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Concentration and distribution of tumor associated antigens TAG-72 and CEA in stomach cancer

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We measured the concentration and distribution of tumor associated antigens, TAG-72 and CEA, in stomach cancer by *in vitro* quantitative autoradiography (IV-QAR). Frozen sections of 33 specimens were incubated with varying concentrations of ^{125}I-labeled CEA-79.1 and B72.3 antibodies specific for carcinoembryonic antigen (CEA) and tumor-associated glycoprotein-72 (TAG-72), respectively. Computer analysis of specific antibody binding gave maximal binding values which were equal to the concentrations of the antigen or epitope. TAG-72 was detected in 25 specimens, at a concentration ranging from 8.4 to 562.9 pmol/g. CEA was detected in 32 of the 33 specimens and its concentration ranged from 8.8 to 525.3 pmol/g. The distribution of TAG-72 by IV-QAR coincided with that of the tumor cells in 41.4% of the pathologic lesions. The distribution of CEA coincided with the tumor cells in 80.5% of pathologic lesions, nearly twice the TAG-72. The concentration of TAG-72 was significantly higher in mucinous adenocarcinoma and mucin containing adenocarcinomas than other types of adenocarcinomas. There was no significant difference in the concentration of CEA among the pathologic types of stomach cancer. In summary, stomach cancer exhibited wide variations in TAG-72 and CEA expression. CEA expression was more frequent and homogeneous than TAG-72.

Key words: carcinoembryonic antigen, tumor-associated glycoprotein-72, autoradiography, stomach cancer

INTRODUCTION

STOMACH CANCER is still the most frequent type of malignant tumor in East Asia, Europe and South America.[1] The only acceptable management of stomach cancer is early diagnosis and surgery.[2,3] Recently, radioimmunoscintigraphy has been used as an investigative tool to detect malignant tumors.[4,5] Monoclonal antibodies with specificity for antigens on stomach cancer cells and colon cancer cells are increasingly being tried as specific probes for use in radioimmunoscintigraphy and radioimmunotherapy.[6,7] Especially B72.3, which is specific for tumor-associated glycoprotein-72 (TAG-72), and anti-carcinoembryonic antigen (CEA) antibodies have been used widely in the radioimmunoscintigraphy of gastrointestinal carcinomas.[7–11] One of the critical factors in successful radioimmunoscintigraphy and immunotherapy is the local concentration and distribution of the target antigen,[12] but a few reports investigated the concentration of these antigens in stomach cancer.

Immunohistochemical methods have been used to analyze the presence and distribution of various antigens in tumors, but immunohistochemistry alone is limited in its use as an objective, sensitive and quantitative diagnostic tool. We previously reported the use of *in vitro* quantitative autoradiography for measuring tumor associated antigens in histologic sections.[13,14] This study evaluates the concentration and distribution pattern of the tumor associated antigens, TAG-72 and CEA, in human stomach cancer.

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MATERIALS AND METHODS

Cancer tissues

Thirty-three specimens of stomach cancer were evaluated. All specimens were from cases of advanced stomach cancer and were obtained by surgical resection.

Monoclonal antibodies

Two monoclonal antibodies (MoAb) to tumor associated antigens were used in the autoradiography. MoAb B72.3 (IgG_1), specific for TAG-72 glycoprotein,[15] was supplied by Dr. Schlom of National Cancer Institute (Bethesda, MD). MoAb CEA-79.1 (IgG_{2a}), was made at Seoul National University.[16] This antibody was specific for CEA, and did not bind nonspecific cross-reacting antigens.[17] Both antigens are located on the cell surface.

These monoclonal antibodies were labeled with ^{125}I by the chloramine T method. One mg of antibody was reacted with 37 MBq (1 mCi) of ^{125}I (New England Nuclear, Boston, MA) by adding 12.5 μg of chloramine T and the reaction was stopped by adding 43.8 μg of sodium thiosulfate. The reaction time was 2 minutes. A PD-10 column (Pharmacia, Piscataway, NJ) was used to separate the radiolabeled antibody from free ^{125}I. The specific activity was around 1.0 mCi/mg. More than 90% of the purified radioactivity was precipitable with 10% trichloroacetic acid.

The immunoreactivity of ^{125}I-labeled B72.3 was determined by solid-phase radioimmunoassay with extracts of LS174T cell, which expressed TAG-72.[18] The immunoreactivity of the ^{125}I-labeled CEA-79.1 was determined by a serial cell binding assay. SNU-C4 human colon cancer cells, which expressed CEA, were reacted with radiolabeled antibody, and immunoreactivity was calculated by the double inverse plot.[19] The immunoreactivity of ^{125}I-CEA-79.1 ranged from 44.6% to 74.4%. In the case of ^{125}I-B72.3, the immunoreactivity ranged from 51.2% to 73.6%.

In vitro *quantitative autoradiography*

The tumor specimens were cut into 20 micron frozen sections with a cryomicrotome. The tissue sections of each tumor were divided into two groups: 1) total binding of specific antibody, and 2) the nonsaturable binding of specific antibody. Sections were fixed in 0.25% glutaraldehyde for 20 minutes. For the nonsaturable binding group, sections were incubated with unlabeled antibody (B72.3 or CEA-79.1) for 30 minutes. All sections were then incubated for 30 minutes in a solution containing 2% bovine serum albumin (BSA) and 10% chicken serum (CS) in phosphate buffer saline (PBS) to reduce the nonspecific binding of the radiolabeled antibody. The individual sections were then incubated for 60 minutes in a solution of ^{125}I-labeled antibody at a concentration ranging from 1.3 nmol/liter to 83.3 nmol/liter. After this incubation, the slides were washed in PBS and dehydrated with ethanol.

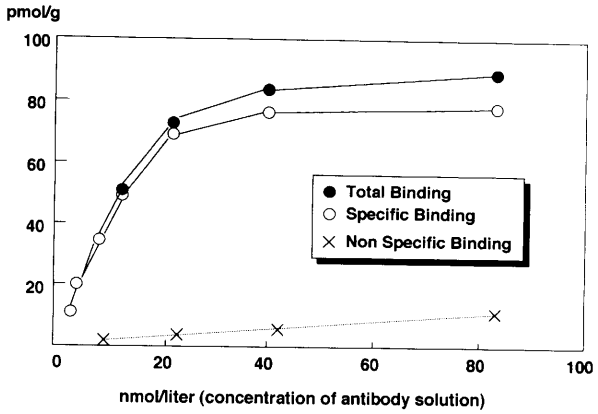

Fig. 1 Binding curves based on the saturation studies. The curves were obtained by plotted the concentration of antibody bound to the tumor sections versus the concentration of ^{125}I-labeled antibody in the incubation solution.

The autoradiographic standard was prepared with ^{125}I-human serum albumin, as described by Mies.[20] The 2 sets of tissue sections were exposed to Kodak SB5 film with standard for two days before they were developed. The autoradiographic films were digitized with a scanning microdensitometer (Amersham, Arlington Heights, IL). The optical density measured from the ^{125}I-labeled human serum albumin standard was plotted against the specific activity of each standard. A polynomial fitting of these data provided a standard curve. We compared the autoradiographic image to a hematoxylin and eosin (H & E) stained adjacent tissue section in order to define the region of interest in the autoradiographic image. The mean optical density of the selected region was obtained and the concentration of radiolabeled antibody in the tumor (μCi/g) was determined from the standard curve. This specific activity was corrected for its immunoreactivity. We calculated the concentration of binding antibody as moles of antibody per grams of tumor (mol/g) from the specific activity value for each ^{125}I-labeled antibody preparation.

Overlying the autoradiographic and H & E section images also allowed us to correlate the distribution of bound radioactivity, i.e., antigen, with tumor cell distribution.

Analysis of binding activity data

The data from the saturation studies were analyzed by the Nonlinear Least Square Fitting method. We used two equations to analyze the saturation curve and obtained values for the dissociation constant (K_d) and the maximum binding of the antibody (B_{max}) (Fig. 1).

The first equation was a saturation plot.

$$\text{Total Binding} = \frac{B_{max} \times [Ab]}{K_d + [Ab]} + a \times [Ab]$$

$$\text{Nonsaturable Binding} = a \times [Ab]$$

The B_{max} is the maximal amount of ^{125}I-labeled antibody which binds specifically to the tissue; [Ab] is the concentration of ^{125}I-labeled antibody in the incubation media; K_d is the antigen/antibody dissociation constant, and a is the slope of the nonsaturable curve. Specific binding was obtained by subtracting the nonsaturable binding curve from the total binding curve.

The second equation was a double inverse plot between the specific binding data and the concentration of labeled antibody.

$$1/\text{Specific binding} = 1/B_{max} + K_d/(B_{max} \times [Ab])$$

A plot of 1/specific binding versus 1/[Ab] will yield a straight line with the value at the y-intercept being equal to $1/B_{max}$. A computer fitting of the curve gave the B_{max}, which is the maximal concentration of ^{125}I-labeled antibody specifically bound to the tumor. The specific activity of the ^{125}I-labeled antibody was used to calculate the maximal pmol/g of the antibody bound to the tumor. The concentration of bound antibody was considered to be equivalent to the concentration of the antigenic epitope. The curve fit and binding calculations were made by means of the RS-1 curve modeling program in this equation.

Statistical analysis

Differences between pathologic types in the concentration of antigen were tested for their significance by Student's-t test. The rates of coincidence between antigen expression and cellular distribution were compared by χ^2-test.

RESULTS

Table 1 shows the results of the quantitative autoradiographic analysis of the tumor sections from stomach cancer specimens. The concentration of TAG-72 could be measured in 25 specimens (75.7%) and was undetectable in 8 specimens (24.3%). CEA was detectable in all specimens except one (96.9%). Out of 40 pathologic lesions, TAG-72 was expressed in 29 (72.5%), and CEA was expressed in 90% of the lesions. The median value of TAG-72 was about one third the concentration (27.9 pmol/g) of that of CEA (101.7 pmol/g). The concentration of TAG-72 ranged from 8.4 pmol/g to 562.9 pmol/g (Fig. 2A), and the concentration of CEA ranged from 8.8 pmol/g to 525.3 pmol/g (Fig. 2B).

The concentration of TAG-72 and CEA in the tumor sections was compared to the tumor's pathologic type (Table 2). The concentration of TAG-72 was higher in mucinous adenocarcinoma than in other types, but the concentration of CEA did not vary greatly between the various types of tumors.

We compared the antigen levels of the various tumors to the presence or absence of mucin, which was determined by H & E stain. Carcinomas with mucinous components had significantly ($p < 0.001$) higher TAG-72 concentrations than carcinomas without mucinous components (Table 3). However, the CEA containing tumors showed no difference between mucin containing and non-containing adenocarcinomas. In our study, the poorly differentiated carcinoma without a mucinous component was the most frequent type (50%). In this type of tumor, the concentration of CEA was significantly ($p < 0.005$) greater than the concentration of TAG-72.

The pattern of the tumor cell distribution, as found in H & E sections, was compared to the pattern of antigen expression in the autoradiographic images. Figure 3 shows a typical example from our study of a case of mucinous adenocarcinoma with diffuse cellular distribution. CEA expression was homogeneous through the tumor cells, but TAG-72 expression was heterogeneous. The pattern of TAG-72 antigen expression coincided with the tumor cell distribution in 41.4% of the cases. There were also signifi-

Table 1 Expressions of TAG-72 and CEA measured by autoradiography

	Total Number	TAG-72	CEA
Patients	33	25 (75.7%)	32 (96.9%)
Lesions	40	29 (72.5%)	36 (90.0%)

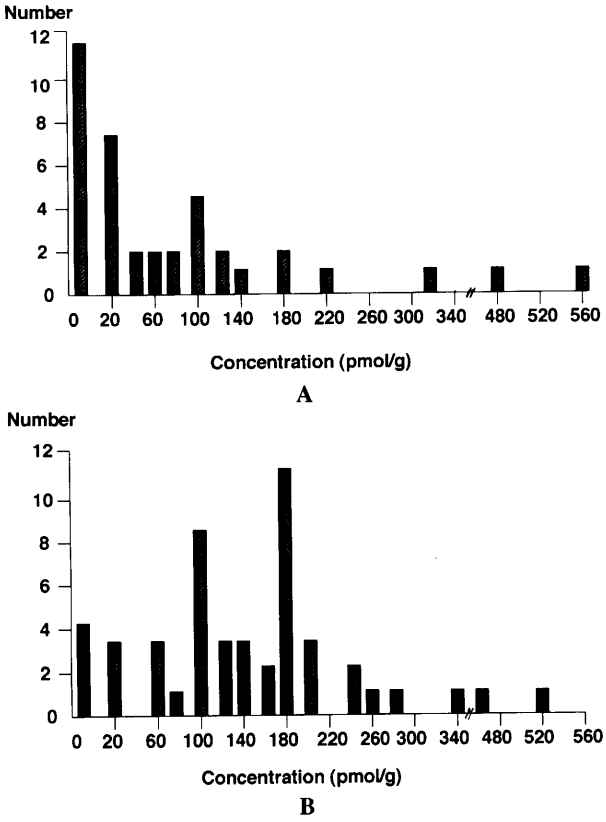

Fig. 2 Frequency histograms showing the autoradiographic measured concentrations of TAG-72 (A) and CEA (B) in tissues of stomach cancer.

Table 2 Concentrations of TAG-72 and CEA according to pathologic types

Pathologic type	Number	Concentration (pmol/g)	
		TAG-72	CEA
Carcinoma			
Well differentiated	4	0	100.1 ± 68.8
Moderately differentiated	8	59.6 ± 78.1	108.7 ± 57.8
Poorly differentiated	20	19.0 ± 35.4*	155.8 ± 162.9*
Mucinous	7	250.5 ± 164.3	140.1 ± 84.1
Signet ring cell	1	102.7	93.9
Intestinal metaplasia	4	48.3 ± 4.1	52.2 ± 13.8

Mean ± Standard deviation
*$p < 0.005$

Table 3 Concentrations of TAG-72 and CEA according to mucin containing nature

Pathology	Number	Concentration (pmol/g)	
		TAG-72	CEA
Adenocarcinoma with mucin	14	178.7 ± 148.4*	122.8 ± 66.6
Adenocarcinoma without mucin	26	23.6 ± 32.3*	143.9 ± 146.8

Mean ± Standard deviation
*$p < 0.001$

Table 4 Coincidence between the expressions of TAG-72 and CEA measured by autoradiography and the cellular distribution measured by H & E staining

	TAG-72	CEA
Lesions	29	36
Coincidence with autoradiography	12 (41.4%)	29 (80.5%)
No coincidence with autoradiography	17 (58.6%)	7 (19.5%)

*$p < 0.005$ by χ^2-test

cant numbers of tumor cells that expressed a low or negligible amount of TAG-72 antigen. The patterns of CEA antigen expression coincided with tumor cellular distribution in 80.5% of the cases; in only 19.5% CEA expression was heterogeneous (Table 4).

DISCUSSION

Radioimmunoscintigraphy and radioimmunotherapy have been studied as new methods to diagnose and treat malignancies. Gastrointestinal carcinomas express a variety of tumor associated antigens including CEA, TAG-72, GICA and DuPan-2.[7,9,10] In this study, we measured the concentration and distribution of CEA and TAG-72 in stomach cancer.

CEA has been described as a Mr. 180,000 glycoprotein complex which is expressed by embryonic colonic mucosa and carcinomas of the gastrointestinal tract. Monoclonal antibody CEA-79.1 was made from CEA, purified from the culture supernatant of a human colon cancer cell line, LS174T.[16] Antibody B72.3 is a murine IgG_1, which was prepared from a membrane-enriched extract of human metastatic breast carcinoma as the antigen, and B72.3 recognizes the $Mr > 10^6$ novel tumor-associated glycoprotein, TAG-72.[15]

It is well known that CEA is expressed frequently in gastric cancer.[21-24] TAG-72 is also expressed in a vast majority of gastric adenocarcinomas.[21,25] Double histochemical staining with antibodies to CEA and TAG-72 revealed that the two antibodies, in combination, reacted with at least 90% of the carcinoma cells in 13 out of 17 tumors.[21] However, Gero et al. reported a poor correlation between the CEA and TAG-72 antigen values in sera obtained from gastric cancer patients, suggesting the complementarity of CEA and TAG-72 measurements in the analysis of gastric cancer.[26] In addition, Ohuchi et al. confirmed that when monoclonal antibodies B72.3 and COL-6 (anti-CEA antibody) were simultaneously reacted with the gastric carcinoma tissue, more carcinomas were detected than with each individual antibody alone.[21] We found that CEA was expressed in 90% of the gastric tumors and TAG-72 was in 72.5% of lesions. In 95% of lesions, at least one of the two antibodies reacted with the stomach cancer specimen.

Previously, Del Vecchio reported the use of *in vitro* quantitative autoradiography for quantitating and evaluating melanoma tumor antigens.[27] In contrast to immunohistochemical techniques, this method yields numerical data which are not subject to the observer's judgment. And different antigen/antibody systems can be directly compared. In this autoradiographic system, we measured, strictly speaking the antigen recognized by the monoclonal antibody we used rather than the antigen itself. By using a standard stick of melanoma cultured cells (H2669), we examined the effects of variable incubation time and temperature with the radiolabeled antibody and found that 1 hour's incubation at room temperature was enough to obtain adequate data.[13] We also compared these autoradiographic data to those obtained by Scatchard analysis and found similar values.[13] Del Vecchio reported the

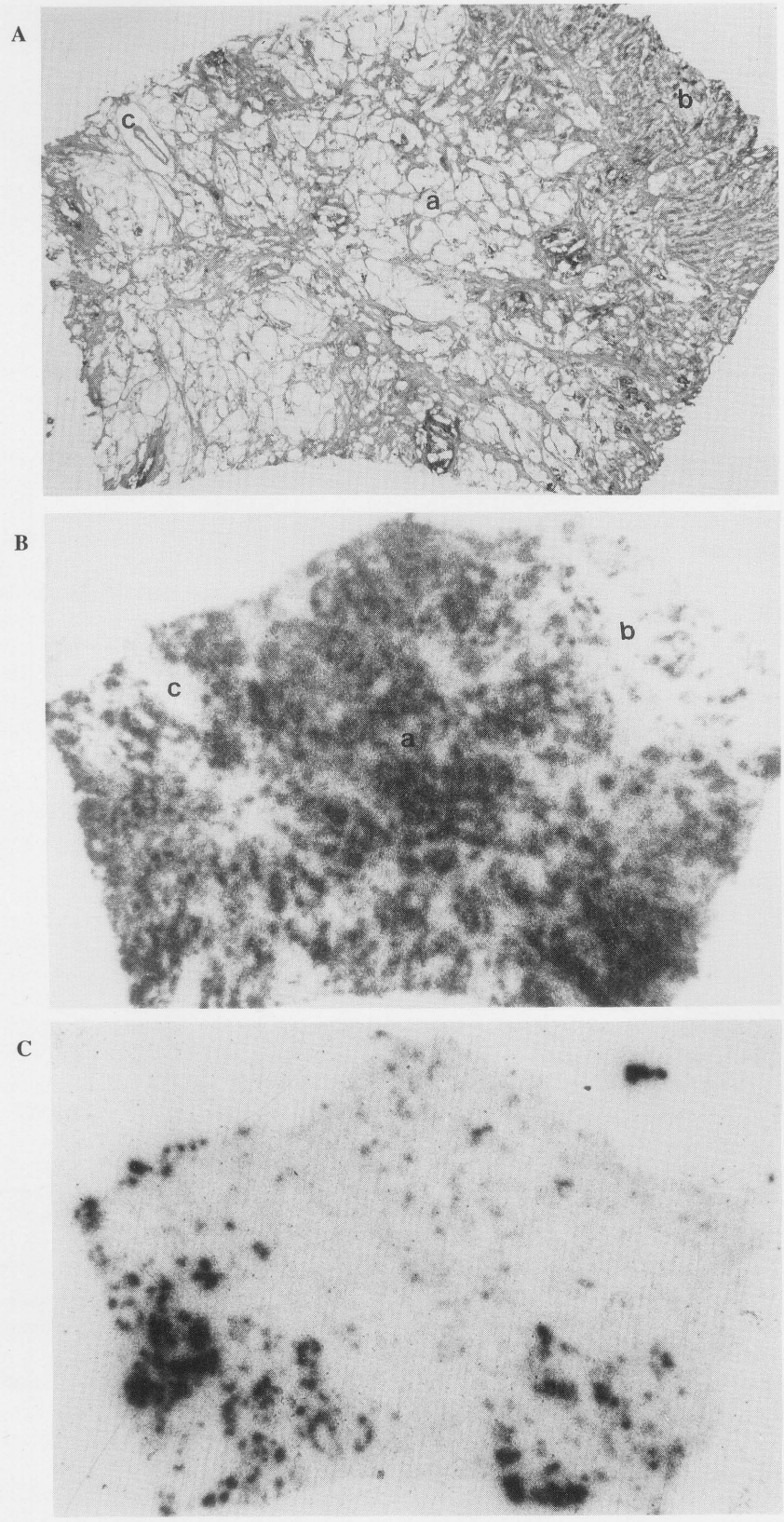

Fig. 3 Autoradiographic images of a mucious adenocarcinoma. A. H & E staining of tumor section showed tumor cells (a), normal mucosa (b) and connective tissues (c); B. Autoradiographic image using ^{125}I-CEA-79.1 showed homogeneous expression of CEA in tumor cells (a), and no expression in the normal mucosa (b) and connective tissues (c); C. Autoradiographic image using ^{125}I-B72.3 showed pathy heterogeneous expression of TAG-72 in tumor cells.

coefficient of variation in quantitative autoradiography was 20%.[27]

In this study we found that the amount of CEA and TAG-72 varied greatly among stomach cancers. This suggests that these two antibodies cannot be used uniformly for radioimmunoscintigraphy or radioimmunotherapy of stomach cancer. The maximal values of both antigens in stomach cancer were similar (around 500–600 pmol/g), but the median value for CEA was higher than that for TAG-72.

Our autoradiographic data showed that the TAG-72 concentration was higher in mucin containing adenocarcinoma. This finding is in accord with previous reports, in which TAG-72 seems to be present on the mucinous protein of adenocarcinoma.[21] Clinical radioimmunoscintigraphy with the B72.3 antibody revealed a greater accumulation of B72.3 in mucinous adenocarcinoma.[10] In contrast, the concentration of CEA expression was similar in both groups. In addition, there was no difference in the concentration of CEA among well differentiated, moderately differentiated and poorly differentiated adenocarcinomas. This finding was previously observed when using an immunoperoxidase staining method.[28] Santeusanio et al. reported results which differ from ours where they found a higher percentage of CEA positivity in well differentiated tumors as compared to moderately differentiated and undifferentiated tumors;[29] this report was not quantitative. The present study demonstrated both CEA and TAG-72 in areas of intestinal metaplasia. Nagura et al. found that the intensity of the CEA immunochemial reaction was related to the severity of metaplasia.[28] Ohuchi detected TAG-72 in benign lesions with intestinal metaplasia.[25] Generally, intestinal metaplasia of the stomach mucosa is believed to be a precancerous lesion. Our findings suggested a relationship between these antigens and carcinogenesis in the stomach.

Here we have shown that a significant number of stomach cancers expressed cellular CEA and TAG-72 heterogeneously. Heterogeneous expression of tumor antigens has been observed previously in gastric carcinomas.[21,30] There is not only antigenic heterogeneity among carcinoma cell populations, but also a temporal modulation of tumor antigen.[21] The CEA antigen was more frequently homogeneous than was TAG-72. However, in light of the degree of antigenic heterogeneity, the use of mixtures of monoclonal antibodies reactive with different antigens may be useful for the application of monoclonal antibodies as diagnostic adjuncts to detect or treat human carcinomas.

In summary, stomach cancer biopsies exhibit a wide variation in TAG-72 and CEA expression with heterogeneity in cellular antigen expression. The CEA expression is more frequent and hemogeneous than TAG-72. These findings suggest that the anti-CEA antibody may be more suitable than the B72.3 antibody for diagnostic immunoscintigraphy and immunotherapy of stomach cancer.

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