Chinese Journal of Cancer Research 8(3):230–234,1996.

CARCINOEMBRYONIC ANTIGEN EXPRESSION IN GASTRIC CANCER AS RELATED TO BIOLOGICAL BEHAVIOUR AND PROGNOSIS*

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Objective: To determine the relationship between carcinoembryonic antigen (CEA) expression in gastric cancer and biological behaviour or prognosis.

Material and Methods: Surgically resected specimens of gastric cancer from 104 patients were obtained. The content and distribution of CEA in gastric cancer were studied by immunohistochemical staining and immunoelectron microscopic technique. The relationship between CEA in gastric cancer and biological behaviour or prognosis were evaluated.

Results: The positivity of CEA was significantly higher in the patients with advanced stage, vascular invasion and lymph node metastasis than that in the patients without. The 5-year survival rate of the CEA (–) group was significantly higher than that of the CEA (+) group. Among the patients with advanced stage or lymph node metastasis, the survival rate was higher in the CEA (–) group than in the CEA (+) group.

Conclusions: Immunostaining for CEA in gastric cancer tissue may be helpful in differentiating among tumors that appear similar by conventional histological methods, thus providing a new means for discernment of invasion, metastasis and prognosis of gastric cancer.

Key words: CEA, Gastric cancer, Prognosis.

The study of correlation between biological behaviour or prognosis and carcinoembryonic antigen (CEA) in gastric cancer has been reported in a very few medical literatures.[1,2] On the basis of light microscopic and immunoelectron microscopic observation of CEA in gastric cancer,[3] we had followed up the patients for five years. This article reports the correlation between biological behaviour or prognosis and CEA in gastric cancer.

MATERIALS AND METHODS

Surgically resected specimens of gastric cancer from 104 patients were obtained. Each of them was fixed in 10% freshly buffered formalin for routine paraffin section and hematoxylin-eosin and in addition, immunohistochemical staining for CEA. Of the 104 cases, 12 were studied by immunoelectron microscopic technique. All the patients were followed up, of which, 19 patients lost connection. The follow up rate was 81.73%.

Immunohistochemistry (PAP)

Following removal of the paraffin, the sections were placed in 3% H_2O_2 at room temperature for 10 minutes so as to remove endogenous peroxidase. Subsequently the section were reacted with normal swine serum at 37 ℃ for 20 min. Then the first antibody (1:100 rabbit anti-human CEA), 2nd antibody (1:20 swine anti-rabbit IgG), and 1:100

Accepted June 21, 1996

* Subsidized by National Natural Science Fund.

rabbit PAP were added successively (all in a humidity chamber at 37 ℃ for 30 min). Finally the sections were dipped in fresh AEC developer for 20 min. At the same time a non-specific serum was used instead of the first antibody as a negative control, a known CEA positive colonic carcinoma as a positive control.

Immunoelectron Microscopy

Small pieces of fresh tumor and peripheral tissues (5×5×2 mm) were immersed rapidly in PLP solution, washed successively with 10%, 15% and 20% sucrose PBS, and frozen quickly in OCT. 20 μ frozen sections were placed on glass slides coated with plastic film and air-dried at room temperature for 30 min. The process of staining, dehydration, immersion and embedded was demonstrating all over the plastic film. Under the light microscope CEA positive pieces were cut out and placed on the tip of an empty Epon 812 embedding block. Ultrathin sections were observed under an electron microscope.

RESULTS

The Distribution of CEA

The contents of CEA were significantly higher in gastric cancer than in "normal" or intestinal metaplastic epithelia surrounding the cancer. CEA positive rate in gastric cancer was 85.85%. The distribution pattern of CEA was divided into membranous type and cytoplasmic type. In well differentiated cancer, CEA was expressed in a polar manner (Figure 1), whereas cells in pooly differentiated cancers CEA polar distribution was entirely lack (Figure 2). Under the electron microscope CEA was seen on the surface microsvilli, lateral and basal surfaces. In addition, CEA was easily recog-nizable in the protein-synthesizing and protein-transporting organelles, i.e., rough endoplasmic reticulum, Golgi complexes, and cytoplasmic vesicles of the cells (Figure 3). The opening of tight junction could be seen among the cancer cells with CEA in it. The desmosomes and gap junctions were decreased or disappeared. Tumor heterogeneity was a notable feature of CEA staining with positively varying from region to rigion and from cell to cell. Areas showing strong CEA positivity were immediately adjacent to, or admixed with, negative staining areas. In some cases, CEA staining was stronger in deep infiltrating cancer cells than in surface cancer cells. Even in some cases, CEA staining in situ was weak, whereas cancer cell thrombi in vessels and tumor nests in lymph node metastases were strongly positive. Thus, 89 (85.58%) of the 104 patients with gastric cancer were in the CEA (+) group and the remaining 15 cases were in the CEA (−) group.

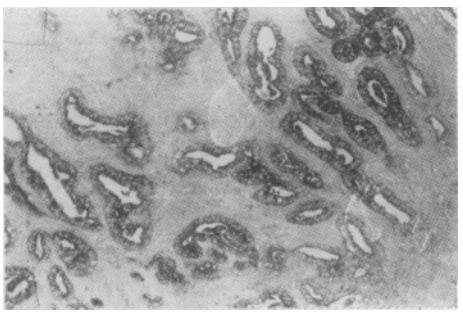

Fig. 1. Well-differentiated adenocarcinoma. CEA material was distributed in cell membrane, especially in the lumina of the glands and on the luminal surface of the tumor cells. PAP × 100

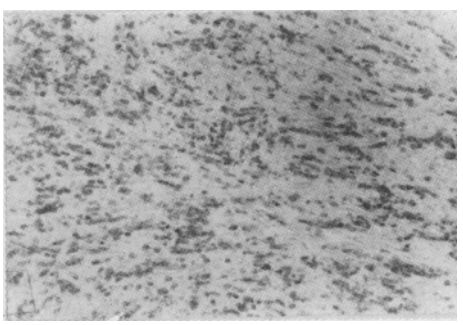

Fig. 2. Poorly-differentiated adenocarcinoma. CEA material was distributed in cytoplasma, losing polar distribution. PAP × 100

Correlation between CEA and Biological Behaviour

Table 1 shows some clinicopathological characteristic of both groups of patients, which might represent biological behaviour. The CEA positive rate was increased with the advance of infiltration depth, in which, the middle or late stage was significantly

higher than the early stage (former $P<0.05$, latter $P<0.01$). Both groups were statistically comparable with respect to vascular invasion and lymph node metastasis. The CEA positive rate was significantly higher in vascular invasion than in no vascular invasion ($P<0.05$), in lymph node metastasis than in those patients whose lymph nodes were free of tumor ($P<0.01$).

Table 1. Some pathological characteristics and CEA status

		No. of CEA (+) (%)	No. of CEA (-) (%)
Stage	Early	5 (45.45)	6 (54.55)
	Middle	14 (82.35)	3 (17.65)
	Late	70 (92.10)	6 (7.90)
Vascular invasion	Positive	68 (90.10)	7 (9.90)
	Negative	21 (72.41)	8 (27.59)
Lymph node metastasis	Positive	64 (91.43)	6 (8.57)
	Negative	16 (66.67)	8 (33.33)

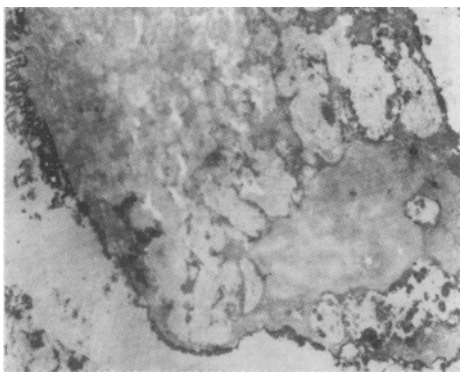

Fig. 3. Signet ring cell carcinoma. CEA material was seen in cell membrane, microvilli and the cytoplasmic membranous structures. TEM × 9000

Correlation between CEA and Prognosis

Table 2 shows the postoperative survival rate for the two groups. The CEA (-) group had a increased survival rate over a period of 5 years as compared with the CEA (+) group. This difference between the two groups was statistically sinificant ($P<0.05$).

Further analysis of the two groups was done in relation to lymph node metastasis and stage. The results are shown in Table 3.

The 5 years survival rate of lymph node metastasis group was significantly lower than that of no lymph node metatasis group ($P<0.01$). In addition, whether lymph node metastasis group or no metastasis group, the 5 years survival rate of CEA (+) group was all lower than that of CEA (-) group, in which the CEA (+) group with lymph node metastasis had the worst prognosis. Among the different stage of gastric cancer, whether CEA (+) group or CEA (-) group, the early cancer had the best 5 years survival rate which was significantly higher than that of advanced cancer ($P<0.01$). In contrast, the post-operative survival rate was low in advanced cancer, especially in the CEA (+) group.

Table 2. Postoperative survivor over 5 years and CEA status

	No. of survivor below 5 years (%)	No. of survivor over 5 years (%)
CEA (+)	41 (51.75)	30 (42.25)
CEA (-)	4 (28.57)	10 (71.43)

DISCUSSION

For a long time, we had decided biological

behaviour and prognosis of tumor with the only simple morphological observation. With the progress of immunohistochemical method, we can identify tumor marker in tissue and combine morphology with function to demonstrate the correlation between the marker and biological behaviour or prognosis.

CEA is a tumor-associated antigen. It is expressed during fetal life, and also in the process of malignant transformation. The increase content and abnormal distribution of CEA in gastric cancer cells are a reflection of morphology and function. The abnormal reflection of cancer cell producing CEA might have some relations with other function and morphology of cancer cell.

Table 3. Lymph node metastasis, stage, CEA status and prognosis

		No. of survivor below 5 years (%)	No. of survivor over 5 years (%)
Lymph node metastasis	CEA (+)	32 (68.09)	15 (31.91)
	CEA (−)	2 (40.00)	3 (60.00)
No lymph node metastasis	CEA (+)	4 (26.67)	11 (73.33)
	CEA (−)	2 (25.00)	6 (75.00)
Early stage	CEA (+)	0 (0.00)	5 (100.00)
	CEA (−)	0 (0.00)	6 (100.00)
Advanced stage	CEA (+)	41 (62.12)	25 (37.88)
	CEA (−)	4 (50.00)	4 (50.00)

Our study revealed that gastric tissue lost its polarity and demonstrated a certain relationship between the degree of morphologic surface polarity and the surface distribution of CEA in cancer cell. Cells in the cancers with well-developed apical microvilli had much more CEA on the microvillus surface than on the basolateral surface, wherease cells in the poorly differentiated cancers had CEA distributed uniformly over the entire cell surface. This correlation suggests that the regulation of morphologic polarity and the polarity of surface membrane components are similarly disturbed in cancer cells.[4]

CEA might be positive or negative in different areas of cancer cells on the same section. It might be interrelated to the heterogenity of tumor cells.[5] Tumor may consist of many tumor cell subsets from many cell lines, and all different in its speed of growth, capacity of metastasis, character of immunity and surface markers. CEA is a tumor marker. Hence it is certain that there is a difference in the content and distribution of CEA. The different content and distribution of CEA in cancer cells might be related to the function, behaviour and sensitivity to the threapy. The study showed that the cancer cells producing more CEA had a malignant behaviour and easily infiltrated and metastasized.

It has been reported by several investigators that the status of CEA staining in tumors or the CEA level in serum was of prognostic significance in patients undergoing radical surgery for some types of cancer.[1,6] However, there is still disagreement as to its value as a prognostic indicator in the treatment of patients with malignant disease. A survival comparison between two groups of patients who had undergone radical gastrectomy for gastric cancer showed that 5-years survival rate was significantly higher in the CEA (−) group than in the CEA (+) group. Among the patients with advanced stage or lymph node metastasis, the survival rate was higher in the CEA (−) group than in the CEA (+) group, in keeping with the Kojima's report.[1] A possible explanation for the observed relationship between primary tumor CEA status and behaviour or prognosis is suggested by the widely accepted concept[7] that the presence of CEA may be associsated with behaviour common to both fetal and malignant cells, enabling them to spread rapidly and to escape destruction by the host immune response. Altered cell-cell interactions, invasiveness, metastasis, and evasion of immunologic dedection are mediated most likely at the cell surface. The abnormal

distribution of membrane glycoproteins in cancer cells might lead to important changes in the behaviour of the cells, i.e., loss of contact inhibition, decrease of cell cohesiveness and mobility.[8]

Whatever the explanation of these resluts, it dose apper that the CEA status of primary tumors in patients with operable gastric cancer may be of some relevance to the prognosis after radical surgery. Our date suggest that CEA staning in tissue sections of gastric cancer may be helpful in differentiating between tumors that appear similar by conventional histological methods, thus providing a new means for obtaining more precise prognostic information.

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