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Carcinoembryonic Antigen (CEA) in Colorectal Cancer − Prognostic Significance of Portal Blood Level −

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The prognostic significance of carcinoembryonic antigen (CEA) values in the drainage vein of the tumor (portal blood levels of CEA) of colorectal cancer patients were evaluated by examining the correlation with the peripheral blood levels of CEA and histopathologic findings of the tumor. 1) Portal blood levels of CEA were significantly increased by the operative procedure. Mean values of CEA in portal blood were higher than those in peripheral blood. Portal blood CEA was correlated with Dukes' staging, and revealed higher positive rates than CEA in peripheral blood in each stage. Elevated CEA levels were noted in those who had cancer infiltration extending through the proper muscle layer. There was a close correlation between portal CEA and CEA content in cancerous tissue (ng/g, wet weight) ($p<0.05$), but no significant correlation between peripheral CEA level and cancerous tissue CEA ($r = 0.372$). The mean values of portal CEA in aneuploidy were significantly higher than those in diploidy. These findings indicate that circulating CEA in peripheral blood might be influenced by the metabolic process of CEA in the liver as well as cancer progression rather than CEA production of the tumor. 2) The 5 year survival rate of the patient's group with a negative rate of portal CEA (93%) was far better than that with a positive rate (57%). This study suggested that the portal blood level of CEA in colorectal carcinoma may be very useful for assessment of the patient's survival.

Introduction

The carcinoembryonic antigen (CEA) of colorectal cancer is the most useful tumor marker to determine prognosis and to monitor patients with resectable colorectal cancer for early recurrence, and it is now widely tested[1-3]. However, about half of the patients with colorectal cancer do not have elevated CEA levels in peripheral blood. It is necessary to find out how CEA is transferred from cancer tissue producing CEA into the circulating blood, since the level of CEA in peripheral blood does not fully reflect the cancer tissue[4-6]. In the present study, we measured the portal blood level of CEA which was drawn from the drainage vein of colorectal carcinoma during operations, and attempted to account for the elevated serum CEA levels by examining the relationship between portal and peripheral bood levels of CEA and CEA content of tumor tissues, and to evaluate the prognostic significance of the portal blood level of CEA in colorectal cancer patients.

Materials and Methods

Patients

One hundred and sixty patients who underwent resection for carcinoma of the colon and rectum at the First Department of Surgery, Nagasaki University School of Medicine since 1978 were examined. There were 102 males and 58 females with a mean age of 60.9 years. Patients with multiple cancer in the large intestine and/or double cancers in other organs were excluded.

Blood Collection from the Portal Vein

A catheter was inserted into the main trunk of the drainage veins from the carcinoma in the colorectum, and blood samples were collected from the marginal vein close to the tumor. To avoid the influence of surgical procedure, blood was collected immediately after laparotomy. Portal blood samples were also collected at the time of resection of the colon and rectum to determine changes of the portal blood levels of CEA caused by surgical procedure.

Radioimmunoassay for CEA was performed by the sandwich method using the Dinabot-RIA Kit. All data were stored on a computer and the chi-square (χ^2) test and Student's test were performed for statistical analysis. P values less than 0.05 (two-tailed) were considered to indicate statistical significance.

CEA Measurement in Tissue Extracts

Fresh tissue samples were obtained from colorectal cancer, metastatic cancer of the liver, regional lymph nodes and normal colonic tissues at oral or anal site at least 5 cm distant from the margin of the tumor. Colorectal cancer or metastatic cancer to the liver were dissected free from surrounding normal tissues, and 0.5-1.0 g of these tumors were minced in 3-5 ml cold normal saline per gram of tissue and homogenized at 4℃ in phosphatebuffered saline, with an Ultrafurrox homogenizer (HITACHI 30PR-52D). After centrifugation at 1,600Xg for 20 min., the supernatant was then tested by micro-immunoassay. The CEA content of tissue was calculated as ng/wet weight of tissue (g).

Results

Changes on Portal CEA Levels during the Operation

To study changes of portal CEA levels caused by surgical manipulation, CEA levels of the portal blood collected on laparotomy and on resection of the colorectum after lymph node dissection were examined in 80 patients. Portal blood levels of CEA on resection of the colon were significantly higher than those on laparotomy ($p<0.01$) (Fig. 1).

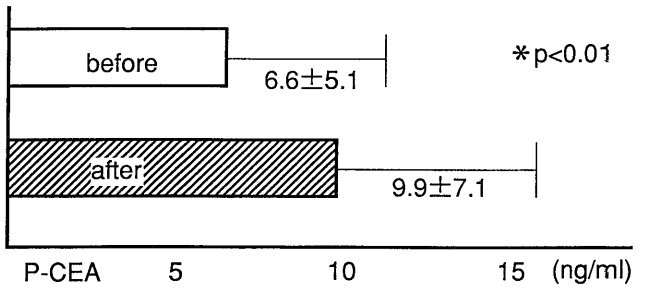

Fig. 1. Changes of the portal CEA levels by the surgical manipulation.

Duke's Staging and Portal and Peripheral Blood Levels of CEA

The relationship between Duke's staging and portal and peripheral blood levels of CEA is shown in Table 1. The positive rate in the portal blood was correlated with Duke's stages, and was higher than that in peripheral blood in all stages ($p<0.05$). The mean value of serial CEA in peripheral blood and in the portal blood in Duke's stage D was significantly higher than those in Duke's stage A and B ($p<0.05$) or Duke's C ($p<0.01$). Age and sex did not appear to be correlated with the CEA titer.

Table 1. Duke's staging and portal and periphral blood levels of CEA.

Duke's stage	No. of Patients	Portal CEA		Peripheral CEA	
		Mean±SD (ng/ml)	Positive rate(%)	Mean±SD (ng/ml)	Positive rate(%)
A	37	3.3±2.7	37.5	2.8±2.2	25.0
B	35	6.6±4.9	65.5	5.6±4.2	45.0
C	58	9.5±7.1	81.4	7.0±5.8	65.1
D	30	25.0±15.3	100*	18.5±13.7*	93.8

*Significant difference between Dukes' A and B ($p<0.05$) and C ($p<0.01$) by the Student's test.

Tumor Differentiation and Portal and Peripheral Blood Levels of CEA

The mean values of portal CEA of moderately differentiated adenocarcinoma were significantly higher than those of well differentiated adenocarcinoma ($p<0.05$) (Table 2). However, there was no significant correlation between cell differentiation of adenocarcinoma and periphral blood CEA. Two out of five patients with poorly differentiated or mucinous carcinoma were positive for portal CEA, but the mean values of CEA were lower than those of moderately differentiated adenocarcinoma.

Table 2. Histology and portal and peripheral blood levels of CEA.

Cell differentiation	No.of patients	Mean CEA values	
		Peripheral (ng/ml)	Portal (ng/ml)
Well	34	3.7±2.0	5.1±3.4
Moderately	103	8.3±7.5*	10.6±8.2*
Poorly	11	7.3±5.9	8.3±6.1
Mucinous	9	9.8±7.1	11.7±9.4

*Significant difference between well and moderately differentiated adenocarcinomas ($p<0.05$).

CEA in Tissue Extract and Serial Levels of CEA

The amount of CEA in tumor tissue ranged from 460 to 29,690 ng/g (wet weight) with a mean value of 7,144 ng/g. Non-cancerous tissues contained one-tenth lower CEA values than cancerous tissues (Table 3). Well and moderately differentiated adenocarcinoma contained greater amounts of CEA than poorly differentiated adenocarcinoma and mucinous carcinoma. High CEA contents were also observed in metastatic lymph nodes or metastatic tumors of the liver. There was a close correlation between tumor CEA (ng/g wet weight) and the portal CEA level ($r = 0.513$), but no significant correlation between peripheral CEA and tumor CEA ($r = 0.372$).

Table 3. CEA content of various tissues in patients with colorectal cancer.

Tissue	No.of patients	tissue CEA (ng/g wet weight)
well	16	9988.8± 8058.6
moderately	59	12090.3±12007
poorly	6	2879.1± 2455.3
mucinous	3	1339.7± 873.6
oral site	84	51003.2± 758.4
anal site	76	1080.8± 700.6
LN(−)	29	603.6± 537.0
LN(+)	17	11127.5± 8976.7
H(+)	5	4338.5± 6132.7

Oral or anal site : normal colorectal mucosa at least 5 cm distant from the edge of tumor. LN(−): no metastatic lymphnode, LN(+): metastatic lympnode, H(+): metastatic tumor of liver

Table 4. DNA ploidy patterns and portal and peripheral blood levels of CEA.

Ploidy	No.of patients	Portal values		Peripheral values	
		Mean±SD	Positive rates(%)	Mean±SD	Positive rates(%)
Diploidy	74	8.7±7.5	42.5	5.8±5.5	39.7
Aneuploidy	101	11.6±9.3*	60.4	6.5±5.2*	54.5

Cut off : Portal 4.0 ng/ml, Peripheral 3.0 ng/ml
*Significant difference between diploidy and aneuploidy patterns($p<0.05$)

Table 5. Liver metastasis and portal and periphral blood levels of CEA.

Liver metastasis	No.of patients	Portal values		Peripheral values	
		Mean±SD	Positive rates(%)	Mean±SD	Positive rates(%)
No metastasis	93	4.2± 3.1	44.1	3.5± 2.9	34.2
Metachronous	23	8.5± 6.1	74.1*	7.6± 5.3	59.7*
Synchronous	23	28.1±20.5	91.3*	16.5±13.0	84.2*

Cut off : Portal 4.0 ng/ml, Peripheral 3.0 ng/ml
*Significant difference between liver metastasis (metachronous or synchronous) and no metastasis ($p<0.01$)

DNA Ploidy Patterns and Portal and Peripheral Blood Levels of CEA

In tumor ploidy patterns of flow cytometric DNA analysis, the mean values of portal and peripheral blood levels of CEA in aneuploidy were significantly higher than those in diploidy (Table 4).

Liver Metastasis and Portal and Peripheral Blood Levels of CEA

Liver metastasis on laparotomy (synchronous) was found in 23 patients. Among 116 patients undergoing curative operations, recurrence of liver metastasis (metachronous) occurred in 23 patients. The other 93 patients showed no recurrence over a 5 year follow-up after operation (Table 5). The positive rate in the portal blood was higher than that in peripheral blood in all groups. The mean values of portal and peripheral blood levels of CEA in metachronous liver metastasis group were significantly higher than those in the no metastasis group ($p<0.05$). The synchronous liver metastasis group also showed significant elevation of portal and peripheral levels of CEA.

Prognosis

The prognosis of patients undergoing curative resections was evaluated on the basis of the Kaplan-Meier method in the group of the patients with positive portal levels of CEA (positive group) and in those with negative portal blood levels of CEA (negative group) (cut off : 4.0 ng/ml). Cumulative survival curves of two groups are shown in Fig. 2. Significant differences ($p<0.01$) was seen between the two groups. The 5-year survival rate was 93% in the negative group and 57% in the positive group.

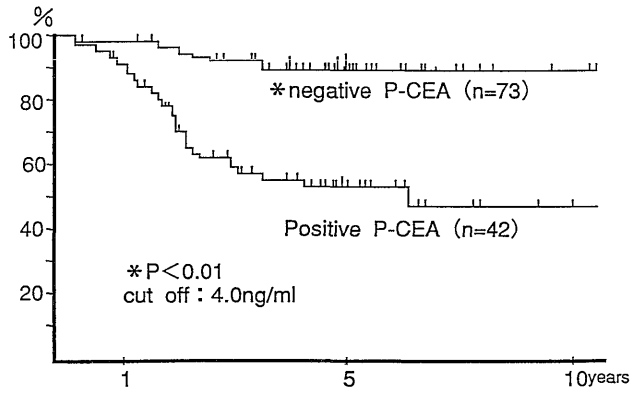

Fig. 2. Cumulative survival rates after curative resection - comparison of positive and negative rates of portal CEA.

Discussion

The present study confirms and extends the application of the CEA assay in the portal blood for monitoring of the disease stage and tumor recurrence in colorectal carcinoma. The CEA level in portal blood were higher than those in peripheral blood, and became elevated values due to the surgical procedure. These findings indicate that most CEA flows from tumor tissues into the peripheral blood vessels via the portal vein. Several factors

contributing to elevated peripheral CEA levels have been pointed out : 1) CEA productivity of the tumor, 2) rlease of CEA from the tumor into the blood stream[7], 3) clearance of circulating CEA[8], and 4) reabsorption of CEA excreted into the intestine[9]. Among these factors, CEA productivity is the most essential. Quantitative studies of extracts of primary and metastatic cancers of the colon and rectum have shown a wide variation in production of CEA. It has been generally agreed that the CEA concentrations of primary and metastatic cancer are higher than that of noncancerous tissue[4,5]. Our data also showed high CEA content of cancerous tissues including primary and metastatic tumors of the liver and metastatic lymph nodes, but very low amounts of CEA in various noncancerous tissues.

The portal CEA levels correlated well with Duke's stage and tumor CEA content, but there were no statistically significant differences between tumor CEA content and peripheral blood levels of CEA. This seems to provide metabolic evidence that CEA flows from the portal vein into the systemic circulation through the liver. Shuster et al.[8], in their study in xenogeneic animals, injected labeled CEA which accumulated specifically in the liver and was degraded and excreted within one hour, with a half life of one day. Some authors have shown that more amount of CEA was produced by more highly differentiated tumors, and we were able to confirm these findings[7,10,11], but our results showed no definite correlation between cellular differentiation and portal CEA levels. Well differentiated adenocarcinoma revealed low portal CEA levels, though it contained a large amount of CEA. This can be explained by the fact that most of the CEA in the tumor was excreted into the lumen of the intestine and only a little was released into the portal vein because well differentiated adenocarcinoma was found to maintain polarity of the cell surface and the structure of normal colonic mucosal cells[12,13]. Moderately differentiated adenocarcinoma with high portal CEA levels and poorly differentiated adenocarcinoma and mucinous carcinoma with low portal CEA levels seems to be correlated with the amount of CEA produced by an individual tumors[12,13]. In general, CEA of cancerous tissue is excreted into the lumen of the intestine, and as the tumor grows, the basal membranes of the epithelium and the vascular system in the stroma are damaged, which causes inhibition of CEA excretion and the transfer of CEA into the portal vein[7,10]. The present study suggests that the pathologic characteristics of the tumor are a major contributing factor to CEA transfer into the blood circulation rather than CEA production of the tumor. On the other hand, many reports have suggested that the DNA content of the tumor was important for prognosis[14-16], and we also recognized in the previous study that there is a close correlation between the DNA ploidy pattern and lymph node metastasis, liver metastasis, and Duke's stage[17]. In the present study, the carcinoma with an aneuploid pattern appeared to contain more CEA than that with a diploid pattern, but these results must be confirmed by additional data based on a large group of patients.

With regard to pathologic findings in the surgical specimens, we confirmed that peripheral blood levels of CEA were significantly correlated with invasiveness of the primary tumor, regional nodal metastasis, and distant metastases. Our primary goal in conducting this study was to confirm that the portal CEA test would provide useful prognostic information which would add to that of traditional pathology staging in patients with resectable colorectal cancer. General agreement has been achieved among many investigators that preoperative peripheral CEA levels adds significant information to Dukes' classification in the estimation of recurrence rates[18-20]. Our study involved a large number of resectable disease patients with essentially complete follow-up for prognostic purposes. We did show a significant correletion between portal CEA and prognosis, and also confirmed that the portal CEA values in patients with recurrence of liver metastasis (synchronous liver metastasis) were higher than those in nonrecurrent patients. A high portal CEA level seems to lead to a poor prognosis even in patients undergoing curative resection to have a high portal CEA level. In conclusion, the CEA values of portal blood in colorectal carcinoma cases were more closely correlated with pathologic characteristics of the tumors than those of the peripheral blood and may be the most useful for assessment of the patient's prognosis.

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