

Detection of circulating tumor cells in patients with gastrointestinal tract cancer using RT-PCR and its clinical implications

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Abbreviations: CEA, carcinoembryonic antigen; RT-PCR, reverse transcriptase-polymerase chain reaction; EGC, early gastric cancer; AGC, advanced gastric cancer; B2 or C2, modified Dukes' classification of colorectal cancer

Abstract

To investigate the relationship between the presence of circulating tumor cells in different stages of gastrointestinal tract cancer and the subsequent relapse or distant metastasis, circulating levels of CEA mRNA was serially examined at an interval of 10.6 ± 4.5 or 13.7 ± 3.0 months in gastric or colorectal cancer patients, respectively. CEA mRNA was measured by means of RT-PCR amplification as an indicator for micrometastatic malignant cells. Seven of twenty-nine respectable gastric cancer patients (24.1%) [EGC: 2/9 (22.2%), AGC IIIa: 1/5 (20.0%), AGC IIIb: 4/15 (26.6%)] were positive for CEA mRNA on the initial test and 10 of 29 patients (34.4%) [EGC: 2/9 (22.2%), AGC IIIa: 1/5 (20.0%), AGC IIIb: 7/15 (46.7%)] were positive on a follow-up test. Only in AGC IIIb, the positive rate for CEA mRNA increased about twice and 6 of 7 positive cases (85.7%) relapsed within 2.6 ± 2.4 months after the follow-up test. In colorectal cancer, 4 of 19 patients (21.1%) [B2: 1/6 (16.7%), C2: 3/13 (23.0%)] were positive on the initial test and 10 of 19 patients (52.6%) [B2: 4/6 (66.7%), C2: 6/13 (46.2%)] were positive on a follow-up test

showing an increase in positive rates during a follow-up, however, no significant correlation between CEA mRNA positivity and subsequent relapse was demonstrated. These results suggest that an early tumor cell dissemination may occur in gastrointestinal tract cancer without subsequent relapse, however, the serial regular examination of CEA mRNA level may contribute to predicting a subsequent relapse in AGC IIIb in gastric cancer.

Keywords: gastric cancer, colorectal cancer, carcinoembryonic antigen

Introduction

The prognosis of patients with malignant tumors depends largely on the absence or presence of metastasis. Metastatic tumor cells being probably more invasive and genetically more unstable than those of a primary site (Ruddon, 1995). Metastasis is a multistep process involving numerous host-tumor interactions, in which hematogenous spread of cancer cells from the primary tumor plays a central role (Liotta and Stetler-Stevenson, 1991).

RT-PCR is the most widely used molecular method for the detection of circulating tumor cells (Pelkey *et al.*, 1996). Among the target genes of this aim for RT-PCR, CEA mRNA seems to be the most frequently used for the detection in the blood of patients with gastrointestinal carcinomas (Funaki *et al.*, 1996; Mori *et al.*, 1996; Jonas *et al.*, 1996; Mori *et al.*, 1998; Castells *et al.*, 1998), since it is expressed in almost all epithelial cells, including cancer cells, but not in nonepithelial cells (Shively and Beatty, 1985).

Most of the available data related to the CEA mRNA detection indicate that the presence of circulating tumor cells correlates with the presence of distant metastasis (Funaki *et al.*, 1996; Mori *et al.*, 1996; Jonas *et al.*, 1996; Mori *et al.*, 1998; Castells *et al.*, 1998) and is expected to be associated with a poor prognosis (Hardingham *et al.*, 1995; Mori *et al.*, 1998). But it is still uncertain of its clinical relevance, because a relatively high proportion of non-metastatic gastrointestinal cancer patients was also positive for CEA mRNA (Mori *et al.*, 1996; Mori *et al.*, 1998; Castells *et al.*, 1998). In our previous study, circulating tumor cells were also detected in both EGC (33.3%) and AGC (18.8%) and in distant metastatic disease (100%) (Noh *et al.*, 1999). Interestingly, two

patients turned into positive result developed an early relapse or multiple distant metastases one or two months later, respectively. Similar cases were reported in lung cancer, in which the patients with positive CEA mRNA without metastasis, developed metastasis within 6 months of analysis (Castaldo *et al.*, 1997). Long-term follow-up data on gastrointestinal tract cancer, however, has not yet been available, and it is, therefore, uncertain whether the presence of circulating tumor cells reliably indicates poor prognosis. In the present long-term follow-up study using CEA mRNA detection by means of RT-PCR, we investigated whether the presence of circulating tumor cells in different stages of gastrointestinal tract reliably indicates subsequent recurrence or distant metastasis.

Patients and Methods

Patient populations

The study population consisted of 57 patients with gastrointestinal tract cancers including 36 gastric (14 EGC and 22 AGC) and 21 colorectal cancer (6 Dukes' B, 15 Dukes' C), undergoing surgery and follow-up from June, 1996 to June, 2000 at Hanyang University Kuri Hospital (Kuri, South Korea). The initial test was performed 11.5 ± 11.1 months (in gastric cancer) and 6.0 ± 1.4 months (in colorectal cancer) after operation respectively in order to avoid a transient introduction of tumor cells into the circulation due to surgical manipulation (Mori *et al.*, 1996) and to escape from the influence of chemotherapeutic agents. After a follow-up study we were able to obtain experimental data from 29 gastric (9 EGC and 20 AGC) and 19 colorectal (6 Dukes' B, 13 Dukes' C) cancer patients. The tumor, node, metastases system (TNM) classification was used for gastric cancer and Dukes' classification for colorectal carcinoma. In this study, patients with distant metastasis were excluded from the beginning, because several reports have demonstrated that CEA mRNA positivity significantly correlates with the presence of distant metastasis, similar to our previous study (Mori *et al.*, 1998; Castells *et al.*, 1998; Noh *et al.*, 1999). All patients who had undergone curative surgery followed postoperative pathological staging. Clinical follow-up studies include abdominal and/or pelvic CT or ultrasonography, gastro- or colonoscopy, chest PA, serum CEA, and bone scan. Fifteen normal healthy subjects were tested as a negative control. Informed consent was obtained from each patient.

Cell lines and peripheral blood samples

As described in our earlier study (Noh *et al.*, 1999), a colon cancer cell line, Colo 201 (purchased from Korean Cell Line Bank, Seoul, South Korea) was used to test the potential sensitivity of RT-PCR in the detection of

circulating tumor cells. Mononuclear cells in 14 ml of peripheral blood obtained from normal healthy donors and patients were separated by Ficoll-Hypaque.

RNA preparation

Total RNA from peripheral mononuclear cells or Colo 201 cells was extracted by thiocyanate, phenol-chloroform method described by Chomczynski and Sacchi (Chomczynski and Sacchi, 1987). RNA was spectrophotometrically quantified at 260/280 nm, and its quality was examined in 1% agarose gel to find intact 28S and 18S RNAs.

RT-PCR

Five micrograms of total RNA were pre-incubated for 10 min at 70°C. After chilling on ice, first-strand cDNA was synthesized in a 25 μ l reaction mixture containing 5 μ l of 5 \times reverse transcriptase reaction buffer (Promega, Madison, WI, USA), 200 μ M dNTP, 100 μ M of random hexamer, 50 units of RNasin (Promega, Madison, WI, USA), 2 μ l of 0.1 M dithiothreitol, and 200 units of Moloney-Murine Leukemia Virus (MMLV) reverse transcriptase (Promega, Madison, WI, USA). The mixture was incubated at 37°C for 60 min, heated at 95°C for 10 min, and then chilled on ice. CEA specific oligonucleotide primers used for nested PCR were synthesized according to published sequence information (Schrewe *et al.*, 1990; Gerhard *et al.*, 1994): A, 5'-TCTGGAACCTCT-CCTGGTCTCTCAGCTGG-3'; B, 5'-TGTAGCTGTTGC-AAATGCTTTAAGGAAGAAGC-3'; and C, 5'-GGGCCA-CTGTCGGCATCATGATTGG-3'. The first and second PCR products exhibited a 160 bp fragment and a 131 bp fragment respectively. The nested PCR was performed according to the method described by Gerhard *et al.* (1994). The first PCR was carried out in a reaction mixture (80 μ l) containing 8 μ l of 10 \times reaction buffer [500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C), and 1.0% Triton X-100], 2.5 unit of *Taq* polymerase (Promega, Madison, WI, USA), 2.5 mM MgCl₂, 200 μ M dNTP, 25 pmole primers A and B, and 8 μ l of template cDNA. Thirty-five cycles of amplification were performed in a thermocycler (Robocycler 40: Stratagene, La Jolla, CA, USA) at 95°C for 1 min, and 72°C for 2 min with a final extension step performed for 10 min at 72°C. Five microliters of the reaction mixture were transferred into a second eppendorf tube containing 200 μ M dNTP, 1.5 mM MgCl₂, 2.5 units of *Taq* polymerase and 20 pmoles of primer B and C. Thirty-five cycles were performed at 95°C for 1 min, 69°C for 1 min, and 72°C for 1 min, with a final extension step for 10 min at 72°C.

To ensure sufficient purity of RNA for RT-PCR, a separate round of PCR with primers specific for the gene β -actin cDNA was carried out in each case. The primer sequences for β -actin cDNA were as follows: 5'-TGACGGGGTCAACCCACTGTGCCCATCTA-3' and

5'-CTAGAAGCATTGCGGTGGACGATGGAGGG-3'.

Each series of RT-PCR included an RNA-omitted sample to rule out the possibility of the genomic DNA contamination, normal healthy blood sample as a negative control, and a total RNA sample of Colo 201 cells as a positive control.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviations. For statistical analysis, age was dichotomized at the median. Differences in relative frequency of CEA mRNA positive cases and correlation between qualitative variables were evaluated using Fisher's exact test. We considered *P* value of 0.05 as the criterion for statistical significance.

Results

Sensitivity of tumor marker CEA mRNA detection

Each dilution of control cancer cells, Colo 201, was mixed with 10^6 mononuclear cells obtained from the peripheral blood of healthy volunteers. As described in our earlier study (Noh *et al.*, 1999), CEA mRNA at a concentration as low as 10^1 Colo 201 cells/ 10^6 normal mononuclear cells could be detected with the same sensitivity as described (Gerhard *et al.*, 1994; Mori *et al.*, 1995).

Results of RT-PCR and clinical data

None of the 15 normal subjects were positive. Among the 29 gastric cancer patients at different stages, 7 patients (24.1%) [EGC: 2/9 (22.2%), AGC IIIa: 1/5 (20.0%), AGC IIIb: 4/15 (26.6%)] were positive for CEA mRNA on the initial test and 10 patients (34.4%) [EGC: 2/9 (22.2%), AGC IIIa: 1/5 (20.0%), AGC IIIb: 7/15 (46.7%)] on follow-up test (Table 1, 2). Representative results are shown in Figure 1. The initial study was done 11.5 ± 11.1 months after curative operation and the follow-up test 10.6 ± 4.5 months after the initial test. During the follow-up, there was no change in positive rates in EGC and AGC IIIa, but positive rates were increased from 26.6% (4 of 15) to 46.7% (7 of 15) in AGC IIIb (Table 2). In addition, none of the patients with EGC and AGC IIIa developed recurrence or distant metastases irrespective of positivity, but 7 of 15 AGC IIIb patients (46.7%) relapsed and 6 of them (85.7%) were positive cases (Table 3). Most of AGC IIIb showing negative results did not develop recurrence or distant metastasis (7 of 8, 87.5%) except one patient, who relapsed one month after the follow-up test (Table 3). Regarding characteristics of relapsed patients, they were all with AGC IIIb and positive for CEA mRNA except one patient, and relapsed within 2.6 ± 2.4 (range 1-8) months after the follow-up test. The average duration between operation date (OPD) and relapse was 25.7 ± 11.0 (range 16-49)

Table 1. Characteristics of gastric cancer patients

Patient	Age	Sex	Status (Initial \rightarrow FU*)	CEA mRNA (Initial test \rightarrow FU test†)
1	63	F	EGC NR	+ -
2	37	M	EGC NR	+ -
3	45	M	EGC NR	- -
4	38	M	EGC NR	- -
5	43	F	EGC NR	- +
6	42	M	EGC NR	- -
7	74	F	EGC NR	- +
8	55	M	EGC NR	- -
9	33	F	EGC NR	- -
10	67	M	IIIb Relapsed‡	- -
11	51	M	IIIb Relapsed	- +
12	52	M	IIIb Relapsed	- +
13	59	F	IIIb NR	- -
14	65	F	IIIa NR	- +
15	62	M	IIIb Relapsed	- +
16	65	M	IIIa NR	- -
17	63	M	IIIa Relapsed	- +
18	60	F	IIIa NR	- -
19	58	M	IIIb Relapsed	+ +
20	34	F	IIIb NR	- -
21	65	M	IIIb Relapsed	- +
22	55	M	IIIa NR	+ -
23	71	M	IIIb NR	- -
24	63	M	IIIb NR	+ -
25	49	F	IIIb NR	- -
26	58	F	IIIb NR	+ -
27	43	M	IIIb NR	+ -
28	63	M	IIIa NR	- -
29	45	M	IIIb NR	- +

CEA, carcinoembryonic antigen; EGC, early gastric cancer; NR, not relapsed; IIIa or IIIb, TNM (tumor, node, metastasis) stage; FU, follow-up; *followed up until June, 2000; † carried out 10.6 ± 4.5 months after the initial study; ‡ distant and local.

Table 2. Positive rates of CEA mRNA in gastric cancer

TNM stage	Initial* (%) (n=29)	FU† (%) (n=29)
EGC	2/9 (22.2)	2/9 (22.2)
AGC IIIa	1/5 (20.0)	1/5 (20.0)
AGC IIIb	4/15 (26.6)	7/15 (46.7)
Total	7/29 (24.1)	10/29 (34.4)

EGC, early gastric cancer; AGC, advanced gastric cancer; IIIa or IIIb, TNM (tumor, node, metastasis) stage; FU, follow-up; *The initial test was done 11.5 ± 11.1 months after curative operation.; † The follow-up test was done 10.6 ± 4.5 months after the initial test.

months.

In colorectal cancer, 4 of 19 (21.1%) [B2: 1/6 (16.7%), C2: 3/13 (23.0%)] were positive on the initial test and 10 of 19 (52.6%) [B2: 4/6 (66.7%), C2: 6/13 (46.2%)] on follow-up test (Table 4, 5). Representative results are shown in Figure 2. The initial study was done 6.0 ± 1.4

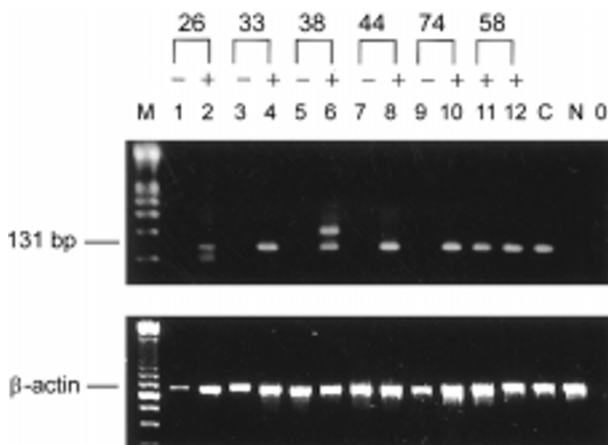


Figure 1. Representative RT-PCR data for CEA mRNA in gastric cancer patients. M, 100 bp ladder DNA size marker; Lanes of the odd numbers, initial test; Lanes of the even numbers, follow-up test; C, Colo 201 as a positive control; N, normal healthy subject; O, RNA-omitted sample as a negative control; Number 26, 33, 38,44, 74, and 58, patient No. 5, 12, 15, 17, 21, and 19, respectively (Table 1); β actin serves as an internal control. The additional bands at 100 and 200 bp regions in lane 2 and 6, respectively, suggest the presence of further subclones or splice variants of CEA in these samples.

Table 3. Results of follow-up test in AGC IIIb cases

	CEA mRNA	
	Positive (%) (n=7)	Negative (%) (n=8)
Relapsed* (n=7)	6 (85.7)	1 (12.5)
NR (n=8)	1 (14.3)	7 (87.5)

CEA, carcinoembryonic antigen; AGC, advanced gastric cancer; IIIb, TNM (tumor, node, metastasis) stage; NR, not relapsed; *distant and local.

months after curative operation and the follow-up test 13.7 ± 3.0 months after the initial test. There was an increase in positive rates during follow-up in all stages tested (Table 5), however, the relapsed was noted only in Dukes' C2 patients, slightly little more in patients with negative CEA mRNA (Table 6). The relapsed patients were all with Dukes' C2 and developed irrespective of positivity or negativity within 1.4 ± 0.8 (range 1-3) months after the follow-up test. The average duration between OPD and relapse was 21.8 ± 8.0 (range 13-32) months. In summary, CEA mRNA positivity significantly correlates with the cancer stages [AGC IIIb vs. EGC and AGC IIIa in gastric cancer ($P=0.005$); C/B2 and C/C2 vs R/B2 and R/C2 in colorectal cancer ($P=0.005$)], but not with age or sex. However, it was noted only in AGC IIIb that CEA mRNA positivity was correlated with the subsequent relapse. In colorectal cancer, positive results were shown more in patients with colon cancer than in those with rectal cancer, but relapse developed more in rectal cancer than in colon cancer, suggesting no correlation between positivity of CEA mRNA and subsequent relapse.

Table 4. Characteristics of colorectal cancer patients

Patient	Age	Sex	Status (Initial \rightarrow FU*)	CEA mRNA (Initial test \rightarrow FU test†)
1	59	M	C/B2 NR	--
2	64	F	C/B2 NR	-+
3	60	M	C/B2 NR	-+
4	57	M	R/B2 NR	--
5	46	M	R/B2 NR	++
6	67	F	C/B2 NR	-+
7	50	F	R/C2 NR	--
8	57	M	R/C2 Relapsed‡	--
9	41	M	R/C2 Relapsed	--
10	38	F	C/C2 NR	-+
11	60	F	R/C2 Relapsed	--
12	34	M	C/C2 NR	-+
13	34	M	C/C2 NR	++
14	45	F	R/C2 NR	++
15	48	F	R/C2 Relapsed	-+
16	24	M	R/C2 NR	--
17	26	M	R/C2 NR	+ -
18	57	F	R/C2 NR	--
19	63	F	C/C2 Relapsed	-+

CEA, carcinoembryonic antigen; C/B2 or C/C2, colon cancer Dukes' B2 or C2; R/B2 or R/C2, rectal cancer Dukes' B2 or C2; NR, not relapsed; FU, follow-up; *followed up until June, 2000; † carried out 13.7 ± 3.0 months after the initial study; ‡ distant and local.

Table 5. Positive rates of CEA mRNA in colorectal cancer

Dukes' classification	Initial* (%) (n=19)	FU† (%) (n=19)
B2	1/6 (16.7)	4/6 (66.7)
C2	3/13 (23.0)	6/13 (46.2)
Total	4/19 (21.1)	10/19 (52.6)

CEA, carcinoembryonic antigen; FU, follow-up; *The initial test was done 6.0 ± 1.4 months after curative operation; † The follow-up test was done 13.7 ± 3.0 months after the initial test.

Discussion

Studies on the detection of micrometastasis to bone marrow or lymph nodes in solid cancers revealed that it can be used as a useful method for predicting prognosis because the risk for tumor relapse increases with the presence of micrometastasis (Dearnaley *et al.*, 1991; Neville, 1991; Schlimok *et al.*, 1991; Pelkey *et al.*, 1996). However, the clinical significance of micrometastasis to blood has not been determined yet in spite of many related studies (Hardingham *et al.*, 1995; Funaki *et al.*, 1996; Jonas *et al.*, 1996; Mori *et al.*, 1996; Castells *et al.*, 1998; Mori *et al.*, 1998). It was reported in 1970 that the vast majority of circulating tumor cells shed from solid tumors do not survive in the blood, and only about 0.1% live long enough to form a distant metastasis (Fidler, 1970). Hence, the presence of tumor cells in the blood does not necessarily indicate that distant metastasis will

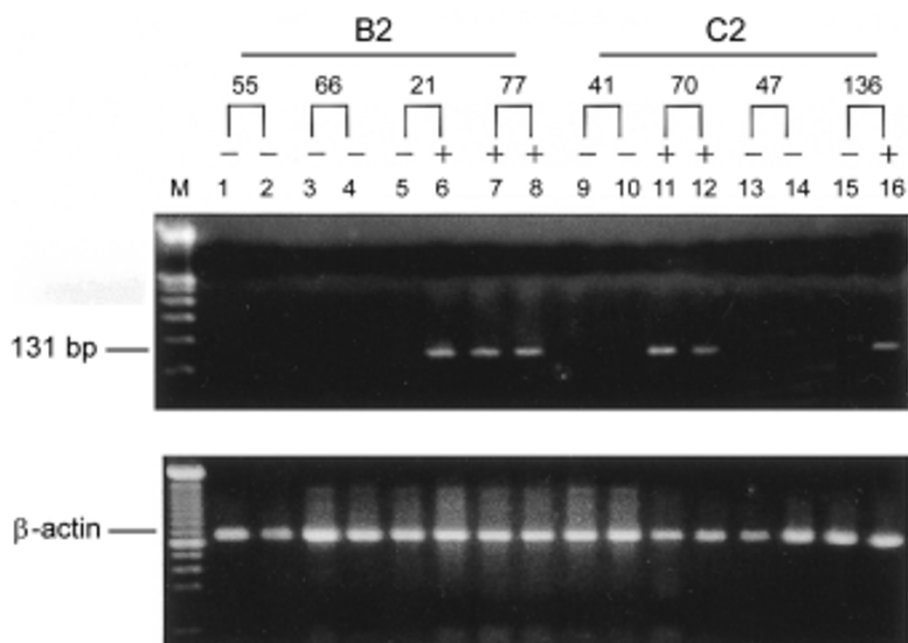


Figure 2. Representative RT-PCR data for CEA mRNA in colorectal cancer patients. M, 100 bp ladder DNA size marker; Lanes of the odd numbers, initial test; Lanes of the even numbers, follow-up test; B2 or C2, Dukes' classification; Number 55, 66, 21, 77, 41, 70, 47, and 136, patient No. 4, 1, 2, 5, 7, 13, 9, and 19, respectively (Table 4); β actin served as an internal control.

Table 6. Results of follow-up test in colorectal cancer C2

	CEA mRNA	
	Positive (%) (n=6)	Negative (%) (n=7)
Relapsed [†] (n=5)	2 (33.3)	3 (42.9)
NR (n=8)	4 (66.7)	4 (57.1)

CEA, carcinoembryonic antigen; C2, Dukes' classification; NR, not relapsed; [†]distant and local.

form (Ruddon, 1995) and more than a simple presence of tumor cells in the blood stream is involved in metastasis formation (Rinker-Schaeffer *et al.*, 1994; Ruddon, 1995; Pelkey *et al.*, 1996). Liotta and Stetler-Stevenson (1991), however, reported hematogenous spread of cancer cells from the primary site constitutes a key point in metastasis.

In our previous study (Noh *et al.*, 1999), we found that the identification of circulating tumor cells by detecting CEA mRNA using RT-PCR was feasible, as shown also by other studies (Funaki *et al.*, 1996; Jonas *et al.*, 1996; Mori *et al.*, 1996; Castells *et al.*, 1998; Mori *et al.*, 1998). Using this method, we found that metastatic gastric cancer patients tested were all positive for CEA mRNA in peripheral blood, but 33.3% of early gastric cancer patients were also positive. The current study also demonstrated that 22.2% of EGC and 66.7% of Dukes' B2 colorectal cancer at the follow-up test were positive for CEA mRNA (Tables 2 and 5), indicating that cancer cells can disseminate even in the early stage of

gastrointestinal cancer and supporting the hypothesis that gastrointestinal cancers might be considered as systemic diseases as breast cancer (Heiss *et al.*, 1995; Mori *et al.*, 1996).

Even though the durations of follow-up until June, 2000 of these EGC and Dukes' B2 colorectal cancer patients are 31.1 ± 12.7 (range 11-48) and 29.5 ± 4.3 (range 25-38) months, respectively, further follow-up with a larger population appears to be needed to elucidate whether the positivity for CEA mRNA in early-stage patients correlates with recurrence or distant metastasis.

Mori *et al.* reported the frequency of positive cases for CEA mRNA increased with advanced stage of disease in gastric or colorectal cancers (Mori *et al.*, 1996; Mori *et al.*, 1998). Interestingly, there have been some reports suggesting the correlation between positivity for CEA mRNA in the peripheral blood and subsequent relapse, *e.g.*, three of 4 positive patients with far-advanced pancreatic carcinoma developed recurrence after curative operation or liver metastasis after mass-reducing operation within 9 months after the analysis (Funaki *et al.*, 1996), two of 4 positive patients without the evidence of distant metastasis developed metastasis within a few months (Castaldo *et al.*, 1997). Also in our previous study, one patients with stage III gastric cancer who was negative for CEA mRNA initially and turned positive during follow-up, developed multiple bone metastasis one month later. Another stage III patient who was positive for CEA mRNA preoperatively revealed early relapse in two months. Most of the available data, however,

simply demonstrated the frequency of positive cases at the different stages of gastrointestinal cancers and small cases showing subsequent relapse, but did not reveal clearly the correlation between the presence of circulating tumor cells and the subsequent relapse due to the lack of long-term study (Funaki *et al.*, 1996; Jonas *et al.*, 1996; Mori *et al.*, 1996; Castells *et al.*, 1998; Mori *et al.*, 1998).

As the first step for the long-term study, we examined CEA mRNA expression in the peripheral blood of patients at around 12 or 6 months after surgery (in gastric or colorectal cancer, respectively), who underwent curative surgery and clinical follow-up (range 12-58 months). About 11 or 14 months after the initial test (in gastric or colorectal cancer, respectively) we performed the follow-up test and clinically followed up the patients until June, 2000. Our results showed that persistent positive cases were rare (4 of 48, 8.3%) as compared with the overall positive rates (Tables 2 and 5), suggesting that tumor cells shed from the primary site intermittently (Ghossein *et al.*, 1995) or circulate in clumps. This meant that inhomogeneous distribution of tumor cells within the circulation could prevent them from being sampled invariably (Jonas *et al.*, 1996). Meanwhile, the frequency of positivity increased in AGC IIIb and colorectal cancer with the duration of disease (Tables 2 and 5), suggesting that shedding of tumor cells increases with advanced stages and duration of disease as well, but the pattern seems to be different according to the kind of gastrointestinal cancers. Accordingly, these phenomena need to be exploited for illuminating the biology of tumor cell invasion into the blood stream. Surprisingly, all the relapsed but one were positive cases with AGC IIIb (6 of 7, 85.7%) and conversely most negative cases with AGC IIIb (7 of 8, 87.5%) did not relapse (Table 3). Only one patient showing negative result developed recurrence of disease in AGC IIIb. None of the patients with EGC or AGC IIIa relapsed irrespective of the results (Table 1).

By contrast, in colorectal cancer, these correlations were not noted. Increasing positive rates during follow-up did not indicate subsequent relapse. On the contrary the relapsed were noted rather more in the patients showing negative for CEA mRNA (Tables 5 and 6).

These results suggest that this analysis should be repeated at some interval to the patients who were diagnosed as being clinically recurrence-free or metastasis-free in AGC IIIb, although it seems to be not so useful in colorectal cancer.

In summary, early tumor cell dissemination occurs in gastrointestinal tract cancer without subsequent relapse and the tumor cells seems to shed from the primary site intermittently. However, the serial regular examination of CEA mRNA appears to predict subsequent relapse in AGC IIIb rather than in colorectal cancer. Finally, simple detection of circulating tumor cells using one target gene like CEA mRNA has limitation to be a reliable prognostic

marker. Therefore, search for the new markers to indicate that carcinoma cells in the bloodstream really form metastasis or concomitant use of other markers related to metastatic potential such as proteases, angiogenesis factors, cell motility regulators or cell adhesion molecules will be desirable to disclose the clinical implications of circulating tumor cells in solid tumors.

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