Detection of carcinoembryonic antigen (CEA) in tissue and gastric secretions of stomach carcinoma

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Gold and Freedman discovered an antigen common to colonic carcinoma and fetal colonic mucosa, and designated this as carcinoembryonic antigen (CEA)[1,2]. At first, this antigen was thought to be specific for cancers of the digestive tract of entodermal origin. However, recent work with highly sensitive immunochemical methods shows that CEA is found in a wide variety of cancers other than those of the digestive tract, and even in non-malignant disorders[3~5].

In our laboratory, immunochemical studies on CEA were performed in order to evaluate its diagnostic significance with respect to gastric cancer. The present report deals with results obtained by the quantitative determinations of CEA in tissue extracts and by detections of CEA in gastric juice and other body fluids.

As reported by Gold and his coworker[1,2], 0.6 M parchloric acid (PCA) soluble fraction was prepared from the saline extract of metastatic cancer tissues from the liver obtained at autopsy from a patient with adenocarcinoma of the stomach. This PCA extract was fractionated by gel filtration using Sephadex G–200. The first peak on the elution pattern was found to be rich in CEA, judging from the immunodiffusion test, hence this fraction is referred to as crude CEA (GcaCEA). Antisera were prepared in rabbits against both the PCA fraction and the crude CEA ; anti-GcaPCA serum to the former and anti-GcaCEA serum to the latter were prepared respectively[6,7]. Absorption of antisera was carried out with polymerized gel of glutaraldehyde with normal human plasma, and saline and PCA extracts of noncancerous gastric mucosa as well as with ABO types of red blood cells[6,7].

The anti-GcaCEA serum formed a dense precipitin arc of CEA in the beta region when allowed to react in immunoelectrophoresis of the PCA extract of gastric carcinoma, indicating, an antibody with a higher titer as

compared with anti-GcaPCA serum. After further absorption with normal tissue components, this gave a single precipitin arc of CEA for cancerous PCA extract, as shown in Fig. 1. A comparative immunodiffusion analysis between this antiserum and the specific anti-CEA serum, kindly supplied by Dr. Burtin, Villejuif, presented a reaction of identity (Fig. 1). Thus, the anti-GcaCEA serum proved monospecific for CEA as far as reacted in immunodiffusion, and was used throughout the present study.

The specificity of the antiserum was also tested with the immunofluorescent antibody technique. When tested for the section of colonic carcinoma, the specific staining showed a centroglandular pattern, which is generally accepted to be characteristic of CEA.

With Mancini's single radial immunodiffusion, CEA concentrations in PCA extracts of tissues were quantitaitvely determined. The calibration curve was obtained using serially diluted standard CEA solution. The standard CEA was prepared by agarose-gel zone electrophoresis of the crude

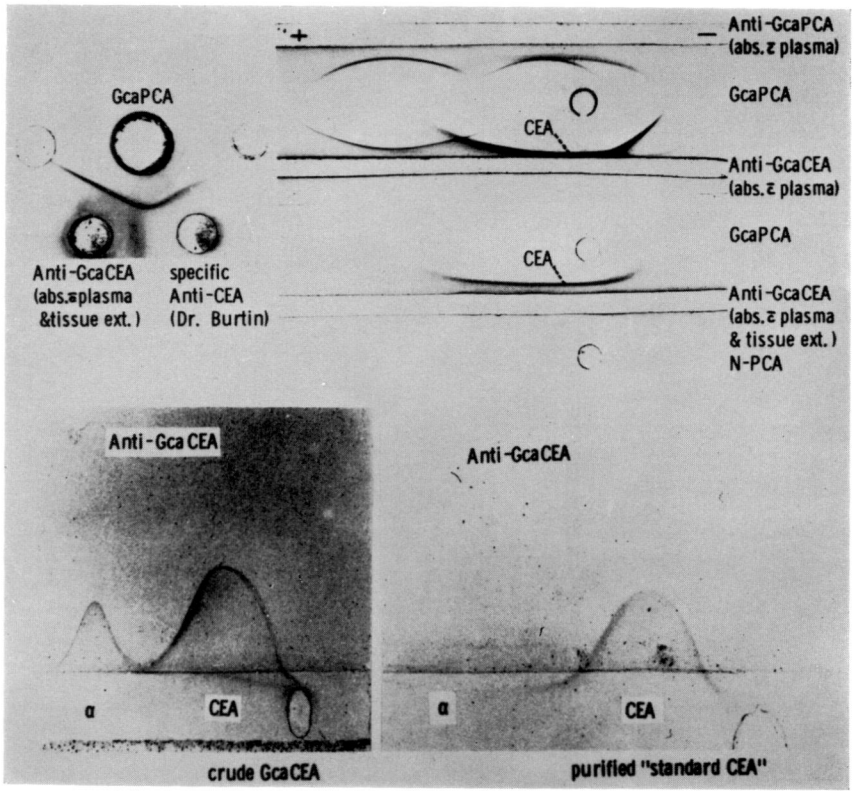

Fig. 1. Identification of CEA and anti-CEA sera by immunodiffusion and immunoelectrophoresis.

CEA, and was found to form a single precipitin line in Laurell's crossed elecirophoresis (Fig. 1) and in other gel-diffusion tests. The CEA concentration was expressed in unit/ml, which is equivalent to μg/ml of protein concentration of the standard CEA. In addition, it appears to be difficult to determine the exact CEA content per weight unit of tissues, thus the ratio of CEA units to the mg protein content of a given PCA extract was calculated.

The CEA to protein ratio (CEA/P ratio) in PCA extracts from cancerous and noncancerous tissues are shown in Fig. 2. Of 20 cases with gastric

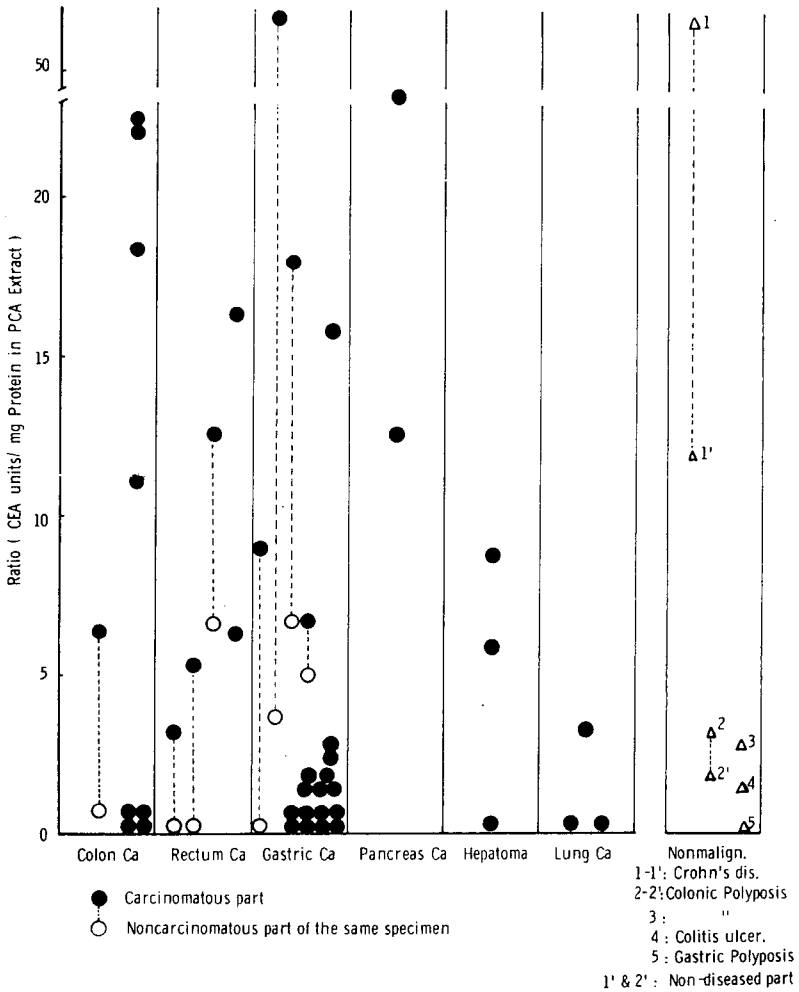

Fig. 2. CEA to protein ratios in PCA extracts of carcinomatous and noncarcinomatous tissues (CEA units: determined by Mancini's method. Protein: Lowry's method).

carcinoma, 12 were positive for CEA and 8 were negative by Mancini's method. There were a wide variety of ratios for gastric carcinoma from 0 to 58.8 with a mean value of 6.0. The CEA/P ratios of nonmalignant gastric mucosa, resected 3 cm or more apart from cancer tissues, were obtained from 4 cases with positive CEA. These mucosal portions showed a remarkable intestinal metaplasia. Of these 4 cases, 3 were positive, with the CEA/P ratios ranging from 3.7 to 6.7. These ratios were markedly lower than those of cancer tissues in respective cases.

Of 10 patients with colonic carcinoma, 6 were positive, with a mean ratio of 8.8. Two cases with carcinoma of the pancreas showed relatively high ratios. There was, however, no significant difference in the mean value of the ratio between gastric carcinoma and other digestive cancers. A small number of cases with primary hepatoma and lung cancer were also positive, though with low CEA/P ratios.

As regards PCA extracts of noncancerous mucosa from patients with

Table 1. *Incidence of CEA in serum, faeces, gastric juice and ascites detected by counter-immunoelectrophoresis*

a) *PCA Fraction of*	No of Cases	Positive	
Serum			
Gastric carcinoma	41	1	
Colonic carcinoma	18	0	
Rectum carcinoma	8	0	
Other cancers	22	0	
Healthy control	10	0	
Faeces			
Gastric carcinoma	5	0	
Colonic carcinoma	2	1	
Healthy control	5	0	
b) *PCA Fraction of*	No of Cases	Positive	%
Gastric Juice			
Gastric carcinoma	18	14	78
Atrophic gastritis	11	2	18
Gastric ulcer	15	1	7
Ascites			
Gastric carcinoma	10	6	60
Primary hepatoma	3	2	
Ovarial carcinoma	1	1	
Liver cirrhosis	5	0	

digestive diseases other than carcinoma, relatively high ratios were found in the diseased parts of intestinal mucosa from cases with Crohn's disease and colonic polyposis, as well as in ulcerative colitis.

In an attempt to develop a method of diagnosis using serum, gastric juice and other body fluids, counter-immunoelectrophoresis was applied for the detection of CEA. Results on incidences of CEA in serum, gastric juice and other body fluids are summarized in Table 1. Only one of 41 serum samples from patients with gastric cancer was positive for CEA. As for the PCA fraction of faeces, one sample from a patient with colonic cancer was positive for CEA. In these instances, CEA in the PCA fraction was concentrated to 15 to 20 times that of the original sample.

In contrast, CEA in the PCA fraction of gastric juice was detected in 14 cases (78%) out of 18 cases with gastric carcinoma. This incidence was remarkably higher than that in atrophic gastritis and gastric ulcer. Gastric juice specimens were collected through gastric tubing while neutralization with phosphate buffer pH 7.2 in vivo and CEA in the PCA fraction was concentrated to about 50 times that of the original sample. We could also detect CEA rather frequently in the PCA fractions of ascites from patients with gastric and other cancers.

It is suggested that the occurrence of CEA may be related to a precancerous changes of tissues, as was pointed out by Fielding and his coworkers[8], and also to an inflammatory response. There is a possibility that CEA may be produced in the gastrointestinal mucosa with enhanced proliferation, and the production may be related to the cell differentiation. From this point of view, our result in which the CEA to protein ratios for gastric mucosa may be elevated with the intestinal metaplasia is of special interest.

Use of counter-immunoelectrophoresis, although not sensitive enough for serum samples, could detect CEA with a relatively high incidence in the PCA fraction of gastric juice and ascites fluid from patients with gastric cancer. This may be of some diagnostic value. Further studies will be required to clarify the significance of CEA in tissues and body fluids, in relation to precancerous changes of tissues.

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