Tumor Res. **27**, 1—14 (1992)

Tumor Markers in Human Gastric Cancer

Kohzoh IMAI and Akira YACHI
Department of Internal Medicine (Section 1), Sapporo Medical College

Key words: Tumor marker, Gastric cancer, Monoclonal antibody, Diagnosis

INTRODUCTION

Many monoclonal antibodies (MoAbs) have been identified over the 15 years since the advent of hybridoma methodology. Antibodies which detect gastric cancer-associated antigens have made a place for themselves as an essential, non-invasive diagnostic tool for the clinician. The name 'tumor markers' has been coined for these antigens as well as cancer-associated proteins, amino acids and glycolipids. The name has been found to apply further afield, to genes, including oncogenes and suppressor oncogenes as well as their mRNAs, if they are expressed or depressed preferentially in tumor cells or tissues.

In this review, we will describe the latest information concerning gastric cancer-associated tumor markers and briefly discuss the significance of these markers from the viewpoint of tumor biology.

TUMOR MARKERS DETECTED IN THE SERUM AND THE TISSUE

Many monoclonal antibodies have been established as an aid in detecting gastric cancer-associated antigens [1, 2]. Among these interesting monoclonal antibodies, some have turned out to be capable of providing precise measurements of circulating antigens in the serum. Table 1 shows the incidence of the antigens in sera from patients with gastric cancer. It is well known that carcinoembryonic antigen (CEA) occurs in 20-30% of the patients with gastric cancer [3]. CA19-9, which carries the sialyl Le^a epitope, shows a 32% incidence [4], while sialyl SSEA-1 bearing molecules, recognized by monoclonal antibody FH6, only show 13% [5]; it seems that gastric cancer cells produce sialyl Le^a bearing molecules more frequently than sialyl SSEA-1 molecules. MoAb B 72.3 has recently been found to recognize the sialosyl Tn epitope [6], and appears in 43% of the sera of the gastric cancer cases [4]. In the case of the YH206 antigen [7, 8], the incidence is 32%, the same as that of CA19-9 [4]. Interestingly, this monoclonal antibody recognizes the high-molecular-weight asialo-type glycoproteins, which belong to the classic mucin molecules [9], while the MoAb

NCC-ST-439 recognizes the sialosyl-type glycoprotein, showing a 19% incidence [10].

Scirrhous-type gastric cancer is still difficult to diagnose using serum markers. Niitsu *et al.* [11], however, found that the procollagen type I shows higher level in the serum in particular from patients with scirrhous-type gastric cancer.

Recently, cDNA of mucin genes has been extensively cloned; as of the beginning of 1992, four mucin genes have been reported, and designated as MUC-1, 2, 3 and 4 [12-15]. We do not know at present to which gene our YH206 antigen belongs. It is interesting, however, that at least one of the mucin gene products carries the asialo-type sugar chain; this epitope can be frequently detected in the sera from gastric cancer patients. It is noteworthy that there was no correlation between CA19-9 and YH206 levels in the sera of gastric cancer patients [9].

Monoclonal antibody MUSE11, which was originated in our laboratory [16], was found to recognize the MUC-1 gene core protein [17]. The binding activity of MUSE11 to a synthetic peptide corresponding to the tandem repeat motif of the MUC-1 gene protein was compared with MoAbs DF3 (Table 2), which is the antibody used in the CA15-3 assay kit for monitoring breast cancer; MoAb MUSE11 showed a higher binding activity than that of MoAb DF3 [18]. It is known that MoAb DF3 detects epitope structure containing sialylated carbo-

Table 1 *Incidence of positivity of various tumor markers in the sera of patients with gastric cancer*

Monoclonal antibody	Antigenic structure	Positive ratio (No. positive/ No. tested)	reference
NS19-9	Sialyl Le^a (CA19-9)	32%(30/94)	4
FH-6	Sialyl SSEA-1	13%	5
B 72.3/CC49	Sialosyl-2 6 α-N-acetyl galactosamyl epitope (TAG-72)	43%(40/94)	4
NCC-ST-439	high-molecular-weight glycoprotein (sialosyl type)	19%(48/249)	10
YH206	high-molecular-weight glycoprotein (asialo-type)	32%(22/69)	8
MUSE11	MUC-1 gene core protein	32%(9/28)	16
c-erbB-2	185 kD glycoprotein	10%(4/40)	submitted

Table 2 *Binding activity of MoAbs MUSE11 and DF3 to a synthetic peptide*

MoAbs	Binding activity (cpm)* to peptide+
MUSE11	42852±794
DF3	14163± 46
YH206	1074± 29
P1-255	2108± 46

* Detected with an ^{125}I-labeled anti-mouse IgG-Fc antibody.
+ A peptide consisting of one repeat and 4 amino acids from the tandem repeat region of mucin core protein. Concentration of the peptide and MoAbs used were 100 μg/ml and 5 μg/ml, respectively.
Values are mean ± SD of triplicated determinations.

Table 3 *Relationship between serum levels of antigen MUSE11 and CA15-3 in patients with gastric cancer*

	CA15-3(+)	CA15-3(−)
Antigen MUSE11(+)	7	11
Antigen MUSE11(−)	2	15

hydrate chains. The serum levels of antigen MUSE11 and CA15-3 were simultaneously measured in 35 specimens including gastric cancers and the relationship between serum levels of antigen MUSE11 and CA15-3 was then evaluated (Table 3) [unpublished observation]. Seven out of 35 serum specimens were tested positive for both antigen MUSE11 and CA15-3. Eleven out of 35 were positive for antigen MUSE11 and negative for CA15-3, whereas only 2 were negative for antigen MUSE11 and positive for CA15-3. These data indicate a higher incidence of abnormal levels of antigen MUSE11 in the sera of gastric cancer patients compared to CA15-3 ($p<0.01$). It is noteworthy that no significant correlation was observed in the serum levels between antigen MUSE11 and CA15-3. In any case, it is of interest that MoAb MUSE11 can detect 32% of the gastric cancer patients (Table 1).

ErbB-2 protein is a 185 kD glycoprotein encoded by the c-erbB-2 gene, which was found by Yamamoto, Toyoshima and their colleagues in 1986 [19]. Several monoclonal antibodies to erbB-2 protein have been established, including our own monoclonal antibody [Ishida, T. *et al.* submitted for publication]. The simultaneous use of two monoclonal antibodies, is neccessary to unambiguously detect erbB-2 protein in the serum. As depicted in Table 3, this method was successful in identifying 10% of the gastric cancer patients. Furthermore, all the patients

with gastric cancer who showed higher levels of erbB-2 protein were found to have well-differentiated adenocarcinoma. Therefore, although it is not very common to find erbB-2 protein in the circulation, when it is found, it is a dependable indicator for this histological type of tumors. It is also possible that the erbB-2 protein-positive patients form a subgroup of patients with well-differentiated adenocacinoma.

Studies of suppressor oncogenes have also borne fruit. For example, Matozaki and his coworkers [20] have studied the p 53 gene, a suppressor oncogene, in 12 human gastric cancer cell lines. After the reverse-transcriptase polymerase chain reaction and direct sequencing, 7 cell lines showed point mutations of the p 53 gene resulting in amino-acid substitutions. Most of them were rare mutations which had not been observed in other types of cancers. Six out of 7 cell lines with mutations of p 53 gene also lost one allele of chromosome 17 p. Immunoblotting of cell lysates with an antibody specific to p 53 demonstrated high levels of p 53 protein in 5 cell lines, all of which contained mutations of the p 53 gene. These results suggest that human gastric cancer tissue may show certain abnormalities either at the genetic level or at the protein/mRNA level. In fact, preliminary results performed by Shiku *et al* (Nagasaki University School of Medicine) showed that 15 out of 30 cases showed point mutations of the p 53 gene and that 12 out of 27 cases showed positive nuclear staining with the antibody to p 53 protein. Eleven of these 12 cases were found to have structural abnormalities in the p 53 gene (personal communication by Prof. SHIKU). Other suppressor oncogenes or the candidates for suppressor oncogenes, such as DCC, APC and PTP will be examined in the near future.

TUMOR MARKERS AND EARLY DETECTION OF GASTRIC CANCER

In many parts of the world, mass endoscopy-based screening for gastric cancer is not feasible because of the relatively low incidence of the disease. Alternative approaches, such as screening using cancer-associated markers, are not, as yet, reliable enough to be used.

To be clinically useful for screening, as distinct from "monitoring" the progress of an established disease, a marker for gastric cancer should also be sensitive enough to allow detection of cancer before it becomes symptomatic or detectable with the usual diagnostic tools. Additional requirements would include that the marker could be assayed with an easily performed test, cause a minimum of tissue damage and discomfort to the patient, have a low cost/effectiveness ratio and give reproducible results.

Farinati *et al*, [21] have identified MoAb CA50, which bears sialosyl fucosyl-lactotetraose corresponding to the sialylated blood group antigen Lewis(a), in

serum, gastric juice and urine of patients undergoing upper gastrointestinal tract endoscopy: this study employed 22 control subjects (no macroscopic or microscopic lesions), 29 patients with chronic atrophic gastritis, 20 with epithelial dysplasia and 16 with gastric cancer. Gastric juices were also tested for pH, protein concentration and specific gravity, and urines for protein concentration and osmolarity. Serum and gastric juices were also tested for CEA levels. Comparison of the results obtained with the two markers revealed that in patients with gastric cancer, CA50 gastric juice levels were statistically higher than in controls; a wide overlap was, however, present among groups, and the sensitivity and specificity were respectively 38% and 85% for serum and 69% and 82% for gastric juice. Sensitivity and specificity were respectively 23% and 89% for CA50 determination in urines. In this case, no statistically significant difference was observed between gastric cancer and control patients. A trend toward higher median values wan observed in advanced with respect to early gastric cancer. A correlation was found between gastric juice and serum CA50 levels, as well as between serum and urine levels of the marker. A correlation was also observed between CA50 values and protein concentration in gastric juice and with osmolarity in urines. Overall, CA50 levels were statistically higher in patients with intestinal metaplasia than in those who had no lesions. Increased CA50 gastric juice levels were also observed in patients with chronic atrophic gastritis and epithelial dysplasia. CA50 levels in gastric juice and urine appear to depend, at least in part, on the concentration of the protein in the fluid. Horinouchi *et al.* [22] investigated alterations of carbohydrate chain antigens using immunohistochemical method in relation to histological malignant changes on 62 cases of gastric atypical epithelial lesions (adenomas) which were diagnosed as Group-III at the first biopsy. These cases were followed up after more than one year. Among the 7 carbohydrate chain antigens which are related to the Lewis antigen, the sialyl Le^x-i antigen showed the most impressive findings; the ratio of positive indications from the first biopsy specimens of Group-III was 6%, however, it rose to 33% in the final biopsy specimens of Group-IV, 50% in the resected specimens of border-line lesions, and 67% in the resected specimens of carcinomas. The results indicate that there exists a close correlation between malignant changes in gastric atypical epithelial lesions and alterations in carbohydrate chains in terms of sialylation.

The monoclonal antibodies to the *ras* oncogene product p 21 have been used to analyse the precancerous lesions. Ohuchi *et al.* [23] showed that dysplastic lesions and noncancerous lesions immediately configuous to cancerous tissue tested positive for p 21 using the monoclonal antibody RAP-5. We have also found a high number of cells expressing p 21 in gastric atypical epithelial lesions of more

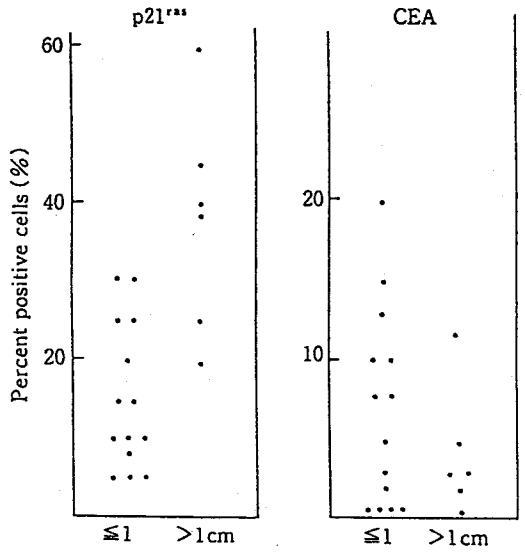

Fig. 1 Percent positive cells of ras p 21 and CEA in the atypical epithelial lesions determined by immunoperoxidase technique

than 1 cm in diameter, using the MoAb rp-12 [Imai *et al*, unpublished observation] which recognizes both the H- and K-*ras* product p 21 (Fig. 1). An *in situ* hybridization assay in conjunction with an H-ras gene probe confirmed this tendency at the mRNA level in our laboratory. Yoshida, Shiku *et al.* [24] have followed this line of investigation using their MoAb RASK-3 which reacted with all the Ki-, N- and Ha-*ras* p 21s, and proposed an interesting hypothesis: They showed a marked expression of p 21 in moderately- to well-differentiated cancer, intestinal metaplasia, and atypical hyperplasia, but not in normal epithelial cells and hyperplastic polyps; this indicates that the expression of p 21 in the epithelial cells of the stomach has increased as a consequence of cellular changes to premalignant status, such as intestinal metaplasia and atypical hyperplasia.

Recently, *Helicobacter pylori* (*H. Pylori*) infection has been believed to be responsible for most cases of gastritis [25]. Investigations into the epidemiology of *H. pylori* infection have been assisted by a large body of information. Moreover, a number of excellent indirect studies have shown a consistant link between the frequency of *H. pylori* and the prevalence of gastric carcinoma in various populations. Recently, the results of three case control studies [26-28] have confirmed and extended the indirect association between *H. pylori* and gastric cancer. We have developed several monoclonal antibodies to *H. pylori* [29]. Because the serum from patients with gastritis reacted strongly with the 25 kD

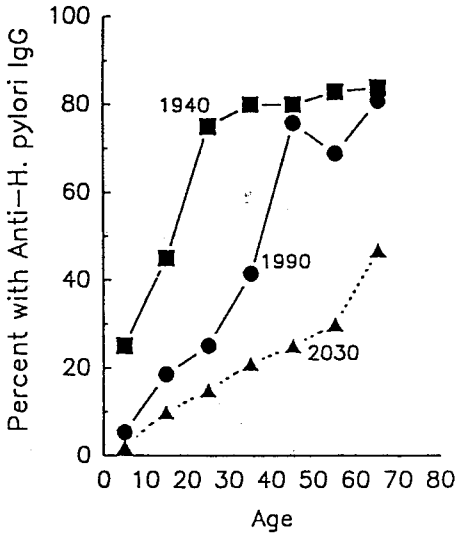

Fig. 2 The effect of westernization on the epidemiology of *Helicobacter pylori* in Japan.
The 1940 curve shows the pattern of incidence in a typical underdeveloped country, which would have been expected from a study in that year.
The 1990 curve shows the current pattern and the 2030 curve represents the prevalence of *H. pylori* that may occur with continued improvements in the standard of living in Japan. IgG, immunoglobulin G.

antigen among the *H. pylori* antigen, we have developed the ELISA system for detecting the serum antibody against the 25 kD antigen [30]. The serum titer of anti-25 kD antibodies (mainly IgG type) was significantly higher in patients with gastritis than in healthy controls. In addition, the titer of IgA type anti-25 kD antibodies in gastric juice correlated closely with the histological grade of gastritis, suggesting that a local immune response to *H. pylori* in gastric mucosa might also be associated with the formation of gastritis [31]. Asaka, Miki., *et al* [32] reported that the serum pepsinogen I and II levels (markers of gastritis and gastric atrophy) were significantly higher in *H. pylori-infected* volunteers than in *H. pylori-uninfected* volunteers. Graham *et al.* [25] suggested an interesting epidemiological way of thinking: their prospective study in Japan (Fig. 2) has given us further insight into the changes in the epidemiology of *H. pylori* associated with westernization of Japanese society, and this change is consistent with ongoing changes in the epidemiology of gastric cancer in Japan [32]. Although we need more data, especially desease-specific features of *H. pylori*, in order to connect *H. pylori* infection and gastric cancer, we must keep in mind

that *H. pylori* provides the proper environment such as chronic atrophic gastritis and/or intestinal metaplasia, for gastric cancer.

TUMOR MARKERS AND PROGNOSIS OF GASTRIC CANCER

Many tumor markers have been used to detect malignancies, and to assess the efficacy of treatment. Predicting prognosis and detecting recurrence are also an important role for tumor markers. In this section, we will introduce some recent reports concerning these points.

A recent study [33] using a MoAb specific to estrogen receptors showed receptors in 28 per cent of gastric adenocarcinomas, particularly in poorly differentiated lesions. Harrison *et al.* [34] described the correlation of estrogen receptor-related protein status with long-term survival in a group of 188 consecutive patients undergoing surgery for gastric cancer; those who tested negative for the presence of this protein had a significant survival advantage over those who tested positive. Prognostic factor analysis with a Cox proportional hazards model showed the tumor stage and the estrogen receptor-related protein to be significantly independent factor in gastric cancer.

In 1970, Bourreille *et al.* [35] reported the first case of α-fetoprotein (AFP)-producing gastric cancer with liver metastasis. There have been a few cases of advanced and early gastric cancer which produced AFP in the literature. The incidence of AFP-producing gastric cancer is reported to be from 1.3 to 15%, depending on the writer (about 5% average) in Japan [36]. Chang Y-C. *et al.* [36] compared 27 cases of AFP-positive gastric cancer with 478 cases of

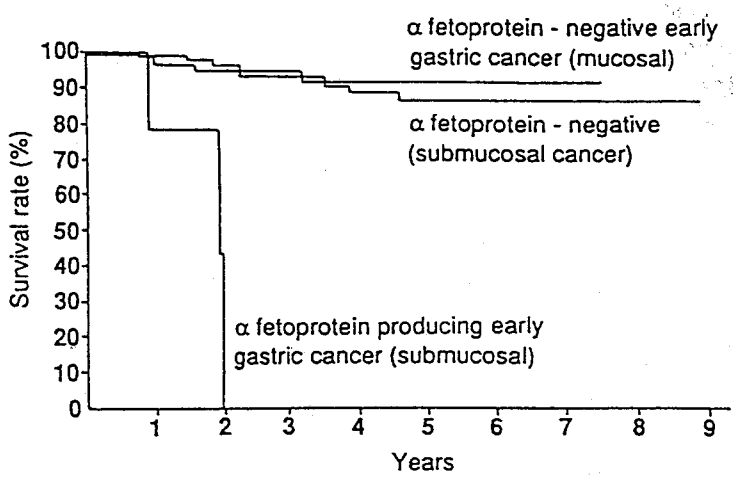

Fig. 3 Survival curves of α fetoprotein producing and α fetoprotein negative early gastric cancers calculated by the Kaplan-Meier method.

AFP-negative gastric cancer. Although sex, age distribution, pathologic type and serum CEA levels were similar between these two groups, Borrmann III-type cancer, lymph node metastasis, and incidence of synchronous and metachronous liver metastasis occurred more often in the AFP-positive group. Liver metastasis occurred in 72% of the AFP-positive patients, all of whom died within 2 years. The long-term survival of the AFP-positive group was clearly worse than that of the others. They also reported three cases of AFP-producing early gastric cancer, in all of whom liver metastasis occurred shortly after curative gastrectomy; all died within two years [37] (Fig. 3). These data suggest that AFP-producing cells are more prone to liver metastasis. Chang discussed the possibility that the liver may offer a suitable environment for AFP-producing tumors to proliferate. It also is possible that AFP-producing cells are associated with early vascular invasion. In any case, AFP-producing gastric cancer seems to have a poor prognosis. Establishment of an AFP-producing gastric cancer cell line [38] may help dissect the mechanisms of this disease process.

Using CA19-9 as a marker, Ikeda *et al.* [39] compared two groups: 57 patients who died of recurrence or metastasis within 2 years (group I) and 58 patients who survived 5 years or more after resection (group II). In undifferentiated type carcinoma, the staining pattern belonging to stromal type was seen in 65% of group I and 23% of group II. Therefore they proposed that the immunohistochemical localization for CA19-9 in tumorous tissues, particularly in undifferentiated carcinoma, will be useful in predicting the prognosis of patients with advanced gastric carcinoma.

Recent studies huve shown that the *erbB-2* proto-oncogene is amplified in 25-33% of human mammary carcinomas and that there is a significant association between *erbB-2* amplification and prognosis, as well as with lymph node metastasis [40]. In gastric carcinoma, however, there have been conflicting results in the literature. Yonemura *et al.* [41] reported that expression of *erbB-2* protein using a polyclonal antibody, was associated with serosal invasion, lymph node metastasis and lymphatic invasion, and that patients with *erbB-2* protein-positive tumors had a 5-fold greater relative risk of death, as compared with those with *erbB-2* protein-negative tumors. Jain *et al.* [42], on the other hand, described apparently opposite results. They claimed that overall, patients with tumors expressing this proto-oncogene had a significantly improved prognosis and that within the group of intestinal-type tumors, those that were *c-erbB-2* positive formed a distinct sub-population which had a better prognosis, suggesting possible differences in etiology. Both Yonemura and Jain used formalin-fixed parafin embedded tissue sections and polyclonal anti-*c-erbB-2* product antisera, although the epitope recognized by each antisera seems to be different. We

would think that the *in situ* hybridization method or Nothern blot analysis may be helpful to dissect these conflicting results.

Yamaguchi *et al.* [43] examined the possibility that urinary pepsinogen I is a tumor marker of stomach cancer after total gastrectomy. Because of the high blood and urine concentration of pepsinogen isozymes derived from gastric mucosa, it is difficult to detect trace amounts of the pepsinogen isozymes produced by gastric cancer cells. However, when the stomach is totally resected, the urinary concentration of "background" pepsinogen isozymes decreases to lower levels. They reported that 22 out of 74 cases who had undergone total gastrectomy for stomach cancer showed positive indications for urinary pepsinogen I, and that 20 of these 22 positive cases had definite clinical signs of recurrence of stomach cancer. This suggests that uninary pepsinogen I will be an useful tumor marker in detecting the recurrence of stomach cancer after total gastrectomy.

Asao *et al.* [44] have reported interesting results after using CEA in peritoneal washings. They determined CEA levels in the peritoneal washings from 120 patients with gastric cancer and 9 patients with benign diseases. Elevated values (>100 ng/g of protein) were observed in 20 of 25 patients with gastric cancer with visible dissemination and 16 of 25 patients with serosal invasion but no dissemination. The same elevation was found in only 9 of 70 patients with no serosal invasion and none of the patients with bening diseases. The 2-year survival rates after curative operations for the patients with and without elevation of CEA levels were 21% and 100%, respectively ($p<0.001$). They concluded from this study that the CEA level in peritoneal washings could be a sensitive detector of invisible peritoneal dissemination and a new predictor for the postoperative prognosis of gastric cancer.

ACKNOWLEDGMENTS

This work was financed by a Grant-in-Aid for Scientific Research on priority Areas and by a Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. YACHI A, IMAI K, BAN T, HINODA Y, WADA T. Serodiagnosis of digestive-tract malignancies employing novel monoclonal antibodies. In: WADA T, AOKI K, YACHI A (ed) Current status of cancer research in Asia, the middle east and other countries, The University of Nagoya Press, Nagoya, 1987, 111-117.
2. IMAI K, MORIYA Y, FUJITA H, TSUJISAKI M, KAWAHARADA M, YACHI A. Immunological characterization and molecular profile of carcinoembryonic antigen by monoclonal anti-

bodies. J Immunol 1984, 132: 2992-2997.

3. KOGA T, KANO T, SOUDA K, OKA N, INOKUCHI K. The clinical usefulness of preoperative CEA determination in gastric cancer. Jpn J Surg 1987, 17: 342-347.
4. GUADAGNI F, ROSELLI M, AMATO T, COSIMELLI M, PERRI P, CASALE V, CARLINI M, SANTORO E, CAVALIERE R, GREINER JW, SCHLOM J. CA 72-4 Measurement of tumor-associated glycoprotein 72 (TAG-72) as a serum marker in the management of gastric carcinoma. Cancer Res 1992, 52: 1222-1227.
5. KANNAGI R, FUKUSHI Y, TACHIKAWA T, NODA A, SHIN S, SHIGETA K, HIRAIWA N, FUKUDA Y, INAMOTO T, HAKOMORI S, IMURA H. Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing fucosyl of sialylfucosyl type 2 chain polylactosamine. Cancer Res 1986, 46: 2619-2626.
6. KJELDSEN T, CLAUSEN HIROHASHI S, OGAWA T, IIJIMA H, HAKOMORI S. Preparation and characterization of monoclonal antibodies directed to the tumor-associated O-linked sialosyi2-6 -N-acetylgalactosaminyl (sialosyl-Tn) epitope. Cancer Res 1988, 48: 2214-2220.
7. HINODA Y, IMAI K, *et al*. Detection of circulating adenocarcinoma-associated antigen in the sera of cancer patients with a monoclonal antibody. Jpn J Cancer Res (GANN) 1985, 76: 1203-1211.
8. HINODA Y, IMAI K, *et al*. A sandwich enzyme immunoassay of an adenocarcinoma-associated antigen, YH206, in cancer sera. Jpn J Cancer Res (GANN) 1897, 78: 607-613.
9. HINODA Y, IMAI K, *et al*. Immunochemical characterization of adenocarcinoma-associated antigen YH206. Int J Cancer 1988, 42: 653-658.
10. OHKURA H, HATTORI N, MIYAZAKI I, SAWABU N, TOBE R, KAWAI K, ABE O, ISHII M, KAWAI T, KAMANO T, KURIHARA M, MIZUMOTO R. Clinical study of the NCC-ST-439 EIA kit using serum from patients with various types of cancer and benign diseases (1). Jpn J Cancer Chemother 1987, 14: (Pt. I) 1901-1906.
11. NIITSU Y, MATSURA K, MORITA Y. Expression of collagen, TGF-β and cadherin in scirrhous-type gastric cancer. Onoclogia 1989, 22: 18-22.
12. SPICER AP, PARRY G, PATTON S, GENDLER SJ. Molecular cloning and analysis of the mouse homologue of the tumor-associated mucin, MUC1, reveals conservation of potential O-glycosylation sites, transmembrane, and cytoplasmic domains and a loss of minisatellitelike polymorphism. J Biol Chem 1991, 266: 15099-15109.
13. TORIBARA NW, GUM JR Jr, CULHANE PJ, LAGACE RE, HICKS JW, PETERSEN GM, KIM YS. MUC-2 human small intestinal mucin gene structure. J Clin Invest 1991, 88: 1005-1013.
14. NIV Y, BYRD JC, HO SB, DAHIYA R, KIM YS. Mucin synthesis and secretion in relation to spontaneous differentiation of colon cancer cells *in vitro*. Int J Cancer 1992, 50: 147-152.
15. PORCHET N, VAN CONG N, DUFOSSE J, AUDIE JP, GUYONNET-DUPERAT V, GROSS MS, DENIS C, DEGAND P, BERNHEIM A, AUBERT JP. Molecular cloning and chromosomal localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. Biochem Biophys Res Commun 1991, 175: 414-422.
16. BAN T, IMAI K, YACHI A. Immunohistological and immunochemical characterization of a

novel pancreatic cancer-associated antigen MUSE11. Cancer Res 1989, 49: 7141-7146.

17. HINODA Y, NAKAGAWA N, OHE Y, KAKIUCHI H, TSUJISAKI M, IMAI K, YACHI Y. Recongition of the polypeptide core of mucin by monoclonal antibody MUSE11 against an adenocarcinoma-associated antigen. Jpn J Cancer Res 1990, 81: 1206-1209.
18. HINODA Y, KAKIUCHI H, NAKAGAWA N, OHE Y, SUGIYAMA T, MASAYUKI T, IMAI K, YACHI A. Circulating tumor-associated antigens detected by monoclonal antibodies against the polypeptide core of mucin. -Comparison of antigen MUSE11 with CA15-3- Jpn J Gastroenterol 1992.
19. YAMAMOTO T, IKAWA S, AKIYAMA T, SEMBA K, NOMURA N, MIYAJIMA N, SAITO T, TOYOSHIMA K. Similarity of protein encoded by the human c-erbB-2 gene to epidermal growth factor receptor. Nature (Lond) 1986, 319: 230-234.
20. MATOZAKI T, SAKAMOTO C, MATSUDA K, SUZUKI T, KONDA Y, NAKANO O, WADA K, UCHIDA T, NISHISAKI H, NAGAO M, *et al.* Missense mutations and a deletion of the p 53 gene in human gastric cancer. Biochem Biophys Res Commun 1992, 182: 215-223.
21. FARINATI F, HOLMGREN J, MARIO FD, CARDIN F, VALLANTE F, FANTON MC, LIBERA GD, NITTI D, PLEBANI M, CRESTANI B, NACCARATO R. CA 50 Determination in body fluids: Can we screen patients at the risk for gastric cancer? Int J Cancer 1991, 47: 7-11.
22. HORINOUCHI H, SATO E, YONEZAWA S, TANAKA S. Alterations of lewis related sugar antigens in gastric atypical epithelial lesions (Adenomas or dysplasia) with relation to their malignant changes —— An evaluation by follow-up cases —— Jpn J Gastroentorol 1991, 88: 19-27.
23. OHUCHI N, HAND PH, MERLO G, FUJITA J, MARIANI-COSTANTINI R, THOR A, NOSE M, CALLAHAN R, SCHLOM J. Enhanced expression of c-Ha-ras p 21 in human stomach adenocarcinomas defined by immunoassays using monoclonal antibodies and in situ hybridization. Cancer Res 1987, 47: 1413-1420.
24. YOHIDA K, HAMATANI K, KOIDE H, IKEDA H, NAKAMURA N, AKIYAMA M, TSUCHIYAMA H, NAKAYMMA E, SHIKU H. Preparation of anti-ras M_r 21,000 protein monoclonal antibodies and immunohistochemical analyses on expression of ras genes in human stomach and thyroid cancers. Cancer Res 1988. 48: 5503-5509.
25. GRAHAM DY, KLEIN PD, EVANS DG, FIEDOREK SC, EVANS Jr DJ, ADAM E, MALATY HM. Helicobacter pylori: epidemiology, relationship to gastric cancer and the role of infants in transmission. Euro J Gastroenterol Hepatol 1992, 4: S1-S6.
26. PRASONNET J, VANDERSTEEN D, GOATES J, SIBLEY RK, PRITIKIN J, CHANG Y. Helicobacter pylori infection in intestinal and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst 1991, 83: 640-643.
27. FORMAN D, NEWELL DG, FULLERTON F, YARNELL JWG, STACEY AR, WALD N, *et al.* Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991, 302: 1302-1305.
28. NOMURA A, STEMMERMANN GN, CHYOU P-H, KATO I, PEREZ-PEREZ GI, BIASER MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991, 325: 1132-1136.
29. YACHI A, SUGIYAMA T, YOSHIDA H, IMAI K, YABANA T, YOKOTA K, OGUMA, K. Preparation of anti-campylobacter pylori monoclonal antibodies and clinical implications. eds.

by TAKEMOTO T, KAWAI K, SHIMOYAMA T: Campylobacter pylori and Gastroduodenal Diseases, Proceedings of The First Tokyo International Symposium on Campylobacter pylori 1989, 99-104.

30. SUGIYAMA T, IMAI K, YOSHIDA H, TAKAYAMA Y, YABANA T, YOKOTA K, OGUMA K, YACHI A. A novel enzyme immunoassay for serodiagnosis of helicobacter pylori infection. Gastroenterology 1991, 101: 77-83.
31. IMAI K, SUGIYAMA T, TAKAYAMA Y, YOSHIDA H, YABANA T, YACHI A, YOKOTA K, OGUMA K. Immunological investigation of Helicobacter pylori infection in gastric mucosal lesions. eds. by TAKEMOTO T, KAWAI K, SHIMOYAMA T: Proceedings of The Trird Tokyo International Symposium on Helicobacter pylori 1990, 13: 26-30.
32. ASAKA M, KIMURA T, KUDO M, TAKEDA H, MITANI S, MIYAZAKI T, MIKI K, GRAHAM DY. Relationship of Helicobacter pylori to serum pepsinogens in an Asymptomatic Japanese Population. Gastroenterology 1992, 102: 760-766.
33. YOZOZAKI H, TAKEKURA N, TAKANASHI A, TABUCHI J, HARUTA R, TAHARA E. Estrogen receptors in gastric adenocarcinoma: a retrospective immunohistochemical analysis. Virchows Archiv [A] 1988, 413: 297-302.
34. HARRISON JD, JONES JA, ELLIS IO, MORRIS DL. Oestrogen receptor D5 antibody is an independent negative prognostic factor in gastric cancer. Br J Surg 1991, 78: 334-336.
35. BOURREILLE J, METAYER P, SAUGER F, *et al*. Existence d'alpha-foeto proteine au cours d' un cancer secondaire du foie d'origine gastrigue. Presse Med 1970, 78: 1277-1278.
36. CHANG Y-C, NAGASUE N, ABE S, TANIURA H, KUMAR DD, NAKAMURA T. Comparison between the clinicopathologic features of AFP-positive and AFP-negative gastric cancers. Am J Gastroenterol 1992, 87: 321-325.
37. CHANG Y-C, NAGASUE N, ABE S, KOHNO H, KUMAR DD, NAKAMURA T. α-Fetoprotein producing early gastric cancer with liver metastasis: report of three cases. Gut 1991, 32: 542-545.
38. TERASHIMA M, IKEDA K, MAESAWA C, KAWAMURA H, NIITSU Y, SATOH M, SAITO K. Establishment of an α-fetoprotein producing gastric cancer cell line in serum-free media. Jpn J Cancer Res 1991, 82: 883-885.
39. IKEDA Y, MORI M, KIDO A, SHIMONO R, MATSUSHIMA T, SUGIMACHI K, FACS, SAKU M. Immunohistochemical expression of carbohydrate antigen 19-9 in gastric carcinoma. Am J Gastroenterolo 1991, 86: 1163-1166.
40. SLAMON DJ, CLARK GM, WONG SG, LEVIN WJ, ULLRICH A, MOGUIRE WL. Human breast cancer: correlation of relapse and survival with amplification of the HE-2/neu oncogene. Science (Washington DC) 1987, 235: 177-182.
41. YONEMURA Y, NINOMIYA I, YAMAGUCHI A, FUSHIDA S, KIMURA H, OHOYAMA S, MIYAZAKI I, ENDOU Y, TANAKA M, SASAKI T. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 1991, 51: 1034-1038.
42. JAIN S, FILIPE MI, GULLICK WJ, LINEHAN J, MORRIS RW. c-erbB-2 Proto-oncogene expression and its relationship to survival in gastric carcinoma: an immunohistochemical study on archival material. Int J Cancer 1991, 48: 668-671.
43. YAMAGUCHI T, TAKAHASHI T, YOKOTA T, KITAMURA K, NOGUCHI A, KAMIGUCHI M,

DOI M, AHN T, SAWAI K, YAMANE T. Urinary pepsinogen I as a tumor marker of stomach cancer after total gastrectomy. Cancer 1991, 68: 906-909.

44. ASAO T, FUKUDA T, YAZAWA S, NAGAMACHI Y, FACS. Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. Cancer 1991, 68: 44-47.