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Clinical Significance of Serum CEA Protein and CEA mRNA After Resection of Colorectal Liver Metastases

M. KIJIMA, S. TOGO, Y. ICHIKAWA, M. MIURA, S. YAMAGISHI, K. MATSUO, K. TANAKA, H. MASUI, T. ISHIKAWA, H. IKE and H. SHIMADA

Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Abstract. *Background: It is difficult to predict the recurrence of colorectal liver metastasis after curative hepatectomy. We investigated the relationship between subsequent metastasis and both CEA protein and CEA mRNA (TaqMan PCR) diachronic levels. Patients and Methods: The subjects were 30 patients with colorectal liver metastases. Serum CEA protein and CEA mRNA assays were performed every month after hepatectomy. Results: Metastasis recurred in 21 of the 30 patients. The CEA mRNA assay showed 26 cases with high (H) levels and 4 with low (L). Among the 15 patients whose protein levels were not elevated (NE group), 6 had recurrence; all 6 belonged to the mRNA H group. None of the 4 patients in the mRNA L group had recurrence. In the protein-elevated (E) group (DFI > 6 months) (n=7), mRNA was elevated in 5 cases (71.4%) 6 months before recurrence, whereas protein was elevated in 1 case. The sensitivity, specificity and accuracy of CEA protein/mRNA for identifying recurrence were 71.4/100, 100/44.4, and 80/83.3%, respectively. Conclusion: CEA mRNA is more sensitive than CEA protein in detecting recurrence. CEA mRNA may be useful for identifying high-risk groups or detecting recurrence at an early stage, when the CEA protein level is still low.*

The liver is the most common target organ for the metastasis of colorectal cancer, and therapeutic tactics for liver metastasis are therefore important (1). Curative hepatectomy for liver metastases, including radical resection after portal embolization or multistep liver resection, is reported to be the best method for improving the overall survival rate (2-4). After hepatectomy for liver metastasis, more than 50% of residual livers show recurrence (5). In a study by Nanko *et al.*, 56% of patients with colorectal liver metastases showed the histological presence of intrahepatic micrometastases, which are thought to be related to residual liver recurrence (6). Early detection of recurrence in residual liver is the critical first step for an early response, such as chemotherapy or preparation for re-resection of the metastasis. However, it is difficult to predict the recurrence of liver metastases after curative hepatectomy.

Carcinoembryonic antigen (CEA), an adhesion molecule belonging to an immunoglobulin superfamily (7), is expressed specifically in epithelial cells and is utilized as a target gene for detecting micrometastasis of cancer cells (8). Reverse transcriptase-polymerase chain reaction (RT-PCR) for CEA can detect occult cancer cells in the lymph nodes, bone marrow and peripheral blood (9-11). We previously reported that quantification of *CEA mRNA* in the peripheral blood of colon cancer patients, using real-time quantitative PCR (ABI PRISM 7700 Sequence Detection System, Perkin-Elmer Applied Biosystems, Foster City, CA, USA), is a good predictor of hematogenous recurrence of colon cancer (12). In the present study, we examined *CEA mRNA* expression levels in the peripheral blood of 30 patients with colorectal liver metastases, and investigated whether serum CEA protein or *CEA mRNA* levels after hepatectomy could help to predict subsequent metastases and detect them at an early stage.

Patients and Methods

Patients. This study included 30 patients with colorectal liver metastases (Table I), who were treated between 1998 and 2002 in the Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Japan. All subjects underwent curative hepatectomy. Based on the Japanese classification of colorectal carcinoma, the patients were classified as H1 (liver metastases limited to one lobe, n=10), H2 (4 metastases or fewer in both lobes, n=6), or H3 (5 or more metastases in both lobes, n=14). CT scans were performed before hepatectomy to evaluate for extrahepatic disease. Four cases (6, 22, 24 and 27) had extrahepatic metastases (lung or adrenal gland) resected. The other cases had disease limited to the liver at the time of hepatic resection. Intraoperative ultrasonography was performed on all

Correspondence to: Miyuki Kijima, Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukuura Kanazawa-ku, Yokohama 236-0004, Japan. Tel: +81-45-787-2650, Fax: +81-45-782-9161, e-mail: miiru@msa.biglobe.ne.jp

Key Words: TaqMan PCR, CEA mRNA, colorectal liver metastasis, recurrence.

Table I. *Relationship between CEA mRNA level and clinical outcome.*

case no.	age	sex	H	first hepatectomy number	first hepatectomy sm (mm)	recurrence residual liver	other sites	CEA protein postoperative*	CEA mRNA postoperative*	outcome	DFI * (months)	follow-up (months)
1	64	M	3	6	10	N	N	NE	L	alive	5.0	5.0
2	63	F	1	1	10	N	N	NE	L	alive	49.3	49.3
3	50	M	1	1	18	N	N	NE	L	alive	46.3	46.3
4	77	M	1	1	0	N	N	NE	H	alive	9.4	9.4
5	73	M	2	2	23	N	lymph nodes	NE	H	alive	11.4	22.5
6	71	M	1	2	1	Y	adrenal gland	NE	H	alive	11.7	15.7
7	51	F	3	13	0	Y	N	NE	H	alive	12.1	34.4
8	70	F	1	1	10	Y	N	NE	H	alive	16.9	16.9
9	66	M	1	1	5	N	N	NE	H	alive	32.3	32.3
10	53	F	3	5	3	N	lymph nodes	NE	H	alive	35.4	41.2
11	66	F	2	2	10	N	N	NE	H	alive	35.8	35.8
12	74	M	3	6	2	Y	local brain	NE	H	alive	3.7	12.5
13	62	M	2	4	0	N	N	NE	H	alive	9.9	9.9
14	68	M	3	5	14	Y	bone	E	H	alive	7.7	17.8
15	49	F	3	5	0	N	lymph nodes	E	H	alive	10.1	24.8
16	66	M	2	3	12	N	local	E	H	alive	38.9	48.5
17	47	F	2	1	0	Y	N	E	H	alive	8.4	20.4
18	55	M	3	5	0	Y	N	E	H	alive	9.8	35.6
19	52	M	3	10	1	N	N	NE	L	alive	45.4	45.4
20	54	F	2	2	30	Y	lung	E	H	alive	3.5	29.6
21	64	M	1	1	20	N	N	NE	H	alive	64.7	64.7
22	73	F	1	13	8	N	lymph nodes lung	E	H	alive	1.0	8.7
23	60	M	1	1	0	Y	dissemination lung	E	H	alive	2.6	7.7
24	40	F	3	7	4	Y	lung	E	H	dead	3.9	16.8
25	72	M	3	5	<5	Y	N	E	H	alive	12.2	24.0
26	59	F	3	17	<5	Y	lung	E	H	alive	3.2	31.2
27	55	F	3	7	<5	Y	lung	E	H	alive	3.8	11.2
28	71	F	3	20	<5	Y	lung bone	E	H	dead	4.2	7.1
29	80	M	1	1	<5	Y	dissemination	E	H	dead	4.8	9.9
30	67	F	3	17	<5	Y	N	E	H	alive	10.9	20.8

* DFI: disease-free interval
* postoperative: at the time of subsequent recurrence or the latest assay
L: low; H: high; high/low: cut-off point 0.9.
E: elevated (more than 2.1 ng/ml/30 days); NE: not elevated.

patients. The Institutional Review Board of the School of Medicine of Yokohama City University approved our study protocol, and documented informed consent was obtained from each patient.

Methods. Serum CEA protein and *CEA mRNA* assays were performed every month after hepatectomy. *CEA mRNA* assay started 0-42 months after hepatectomy. The median follow-up period was 25.2 months (range, 5.0 to 64.7 months). Serum CEA protein assay was performed by enzyme-linked immunosorbent assay (ELISA) (AIA-Pack, Tosoh Co., Yamaguchi, Japan; cut-off point, 5.7 ng/ml). The clinicians following up the patients were aware of the assay findings 1-2 months after measurement, but the routine CT scans were performed every 3 months after hepatectomy. So, neither the CEA protein nor *CEA mRNA* level influenced the intensity of surveillance for recurrence.

Blood samples. Ten ml of peripheral blood was obtained from each patient at each examination and stored in 1.5 ml EDTA-ACD solution at 4˚C. The samples were processed within 48 hours. The mononuclear cell fraction was separated from the samples using the Ficoll-Paque method of specific gravity separation (13). The total RNA of the fraction was extracted by QIAGEN (QIAGEN Pty Ltd., Victoria, Australia).

RT-PCR. Preparation of RNA from blood samples and quantitative RT-PCR (TaqMan PCR) were performed following methods described previously (12). cDNA was synthesized from 1 μg of total RNA. The quantitative assay of relative mRNA abundance was designed and optimized following the guidelines of Perkin-Elmer Applied Biosystems. During RNA amplification, cleavage of the probe by TaqDNA polymerase separates the reporter from the quencher, resulting in an increasing fluorescence signal. To standardize the amount of each sample of mRNA applied to the reaction, we utilized beta-actin, a housekeeping gene. To quantify the amount of specific mRNA in the samples, a standard curve was

generated for each run of the KATO-III gastric cancer cell line strongly expressing CEA. The relative expression levels of CEA were obtained by normalizing the amount of *CEA mRNA* divided by that of beta-actin (14, 15). The assay was determined to be able detect 10^1 contaminated cancer cells in 10^7 mononuclear cells.

Results

Diachronic change of CEA mRNA and CEA protein levels. We determined 0.9 as the cut-off point for *CEA mRNA*, as described previously (12). Patients whose expression levels were less than 0.9 were assigned to the low expression group, while those whose levels were 0.9 or above were considered to have high expression.

CEA mRNA and CEA protein levels were classified according to diachronic change. Two patterns of diachronic change appeared in the *CEA mRNA* levels of the 30 patients: high (H) (26 cases, 86.7%) and continuously low (L) (4 cases, 13.3%). Two patterns also emerged in diachronic change in CEA protein levels: elevated (E) (15 cases, 50.0%) and not elevated (NE) (15 cases, 50.0%). We defined CEA protein levels of more than 2.1 ng/ml/30 days as "elevated" (16).

During the observation period, 21 cases (70%) had subsequent recurrence (Rec group) in the liver or in other organs such as the lung, local sites, or bone (Table I).

Correlations between both CEA protein and CEA mRNA patterns and recurrence. The correlations between both CEA protein and *CEA mRNA* patterns and recurrence is shown in Table II (a, b). None of the 4 cases in the L group of mRNA suffered recurrence in the follow-up period after hepatectomy, which ranged from 5.0 to 49.3 months. In the mRNA H group, 21 out of 26 cases (80.8%) had subsequent recurrence. All 15 cases in the CEA protein E group showed subsequent recurrence. Of the 15 cases in the CEA protein NE group, 6 (40.0%) had subsequent recurrence; all 6 cases belonged to the H group of *CEA mRNA* (Table II (c)). The sensitivity of CEA protein and *CEA mRNA* for identifying subsequent recurrence was 71.4% and 100%, respectively; specificity was 100% and 44.4%, and accuracy was 80% and 83.3%.

Comparison of the timing of CEA mRNA and CEA protein elevation at the subsequent recurrence. In the CEA protein E group subjects whose disease-free intervals (DFI) were more than 6 months (n=7), elevation of *CEA mRNA* and CEA protein levels at 1, 3 and 6 months before subsequent recurrence were compared (Figure 1). *CEA mRNA* levels were elevated in 5 cases (71.4%) at 6 months before subsequent recurrence; CEA protein was elevated in 1 case (14.3%). At 1 month before subsequent recurrence, mRNA was elevated in all 7 cases; and CEA protein was elevated in 6 cases (85.7%).

Table II. *(a) Correlation between CEA protein and recurrence. (b) Correlation between CEA mRNA and recurrence. (c) CEA mRNA pattern in the recurrence group (CEA protein; NE).*

a)	CEA protein		
	NE (n=15)	E (n=15)	total
recurrence (-)	9	0	9
recurrence (+)	6	15	21

sensitivity: 71.4% (15/21)
specificity: 100% (9/9)
accuracy: 80%(24/30)

b)	CEA mRNA		
	L (n=4)	H (n=26)	total
recurrence (-)	4	5	9
recurrence (+)	0	21	21

sensitivity: 100% (21/21)
specificity: 44.4% (4/9)
accuracy: 83.3%(25/30)

c)	CEA mRNA		
	L	H	total
Recurrence group (CEA protein; NE)	0	6	6

NE: not elevated
E: elevated (more than 2.1 ng/ml/30 days)
L: low
H: high (cut-off point; 0.9)

Case Report

Case 16 (66-year-old man): A hepatectomy was performed because of H2 liver metastases. The *CEA mRNA* assay was started 29 months later. At that time, CT scans revealed no subsequent recurrence and the CEA protein level showed no elevation. *CEA mRNA* levels became elevated 32 months post-surgically. At that time, the CEA protein level was not elevated and CT findings were normal. At 34 months after surgery, the CEA protein level was elevated, and at 38 months local recurrence was detected by abdominal CT (Figure 2).

Discussion

RT-PCR is a sensitive technique that can detect circulating colorectal cancer cells. This method has detected

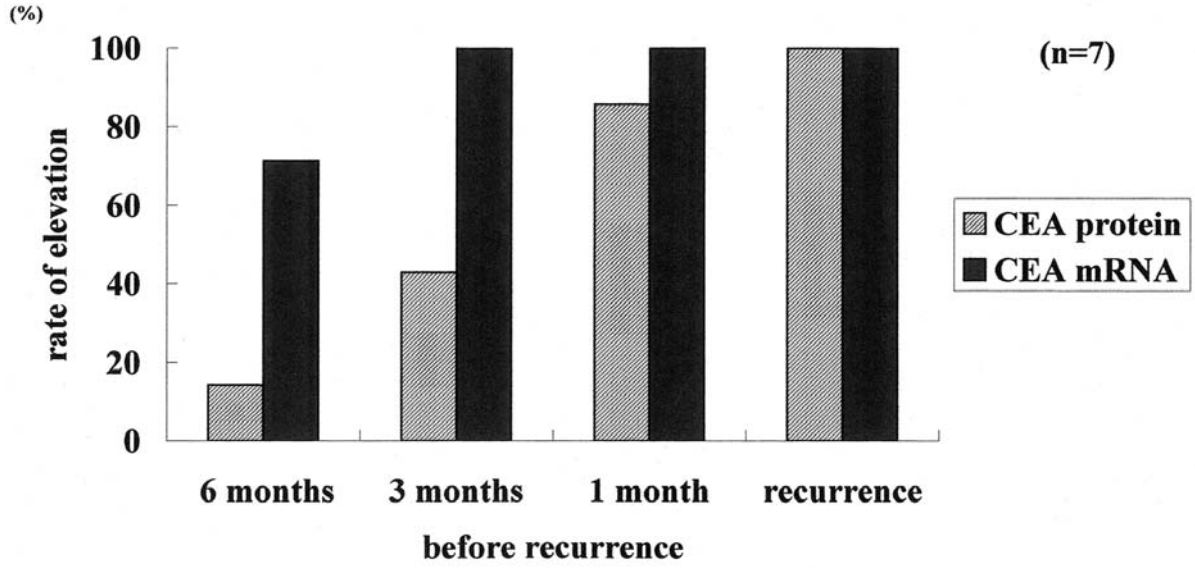

Figure 1. *Timing of elevation of CEA protein and CEAmRNA. CEA protein E group whose DFI> 6 months (n=7); CEA mRNA levels were elevated earlier than CEA protein before subsequent recurrence.*

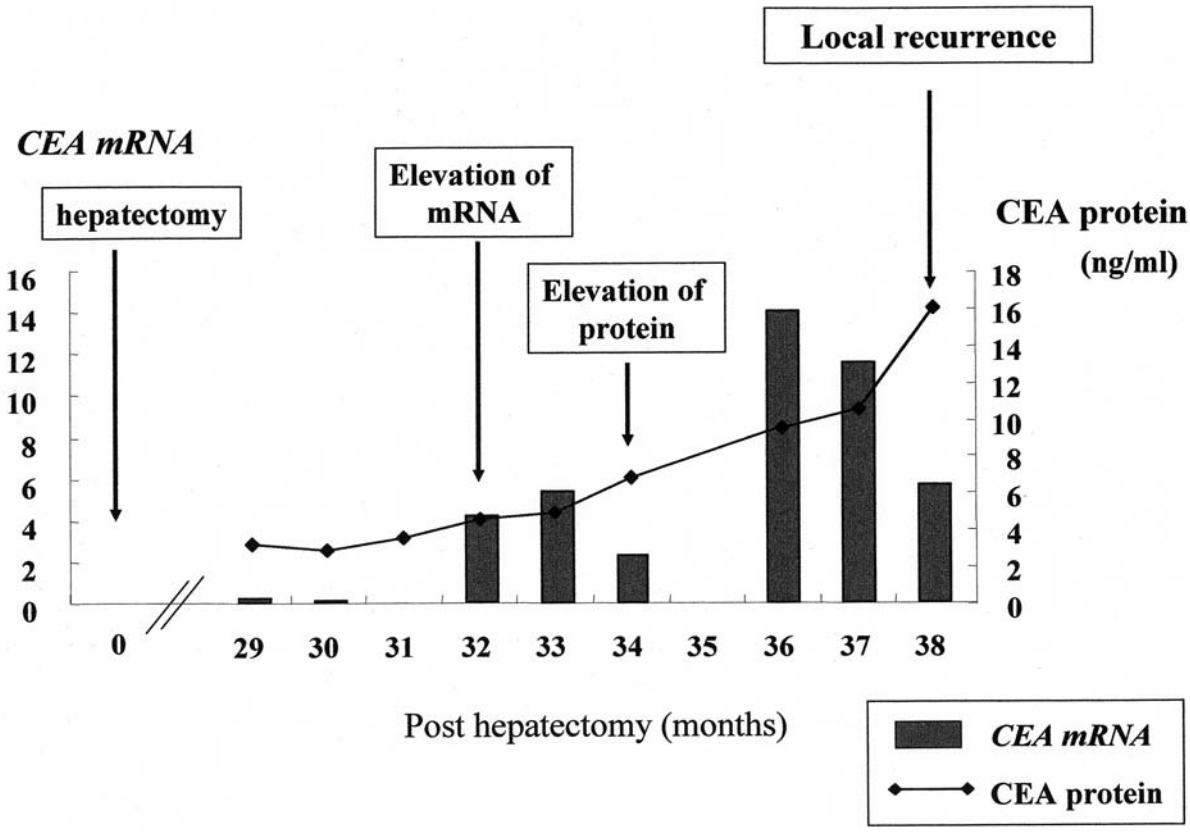

Figure 2. *Time course of case 16.*

micrometastases in bone marrow, lymph nodes, surgical specimens and peripheral blood (9-11, 17).

Although *CEA mRNA* cannot be detected in non-epithelial cells, it can be detected in almost all epithelial cells, including cancer cells. Mori *et al.* detected *CEA mRNA* in the peripheral blood of patients with gastrointestinal carcinoma during surgery by tumor manipulation (17).

It is known that metastasis is generated through a complex host-tumor interaction system, and less than 0.01% of circulating malignant cells are thought to successfully establish metastatic colonies (18). Although it remains controversial whether circulating tumor cells will become metastatic tumors, recent studies support the hypothesis that perioperative detection of *CEA mRNA* in peripheral blood could be a predictor of subsequent recurrence after hepatectomy (19).

A real-time quantitative RT-PCR method has been developed to quantify a very small amount of mRNA (14, 15), and several studies have been conducted using this method. Miyake *et al.* reported that *CEA mRNA* expression levels in the lymph nodes of colorectal cancer patients were correlated to histopathological findings (20). Utsunomiya *et al.* reported that cystatin-like metastasis-associated protein (CMAP) mRNA expression levels were correlated to both liver metastasis and prognosis in patients with colorectal cancer (21). Additionally, we previously reported that serum *CEA mRNA* levels were significantly higher in patients with Dukes' D colorectal cancer than in other clinical stages (12). Nevertheless, there has been no report to date on the relationship between diachronic *CEA mRNA* change and prognosis after hepatectomy.

In the present study, we evaluated the relationship between diachronic changes in the *CEA mRNA* expression level and clinical course and, furthermore, examined whether *CEA mRNA* or CEA protein is more sensitive in detecting subsequent recurrence. We found that patients in the H group of mRNA had a high recurrence rate, while those with low *CEA mRNA* levels had no re-recurrence (maximum disease-free interval: 49.3 months). Of the 15 cases in the CEA protein NE group, 6 (40%) had subsequent recurrence; all 6 cases belonged to the H group of *CEA mRNA*. It is, thus, possible that measurement of the *CEA mRNA* level may help identify patients at high risk of recurrence after hepatectomy even if the CEA protein level is low. In the H group of mRNA, 5 cases (5/26=19.2%) had no recurrence. However, the reasons for this result are unclear and it is important to bear in mind that our follow-up period was limited to 2 years. It is also possible that these cases gave false-positive results due to contamination of epithelial cells. RT-PCR is so sensitive that cancer-free specimens may easily test positive due to contamination (22). Because epidermal cells from drawing blood might contaminate specimens, the first few samples should be discarded.

In the CEA protein E group subjects whose disease-free intervals (DFI) were more than 6 months (n=7), *CEA mRNA* levels were elevated in 5 cases (71.4 %) at 6 months before subsequent recurrence; CEA protein levels were elevated in 1 case (14.3%). It is clearly advantageous to be able to detect recurrence at an early stage, since this offers the opportunity to perform a rehepatectomy. *CEA mRNA* has the advantage of being more sensitive than CEA protein in detecting recurrence and, therefore, might be more useful for early diagnosis. When mRNA levels show an H pattern after hepatectomy, further examination (*e.g.*, PET) in addition to the routine CT scans or adjuvant chemotherapy might be indicated.

In conclusion, *CEA mRNA* may be useful in identifying patients at high risk of recurrence after hepatectomy or to detect recurrence at an early stage when the CEA protein level is still low.

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