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Concentration and Distribution of Tumor Associated Antigens, TAG-72 and CEA, in Stomach Cancer

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위암조직에서 종양관련항원 TAG-72와 CEA의 농도 및 분포

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악성종양의 진단 및 치료에 있어서 특정 종양에 대한 항체를 이용하는 연구가 활발히 진행되고 있다. 단세포군항체를 이용한 방사면역신티그라피로 암의 조기 진단 및 영상화가 가능하고 나아가 방사면역치료는 암의 선택적 치료에 도움이 될 수 있다. 위암은 우리나라에서 가장 흔한 악성종양으로 방사면역신티그라피와 방사면역치료법이 새로운 방법으로 모색되고 있다. 이러한 진단 및 치료법의 성공여부를 결정하는 중요한 인자의 하나가 종양조직내에서 종양관련 항원의 농도와 분포이다. 따라서 본 연구에서는 단세포군 항체를 이용한 방사면역학적 방법의 임상 이용을 위한 기초 연구의 일환으로 in vitro quantitative autoradiography를 이용하여 종양 관련항원인 TAG-72와 CEA의 위암조직내 농도 및 분포를 측정하였다.

33예의 위암조직에서 얻은 동결절편을 1.3~83.3 nmol/liter의 ^{125}I 표지 항 TAG-72 단세포군 항체 B-72.3과 항 CEA 단세포군 항체 CEA-79로 반응시킨 후 이 표본들의 자가방사법 디지탈 영상을 H & E 염색과 immunoperoxidase 염색 표본과 비교하였으며, 특정 단세포군 항체의 결합에 대한 컴퓨터 분석으로 조직내 항원의 농도와 분포를 측정하였다.

TAG-72는 25예(75.7%)의 조직에서 검출되었으며 그 농도는 8.4~525.3 pmol/gram 이었고, CEA는 32예(96.9%)에서 검출되었으며 그 농도는 8.8~592.9 pmol/gram 이었다. CEA의 위암 조직내 발현농도는 중앙치가 101.7 pmol/gram 으로 TAG-72의 중앙치인 27.9 pmol/gram 보다 높았다. TAG-72의 조직내 분포는 41.4%에서 병변 부위의 암세포 분포와 일치하였고, CEA의 분포는 병

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변 부위의 80.5%에서 암세포와 일치하는 소견을 나타내었다. TAG-72의 농도는 점액성 선종(mucinous adenocarcinoma)과 점액함유 선종(mucin containing adenocarcinoma)에서 다른 선종보다 더 높았다. CEA의 농도는 위암의 병리학적 종류에 따른 유의한 차이가 없었다.

이상의 결과로 위암조직에서 TAG-72와 CEA 항원이 다양하게 발현됨을 알 수 있었고 CEA는 TAG-72 보다 더 빈번하게 균일한 분포로 발현하는 것으로 나타났다.

INTRODUCTION

Stomach cancer is the most frequent type of malignant tumor in many Asian countries including Korea, Japan and China[1]. There is no acceptable management of stomach cancer except early diagnosis and surgery[2,3]. Recently radioimmunoscintigraphy has been investigated as a new method to detect malignant tumors[4,5]. Monoclonal antibodies with specificity for antigens on stomach cancer as well as colon cancer are increasingly being tried as specific probes for radioimmunoscintigraphy and radioimmunotherapy[6,7]. Large amount of specific monoclonal antibodies are now available and it is important to find the appropriate antibodies among the abundant antibodies. Monoclonal antibody B-72.3 specific for TAG-72 and anti-CEA have been used widely in the radioimmunoscintigraphy for gastrointestinal carcinomas[7~11]. However, there were a few reports to investigate the concentration of these antigens in stomach cancer.

To analyze the presence and distribution of antigen in tumor, immunohistochemical methods have been used. However, these methods are not objective, not sensitive and not quantitative in nature. In a previous report we showed that in vitro quantitative autoradiography can be used to measure tumor associated antigen in histologic sections[12,13]. Using this method we evaluated the concentration and distribution pattern of tumor associated antigens, TAG-72 and CEA, in human stomach cancer.

MATERIALS & METHODS

1. Stomach Cancer Tissues

Thirty three specimens of stomach cancer were evaluated. All tumors were cases of advanced stomach cancer and obtained by surgical biopsy.

2. Monoclonal Antibodies

Two monoclonal antibodies (MoAb) to tumor associated antigens were used in the autoradiography. MoAb B-72.3 (IgG1), specific for TAG-72 glycoprotein, was supplied Dr. Schlom of NCI (Bethesda, MD). MoAb CEA-79 (IgG1), specific for CEA, was made at Seoul National University. Both antigens are on the cell surface.

Labeling of these antibodies with I-125 was performed with chloramine T method. One mg of antibody was reacted with 1 mCi of I-125 (New England Nuclear, Boston, MA) by adding 12.5 ug of chloramine T and stopped by adding 43.8 ug of sodium thiosulfate. The reaction time was 2 minutes. A PD -10 column (Pharmacia, Piscataway, NJ) was used for the separation of radiolabeled protein and free I-125. Specific activity was around 1.0 mCi/mg. Greater than 90% of the purified antibody was precipitable with 10% trichloroacetic acid.

Immunoreactivity of the radiolabeled antibody was determined by serial cell binding assay. In 200 ul, 0.25 million to 10 million SNU-C4 human colon cancer cells which expressed CEA were reacted with 5 ng of radiolabeled antibody. Nonspecific binding was measured by adding 25 ug of unlabeled antibody. After 2 hours incubation, all tubes were centrifuged. Cell pellets were counted and expressed as a percent-

age of total count (corrected with nonspecific binding). The immunoreactivity was calculated by double inverse plot, as described by Lindmo et al[14]. The immunoreactivity of I-125 CEA-79 ranged from 44.6% to 74.4%. In case of I-125 B-72.3, it ranged from 51.2% to 73.6%.

3. In Vitro Quantitative Autoradiography[15]

Twenty micron frozen sections were cut in a cryomicrotome. The tissue section were arranged into two groups: Total binding of specific antibody, 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 MoAb (B-72.3 or CEA-79) for 30 minutes. Next, all sections were incubated for 30 minutes in a solution containing 2% bovine serum albumin (BSA) and 10% chicken serum (CS) in phophate buffer saline (PBS) to reduce nonspecific binding of radiolabeled antibody. Then individual sections were incubated for 60 minutes in solution of I-125 MoAb ranged from 1.3 nmol/liter to 83.3 nmol/liter. Slides were washed in PBS and dehydrated with ethanol.

Autoradiographic standard was prepared with I-125 human serum albumin as Mies described[16]. The 2 sets of tissue section were exposed to the Kodak SB5 film with standard. Two days later, the films were developed. The autoradiographic films were digitized using a scanning microdensitometer (Amersham, Arlington Heights, IL). The optical density measured from I-125 human serum albumin standard were plotted against the specific activity of each standard. A polynomial fitting of these data provided a standard curve. We compared autoradiographic image to hematoxylin and eosin stained histologic slide, and defined the region of interest in autoradiographic image. The mean optical density of the selected region was obtained and the uCi/g of tumor were determined from the standard curve. We calculated the concentration of binding antibody as mol/g from the specific activity value for each I-125 MoAb preparation.

4. Analysis of Binding Data

The data of the saturation study were analyzed using nonlinear least square fitting method. We used two equations to analyze the fitting curve and obtained value (Fig. 1).

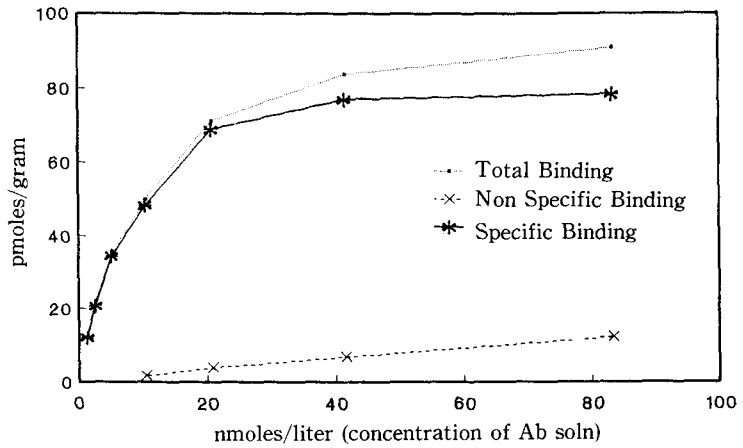

Fig. 1. Binding curves developed from saturation study. The curves were obtained by relating concentration of antibody bound to tumor sections vs. concentration of ^{125}I labeled MoAb in incubation solution.

The first was a saturation plot.

$$\text{Total Binding}=\frac{Bmax\times[Ab]}{Kd+[Ab]}+a\times[Ab]$$

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

Where Bmax is the maximal amount of I-125 labeled MoAb which binds specifically to tissue, [Ab] is the concentration of I-125 labeled MoAb in the incubation media, Kd is the antigen/antibody dissociation constant, and a is the slope of the nonsaturable curve. Specific binding was obtained by substracting nonsaturable binding curve from the total binding curve. With these curves we evaluate the uncertainty of the data.

The other was a double inverse plot between specific binding and the concentration of labeled MoAb.

$$1/\text{Specific binding}=1/Bmax+Kd/Bmax\times[Ab]$$

A plot of 1/specific binding versus 1/[Ab] will yield a straight line with the value of the y-intercept equal to 1/Bmax. Computer fitting of the curve gave Bmax, the maximal concentration of I-125 labeled antibody was used to calculate the maximal pmol/gram of antibody bound to tumor. This value was taken to be the same as the concentration of antigenic epitope.

The fitting and calculation were made with IBM personal computer AT and RS-1 curve modeling program.

5. Immunoperoxidase Staining

Adjacent sections of tissue were processed for immunoperoxidase staining. The avidin-biotin complex method was used for both MoAbs. The intensity of the chromogen reaction was graded 0 to 4 plus.

RESULTS

Table 1 showed the results from the quantitative autoradiographic analysis of tumor sections from stomach cancer specimens. TAG-72 concentration could be measured in 25 specimens (75.7%) and undetectable in 8 specimens. CEA was detectable in all specimens except one (96.9%). Out of 40 pathologic lesions, TAG-72 was expressed in 29 (72.5%). However, CEA was expressed in 90% of lesions. The median value of TAG-72 was 27.9 pmol/gram (Table 2), and that of CEA was higher (101.7 pmol/g). Figure 2 is frequency histogram of concentrations of TAG-72 and CEA. The concentration of TAG-72 ranged from 8.4 pmol/g to 525.3 pmol/g, and the concentration of CEA from 8.8 pmol/g to 592.9 pmol/g. The concentrations of these antigens varied over 2 orders of magnitude.

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%)

Table 2. Concentrations of TAG-72 and CEA in Stomach Cancer

	TAG-72 (pm/g)	CEA (pm/g)
Median value	27.9	101.7
Range	0 - 525.3	0 - 592.9

The concentrations of TAG-72 and CEA were compared to the pathologic type of tumor. Table 3 showed that the concentration of TAG-72 was higher in mucinous adenocarcinoma than other types. However, as shown in table 4, the concentration of CEA did not vary greatly from one type of tumor to another.

We related the antigen levels of various tumors to the presence or absence of mucin. Carcinomas with mucinous components had higher values of TAG-72 concentration than carcinomas without mucinous components (Table 5). In our series, the poorly differentiated carcinoma without a mucinous component was the most frequent type and in this tumor the

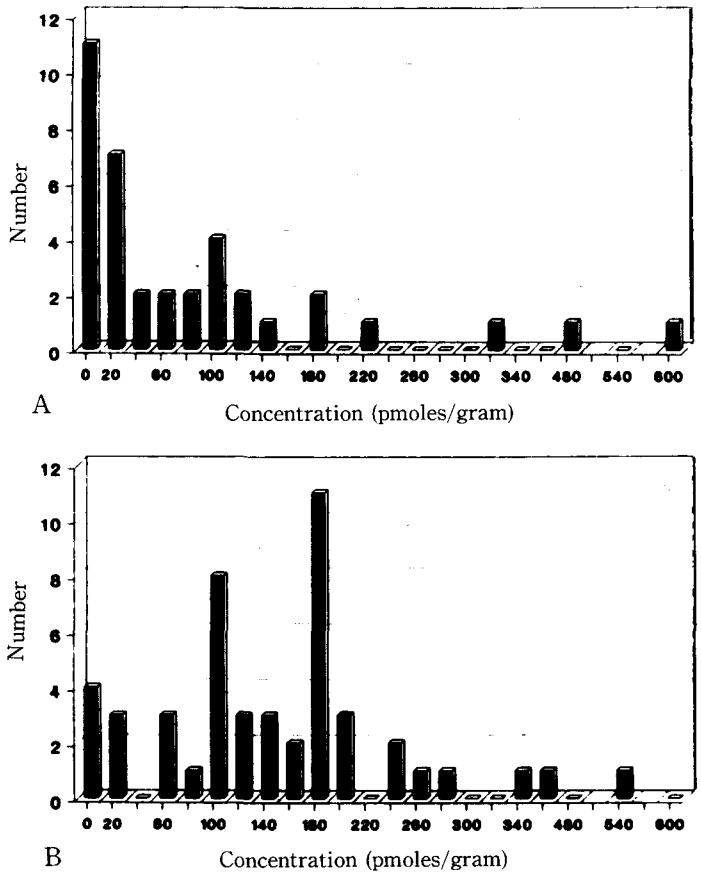

Fig. 2. Frequency histograms showing the autoradiographic measured concentration of TAG-72 (A) and CEA (B) in tissues of stomach cancer.

Table 3. Concentrations of TAG—72 According to Pathologic Types

Pathologic type	Number	Concentration (pm/g)
Carcinoma		
Well differentiated	3	0
Moderately differentiated	7	59.6± 78.1
Poorly differentiated	19	19.0± 35.4
Mucinous	7	250.5±164.3
Signet ring cell	1	102.7
Intestinal metaplasia	4	48.3± 4.1

Table 4. Concentrations of CEA According to Pathologic Types

Pathologic type	Number	Concentration (pm/g)
Carcinoma		
Well differentiated	4	100.1± 68.8
Moderately differentiated	7	108.7± 57.8
Poorly differentiated	19	155.8±162.9
Mucinous	7	140.1± 84.1
Signet ring cell	1	93.9
Intestinal metaplasia	4	52.2± 13.8

concentration of CEA was significantly greater than was the concentration of TAG-72. In the table 5, we summarized the antigen concentration according to mucin containing nature. In TAG-72, the concentra-

tion of mucin containing adenocarcinomas was significantly higher than the concentration of carcinomas without mucin. However in CEA, there was no difference between 2 groups.

The pattern of tumor cell distribution as found in H & E sections was compared to the pattern of antigen expression in the autoradiographic images (Table 6). When tumors were evaluated for TAG-72, the pattern of antigen expression coincided with the cellular distribution in 41.4% of the cases. There were significant numbers of cells that expressed low or negligible amount of antigen. Evaluation of CEA showed that patterns of antigen expression and cellular distribution coincided in 80.5% of the cases. That is CEA expression was heterogeneous in only 19.5% (Table 7).

The typical example was shown in figure 3, which was a case of poorly differentiated adenocarcinoma with diffuse cellular distribution.

Table 5. Concentrations of TAG–72 and CEA According to Mucin Containing Nature

Pathology	No.	TAG–72 (pm/g)	CEA (pm/g)
Adenocarcinoma with mucin	13	178.7±148.4*	122.8± 66.6
Adenocarcinoma without mucin	25	23.6± 32.3*	143.9±146.8

* $p < 0.0001$

Table 6. Comparison of Autoradiographic (ARG) Image Patterns of TAG–72, CEA and Tumor as Defined in Sections Stained with H & E

H & E	ARG	TAG–72	CEA
Diffuse			
	Diffuse	9	22
	Focal	0	0
	Irregular	14	6
Focal			
	Focal	0	2
	Irregular	3	1
Scattered			
	Irregular	3	5

Table 7. Coincidence Between Expressions of TAG–72 and CEA Measured by Autoradiography (ARG) and Cellular Distribution Measured by H & E Staining

	TAG–72	CEA
Lessions	29	36
Coincided with ARG	12 (41.4%)	29 (80.5%)
Not coincided with ARG	17 (58.6%)	7 (19.5%)

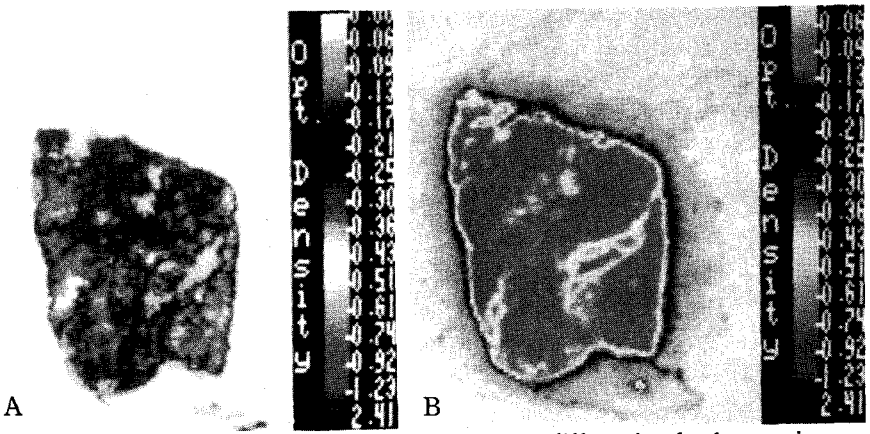

Fig. 3. Autoradiograhic (ARG) images of a poorly differentiated adenocarcinoma with diffuse cellular distribution. A. ARG image using ^{125}I-B-72.3 showed irregular heterogeneous expression of TAG-72; B, ARG image using ^{125}I-CEA-79 showed homogeneous expression of CEA.

DISCUSSION

Worldwide the most common neoplasm is still cancer of the stomach, incidence rates of which are high in East Asia, Europe and South America. This malignancy has a very poor prognosis, and mortality rate is extremely high[2,3].

Radioimmunoscintigraphy and radioimmunotherapy have been studied as new methods to diagnose and treat malignancy. Gastrointestinal carcinomas express a variety of tumor-associated antigens including CEA, TAG-72, GICA and DuPan-2. In this study, we measure 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 was made from CEA, which were purified from culture supernatant of human colon cancer cell line, LS174T[17].

MoAb B72.3 is a murine IgG_1, which was prepared using a membrane-enriched extract of human metastatic breast carcinoma as antigen and recognizes a $Mr > 10^6$ novel tumor-associated glycoprotein, TAG-72[18]. TAG-72 is expressed in several epithelium-derived malignancies, including the vast majority of adenocarcinomas of the stomach, colon and breast, non-small cell lung carcinoma and ovarian carcinomas[19].

It is well known that CEA is expressed frequently in gastric cancer[20~22]. And TAG-72 is also expressed in vast majority of gastric adenocarcinoma[19,23]. Double histochemical staining with both antibodies for CEA and TAG-72 revealed that two antibodies in combination, react with at least 90% of carcinoma cells in 13 of the 17 tumors[19]. However, Gero et al. reported a poor correlation between the CEA and TAG-72 values of sera obtained from gastric cancer patients, suggesting the complementarity of CEA and TAG-72 measurements in the analysis of gastric cancer[24]. In addition, Ohuchi et al. confirmed that when monoclonal antibodies B-72.3 and COL-6 (anti-CEA antibody) were simultaneously reacted with the gastric carcinoma tissue, more carcinomas were detected as compared to the number of carcinomas shown to be reactive with the individual antibody[19]. In our experiment, CEA and TAG-72 were expressed in 90% and 72.5% of lesions, respectively. And either one of two antibodies was reactive with stomach cancer in 95% of lesions.

Previously, Del Vecchio reported in vitro quantitative autoradiography as a new method to quantitate and evaluate the tumor antigen[15]. Contrary to the immunohistochemical technique, this method yields numerical data which are not dependent on observer judgement and the results for different tissues, using the same or different antibodies or from different antigen/antibody systems can be directly compared. By using standard stick of melanoma cultured cells, we compared the effects of variable incubation time and temperature with radiolabeled antibody and found that 1 hour incubation in room temperature was enough for the adequate data[13]. Del Vecchio raported the coefficient of variation in this method was 20%[15].

In this study, we found that wide variation of CEA and TAG-72 in stomach cancer. This suggested that we can not use these antibodies uniformly for the radioimmunoscintigraphy and radioimmunotherapy. The maximal values of both antigens in stomach cancer were similar (around 500-600 pmol/gram). The median value, however, was higher in CEA than in TAG-72.

This autoradiographic data showed that the concentration TAG-72 was higher in mucin containing adenocarcinoma. This finding is in accord with the previous reports, in which TAG-72 was seemed to be in the mucinous protein of adenocarcinoma[19]. Clinical radioimmunoscintigraphy using B-72.3 antibody revealed that higher accumulation of the antibody in mucinous adenocarcinoma[25]. In case of CEA, the

concentration of expression was not different between two groups. Additionally, there was no difference in the concentration of CEA among well differentiated, moderately differentiated and poorly differentiated adenocarcinomas. This finding was previously observed by immunoperoxidase staining method[26]. However, Santeusanio reported a high percentage of CEA positivity was seen in well-differentiated tumors[27]. But this report was not quantitative. CEA and TAG-72 were demonstrated in areas of intestinal metaplasia. Nagura et al. reported the intensity of the immunochemical reaction of CEA was related to the severity of metaplasia[26], and Santeusanio found a faint CEA-positivity was observed in intestinal metaplasia[27]. Ohuchi reported that TAG-72 was detected in benign lesions with intestinal metaplasia[23]. Generally intestinal metaplasia of the stomach mucosa are seemed to be precancerous lesion. Thus this finding suggested the relation between these antigens and carcinogenesis in the stomach.

In this experiment, heterogeneity of cellular CEA and TAG-72 expressions was exhibited by a significant number of stomach cancer. This kind of heterogeneity of antigen expression was observed in other reports[19,28,29].

There is not only antigenic heterogeneity among carcinoma cell populations, but also a temporal modulation of tumor antigen[19]. The CEA antigen was more frequently homogeneous than was TAG -72. However, in the light of the degree of antigenic heterogeneity, the use of mixtures of monoclonal antibodies reactive with different antigens, may be essential for the application of monoclonal antibodies as immunological adjuncts to detect or treat human carcinomas.

In summary, stomach cancer exhibited wide variation of TAG-72 and CEA expression with heterogeneity in cellular antigen expression. However, the CEA expression was more frequent and hemogeneous than TAG-72. These findings suggested that anti-CEA antibody may be more suitable for immunoscintigraphy and therapy of stomach cancer.

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