

Normalization of Serum p53 Antibody Levels in Patients after Curative Resection for Colorectal Cancer

HIDEJIRO KAWAHARA¹, KAZUHIRO WATANABE¹, HIROYA ENOMOTO¹,
YOICHI TOYAMA¹, TADASHI AKIBA¹ and KATSUHIKO YANAGA²

¹Department of Surgery, Kashiwa Hospital, School of Medicine, Jikei University, Chiba, Japan;

²Department of Surgery, School of Medicine, Jikei University, Tokyo, Japan

Abstract. *Background:* The aim of this study was to evaluate the significance of high serum p53 antibody (p53Ab) levels in relation to curative resection of colorectal cancer. *Patients and Methods:* Between 2007 and 2010, 24 patients with colorectal cancer with higher-than-normal preoperative serum p53Ab, measured by enzyme-linked immunosorbent assay, were enrolled in this study. After curative resection, their serum p53Ab and carcinoembryonic antigen (CEA) levels were measured at one, six, 12, 18, and 24 months after surgery. The relationship between clinicopathological features and the presence of serum p53Ab was evaluated. *Results:* None of the patients developed recurrence up to 24 months after the surgery. The positive rate for CEA was 33.3% before surgery, 16.7% at one month after surgery, and 0% at six months and more, while the rate for p53Ab was 75% at six months, 70.8% at 12 months, 58.3% at 18 months, and 54.2% at 24 months after surgery. The positive rate for serum p53Ab at 24 months after the surgery correlated with the one before and that at one month after the surgery. *Conclusion:* For patients with colorectal cancer and high preoperative serum p53Ab levels, serum p53Ab does not seem to be a useful marker of recurrence after curative resection, since normalization of serum p53Ab levels requires years after surgery.

The expression of the p53 protein has been reported in a number of human tumor types, including those of breast, lung, esophagus, stomach, and colorectum (1-6). p53 protein is detectable by immunohistochemical staining, and the overexpression of mutant p53 protein has been found to

induce serum p53 antibody (serum p53Ab) in 30-48% of patients with esophageal carcinomas (7-12). Serum p53Ab as a serological marker of cancer has been recently gaining attention. In patients with esophageal carcinoma, the presence of p53Ab in the serum has been reported to be associated with poor prognosis, low histological grade, and high incidence of lymph node metastasis (13-15). On the other hand, in patients with colorectal carcinomas, little is known about the correlation between serum p53Ab and clinicopathological features of colorectal cancer. This study was undertaken to evaluate the significance of high p53Ab levels in serum, in relation to curative resection of colorectal cancer.

Patients and Methods

Patients. Twenty-four patients with colorectal cancer who underwent selective colorectal resection between January 2007 and December 2010 at the Department of Surgery, Kashiwa Hospital, Jikei University School of Medicine, with higher-than-normal preoperative serum p53Ab by enzyme-linked immunosorbent assay (ELISA) were enrolled in this study. After curative resection, their serum p53Ab and carcinoembryonic antigen (CEA) were measured at one, six, 12, 18, and 24 months after surgery. The relationship between clinicopathological features, serum CEA and presence of p53Ab in serum were evaluated. The tumor, node, and metastasis (TNM) classification (16) was established based on pathological examinations of the resected specimens. Serum samples were obtained before surgery and at one, six, 12, 18, and 24 months after surgery, and stored at -80°C until assayed. To examine postoperative recurrence, computed tomography was performed every six months after surgery.

Enzyme immunoassay for p53Ab in the serum. p53Ab in the serum was assessed by ELISA with the MESACUP anti-p53 Test (MBL, Nagoya, Japan). In brief, serum samples were added to the wells of a microtiter plate coated with wild-type human p53 or a control protein and incubated for one hour. A conjugated second antibody was added, the samples were incubated for another hour, and then the substrate solution was added. After addition of the stop solution, the color reaction was measured immediately by the absorption at 450 nm using a photospectrometer. The cut-off value was determined as 1.30 U/ml.

Correspondence to: Hidejiro Kawahara, MD, Ph.D., Department of Surgery, Kashiwa Hospital, Jikei University School of Medicine, 163-1 Kashiwashita, Kashiwashi, Chiba 277-8567, Japan. Tel: +81 471641111 Ext. 3421, Fax: +81 471633488, e-mail: kawahide@jikei.ac.jp

Key Words: Serum p53 antibody, colorectal cancer, tumor marker, recurrence, curative resection.

Table I. Clinicopathological features of the patients.

Variable	Value
Gender, n(%)	
Male	17 (71)
Female	7 (29)
Age, median years (range)	64 (37-80)
Tumor diameter, median mm (range)	35 (10-90)
Location of the tumor, n(%)	
Colon	17 (71)
Rectum	7 (29)
Differentiation, n(%)	
Well	11 (46)
Mod	13 (54)
Depth of the tumor, n(%)	
T1	5 (21)
T2	2 (8)
T3	17 (71)
Status of lymph nodes, n(%)	
Negative	19 (79)
Positive	5 (21)

Well: Well-differentiated adenocarcinoma; Mod: moderately-differentiated adenocarcinoma.

Assays for serum CEA. The serum tumor marker CEA was also tested in all patients. Serum CEA concentrations were measured with the CEA-IIenzyme immunoassay (EIA) kit (Roche Diagnostics, Tokyo, Japan). The cut-off value for serum CEA was 5.0 ng/ml.

Statistical analysis. Statistical significance was determined by the Fisher's exact test, Student's *t*-test, Mann-Whitney's *U*-test or the Chi-square test. *p*-Values less than 0.05 indicate significance. All statistical analyses were performed using SPSS version 18.0 (IBM Software Group, Chicago, USA).

Results

Clinicopathological features of the patients (Table I). The median patient age was 64 years (range=37-80 years), and seven of these were females. Their median tumor diameter was 35 mm (range=10-90 mm). The tumor was located in the colon in 17 (71%) and in the rectum in seven (29%) patients. All the patients had adenocarcinoma, consisting of well-differentiated in 11 (46%) and moderately-differentiated in 13 (54%) patients. The depth of the tumor was T1 in five (21%), T2 in two (8%), and T3 in 17 (71%) patients. Lymph node metastasis was positive in five patients (21%).

Monitoring of serum 53Ab (Figure 1). The median serum p53Ab levels before surgery was 9.7 U/ml (range=3.1-684 U/ml). The median serum p53Ab levels at one, 6, 12, 18, and 24 months after surgery were 4.1 U/ml (range=1.2-384 U/ml), 3.5 U/ml (range=0.4-198 U/ml), 2.3 U/ml (range=0.4-

Table II. Comparison between pre-operative serum carcinoembryonic antigen (CEA) and serum p53 antibody levels after surgery.

Serum p53 antibody	Pre-operative CEA		<i>p</i> -Value
	≤5.0 ng/ml	>5.0 ng/ml	
One year after surgery			
≤1.3 U/ml	3 (19%)	4 (50%)	0.167
>1.3 U/ml	13 (81%)	4 (50%)	
Two years after surgery			
≤1.3 U/ml	7 (44%)	5 (63%)	0.667
>1.3 U/ml	9 (56%)	3 (37%)	

174 U/ml), 2.0 U/ml (range=0.4-82 U/ml), and 1.7 U/ml (range=0.4-78 U/ml), respectively. The serum p53Ab levels fell steadily after surgery.

Correlation between serum p53Ab and CEA (Table II, Figure 2). The positive rate for serum CEA was 33.3% before surgery, 16.7% at one month after surgery, and 0% at six months and more. On the other hand, the positive rate for serum p53Ab was 75.0% at six months after surgery, 70.8% at 12 months, 58.3% at 18 months, and 54.2% at 24 months after surgery. The serum p53Ab levels in half or more of all patients was higher than normal levels even, when more than two years had passed after surgery.

As to the relationship between preoperative serum CEA and p53Ab at one or two years after surgery, no relationship was found between the two.

Multivariate logistic regression analysis for clinicopathological factors and serum p53Ab after the surgery (Table III). To determine the variables associated with serum p53Ab levels after surgery, seven variables (age, gender, tumor diameter, tumor location, histological type, depth of tumor, and lymph node metastasis) were analyzed by use of the multivariate logistic regression. There was no relationship between any of these factors and postoperative serum p53Ab at one year or two years after surgery.

Correlation between serum p53Ab levels at two years after surgery and before or at one month after surgery (Table IV). The relationship between serum p53Ab level at two years after surgery and that before and at one month after surgery were evaluated. Eight (67%) out of the twelve patients with higher-than-normal serum levels of p53Ab at two years after surgery had a preoperative serum level of p53Ab of over 10-times the normal value. Similarly, 10 (83%) out of the 12 patients whose serum p53Ab level was higher-than-normal at two years after surgery had more than 5 U/ml higher level of p53Ab in serum at one month after surgery. Therefore,

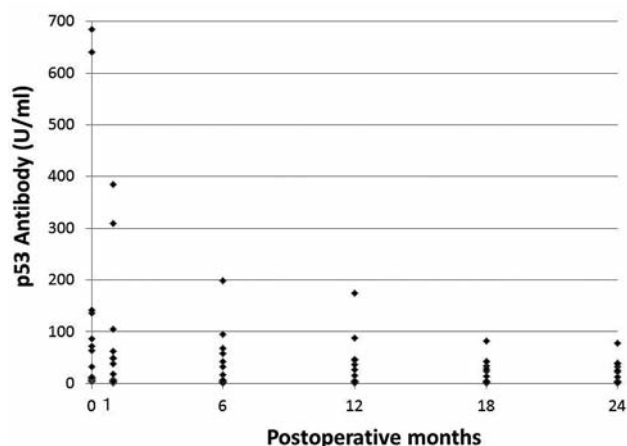


Figure 1. Levels of serum p53 antibody before and after curative resection of colorectal cancer. The levels decreased steadily and gradually after surgery.

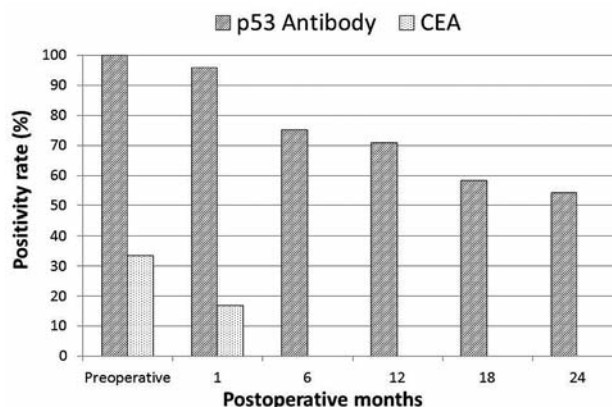


Figure 2. Correlation between positivity for serum p53 antibody and serum carcinoembryonic antigen (CEA) after curative resection of colorectal cancer. Postoperatively, the serum CEA levels decreased much faster than those of serum p53 antibody.

Table III. Multivariate logistic regression analysis for clinicopathological factors and serum p53 antibody levels after surgery.

Variable	p-Value	Odds ratio	95% Confidence interval
Value at one year after surgery			
Age	0.102	0.844	0.689-1.034
Gender	0.136	0.017	0.001-3.608
Tumor diameter	0.932	1.003	0.942-1.068
Tumor location	0.394	0.181	0.007-4.421
Pathological type	0.620	1.792	0.179-17.991
Depth of tumor	0.205	0.294	0.044-1.956
Lymph node metastasis	0.552	3.209	0.069-149.747
Value at two years after surgery			
Age	0.532	0.973	0.893-1.061
Gender	0.379	2.827	0.279-28.651
Tumor diameter	0.157	1.062	0.977-1.153
Tumor location	0.414	0.403	0.046-3.569
Pathological type	0.891	1.153	0.149-8.887
Depth of tumor	0.621	0.709	0.182-2.759
Lymph node metastasis	0.894	1.189	0.093-15.171

there was a strong correlation between higher-than-normal serum p53Ab levels at two years after surgery and those before or at one month after surgery ($p < 0.01$).

Discussion

The accumulation of p53 protein in the nucleus of malignant cells induces the production of serum antibodies against the p53 protein (7, 17-19). Thus, serum p53Ab as a serological

Table IV. Comparison of serum p53 antibody levels.

	Levels at two years after surgery		p-Value
	≤1.3 U/ml	>1.3 U/ml	
Levels before surgery			
<13 U/ml	12 (100%)	4 (33%)	<0.01
≥13 U/ml	0 (0%)	8 (67%)	
Levels at one month after surgery			
<5 U/ml	12 (100%)	2 (17%)	<0.01
≥5 U/ml	0 (0%)	10 (83%)	

marker of cancer has been the focus of much attention (11, 20-25). In 2003, Shimada *et al.* (26) conducted a multi-institutional collaborative study of surveillance of serum p53Ab in 1,085 patients with various types of malignant tumors using the same ELISA kit (MESACUP anti-p53Ab Test, MBL) for serum p53Ab measurement. Based on the results of their study, the Japanese Ministry of Health, Labor and Welfare approved the use of the kit for serum p53Ab as a tumor marker for cancer of the colon, esophagus, and breast in 2007, with the cost of serum p53Ab measurement covered by the National Health Insurance System.

In our previous study using the same kit, there was no correlation between the presence of p53Ab in serum and clinicopathological factors including gender, age, location of tumor, tumor diameter, histological grades, lymph node metastasis, lymphatic invasion and venous invasion (27). Since the serum p53Ab-positive rate was approximately 30%, regardless of the depth of tumor invasion, serum p53Ab does

not seem to be a marker of tumor progression. On the other hand, by using the same kit, Ochiai *et al.* (28) reported that the positive rate for serum p53Ab was significantly higher in patients with cancer involvement of the lymphoid tissues. In examinations using other measurement kits, some studies have noted a correlation between preoperative serum p53Ab and a poor outcome in patients with cancer (29-35), while others reported different results (36-39). In patients with colorectal carcinoma, the correlation between serum p53Ab and clinicopathological features or prognosis of colorectal cancer is yet to be clarified.

In the present study, the significance of serum p53Ab as a marker for detecting recurrent colorectal cancer after curative resection was investigated. Normalization of serum CEA levels in all patients required at most six months after surgery. On the other hand, the serum p53Ab levels in half or more of all patients was higher-than-normal even when more than two years had passed after surgery. However, no patient developed postoperative recurrence. Thus, serum p53Ab does not seem to be a useful marker of recurrence after curative resection of colorectal cancer in patients with high serum p53Ab levels before surgery because normalization of their serum p53Ab levels may require very long time. A continuous and steady decrease in serum p53Ab level may make it difficult to reveal recurrence after surgery. Furthermore, in patients with unknown serum p53Ab levels prior to or one month after surgery, the significance of postoperative measurement of serum p53Ab levels is limited.

Conclusion

For patients with colorectal cancer, serum p53Ab does not seem to be a useful marker of recurrence after curative resection, since normalization of their serum p53Ab levels requires years after surgery.

Conflicts of interest

We declare that we have no conflicts of interest.

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Received March 15, 2013

Revised April 15, 2013

Accepted April 16, 2013