peritoneal recurrence-free survival was not different between CEA (CK20) mRNA-positive and -negative CRC patients, in quite contrast to GC cases. Multivariate analysis showed that CEA mRNA was an independent prognostic factor for overall survival in GC patients, but not in CRC patients. These results suggest that the rare peritoneal recurrence in CRC patients is not due to the low incidence or the small number of intraperitoneal free cancer cells, but more likely reflects due to the low-peritoneal metastatic potential of CRC cells.

Keywords Quantitative RT-PCR · Peritoneal metastasis · Colorectal cancer · Gastric cancer · Peritoneal lavage cytology

Abbreviations

RT-PCR	Reverse transcription-polymerase chain reaction
qRT-PCR	Quantitative RT-PCR
GC	Gastric cancer
CRC	Colorectal cancer
CEA	Carcinoembryonic antigen
CK20	Cytokeratin 20

Introduction

Major routes of metastatic spread in colorectal cancers (CRC) are hematogenous metastasis to the liver and lung and regional lymph node metastasis. Peritoneal dissemination is less frequent and therefore prognostically less

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important than the other two routes [1]. In contrast, peritoneal metastasis in gastric cancer (GC) is the most frequent pattern of recurrence after curative surgery and it is the most important prognostic factor [2]. The reason for this remarkable difference in peritoneal metastasis between CRC and GC despite the fact that the tumor originates from the same gastrointestinal tract remains largely unknown. Peritoneal metastasis consists of two steps; first, exfoliation of free cancer cells from the serosal surface of the primary tumor into the peritoneal cavity. Second, attachment of intraperitoneal free tumor cells to a preferable site in the peritoneal cavity such as the omentum and mesenterium and subsequent growth and dissemination into the peritoneal cavity [3]. Therefore, the low incidence of peritoneal recurrence in CRC patients may be either due to the low incidence and little exfoliation of free cancer cells from the primary tumors or low-metastatic potential of CRC cells in the peritoneal cavity. For the development of a new preventive modality for peritoneal recurrence in both GC and CRC patients, it is very important to understand the reason for this rare peritoneal recurrence in CRC patients.

Detection of free cancer cells in the peritoneal cavity in gastric, colorectal, pancreatic, and ovarian cancer patients has been performed with peritoneal lavage cytology using Papanicolaou staining [4–6]. The conventional cytology is a reliable and specific method, but has limited usefulness due to its lack of sensitivity. Recent advances in PCR and non-PCR technology such as real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) have allowed sensitive and quantitative detection of free cancer cells in the peritoneal cavity [7–9]. We applied real-time quantitative RT-PCR (qRT-PCR) for the first time to quantify free cancer cells in the peritoneal washes in GC patients and declared the prognostic significance of intra-abdominal carcinoembryonic antigen (CEA) mRNA levels [10, 11]. To date, evidences that CEA mRNA levels are a reliable prognostic factor for assessment of the peritoneal recurrence risk after curative resection in GC patients have been accumulating from a prospective study [12], as well as many retrospective studies [13, 14]. In CRC, however, the prognostic significance of intraperitoneal free cancer cells remains somewhat controversial. Several investigators have reported that overall survival of patients with either cytology or RT-PCR positive for the peritoneal washes were worse than the negatives in CRC patients, but most of them are small-scale study [15–18]. On the other hand, it was reported that intraperitoneal free tumor cells do not influence overall survival of the CRC patients [19, 20]. Furthermore, prognostic significance in terms of peritoneal recurrence-free survival of free cancer cells in the peritoneal washes of CRC patients remains to be elucidated [21].

In the present study, we quantitatively measured intraperitoneal free cancer cells using dual marker qRT-PCR and compared peritoneal recurrence between CRC and GC patients with curative resection. We found that exfoliation of free cancer cells into the peritoneal cavity occurs in CRCs essentially to the same extent as in GCs, but the rate of peritoneal recurrence was remarkably low in the former. Possible reason for the low-peritoneal recurrence in the CRC patients as compared with GCs will be discussed.

Materials and methods

Patients

A total of 128 CRC patients and 131 GC patients were enrolled in this study. All patients underwent operation at the Department of Gastroenterological Surgery, Aichi Cancer Center Central Hospital. In CRCs, total 128 patients include 126 primary cancer (64 colon cancer and 62 rectal cancer patients) and two patients with peritoneal recurrence disease. These patients underwent curative and non-curative operation from June 2001 to August 2003. Median follow-up period was 672 days, ranging from 119 to 1,147 days. The population included 19 patients with synchronous liver metastases, six patients with peritoneal metastases at laparotomy, four patients with positive cytology (three primary and one recurrence), and three patients with distant metastases (two lungs and one bone). Lymph node metastases were observed in 63 patients. There were 12 patients with T1 (mucosal to submucosal invasion), 93 patients with T2 (muscularis propria to subserosal invasion), 18 patients with T3 (serosal invasion), and three patients with T4 (invasion to adjacent tissues). The definitions of pT category (depth of cancer invasion) of the UICC classification for GC and for CRC are different. Therefore, the pT category used in this study was graded according to the UICC classification for GC based on the histological examination of resected specimens to compare CRC and GC using the same criteria. Among these 128 CRC patients, curative operations with R0 resection were performed in 102 patients, some of whom (42 patients) had lymph node metastasis. There were 12 patients with T1, 83 patients with T2, five patients with T3, and two patients with T4. Patient characteristics are shown in Table 1.

In GCs, the population included 126 patients with curative resection and five patients with peritoneal metastasis. These patients underwent operation from July 2001 to August 2003. Median follow-up period was 669 days, ranging from 141 to 1,306 days. Population characteristics of 126 GC patients with curative resection are also summarized in Table 1. Preoperative and intraoperative chemotherapy and radiation therapy were not performed in this series. The sites of the recurrence were judged based on

Table 1 Characteristics of colorectal cancer and gastric cancer patients enrolled in this study

	Colorectal cancer patients		Gastric cancer patients	
	All patients[a] n = 126 (%)	Curative operation n = 102 (%)	All patients[a] n = 132 (%)	Curative operation n = 126 (%)
Age (mean)	63.3	61.6	62.9	62.5
Gender				
Male	71 (56.3)	48 (47.1)	89 (67.4)	84 (66.7)
Female	55 (43.7)	54 (52.9)	43 (32.6)	42 (33.3)
Depth of tumor invasion[b]				
T1	12 (9.5)	12 (11.8)	50 (37.9)	50 (39.7)
T2	93 (73.8)	83 (81.4)	39 (29.5)	38 (30.2)
T3	18 (14.3)	5 (4.9)	35 (26.5)	33 (26.2)
T4	3 (2.4)	2 (2.0)	8 (6.1)	5 (4.0)
Lymph node metastasis				
Positive	66 (52.4)	42 (41.2)	65 (49.2)	59 (46.8)
Negative	60 (47.6)	60 (58.8)	67 (50.8)	67 (53.2)
Histologic type				
Well	10 (7.9)	10 (9.8)	19 (14.4)	18 (14.3)
Moderately	103 (81.7)	85 (83.3)	35 (26.5)	35 (27.8)
Poor	13 (10.3)	7 (6.9)	78 (59.1)	73 (57.9)
Hepatic metastasis				
Positive	19 (15.1)	0 (0)	1 (0.8)	0 (0)
Negative	107 (84.9)	102 (100)	131 (99.2)	126 (100)
Distant metastasis				
Positive	3 (2.4)	0 (0)	1 (0.8)	0 (0)
Negative	123 (97.6)	102 (100)	131 (99.2)	126 (100)
Visible peritoneal metastasis				
Positive	6 (3.2)[c]	0 (0)	5 (3.8)	0 (0)
Negative	122 (96.8)	102 (100)	127 (96.2)	126 (100)

[a] All patients; patients with curative and non-curative operation

[b] T1; mucosal to submucosal invasion, T2; muscularis propria to subserosal invasion, T3; serosal invasion, T4; invasion to adjacent tissue (pT category based on the UICC classification for gastric cancer)

[c] Four primary with synchronous peritoneal metastasis and two recurrence with metachronous metastasis

radiological or cytopathological evidence. Local recurrences of rectal cancer were distinguished from the peritoneal metastases, because of possibly inadequate local excision or unresected lymphatic permeation. Patient's written informed consent was obtained from all patients examined in this study.

Cell lines

In this study, ten GC cell lines including GCIY, MKN-28, MKN-45, MKN-74, HSC-43, GLM-1, GLM-2, GLM-4, NUGC-4, and KATO-III, and ten CRC cell lines including LS174T, COCM-1, COLM-1, COLM-2, COLM-3, COLM-4, COLM-5, COLM-6, CaCo-2, and HT-29 were used to compare CEA mRNA expression between CRC and GC cells. Primary mesothelial cells were chosen as negative controls. Human CRC cell lines, LS174T and COCM-1, and GC cell lines, NUGC-4, GCIY, MKN-28, MKN-45, and MKN-74, were obtained from RIKEN cell bank (Tsukuba, Japan). GLM-1, GLM-2, GLM-4, COLM-1, COLM-2, COLM-3, COLM-4, COLM-5, and COLM-6 cell lines were established in our laboratory from liver metastasis [22, 23]. HSC-43 was kindly provided by Dr. Yanagihara (National Cancer Center Research Institute, Tokyo, Japan). These cell lines were cultured in the same method as described previously [22].

Peritoneal washes

Peritoneal washes were obtained during laparotomy. At the beginning of each operation, 100 ml saline was introduced into the Douglas cavity and paracolic cavity near the tumors. After gentle stirring, these fluids were aspirated into the sterile tube. One half of each wash was sent to the

Division of Cytology at the Central Clinical Laboratory, Aichi Cancer Center Hospital for routine cytopathology with conventional Papanicolaou staining. The other half of the wash was sent to the Division of Oncological Pathology, Aichi Cancer Center Research Institute to measure CEA, and cytokeratin 20 (CK20) mRNA levels. Intact cells collected from the lavages by centrifugation at 1,800 rpm for 10 min were washed with phosphate buffer saline (PBS), dissolved in ISOGEN-LS, RNA extraction buffer (Nippon Gene, Tokyo, Japan) and stored at –80°C until analysis.

cDNA synthesis

Frozen peritoneal wash samples and cell lines in ISOGEN-LS were thawed and total RNA was extracted using guanidinium–isothiocyanate–phenol–chloroform method. Since cells are usually few in wash fluids, we added 2 μl of glycogen solution (20 mg/ml) (Boehringer, Mannheim, Germany) per tube as a carrier to improve RNA recovery before isopropanol precipitation. Extracted total RNA (up to 5 μg) was incubated with 50 ng of random hexanucleotide primer (Invitrogen, Carlsbad, CA, USA) in a volume of 9 μl for 10 min at 70°C. After chilling on ice, 4 μl of five fold synthesis buffer, 2 μl of 100 mM dithiothreitol, 4 μl of 2.5 mM each dNTP, and 1 μl of SuperScript II RNase H^- reverse transcriptase (200 U/μl, Invitrogen) were added. The reaction mixture was incubated at 42°C for 40 min and terminated by heating at 70°C for 15 min. The resultant first-strand cDNA was stored at –80°C until analysis.

Real-time quantitative RT-PCR

Single-step real-time qRT-PCR was performed using CEA- and CK20-specific oligonucleotide primers and two fluorescent hybridization probes (donor and acceptor) on the LightCycler instrument (Roche Diagnostics, Mannheim, Germany). To quantify and prove the integrity of the isolated RNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also analyzed by real-time RT-PCR using the hybridization probe method. The sequences of the primers and probes for CEA, CK20, and GAPDH used in this study are the same as described previously [12, 24]. All primers and probes were synthesized and purified by reverse-phase HPLC at Nihon Gene Research Laboratories (Sendai, Japan).

Amplification by PCR using a LightCycler proceeded in a 10 μl volume consisting of master mix containing Taq DNA polymerase, dNTP mixture and buffer (LightCycler DNA Master hybridization probes, Roche Diagnostics), 4.0 mM $MgCl_2$, 0.5 μM sense and anti-sense primer, 0.4 μM of each probe, and 1 μl of template cDNA in the LightCycler capillaries. Before amplification, primer elongation was blocked by adding 0.1 μl of anti-Taq DNA polymerase antibody (TaqStart antibody, Clontech Lab., CA, Palo Alto, USA) to the reaction mixture at room temperature for 5 min. Antibody was inactivated at 95°C for 90 s and then CEA and CK20 was amplified by 50 cycles at 95°C (0 s) for denaturation, 50°C (55°C for CK20) (10 s) for annealing, and 72°C (10 s) for extension. The same temperature profile was used to amplify GAPDH except for the extension step, which was 72°C for 20 s. Six external CEA and CK20 mRNA standards were prepared by tenfold serial dilution ($1–10^5$ cells) of cDNA equivalent to 1×10^6 COLM-2 cells (a colon cancer cell line that highly expresses CEA and CK20) spiked into 1×10^7 peripheral blood leukocytes. Each run consisted of external standards, a negative control without a template and patient samples with unknown mRNA concentrations. The higher CEA and CK20 mRNA value of two washes (Douglas cavity and Paracolic cavity) from each patient was selected. If at least one CEA (CK20) mRNA value from the two washes was above the cut-off value, the patient was considered positive for CEA mRNA.

Cut-off value for CEA and CK20 mRNA

A cut-off value for CEA mRNA (0.1) was previously determined based on the Receiver Operating Characteristic (ROC) curve analysis performed as a retrospective study of GC patients using qRT-PCR. CEA mRNA value more or less than 0.1 was judged as positive or negative for qRT-PCR, respectively. A cut-off value for CK20 mRNA (0) was determined as reported previously [25].

Statistical analysis

The CEA and CK20 mRNA values and the mRNA positivity rates among each pT category were compared using the Kruskal–Wallis test and Fisher's exact test, respectively. Survival was analyzed by Kaplan–Meier curves with death and a clinical diagnosis of peritoneal recurrence as endpoints. Cancer deaths resulting from other types of metastasis in the absence of clinical signs of peritoneal recurrence were treated as censored. Multivariate analysis using the Cox regression hazards model identified independent prognostic factors. Tumor grade, lymph node metastasis, and depth of tumor invasion were selected as covariates, along with CEA mRNA status.

Results

CEA and CK20 mRNA level in the peritoneal washes of colorectal and gastric cancer patient

Real-time qRT-PCR method allowed sensitive and quantitative detection of CEA (CK20) mRNA ranging from 1 (10) to 1 × 10^5 COLM-2 colon carcinoma cells expressing CEA and CK20. In peritoneal washes, CEA mRNA was detected in 29 (23.0%) of 126 CRC patients, but not in the benign counterparts. The average values of CEA mRNA (T1: 1.1, T2: 24.7, T3: 340.3 and T4: 106.8) (Fig. 1a) and CK20 mRNA (T1: 0, T2: 19.8, T3: 20.7 and T4: 728.0) in CRC patients were correlated with the depth of tumor invasion according to the pT category of UICC classification for GC. Similar correlation was observed in GC cases for average CEA mRNA values (T1: 0.07, T2: 5.6, T3: 48.8 and T4: 8800.0) (Fig. 1b) and CK20 mRNA values (T1:0, T2: 0.97, T3: 10.7 and T4: 282.5). Positivity rate for CEA mRNA and CK20 mRNA with the depth of tumor invasion (Fig. 2) were not significantly different between CRC and GC patients at any stages (CEA, T1: $P = 0.83$, T2: $P = 0.13$, T3: $P = 0.09$, T4: $P = 0.5$; CK20, T1: $P > 0.99$, T2: $P = 0.73$, T3: $P = 0.45$, T4: $P > 0.99$), indicating that in CRC patients, tumor cells also exfoliated into the peritoneal cavity from primary tumor at a level comparable to that of GC patients in terms of tumor cell numbers and incidence. Based on eight patients with benign disease and six CRC patients with macroscopic peritoneal deposits, sensitivity of qRT-PCR (CEA and CK20) and cytology was calculated to be 100% (6/6) and 50% (3/6), respectively, and specificity was 100% (8/8) and 100% (8/8), respectively.

CEA and CK20 mRNA expression in colorectal cancer and gastric cancer cell lines

To compare CEA and CK20 mRNA expression between CRC and GC, we measured the mRNA of ten CRC and ten GC cell lines by qRT-PCR. Mean (±SD) CEA/GAPDH ratios in CRC and GC cell lines were 170.4 ± 189 (range 0.31–490.4) and 225.8 ± 326 (range 0–798.7), respectively. CEA mRNA was not detected in primary mesothelial cells as negative control. Although CEA mRNA expression of some poorly differentiated GC cell lines was lower than that of CRC cell lines, average CEA and CK20 mRNA expression per cell base was not significantly different between gastric and CRC cell lines ($P = 0.3$ and 0.16, respectively) (Fig. 3a, b, c and d).

Clinicopathological features of CEA and CK20 mRNA positive colorectal cancer patients

Table 2 shows the clinicopathological features of CEA mRNA-positive patients among 126 primary CRC patients. The univariate analysis showed that CEA mRNA positivity in the peritoneal washes correlated with the depth of tumor invasion (pT) ($P < 0.0001$), peritoneal metastasis ($P < 0.0001$), histology ($P < 0.0001$), hepatic metastasis ($P < 0.0001$), and lymphatic metastasis ($P = 0.004$).

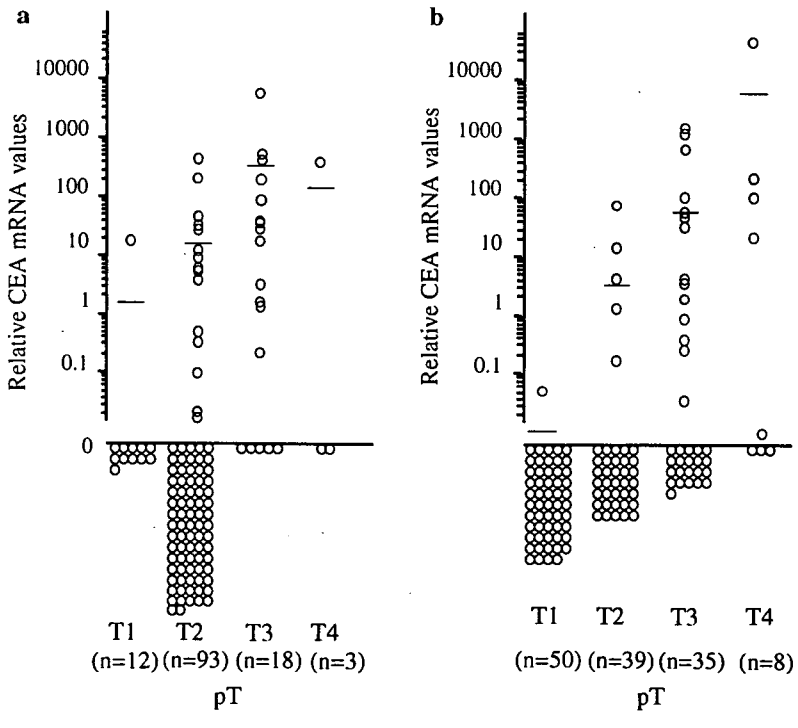

Fig. 1 Relative CEA mRNA values of peritoneal washes from colorectal cancer patients (**a**) and gastric cancer patients (**b**) measured by qRT-PCR according to the depth of tumor invasion (pT category)

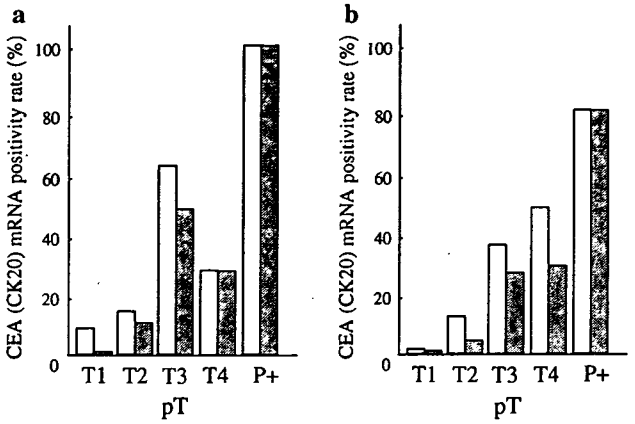

Fig. 2 CEA and CK20 mRNA positivity rate of peritoneal washes from colorectal cancer patients (a) and gastric cancer patients (b). CEA mRNA (*white bar*), CK20 mRNA (*gray bar*). P+ indicates six patients with synchronous and metachronous peritoneal metastasis. Positivity rate for CEA mRNA and CK20 mRNA with pT category was not significantly different between colorectal and gastric cancer patients at any stages ($P > 0.09$)

Among these, however, only histology ($P = 0.01$) and pT ($P = 0.002$) remained a significant covariate correlating CEA mRNA by multivariate analysis (Logistic analysis). In CK20 mRNA-positive CRC patients, only pT ($P = 0.004$) and lymphatic metastasis ($P = 0.008$) remained significant by multivariate analysis (data not shown).

Prognostic significance of CEA mRNA in peritoneal washes of colorectal and gastric cancer patients

The recurrence patterns of CRC and GC patients who underwent curative resection is shown in Table 3. The recurrence rate was almost the same between colorectal and GC patients (13.7% = 14/102 vs. 15.1% = 19/126), but the site of recurrence differed remarkably. In GC patients, peritoneal recurrence accounted for more than half of the recurrences (10/19), whereas virtually no peritoneal recurrence was observed in CRC patients. Overall survival was significantly worse ($P = 0.008$) in CEA mRNA-positive patients and marginally worse ($P = 0.08$) in CK20 mRNA-positive patients than the mRNA-negative patients in CRC patients (Figs. 4a, 5a), although the extent of significance is less than that of GC cases (Fig. 4c). Peritoneal recurrence-free survival of CEA mRNA-positive GC patients was also significantly worse than the negatives (Fig. 4d), but, this was not the case at all with CRCs in which no peritoneal recurrence was observed in CEA and CK20 mRNA-positive patients (Figs. 4b, 5b). Even three patients who were classified as positive by conventional cytology have not developed peritoneal metastasis in the CRC cases.

Multivariate analysis of prognostic factors

A Cox regression analysis with overall survival as an endpoint was performed to determine independent prognostic factors among covariates including tumor grade, pT, lymph node metastasis, cytology, and CEA mRNA status of peritoneal washes of patients who underwent curative resection. Although CEA mRNA of peritoneal washes ($P = 0.04$) was an independent prognostic factor in GC patients, lymph node metastasis ($P = 0.008$), but not CEA mRNA of peritoneal washes ($P = 0.23$), proved to be an independent prognostic factor in CRC patients (Table 4).

Discussion

As for genetic markers for detection of micrometastasis in CRCs, a number of candidates genes have been reported. Among these, CEA and CK20 is reportedly the most useful genetic marker for RT-PCR to detect free tumor cells in the peritoneal lavage in CRC patients [16, 26]. In fact, we found that CRC cell lines expressed CEA and CK20 mRNA at a similar level. CEA mRNA values in the peritoneal washes of CRC patients were also comparable to CK20 mRNA. Furthermore, neither CEA nor CK20 mRNA was detected in the peritoneal washes of eight patients with benign disease and of the peripheral blood leukocytes from ten healthy volunteers. These findings indicate that CEA and CK20 mRNA can be equally used as reliable parameters for the number of intraperitoneal tumor cells in CRC patients.

To date, few survival analyses were performed with an adequate sample size specific to peritoneal washes in CRC patients [16–20]. In the present study, to resolve some controversy on the prognostic significance of intraperitoneal free tumor cells in CRC patients, we conducted above-mentioned, dual marker qRT-PCR analysis on the relationship between free tumor cells and peritoneal recurrence with a sufficient sample size. We here demonstrated that the incidence of peritoneal recurrence was not different between CEA (CK20) qRT-PCR-positive and -negative CRC patients. Previously, Yamamoto et al. and Kanellos et al. reported that the peritoneal recurrence rate was significantly increased in patients with positive cytology than the negatives and that cytology was an independent prognostic predictor of cancer-specific survival [21, 27]. In contrast, Vogel et al. reported in their immunohistochemical study that microscopic intraperitoneal free tumor cells do not influence survival time after R0 resection in CRC patients [19]. Our present results are consistent with the Vogel's work. This is probably because the detection sensitivity of Vogel's work (62–73%) and ours (60%) were much higher than with conventional cytology (6–30%) in

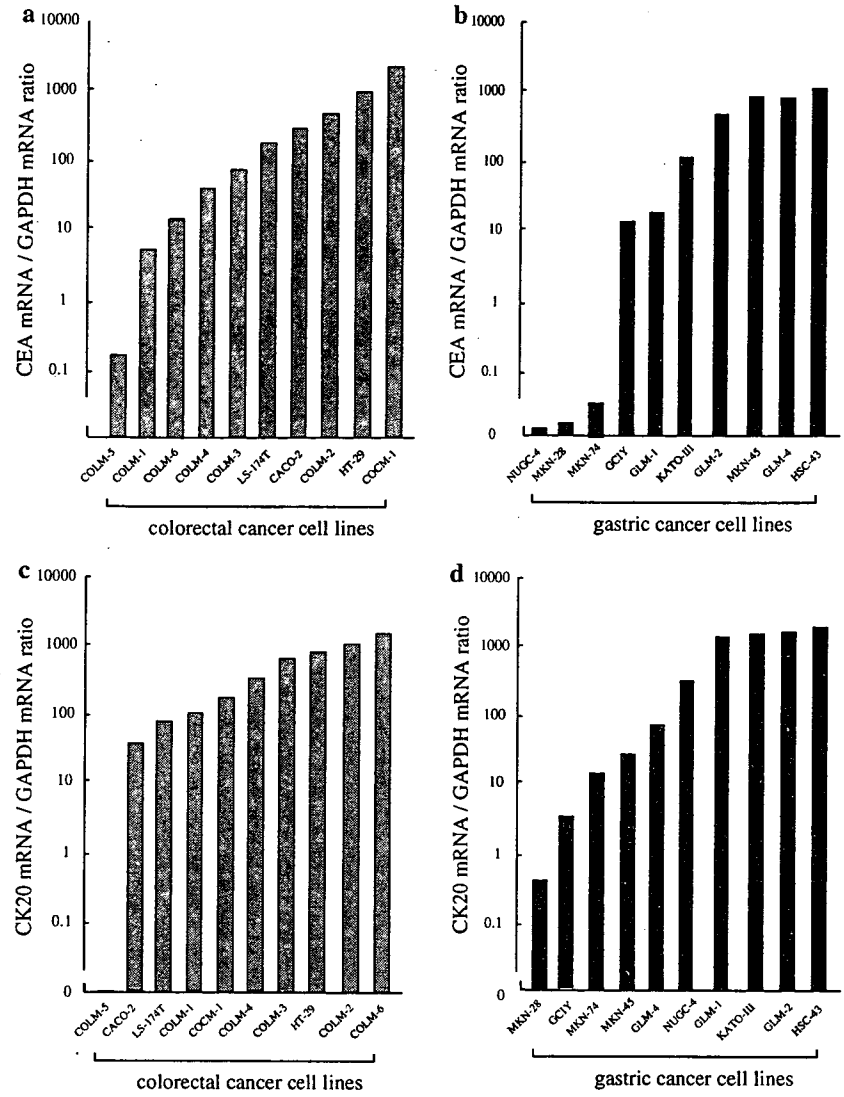

Fig. 3 Comparison of CEA and CK20 mRNA expression between colorectal (**a, c**) and gastric cancer cell lines (**b, d**). Relative CEA mRNA expression was calculated as CEA mRNA value relative to GAPDH mRNA value (CEA/GAPDH ratio). Average CEA and CK-20 mRNA were not significantly different between colorectal and gastric cancer cell lines ($P = 0.30$ and 0.16, respectively)

patients with serosal invasion. Abundant free tumor cells detectable by low-sensitive cytology may lead to at least in part peritoneal recurrence, but a small number of intraperitoneal free tumor cells detected only by high-sensitive immunohistochemistry (Vogel's work) or qRT-PCR (our study) do not result in peritoneal recurrence in CRC patients, suggesting that prognostic significance depends on the number of disseminated intraperitoneal free tumor cells.

The most important finding in the present study results from comparative analysis of the intraperitoneal free tumor cells between CRC and GC patients. We clearly demonstrated that although CRC cells exfoliated into the peritoneal cavity at a level similar to GC in terms of incidence and cell number, the patient outcome was completely different between the two cancers. Peritoneal recurrence occurred in ~50% of the CEA mRNA-positive GC patients, but never at all in CRC patients with curative resection. In the present study, indeed, overall survival was worse in CEA (CK20) mRNA-positive CRC patients than negatives similar to the previous report by Vogel et al. [19], but multivariate survival analysis showed that only lymph node metastasis was an independent prognostic factor, suggesting that shorter overall survival with CEA (CK20) mRNA-positive CRC patients is associated with lymph node metastasis, not peritoneal recurrence. These results strongly suggest that the rare peritoneal recurrence in CRC patients is not due to low incidence or a small number of intraperitoneal free cancer cells, but to the low-metastatic potential of intraperitoneal CRC cells, in quite contrast to GC cells.

A number of pathological factors and genes such as adhesion molecules have been reported to be associated with metastasis in CRCs [28]. Among these factors, specific histological type such as poorly differentiated adenocarcinoma and mucinous carcinoma have a known

Table 2 Univariate and multivariate analysis of 126 colorectal cancer patients

	Univariate			Multivariate		
	CEAmRNA-	CEAmRNA+	*P*	Hazard ratio	95% CI	*P*
Histologic type						
Well + moderately	93	20	<0.0001	1	1.562–34.12	0.01
Poor + mucinous	4	9		7.0		
Depth of tumor invasion[a]						
T1–T2	90	15	<0.0001	1	2.269–35.87	0.002
T3–T4	7	14		8.7		
Hepatic metastasis						
Negative	89	18	<0.0001	1	0.443–8.177	0.3
Positive	8	11		2.0		
Lymphatic metastasis						
Negative	57	7	0.004	1	0.706–7.052	0.2
Positive	40	22		2.2		
Distant metastasis						
Negative	96	27	0.07	1	0.024–105.3	0.9
Positive	1	2		1.4		
Cytology						
Negative	96	27	0.07	1	0.147–83.22	0.5
Positive	1	2		2.7		

[a] T1; mucosal to submucosal invasion, T2; muscularis propria to subserosal invasion, T3; serosal invasion, T4; invasion to adjacent tissue (based on the UICC classification for gastric cancer)

Table 3 Pattern of recurrence of colorectal cancer and gastric cancer patients who underwent curative resection

	Colorectal cancer patients				Gastric cancer patients			
	Total	(%)	PCR+	PCR–	Total	(%)	PCR+	PCR–
Recurrence[a]	14	(13.7)	5	9	19	(15.1)	9	10
Liver	10	(9.8)	3	7	3	(2.4)	0	3
Lung	4	(3.9)	0	4	1	(0.8)	1	0
Peritoneum	0	(0)	0	0	10	(7.9)	8	2
Local	2	(2.0)	1	1	1	(0.8)	0	1
Bone	2	(2.0)	1	1	2	(1.6)	1	1
Lymph node	0	(0)	0	0	7	(55.6)	3	4
No recurrence	88	(86.3)	11	77	107	(84.9)	10	97
Total	102	(100)	16	86	126	(100)	19	107

[a] Primary recurrence site

tendency to disseminate into the peritoneal cavity as compared with differentiated adenocarcinoma [29–32]. In fact, CEA mRNA positivity rate of peritoneal washes in CRC patients was found to be significantly higher in the (poorly differentiated + mucinous) type (69% = 9/13) than differentiated type (18% = 20/113) in the present study. To elucidate whether the peritoneal metastatic potential differs depending on the histological type of CRC, we tested peritoneal metastatic capability of two moderately differentiated colonic cancer cell lines (COLM-2 and COLM-3), one poorly differentiated colonic cancer cell line (COLM-5), one mucinous colonic cancer cell line (COLM-6), and two moderately differentiated and poorly differentiated GC cell lines (MKN-28 and GCIY) in nude mouse xenograft models. Our preliminary results showed that COLM-2 and COLM-3 cell lines produced a small peritoneal metastasis at omentum 2 months after intraperitoneal injection (average tumor weight 0.42 and 0.28 g, respectively), whereas COLM-5 and COLM-6 cell lines generated relatively large intraperitoneal metastatic tumors (average tumor weight 2.05 and 1.25 g, respectively). GC cell lines (MKN-28 and GCIY) formed large metastatic tumors in the

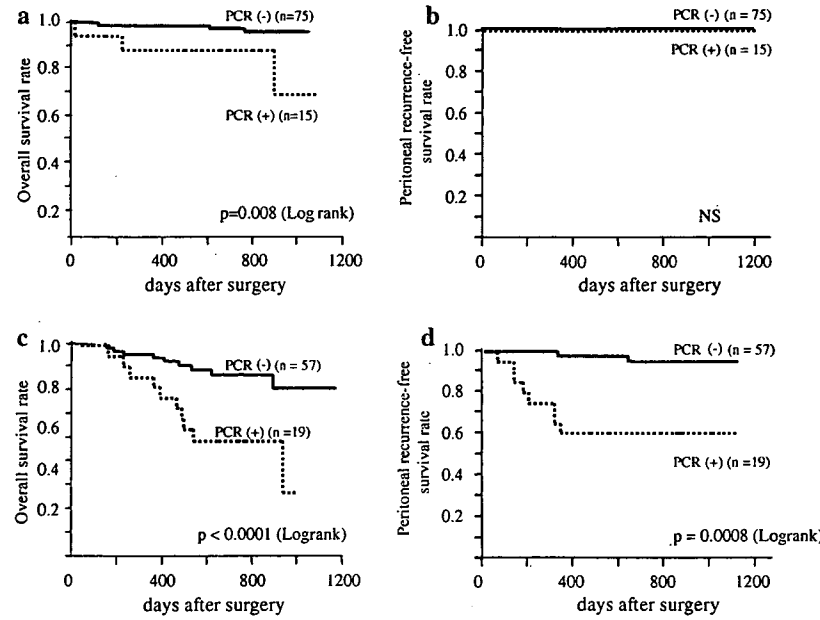

Fig. 4 Comparison of overall (**a, c**) and peritoneal recurrence-free survival (**b, d**) between 90 curatively resected advanced colorectal (**a, b**) and 76 advanced gastric cancer patients (**c, d**) with positive and negative for CEA mRNA

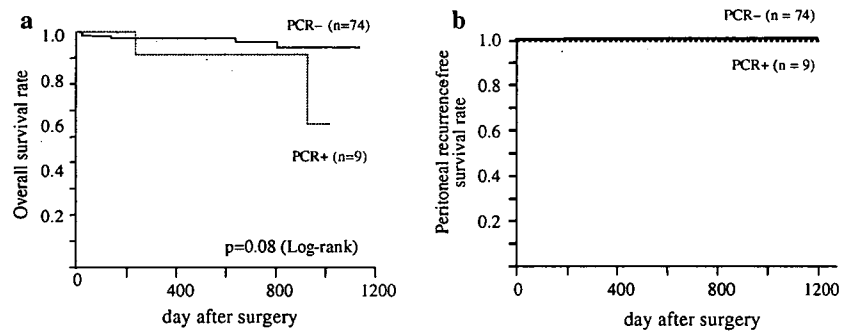

Fig. 5 Overall survival (**a**) and peritoneal recurrence-free survival (**b**) of 83 curatively resected advanced colorectal cancer patients with positive and negative for CK20 mRNA

Table 4 Multivariate analysis of prognostic factors in 102 colorectal cancer and 126 gastric cancer patients who underwent curative resection with overall survival as an end point

Variable	Colorectal cancer (n = 102)			Gastric cancer (n = 126)		
	Risk ratio	95% CI	P	Risk ratio	95% CI	P
Histology						
Differentiated	1		0.20	1		0.09
Undifferentiated	2.00	0.662–5.193		3.63	0.804–16.388	
Depth of tumor invasion[a]						
T1–T2	1		0.30	1		0.001
T3–T4	1.72	0.651–4.195		12.2	2.646–56.510	
Lymph node metastasis						
Negative	1		0.008	1		0.86
Positive	2.67	6.25e200		1.12	0.327–3.780	
CEA mRNA						
Negative	1		0.23	1		0.04
Positive	1.72	0.651–7.680		2.81	1.034–7.626	

[a] T1; mucosal to submucosal invasion, T2; muscularis propria to subserosal invasion, T3; serosal invasion, T4; invasion to adjacent tissue (based on the UICC classification for gastric cancer)

peritoneum (average tumor weight 2.92 and 3.48 g, respectively). These results indicate significantly slower metastatic growth potentials of moderately differentiated colonic cancer cell lines than poorly differentiated/mucinous colonic cancer cell lines and GC cell lines (unpublished results), suggesting the low-metastatic ability of differentiated type CRC cells experimentally, even if it is still preliminary. Taken together, these results strongly suggest that low-peritoneal metastatic potential of CRCs are largely due to the low-metastatic potential of well to moderately differentiated cancers, a major subtype of CRCs.

In conclusion, we quantitatively demonstrated for the first time that the rare peritoneal recurrence in CRC patients is not due to the low incidence of exfoliation into the peritoneal cavity and small number of intraperitoneal free tumor cells. The present clinical findings and experimental evidence with CRC and GC cell lines further suggest that intraperitoneal free CRC cells have a low risk for generating peritoneal recurrence if the number of free tumor cells is limited within a range detectable only by sensitive qRT-PCR and histology of tumor cells is restricted to the well to moderately differentiated type. Understanding the reason for the low-metastatic potential of the differentiated type CRC s may provide fresh insight into the development of a new therapeutic modality against gastric as well as CRC peritoneal metastasis.

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特集 Stage IV 大腸癌と診断したらどうするか

肝転移を伴う Stage IV 大腸癌の治療方針

Treatment policy for stage IV colorectal cancer with liver metastases

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肝転移を伴うStage IV大腸癌では，まず原発巣の治癒切除を行い，次いで肝転移巣に対する治療を行う．

肝転移に対する治療は転移巣の完全切除が第一選択である．完全切除が行えない症例では切除と凝固療法を併用したり，neoadjuvant chemotherapy を行い腫瘍縮小を待って切除を行う．残肝量が少なくなる症例では門脈塞栓を行って肝肥大を，あるいは二期手術を計画して転移巣の切除を目指す．

切除以外では，ラジオ波や凍結などの凝固療法が有望である．外国では放射線外照射も行われる．

はじめに

当院における1965～1999年の大腸癌手術例は3,235例で，そのうち414例(12.3%)が肝転移のためにStage IVとなった．これは全Stage IV症例中の56%に当たる．同時期の治癒切除例2,491例の術後の再発でも肝転移再発が7.5%で最も多い再発であり，肝転移への対応は大腸癌治療の上で重要な位置を占める．

肝転移を伴うStage IV大腸癌に対する治療戦略は，原発巣が切除できるものは原発巣による症状があるものはもちろん，症状がないものについても持続する出血や将来おこるであろう狭窄を予防するためにまず原発巣を切除する．原発巣を切除して，遺残する転移巣を肝転移のみにすれば，肝切除を初めとする局所療法や全身化学療法などいろいろな治療法を選択できる．原発巣を切除できない症例に対しては全身化学療法を行う．

本稿では原発巣を切除した上での肝転移に対する局所療法について解説する．

I. 肝 切 除

1．肝転移切除の現状

肝転移無治療例では5年生存は期待できず[1)2)]，非切除例の5年生存率が5%以下であるのに対し，肝切除例の5生率は20%～50%[3)]で原発巣と

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Key words：大腸癌／肝転移／肝切除／凝固療法／切除不能肝転移の治療

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表1 肝転移切除後の予後不良因子(文献4より)

1．原発巣因子	3．肝転移切除後の予後因子：手術因子
根治度 C	断端陽性
リンパ節転移陽性(転移個数多)	tw＜10 mm
組織型 低分化／粘液	肝転移巣の遺残
ly 2～3	
budding あり	4．肝転移切除後の予後因子：背景因子
	術前遠隔転移
2．肝転移巣因子	肝転移時の他臓器転移
肝転移組織型 低分化／粘液	同時性
肝転移個数(多発)	無病期間＜1年
局在(両葉)	肝切除前 CEA 高値
肝転移程度(H_3)*	肝切除後 CEA 高値
腫瘍最大径	肝切除後 CA19-9 高値
衛星病変あり	
肝転移進展因子	
門脈腫瘍塞栓，肝静脈腫瘍塞栓	
胆管内腫瘍進展，門脈浸潤	
神経周囲浸潤	
腫瘍周囲偽皮膜形成	
liver cell entrapment	
肉眼型	
肝所属リンパ節転移陽性	

*大腸癌取扱い規約第6版による

同様に外科的完全切除以外に根治的な治療法はない．現在では肝以外の臓器に転移があっても，それが完全切除できれば肝転移，他臓器転移ともに切除することが多い．

肝切除後の再発は残肝再発が40%，次いで肺転移が20%に見られて[4]，肝切除後はこの2つの再発の予防法が現在の課題である．

肝切除後の予後に関係する因子を表1に示した．切除後の予後不良因子として異論がないのは，剝離断端の癌露出，肝所属リンパ節転移陽性，衛星病変など肝転移進展因子陽性，肝外転移であり，さらに予後に大きく影響を与える因子として肝転移個数，肝切除断端距離(tw)，肝転移切除後のCEA値とCA19-9値などがあげられる．大腸癌取扱い規約第7版で新たに採用された肝転移の進行度分類における予後規定因子は，肝転移巣の転移個数と最大径および原発巣のリンパ節転移度である[3)5)]．

肝転移巣切除後の残肝再発についても根治の可能性があれば，初回手術と同じ基準で切除の対象となる．再肝切除の成績は5生率は30～50%[6)-9)]と良好であり再肝切除は肝転移の治療成績を向上させる重要な因子である．

2．肝切除の適応

肝切除の適応基準として，①外科切除のリスクが良いこと，②原発巣がコントロールされていること，③適度な残肝量を残して肝転移巣が完全に切除できること，④肝転移以外の遠隔転移がないこと，⑤肝所属リンパ節転移がないことが一般にあげられ，さらに肝転移巣の条件として，⑥肝転移個数4個以下，⑦切除断端距離(tw)を10 mm以上切除できることが手術のstandard criteriaとされてきた．肝転移症例のうち，切除可能なものは25～50%である[10)]．

以下，手術に関係するいくつかの問題点について考察する．

3．切除時期

同時性肝転移に対しては，原発巣と同時に切除する者と，まず原発巣を切除して，その後3ヵ月ほど待って肝転移巣を切除する者とがある．

同時切除を行う理由は，①経過観察をしても予後に変わりはなく，②術中超音波検査で小病巣も

把握できるから遅らせる必要はない，③3ヵ月遅らせることで肝転移巣からの二次転移の危険性がある，④多発肝転移に対し3ヵ月遅らせることで肝切の時期を逃すなどである．

異時切除を行う理由は，①肝転移状況の精査，②肝外転移の精査，③肝切除を同時に行うことによる死亡率や合併症率が高い，④同時に行うと微小転移を診断できないことがあるので隠れた転移巣が明らかになるまで待って肝切除を行う，というものである．また，最大径2 cm 以下の小さな病変では他に検査で描出できない微小病変が隠れていることがあるので，3ヵ月待って新しい病変の出現を待って一括して切除し，一方最大径5 cm 以上のものや肝静脈，下大静脈，肝門に近いものは切除の機会を逃さず直に手術する意見もある[11]．また安野ら[11]は，1年以内の再発例も3ヵ月間経過観察をするとしている．

Capussotti ら[12]は予後不良因子(男，原発巣リンパ節転移3個以上，隣接臓器浸潤)を有する症例では neoadjuvant chemomtherapy を行い，肝転移の進展がない症例に肝切除を行うとしている．

原発巣切除は肝転移巣切除に先行して，あるいは同時に行われるのが一般的であるが，Mentha ら[13]は多発肝転移や大きな肝転移では原発巣手術後に肝転移巣が進展する危険性があるので，大腸癌の狭窄症状がない症例ではまず neoadjuvant chemotherapy を行い，6コース(肝転移奏効例では3コース)後にまず肝切除を行い，その3～8週後に原発巣の手術を行う方法を提唱している．

4．肝転移個数と大きさから見た適応

一般に単発例は多発例よりも予後が良く，転移個数の多いものはそれ以下のものと比べて予後は不良である．Sasson ら[14]は転移個数が多いものでは切除断端距離を十分に取れないことが予後不良の理由ではないかと推測している．一方，転移個数と予後とは関係ないとする報告も少なくない．

肝転移巣最大径は3 cm ごと，4 cm で分類するもの，5 cm で分けるものなど幾つかの分類があり，小さいものの予後が良いとされるが，切除断端の距離(tw)を十分にとって切除すれば予後には関係ないとする報告もある．

5．切除断端距離(tw)

切除断端に癌が露出しているものの予後は不良である．さらに，切除断端が陰性でも tw が10 mm 以下の症例の予後は不良とされ，tw を10 mm 以上取ることは肝切除時の主要な目標であった．

一方，肝転移巣周辺の衛星病変の頻度は少なく[15][16]，存在する範囲も転移巣からわずかの距離であり，肝切除前の転移存在診断が確実に行えるようになった現在では断端(－)であれば切除距離には関係しないとする報告も多い[17]-[19]．

6．肝切除術式

肝切除術式は局所切除，区域切除，葉切除，拡大葉切除(3区域切除)などが行われ，大きく分けて，解剖学的肝系統切除と非解剖学的肝局所切除とに分類される．基本術式が解剖学的系統切除か非解剖学的部分切除かについては解決していない．

系統切除派の意見は，3 cm 以上の転移巣では衛星病変や肝転移進展因子が高頻度に出現するが，系統切除はこれらを一括して切除できて予後が良いとするものである．

部分切除派の意見は，肝転移巣では非連続性進展の頻度は低いので，術中超音波検査を行って断端(－)あるいは surgical margin を十分にとって局所切除を行えば局所切除で良く，残肝量を多くして再肝切除に備えるというものである．さらに部分切除の予後は系統切除と差はない，合併症が系統切除と比べて少ないと主張している．ただし局所切除症例は小さな転移巣が選ばれるという selection bias を考慮しなくてはならない．

7．肝所属リンパ節郭清

肝門部リンパ節転移は他部位へ転移している

signalであるとされその予後は不良で，1988年にUS Registry of Hepatic metastasesが肉眼的リンパ節転移(859例中24例)を切除して5生例が1例のみだったこと(Eliasら[20]より引用)から肝門リンパ節転移例は手術適応外とすることが世界的なconsensusとなった．

Eliasら[20]は最近の報告から肝所属リンパ節の転移率1～7%，転移切除例の5生率0～27%，系統的en blocリンパ節郭清を行った場合の転移率13～25%，5生率0～42%とまとめている．またRodgersら[21]は15報告例から145例の肝門部リンパ節転移陽性例を集積し，その5年生存率は5%であると報告した．Katoら[4]の報告では転移陽性18例の5生率は12.5%である．転移陽性例の5生率は低いものの肝門部リンパ節郭清を行うことで生存期間が延長するという報告も多い．Sakaguchiら[22]は初回手術のリンパ節転移陽性例の予後は悪いが，再肝切除時に肝門部リンパ節に転移がある例では郭清効果があるとしている．

Jaeckら[23]は160例に郭清を行い17例のリンパ節転移をArea 1(肝十二指腸靱帯・膵後部)とArea 2(総肝動脈・腹腔動脈転移)に分け，Area 1の転移例(8例)の3生率38%に対してArea 2の転移例(9例)では1年以上生存例がなかった．

郭清範囲について，Eliasら[24]は100例の肉眼的に転移陰性と判断したもののうち14例が顕微鏡的に転移陽性であり，肝門部～腹腔動脈まであらゆる部位に転移していたと報告しており，Kaneら[25]はisosulfan blue dyeを腫瘍周囲に注入して注入前に判らなかったリンパ節の染色を7例中3例(全例転移なし)に認めた．転移リンパ節が必ずしも腫大しているわけではないので郭清する以上はJaeckの言うAreal・2の系統的郭清を行うべきと考えられる．

転移の有無に関係なく郭清例と非郭清例の生存率を比べると両者間に差はなく[4]，予防的肝門部リンパ節郭清の意義についての評価は定まっていない．山本ら[26]はリンパ節転移例の予後は不良であり，予防的郭清は残肝再発が多い大腸癌肝転移では(再肝切除が行いにくくなり)むしろ弊害が多いと述べている．Jaeckら[23]は全例にroutineに行うのではなく，リンパ節転移の危険性が高い転移個数3個以上，転移巣がsegment 4および5に存在するもの，低分化腺癌例に行うとしている．

8．補助療法

肝切除後の再発は残肝再発が最も多く次いで肺転移再発が多い．したがって，肝切除後の残肝再発と，肺転移を主とした他臓器転移の予防が重要となる．肝切除後の補助療法は主に残肝再発の予防を目的として5-FUを主体とした肝動注療法が行われてきたが，肝局所再発は抑えるものの全身転移が押さえることができず，補助療法を行わなかったものと生存率は変わらなくて有効性は確立していない．そこで肝動注と全身化学療法の併用が試みられているが，まだ長期経過例で有用性を示す報告はない．厚生労働省の第三次対がん総合戦略事業・がん臨床研究事業(H16-032)では，肝切除後の補助療法として現在進行大腸癌に対して最も有効とされる5-FU／leucovorin／oxaliplatin併用療法(mFOLFOX6)の有効性を検証する比較試験を開始した．

9．切除不能肝転移例の対応

1) Neoadjuvant chemotherapy

Bismuthら[27]は5FU／folinic acid／oxaliplatin併用療法を切除不能53例に行い，腫瘍の縮小を待って局所的に根治切除が可能となった時点で肝切除を行った．46例は肉眼的根治切除が行え，7例は非治癒切除となったので門脈塞栓術により残肝の増大を図り化学療法を続けて第2期切除を行った結果5生率は40%，残肝再発66%，肝外再発47%と報告した．

これ以後，腫瘍が大きい，多発肝転移，転移場所が悪いなどの理由で切除不能となった症例に対してneoadjuvant chemotherapyを行い，腫瘍の縮小が得られたものに切除を行った多くの報告がある．Neoadjuvant chemotherapyによる肝転移の切除率は腫瘍縮小率に相関し，完全切除率

は3.4～47%である[28][29]．肝動注療法は全身化学療法と比べて延命効果では変わりはないが，腫瘍縮小率が高いのでneoadjuvantとして有効である[30]．全身化学療法と肝動注療法の併用の報告も多い．最近の報告では，全身化学療法に用いられる薬剤は5-FU, leucovorin, oxaliplatin, CRT-11, gefitinibなどであり[28][29]，肝動注ではFUDR／dexamethason[31]，mitomycin C[32]，CDDP[33]などである．Neoadjuvant chemotherapy後に肝切除可能となった症例でははじめから切除可能だった症例よりも再発率は高い[34]．

2）残肝量が少ない症例の対応

肝切除の適応に残肝の予備能が十分であることがある．残肝容積が25%以下の場合90%が肝機能障害を起こし[35]，肝炎や肝硬変など慢性の肝疾患がある患者，あるいは大量の化学療法を受けた患者では40%以上の残肝量が要るとされる[36]．正常肝の場合，非腫瘍部の肝を40%以上温存できない場合[37]，あるいは非癌部肝切除率が50～70%[38]に及ぶ場合には術前に片側の門脈塞栓術あるいは門脈結紮を行い予定残肝容積増大を促す適応となる．術前に門脈塞栓を行った場合の肝増量は8%である[36]．Selznerら[39]は門脈結紮と肝動注を行い，6ヵ月後も腫瘍の増大がなくて11例中4例に完全切除が可能となったと報告している．

3）Two stage operation

Bismuthら[27]およびAdamら[40]は多発肝転移で切除不能と思われる症例に対して術前化学療法，門脈塞栓術を行ってもone stageで完全切除ができない場合はtwo stage手術を提唱している．第Ⅰ期手術ではできるだけ多くの転移巣を切除して，残肝の肥大を待つ間全身化療で遺残腫瘍の増大と転移を防ぎ，肝肥大が起きて完全切除ができるようになれば第Ⅱ期手術を行うものである．3生率は35%であるが第Ⅰ期手術ではなかった術死が15%にあり合併症もⅡ期手術では多い．

4）肝切除と凝固療法の併用

多発転移に対しては，RFAと肝切除の併用が行われており，Curleyら[41]の報告では肝切除の5生率65%，肝切除とRFAの併用36%，RFA単独22%であった．注意しなくてはならないのは併用療法が肝切除単独やRFA単独治療と比べて術後の合併症が20%前後と高く，手術死亡もあることである[41][42]．

Eliasら[43]は肝切除量が大きくなって残肝機能を維持できない21症例に対して，切除線上の転移巣をRFAで焼灼して壊死させ，その壊死部上で肝を切除し，小転移巣はRFAで焼灼した結果，術死の1例を除いて切離線上の局所再発はなかった(median follow up 19.4ヵ月)と報告している．

II. 凝固療法

凝固療法にはエタノール注入，マイクロ波熱凝固療法，ラジオ波熱凝固療法(RFA)，凍結療法などがある．

熱凝固療法は，本邦では1990年頃からマイクロ波熱凝固療法が行われていたが，1995年以降は主にRFAが行われるようになった[44]．RFAの1回の凝固で治療できる範囲は3 cm以下であり[45]，それよりも大きいものは凝固を繰り返すこと[41]で対応する．3 cm以下の症例の局所再発率は低い[46]．転移巣への到達ルートは経皮的，腹腔鏡下，開腹の3ルートがある．経皮的は侵襲が少ないが再発率が高く，合併症率も高い．完全凝固できた場合の5年生存率は20%前後[41][47]で，Machiら[48]は30%と高い生存率を報告しており，現在では肝切除に次ぐ治癒率が期待されている．

凍結療法は本邦ではあまり行われないが，Seifertら[49]の報告では凍結療法単独または凍結療法と手術の併用で26%の5年生存率を上げている．RFAよりも合併症率が高いが，RFAが3 cm以下のものが対象となるのに対して大きいものにも有効である[50][51]．8 cm以上になると局所制御率は低くなる[50]．

また，肝切除の補助療法として断端陽性例あるいはTWが1 cm未満の症例に同部の凍結療法，マイクロ波凝固壊死療法やラジオ波熱凝固療法などの凝固療法を行う報告もある．

III. 放射線外照射

肝転移に対する放射線外照射は全肝照射となるために本邦ではほとんど行われないが，外国では試みられており[52]，最近は転移巣に限局した高線量照射が可能となって[53]，肝動注あるいは全身化学療法を併用して良い成績をあげている[54)55]．Malikら[52]のreviewによれば，照射による肝障害は35 Gy以上で出現し，重度な肝障害の出現を5％以下に留める照射線量は肝の照射範囲が1／3で55 Gy，全肝照射では40 Gyとされ，全肝照射の安全域は30～35 Gyである．外照射30 Gyでは奏効率90%，生存期間4ヵ月であり，外照射25 Gy＋5FUでは奏効率90%，生存期間10ヵ月であることから，Malikら[52]は全肝に30 Gy＋腫瘍部に10～20 Gyの照射と5FUの併用を推奨している．その他，腫瘍部への陽子線治療[56]あるいは放射線sphereを肝動脈内へ投与・塞栓する報告もある[57]．

おわりに

肝転移を伴うStage IV大腸癌に対してはまず原発巣を切除し，肝転移巣に対しては切除を第一選択とする．肝転移切除不能例に対しては抗がん剤の肝動注療法を行うのが今までの治療戦略だった．現在の検討課題はいかにして切除例の治癒度を高め，いかにして切除不能例を治癒切除が可能な状況にするかという点である．

抗がん剤の肝動注療法はneoadjuvant therapyも含めて肝転移に対する重要な局所治療であるが，他稿に譲った．

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