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Original article

Titration of serum p53 antibodies in patients with gastric cancer: a single-institute study of 40 patients

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Abstract

Background. **Alterations of the *p53* tumor suppressor gene are the most commonly observed genetic abnormalities in many different types of human malignancies. The accumulation of mutant *p53* often leads to the production of p53 antibody (p53-Ab) in the sera of patients with various cancers. To evaluate the clinical implications of serum p53-Abs in patients with gastric cancer, we compared p53-Abs with conventional tumor markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen (CA)19-9.**

Methods. **Serum samples were obtained preoperatively from 40 patients with histologically confirmed gastric adenocarcinoma, including 28 (70%) patients in stage Ia. The serum p53-Abs were assessed by enzyme-linked immunosorbent assay, using a new version of a highly specific, quantitative p53-Abs Kit (MESACUP Kit II).**

Results. **p53-Abs were detected in 6 (15%) of 40 patients with gastric cancer, including 3 patients with early gastric cancer. Seven (17.5%) of the 40 patients were positive for CEA in serum. However, none of 7 patients with high CEA levels were positive for p53-Abs. No significant correlation of p53-Abs with patient age, sex, pathological parameters, tumor markers such as CEA and CA19-9, or poor survival (P = 0.116) was observed.**

Conclusion. **Although we employed the latest version of the p53-Abs Kit, the sensitivity of serum p53-Ab in gastric cancer patients was relatively low. No correlation was found between the presence of p53-Ab and the staging of cancer or survival. However, serum p53-Ab was detectable in patients with gastric cancer even in the early stages of disease. In addition, it may be independent of CEA and CA19-9.**

Key words Gastric cancer · p53 Antibody · Tumor marker

Introduction

Conventional tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA)19-9, squamous cell carcinoma antigen (SCC-Ag), tissue polypeptide antigen (TPA), and cytokeratin fragment (CYFRA)21-1, are not suitable for the screening or monitoring of patients with malignant tumors, because of low sensitivity and specificity. It has been suggested that oncogenes and tumor suppressor genes and their products may be useful in biochemical tests for cancer [1]. The tumor suppressor *p53* gene, located on chromosome 17p13.1, frequently undergoes mutation in the genesis of human cancer [2]. The frequency of *p53* mutations in all malignant tumors was reported to be at least 50% [3, 4]. The mutated *p53* gene leads to the synthesis of a mutant protein with a longer than normal half-life, and massive overexpression of the protein products [5, 6]. The accumulation of mutant p53 protein has been found to be immunogenic in cancer patients and to result in the production of p53 antibody (p53-Ab) in serum [7].

The p53-Ab in the sera of cancer patients can be detected by immunoprecipitation or Western blotting, or by enzyme-linked immunosorbent assay (ELISA) [7–10]. Circulating p53-Abs in patients have been reported for various types of carcinomas [9, 10], including breast cancer, hematopoietic malignancy, esophageal cancer, colon cancer, ovarian cancer, lung cancer, pancreatic cancer, and gastric cancer [11–15]. Several studies have demonstrated that the p53-Ab in sera served as an early marker of malignant disease, as an indicator for monitoring patients with malignant tumors during treatment, and as a prognostic factor for patients with several types of tumors [11, 16–18]. Because these studies attempted to evaluate the clinical value of p53-Ab under different conditions, the role of p53-Ab in patients with malignant tumors has not been clearly established yet.

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Gastric cancer is widely prevalent in the world and is one of the leading malignancies in terms of incidence and cause of cancer death in East Asia and South America. In Japan, the mortality rate of gastric cancer is showing a decreasing trend, reflecting advances in medical technology, such as early detection and treatment with an endoscope. It is necessary to evaluate the clinical usefulness of new early diagnostic markers of malignancies (for example, in gastric cancer) which could be found in the early stage of tumorigenesis. In this regard, reports on p53-Ab in the sera of patients with gastric cancer have not been adequate [15, 19–22].

In this study, we examined 40 patients with gastric cancer, including 28 (70%) patients in the early stages of the disease, for the presence of circulating antibodies against the tumor suppressor protein p53 and we examined these findings in relation to conventional tumor markers, tumor characteristics, and the clinical status of the patients. The serum levels of p53-Abs were assessed by ELISA, using a new version of a highly specific, quantitative p53-Ab Kit [23].

Patients and methods

Patients

Forty patients with primary gastric cancer who underwent gastric resection at the Department of Surgery, Division of Digestive Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan, between July and December 2000 were enrolled in this study. Written informed consent was obtained from each patient. No patients had received preoperative radiotherapy or chemotherapy. There were 28 (70%) male and 12 (30%) female patients, with an average age of 60.6 years (range, 28–86 years).

Serum and tumor samples

Serum samples were collected from each patient before and 28 days after surgery. Samples were stored at –80 °C until they were assayed. After resection, the tumor specimens were subjected to routine processing for the control of resection margins; also, exact histological investigation included an evaluation of staging in accordance with the International Union Against Cancer (UICC)/TNM classification.

Enzyme immunoassay for serum p53 antibodies

Serum p53-Ab levels were assessed by (ELISA) with the anti-p53 EIA Kit II (MESACUP anti-p53 Test; Medical and Biological Laboratories (MBL), Nagoya, Japan). In brief, the samples were added, for 1 h at 37 °C, to microtiter wells coated with wild-type human p53 protein or a control protein to detect nonspecific interactions. After washing, a peroxidase-conjugated goat antihuman immunoglobulin G that binds p53-Ab was applied for 1 h at 37 °C. Then substrate solution was added for 30 min at 37 °C. After the addition of stop solution, color development was assessed by measuring absorption at 450 nm, using a photospectrometer. Levels of p53-Abs were determined from a calibration curve constructed from the specific signals of standards. The cutoff value for serum p53-Abs was 1.3 U/ml. The specificity of this assay is greater than 95.5% [23].

CEA and CA19-9 assays

Serum CEA concentrations were measured with an immunoradiometric assay, using a CEA RIABEAD Kit (Abbott Japan, Tokyo, Japan). Serum CA19-9 concentrations were also measured with an immunoradiometric assay, using a CA19-9 RIA Kit (TFB, Tokyo, Japan). According to the manufacturers, the cutoff values for serum CEA and CA19-9 were 2.5 ng/ml and 37 U/ml, respectively.

Statistical analysis

Fisher's exact test, Student's *t*-test, and the Mann-Whitney *U*-test were used to determine the significance of differences between two groups. Survival curves were plotted using the Kaplan-Meier method. The logrank test was adopted to compare two groups. Cox regression analysis was performed to determine which factors would be useful in predicting overall survival. A P value of less than 0.05 was considered significant.

Results

Detection of serum p53 antibody in gastric cancer

We tested serum samples from 40 patients with gastric cancer for the presence of p53-Abs. Six (15.0%) of the 40 patients were positive for serum p53-Abs: the mean age of this group was 63 years (range, 40–77 years), and the male/female ratio was 2:1. The other 34 (85.0%) patients were negative for serum p53-Abs: their mean age was 60.2 years (range, 28–86 years), and the male/female ratio was 2.4:1. Based on the UICC/TNM classification, 3 of the 6 p53Ab-positive patients were in stage Ia; none of the 6 patients was in stage IV, but p53-Abs were also detected at stages II, IIIa, and IIIb. No significant differences between the p53Ab-positive and -negative groups were observed in age, sex, or tumor staging (Table 1).

We analyzed the histopathological factors of tissue type, tumor invasion, lymph node metastasis, and dis-

Table 1. Correlation between the presence of serum p53 antibody (Ab) and clinicopathological features in gastric cancer

Variables	Total	Serum p53 antibody		*P* value
		Positive	Negative	
Number of patients	40	6	34	
Age (years)	60.6	63	60.2	0.64
Sex (M:F)	2.3:1	2.0:1	2.4:1	0.88
Stage				
Ia	28	3	25	
Ib	3	0	3	
II	4	1	3	0.3
IIIa	1	1	0	
IIIb	2	1	1	
IV	2	0	2	

Table 2. Correlation between the presence of serum p53-Ab and histopathological findings in gastric cancer

	Serum p53 antibody		*P* value
	Positive	Negative	
Tissue type			
Differentiated	2	21	0.272
Undifferentiated	4	13	
Tumor invasion			
Mucosa or submucosa	3	25	0.363
Deeper than submucosa	3	9	
Lymph node metastasis			
Negative	3	31	0.111
Positive	3	3	
Distant metastasis			
Negative	6	33	0.909
Positive	0	1	

tant metastasis. Four (66.7%) of the 6 p53Ab-positive patients had histologically undifferentiated adenocarcinomas, compared to 13 (38.2%) of the 34 p53Ab-negative patients ($P = 0.272$). Three (50%) of the 6 patients with lymph node metastasis were positive for serum p53-Ab, whereas only 3 (8.8%) of the 34 patients without lymph node metastasis were positive ($P = 0.111$). There were no significant differences in these factors between the groups who were positive and negative for p53-Ab (Table 2).

Sensitivity of serum CEA, CA19-9, and p53 antibody in gastric cancer

The correlation between the presence of serum p53-Ab and the two tumor markers CEA and CA19-9 was analyzed. The sensitivities of CEA and CA19-9 in this study were 17.5% (7/40) and 10% (4/40), respectively. The 7 patients positive for CEA did not express p53-Abs, and CEA was not detected in any p53-Ab-positive patients (Table 3).

Table 3. Correlation between the presence of serum p53-Ab and tumor markers in gastric cancer

	Serum p53 antibody		*P* value
	Positive	Negative	
CEA			
Positive	0	7	0.426
Negative	6	27	
CA19-9			
Positive	1	3	0.762
Negative	5	31	

We analyzed the sensitivity of serum p53-Ab and CEA according to stage based on the UICC/TNM classification. Three (10.7%) of the 28 patients in stage Ia were positive for serum p53-Ab, whereas none (0%) of these 28 patients was positive for CEA. In stage IV, both patients were positive for CEA, but neither was positive for p53-Ab (Table 4).

Three (50%) of the 6 p53Ab-positive patients became negative postoperatively, while 5 (71.4%) of the 7 CEA-positive patients became negative postoperatively (Table 4).

Detection of serum p53 antibody in stage Ia gastric cancer

We focused on the stage-Ia patients to investigate the clinical usefulness of the levels of serum p53-Ab as a marker for the early detection of gastric cancer. Table 5 demonstrates that only p53-Ab was positive in patients with stage Ia gastric cancer, whereas CEA and CA19-9 were not positive. No significant differences between the p53Ab-positive and -negative groups were observed in regard to tissue type or tumor invasion.

Survival rates

The 4-year survival rates for patients with sera that was positive or negative for CEA, CA19-9, and p53-Ab are shown in Table 6. The median follow-up time for all 40 patients was 31.7 months (range, 1–48 months). The 4-year survival rate was 82.9% for the p53Ab-negative patients and 60% for the p53-Ab-positive patients. However, there was no significant difference in the rate of survival between the p53-Ab-positive group and the p53Ab-negative group ($P = 0.116$). In contrast, the overall survival of patients positive for CEA was significantly shorter than that in the CEA-negative patients ($P = 0.0008$) (Table 6).

Table 4. Correlations between sensitivity of serum CEA and p53 Ab according to clinical stage

	Serum p53 antibody	CEA	*P* value
Stage			
Ia	10.7% (3/28)	0% (0/28)	0.49
Ib	0% (0/3)	66.7% (2/3)	0.19
II	25% (1/4)	50% (2/4)	0.56
IIIa	100% (1/1)	0% (0/1)	0.31
IIIb	50% (1/2)	50% (1/2)	1
IV	0% (0/2)	100% (2/2)	0.12
Negative conversion post-surgery	50% (3/6)	71.4% (5/7)	0.52

Table 5. Correlation between the presence of serum p53-Ab, histopathological findings, and tumor markers in stage Ia patients

	Serum p53 antibody		
	Positive	Negative	*P* value
Tissue type			
Differentiated	2	19	0.853
Undifferentiated	1	6	
Tumor invasion			
Mucosa	2	15	0.906
Submucosa	1	10	
CEA			
Positive	0	0	0.49
Negative	3	25	
CA19-9			
Positive	0	0	0.49
Negative	3	25	

Table 6. Association between 4-year survival rates and tumor markers in patients with gastric cancer

	Survival rate (%)	*P* value
CEA		
Positive	25.7	0.0008
Negative	92.3	
CA19-9		
Positive	50	0.118
Negative	85.4	
p53-Ab		
Positive	60	0.116
Negative	82.9	

However, Cox regression analysis of all factors listed in Tables 1 and 2 revealed that lymph node metastasis, but not p53 Ab or CEA, was an independent prognostic factor in gastric cancer ($P < 0.05$).

Discussion

At present, there is no satisfactory tumor marker for the diagnosis or monitoring of malignant disease. It is expected that a new biological marker which shows high sensitivity and specificity and can be used with relative ease will be established.

p53-Ab is an autoantibody induced by mutation of the *p53* tumor suppressor gene, and has been detected in the sera of patients with various types of cancers. Since its initial description more than 20 years ago, the usefulness of serum p53-Ab in patients with various cancers has been reported [9–15]. Gastric cancer remains a major cause of cancer-related deaths in the world. Serum CEA is generally used for the diagnosis and monitoring of gastric cancer, but only a limited proportion of patients benefit. Therefore, potential new biological markers, such as p53-Ab, E-cadherin, or hepatocyte growth factor (HGF) for patients with gastric cancer, have received attention [24–26]. Because gastric cancer can be diagnosed at an early stage by endoscopy, it is suitable for testing a potential biological marker for early diagnosis. Nevertheless, only a small number of reports regarding the evaluation of p53-Abs in the sera of patients with gastric cancer have been published to date [15, 19–22].

The present study demonstrated that, in 15% (6 of 40) of patients, gastric cancer was detectable by p53-Ab ELISA assay preoperatively. This is comparable with previous observations in patients with gastric cancer [15, 19–23]. No significant correlation between p53-Abs and either tumor stage, tissue grade of differentiation, depth of tumor invasion, lymph node metastasis, or distant metastasis was observed. The positive rate for CEA and CA19-9 in the sera of patients with gastric cancer was 17.5% (7 of 40) and 10% (4 of 40), respectively, which is similar to results reported by other groups [27, 28]. Most interestingly, the 6 patients positive for p53-Abs did not show high levels of CEA, and only 1 patient positive for p53-Ab showed a high CA19-9 level. The presence of p53-Ab was not associated with serum CEA

or CA19-9 ($P = 0.426$ and $P = 0.762$, respectively). It was supposed that p53-Ab might be an independent marker of CEA or CA19-9. The positivity rate for the diagnosis of gastric cancer increased to 32.5% when p53-Ab and CEA were combined in this study.

Because alterations in the *p53* gene result in an accumulation of the protein in tumor cells, the presence of serum p53-Ab was described as an early event that could predate the diagnosis [29]. Our results demonstrated that, of 28 patients with stage Ia gastric cancer tested preoperatively, 3 were positive for p53-Ab in serum, whereas none was positive for serum CEA or CA19-9. A *p53* mutation may be not only an advanced-stage phenomenon but may also be an early event of carcinogenesis. Several studies have reported that p53-Ab can be found in the serum of individuals at high risk of developing cancer, including heavy smokers and workers exposed to vinyl chloride [16, 29, 30]. In contrast, no association between *p53* abnormalities (overexpression/mutation) and *Helicobacter pylori* infection was found in patients with gastric adenocarcinoma; therefore, mutations of the *p53* gene do not seem to be a predominant event in gastric carcinogenesis [31]. These contradictory findings might be explained by a report that 39.1% of patients with gastric cancer positive for p53-Ab in sera had tumor tissues that stained negative for p53 protein [19].

Although there have been several reports that the presence of p53-Ab in serum was a prognostic factor for patients with various types of malignancies, the prognostic value of p53-Abs in patients with gastric cancer is still controversial [15, 19, 21]. We did not find a significant correlation between the presence of p53-Abs in the sera of patients with gastric cancer and overall survival, despite the finding that the 4-year survival rate was about 20% higher in the p53-Ab-negative patients than that in the -positive patients. On the other hand, high levels of CEA could be associated with prognosis. However, Cox regression analysis revealed that lymph node metastasis, but not p53 Ab or CEA, served as an independent prognostic factor in gastric cancer in this series.

The p53-Abs circulating in patients with various types of cancer can be detected by several methods, including immunoprecipitation, Western blotting, and ELISA [7–10]. Because none of these methods give satisfactory rates of detection, further improvement is needed. We employed the latest version of an ELISA kit, which has the advantage of quantitative analysis, for the detection of p53-Ab. Using this assay, Shimada et al. [23], in a multiinstitutional study, reported 20.4% positivity for p53-Abs in 1085 patients with 15 types of malignant tumors, and they determined a cutoff value of 1.3 U/ml with over 95.5% specificity by analyzing serum samples of 205 healthy controls. This assay could thus contribute to achieving high true-positive rates with low false-positive rates. Recently, a new protocol for the rapid and sensitive detection of p53-Abs in serum by immunomagnetic electrochemiluminescence (IM-ECL) was developed [32]. Further study will be needed to fully elucidate the importance of this detection method.

Here, we measured circulating p53-Ab levels in the sera of 40 patients with gastric cancer using a new version of the p53-Abs ELISA kit. The presence of p53-Ab was demonstrated in 15% (6 of 40) of the patients with gastric cancer preoperatively. No correlation was found between the presence of p53-Ab and the staging of cancer or survival. However, circulating p53-Ab was detectable in patients with early-stage gastric cancer, and was independent of the currently available tumor markers CEA and CA19-9.

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