Clinical Value of Serum p53 Antibody in the Diagnosis and Prognosis of Colorectal Cancer

MASAKI KUNIZAKI[1], TERUMITSU SAWAI[1], HIROAKI TAKESHITA[1], TETSURO TOMINAGA[1], SHIGEKAZU HIDAKA[1], KAZUO TO[1], TAKURO MIYAZAKI[1], RYUJI HAMAMOTO[2], ATSUSHI NANASHIMA[1] and TAKESHI NAGAYASU[1]

[1]Division of Surgical Oncology, Department of Translational Medical Sciences,
Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan;
[2]Laboratory of Genome Technology, Human Genome Center, Institute of Medical Science,
The University of Tokyo, Tokyo, Japan

Abstract. *Background: Serum p53 antibody (s-p53Ab), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) were investigated to evaluate the significance of these singly and combined tumor markers in the diagnosis and prognosis of colorectal cancer (CRC). Patients and Methods: Preoperative serum samples were obtained from 170 patients with histologically confirmed CRC, including 28 (16%) with stage I. s-p53Ab was assessed using the MESACUP Kit II, that is a new and highly specific version of a quantitative p53-Abs enzyme-linked immunosorbent assay. Results: s-p53Ab was detected in 30.6% (52 out of 170) of patients with CRC, including 31.9% (29/91) of patients with early-stage CRC. The positive rates for CEA and CA19-9 of patients with CRC were 28.8% (49/170) and 22.9% (39/170), respectively. Combining use of s-p53-Ab with CEA increased the positive rate of a diagnosis of CRC to 48.8%. Positivity for s-p53Ab in CRC did not correlate with overall survival. On the other hand, Cox regression analysis of this series revealed that high levels of CEA served as an independent prognostic factor for CRC. Kaplan-Meier analysis revealed significant differences between patients with elevated s-p53Ab and CEA and those with elevated levels of either one or neither of these factors (p<0.001). Conclusion: The diagnostic rate of s-p53Ab was better than that of CEA and CA19-9 in patients with early-stage CRC. Combined detection of s-p53Ab and CEA can improve diagnostic sensitivity and may permit more accurate stratification of patients with CRC.*

Colorectal cancer (CRC) is highly prevalent and is one of the leading malignancies in terms of incidence and causes of cancer death worldwide (1). The mortality rate and number of patients with CRC is increasing in Japan, despite advances in medical technology, endoscopic surgery, chemotherapy, and early detection. The clinical value of new diagnostic markers of early tumorigenesis requires evaluation. Malignant tumor progression has been detected using conventional tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, squamous cell carcinoma antigen, cytokeratin fragment 21-1, alpha-fetoprotein, and protein induced by vitamin K antagonist II. However, the sensitivity and specificity of these markers are too low for screening or monitoring patients with malignant tumors. Thus, new useful biological serum markers are needed to detect CRC. Circulating p53 antibody has been identified in patients with cancer of the breast, esophagus, ovary, lung, stomach, and CRC (2, 3). Several studies have demonstrated that serum p53 antibody (s-p53Ab) can serve as an early marker of malignant disease, as an indicator of treatment effects in patients with malignant tumors, and as a prognostic factor for patients with several types of tumors (4-8). The diagnostic performance of individual serum biomarkers of malignant tumors, such as ovarian or pancreatic cancer has been improved by combining data derived from multiple biomarkers (9, 10). Although, few studies have examined the clinical implications of s-p53Ab with those of the conventional tumor markers, CEA and CA 19-9 in patients with CRC. Therefore, the present study evaluated the clinical relevance of s-p53Ab and investigated whether its diagnostic value could be improved through combination with other biomarkers in 170 patients with CRC, including 91 (53.5%) with the early stages of the disease.

Correspondence to: Masaki Kunizaki, Division of Surgical Oncology, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel: +81 958197304, Fax: +81 958197306, e-mail: makuni49@nagasaki-u.ac.jp

Key Words: Colorectal cancer, serum p53 antibody, tumor marker.

Table I. *Association between the presence of serum-p53 antibody (s-p53Ab) and clinicopathological features in patients with colorectal carcinoma.*

Variable	s-p53Ab (n=170)		
	Positive n=52	Negative n=118	p-Value
Gender (Male/female)	29/23	61/57	0.624
Age: <70/≥70 years	31/21	75/43	0.625
BMI: <25/≥25 kg/ m^2	39/13	89/29	0.953
Tumor size: <5/≥5 cm	28/24	68/50	0.647
Tumor site: Colon/rectum	28/24	63/55	0.956
Histological type: tub1/tub2/por	20/31/1	46/62/10	0.425
Lymphatic invasion: 0/1,2,3	1/51	20/98	0.013
Venous invasion: 0/1,2,3	5/47	22/96	0.209
Nodal stage: N0/N1-3	30/22	68/50	0.994
Tumor depth: T1/T2/T3/T4	2/11/23/16	19/11/54/34	0.090
Liver metastasis: Yes/no	6/46	15/103	0.969
TNM stage: 0/I/II/III/IV	0/11/18/12/11	9/17/36/39/17	0.299
C-reactive protein:			
<1.0/≥1.0 U/ml	41/11	105/13	0.080
Albumin: <3.5/≥3.5 g/dl	11/41	14/104	0.115
mGPS: 0/1,2	41/11	105/13	0.080
GPS: 0/1,2	37/15	96/22	0.137
CEA: <5/≥5 ng/ml	35/17	86/32	0.460
CA19-9: <37/≥37 U/ml	42/10	89/29	0.445

BMI: Body mass index; tub1: well-differentiated adenocarcinoma; tub2: moderately differentiated adenocarcinoma; por: poorly differentiated adenocarcinoma; mGPS: modified Glasgow prognostic score; GPS: Glasgow prognostic score; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

Table II. *Association between sensitivity of serum-p53 antibody (s-p53Ab), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 CA19-9) according to clinical stage in patients with colorectal cancer.*

Stage	s-p53Ab	CEA	CA19-9	p-Value
0	0% (0/9)	0% (0/9)	0% (0/9)	0.959
I	39.3% (11/28)	7.1% (2/28)	3.6% (1/28)	0.002
II	33.3% (18/54)	24.1% (13/54)	13.0% (7/54)	0.076
III	23.5% (12/51)	25.5% (13/51)	27.5% (14/51)	0.902
IV	39.3% (11/28)	75% (21/28)	60.7% (17/28)	0.050
Total	30.6% (52/170)	28.8% (49/170)	22.9% (39/170)	0.255

Table III. *Association between clinical stage and sensitivity of biomarker combinations.*

Stage	s-p53Ab+CEA	CEA+CA19-9	s-p53Ab+CA19-9	p-Value
0	0% (0/9)	0% (0/9)	0% (0/9)	0.959
I	46.4% (13/28)	7.1% (2/28)	42.9% (12/28)	0.002
II	48.1% (26/54)	33.3% (18/54)	44.4% (24/54)	0.268
III	39.2% (20/51)	35.3% (18/51)	43.1% (22/51)	0.720
IV	85.7% (24/28)	82.1% (23/28)	78.6% (22/28)	0.784
Total	48.8% (83/170)	35.9% (61/170)	47.1% (80/170)	0.033

s-p53Ab: Serum-p53 antibody; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

Patients and Methods

Patients. We retrospectively reviewed a database of 170 patients (average age=63.8 years; range=16-90 years; male, n=90; female, n=80) with primary CRC who underwent colorectal resection at the Division of Surgical Oncology, Nagasaki University Hospital, between June 2000 and December 2004. Written informed consent was obtained from each patient, none of whom had received preoperative radiotherapy or chemotherapy. The current study was approved by the Ethics Review Board (number 09062633) of our Institution and complied with the standards of the Declaration of Helsinki.

Serum and tumor samples. Resected tumor specimens were routinely processed to control resection margins. Histological assessment included staging in accordance with the International Union Against Cancer (UICC7)/TNM classification (11). Serum samples collected from all patients before surgery were stored at −80˚C.

Enzyme immunoassay for s-p53Ab. Levels of s-p53Ab were assessed by enzyme–linked immunosorbent assay (ELISA) using the MESACUP anti-p53 Test anti-p53 detection Kit (Medical and

Biological Laboratories, Nagoya, Japan). In brief, samples were incubated for 1 h at 37˚C in microtiter wells coated with wild-type human p53 protein or a control protein to detect non-specific interactions. The wells were washed and then a peroxidase-conjugated goat antihuman immunoglobulin G that binds p53Ab was applied for 1 h at 37˚C followed by substrate solution for 30 min at 37˚C. Stop solution was added and then color development was assessed by measuring absorption at 450 nm using a spectrophotometer. Levels of s-p53Ab were determined from a calibration curve constructed from the specific signals of standards. s-p53Ab levels ≥1.3 U/ml were considered as positive, according to the manufacturer's suggestion (2).

Values for CEA, CA19-9, Glasgow prognostic score (GPS), and modified GPS. Routine factors including albumin, serum C-reactive protein, and tumor markers such as CEA and CA19-9, were measured on the same day to exclude any inflammatory effect of preoperative sequential examinations such as colonoscopy or a barium enema. The positivity values for serum CEA and CA19-9 according to our institutional criteria were 5.0 ng/ml and 37 U/ml, respectively. Glasgow prognostic score (GPS) and the modified glasgow prognostic score (mGPS) were estimated as described elsewhere (12, 13).

Table IV. *Univariate and multivariate analyses of survival using Cox proportional hazard models in patients with colorectal cancer.*

Variable	Univariate			Multivariate		
	p-Value	HR	95% CI	*p*-Value	HR	95% CI
Sex: Male/female	0.702	1.107	0.657-1.864			
Age: <70/≥70 years	0.701	1.109	0.653-1.883			
BMI: <25/≥25 kg/m^2	0.890	0.963	0.569-1.631			
Tumor size: <5/≥5 cm	0.447	1.224	0.727-2.059			
Tumor site: Colon/rectum	0.940	1.020	0.606-1.718			
Histological type: tub1,tub2/por	0.040	2.428	1.040-5.665			
Lympatic invasion: 0/1,2,3	0.063	3.013	0.942-9.639			
Venous invasion: 0/1,2,3	0.147	1.872	0.803-4.362			
TNM stage: 0,I,II/III,IV	<0.001	3.796	2.124-6.783			
C-Reactive protein: <1.0/≥ 1.0 mg/dl	0.015	2.166	1.166-4.023			
Albumin: <3.5/≥3.5 g/dl	<0.001	2.731	1.509-4.943			
mGPS: 0/1,2	0.015	2.1658	1.166-4.023			
GPS: 0/1,2	0.002	2.385	1.382-4.1142	0.060	1.755	0.977-3.153
CEA: <5/≥5 ng/ml	<0.001	1.577	1.323-1.878	0.003	2.545	1.361-4.758
CA19-9: <37/≥37 U/ml	<0.001	2.572	1.506-4.392	0.104	1.662	0.901-3.066
s-p53Ab: <1.3/≥1.3 U/ml	0.965	0.988	0.559-1.742	0.889	0.960	0.538-1.713

BMI: Body mass index; tub1: well-differentiated adenocarcinoma; tub2: moderately differentiated adenocarcinoma; por: poorly differentiated adenocarcinoma; mGPS: modified Glasgow prognostic score; GPS: Glasgow prognostic score; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; s-p53Ab: serum-p53 antibody; HR: hazard ratio; Ci: confidence interval.

Statistical analysis. The significance of differences between two groups was determined using the Chi -square test and the Mann-Whitney *U*-test. Hazard ratios with 95% confidence intervals were calculated after univariate or multivariate analysis using the Cox proportional hazards model. Multivariate analysis included tumor markers with *p*<0.05 in the univariate analysis to assess those most closely associated with overall survival. Survival curves for the two groups were compared using Kaplan–Meier analysis and the log-rank test. Overall survival was calculated from the date of surgery and deaths that occurred before December 31, 2015 were included in this analysis. All data were statistically analyzed using JMP®10 (SAS Institute, Inc., Cary, NC, USA) at a significance level of *p*<0.05.

Results

Correlation between the presence of s-p53Ab and clinicopathological features in colorectal carcinoma (Table I). We found that serum samples from 52 (30.6%) out of 170 patients with CRC were positive for s-p53Ab. The mean age of this group was 65.0 (range=43-81) years, and the male/female ratio was 1.3:1. The remaining 118 (69.4%) patients were negative for s-p53Ab; and their mean age was 63.3 (range=16-90) years and the male/female ratio was 1.07:1. Table I shows clinicopathological features of these patients stratified by the presence of s-p53Ab. No significant relationships, except lymphatic invasion, were identified regardless of being positive or negative for s-p53Ab.

Sensitivity of serum CEA, CA19-9, and s-p53Ab in patients with CRC. The detection sensitivity of single serum tumor markers was the highest for s-p53Ab (Table II). The sensitivity of s-p53Ab, CEA, and CA19-9 according to UICC7/TNM stage was as follows. Among 28 patients with stage I CRC, 11 (39.3%), 2 (7.1%), and 1 (3.6%) were positive for s-p53Ab, CEA, and CA19-9, respectively (*p*=0.002). We investigated the clinical relevance of s-p53Ab level as a marker of early CRC in patients with stages 0, I, and II CRC. In patients with early-stage CRC, the s-p53Ab positivity rate (31.9%) was higher than that for CEA (16.5%, *p*=0.015) and CA19-9 (8.8%, *p*<0.01). We evaluated the benefit of testing for s-p53Ab in addition to measurements of the other two tumor markers. The detection sensitivity of CEA plus s-p53Ab, CEA plus CA19-9, and s-p53Ab plus CA19-9 combinations in 170 patients was 52 (30.6%), 49 (28.8%), and 39 (22.9%), respectively, with CEA plus s-p53Ab being the most sensitive. Table III shows the detection sensitivity of CEA plus s-p53Ab, CEA plus CA19-9, and s-p53Ab plus CA19-9 according to UICC7/TNM stage. The rate of positivity for CEA+s-p53Ab (46.4%) was significantly higher than that of CEA plus CA19-9 (7.1%) and s-p53Ab plus CA19-9 (42.9%) in those with stage I disease, suggesting that s-p53Ab plus CEA was more sensitive for detecting CRC at an early stage.

Characteristics of patients and their surgical outcomes. The major clinicopathological factors of sex, age, and BMI

(patient factors); tumor size, tumor site, histological type, lymphatic invasion, venous invasion, nodal stage, tumor depth, liver metastasis, and TNM stage (tumor factors); CRP, albumin, mGPS, and GPS (inflammation factors); CEA, CA19-9, and s-p53Ab (tumor markers) were investigated. Univariate and multivariate analyses were performed to evaluate the relationship between clinicopathological factors and overall survival. The result of univariate analyses demonstrated that TNM classification (0, I, II/III, IV) was the most sensitive predictor of postoperative mortality (hazard ratio=3.796; 95% confidence interval=2.124-6.783; *p*<0.001) (Table IV). All tumor markers, but not s-p53Ab, were positively associated with postoperative mortality. Multivariate analyses of GPS, CEA, CA19-9 and s-p53Ab found only CEA maintained its association with postoperative mortality. The median and minimum follow-up terms for survivors were 2,847 and 1,825 days, respectively. Kaplan-Meier analysis revealed significantly poorer survival for patients with elevated levels of both s-p53Ab and CEA compared to those with no elevation of these factors or elevation of only one of them.

Discussion

Tumor markers are useful for managing patients with CRC. CEA is a glycoprotein produced by columnar and goblet cells in normal colorectal cells and in CRC cells, with a half-life of 3-11 days. Recommendations indicate that levels of CEA should be assessed the most frequently when CRC is suspected (14). CA19-9 is also a high-molecular-weight glycoprotein that is detectable in human blood (15). Although CRC can generally be diagnosed and monitored using serum CEA and CA19-9, only a limited number of patients can benefit from this strategy. Therefore, potential new biological markers of CRC, such as p53Ab, E-cadherin, endothelial leukocyte adhesion morecule-1 (ELAM-1), or hepatocyte growth factor (HGF) have recently been applied (16, 17). The autoantibody p53Ab is induced by mutation of the p53 tumor-suppressor gene, and it has been detected in the sera of patients with various types of cancer. Since its initial discovery during the late 1970s, s-p53Ab has become a useful marker of various cancer types (2-8). Because CRC can be diagnosed at an early stage thanks to advances in medical technology, it is suitable for testing potential biological markers for early diagnosis. Nevertheless, only few studies that have evaluated s-p53Abs in patients with CRC have been published. The present study showed that CRC was preoperatively detectable using an s-p53Ab ELISA assay in 52 (30.6%) out of 170 patients, which is comparable with previous findings of patients with CRC (4, 5). The presence of s-p53Ab did not significantly correlate with clinicopathological characteristics. Among 28 patients with stage I CRC, 11 (39.3%), two (7.1%), and one (3.6%) were
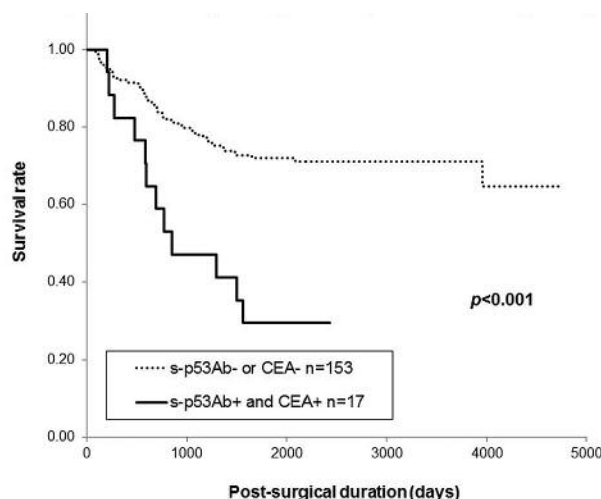

Figure 1. *Overall survival among patients with colorectal cancer according to positivity for erum p53 antibody (s-p53Ab) and carcinoembryonic antigen (CEA). Positivity for both markers significantly reduced survival.*

positive for s-p53Ab, CEA, and CA19-9, respectively (*p*=0.002). We investigated the clinical relevance of s-p53Ab levels as a marker of early CRC in patients with stages 0, I, and II CRC. Table II shows that among these 91 patients with stage 0-II CRC, 29 (31.9%), 15 (16.5%), and 8 (8.8%) were positive for s-p53Ab, CEA, and CA19-9, respectively (*p*<0.001). Notably, the 80.8% of those positive for s-p53Ab did not have a high CA19-9 level and only 32.7% of those positive for s-p53Ab had a high CEA level. The presence of s-p53Ab was not associated with serum CEA or CA19-9 (*p*=0.460 and *p*=0.445, respectively). We supposed that s-p53Ab could serve as an independent marker of CEA or CA19-9 and an early marker in CRC detection. The positive rate for a diagnosis of CRC increased to 48.8% when p53-Ab was combined with CEA (*p*=0.033). Because changes in the p53 gene result in protein accumulation in tumor cells, the presence of s-p53Ab has been described as an early event that could predate diagnosis (18-21). Although several studies have found that s-p53Ab is a prognostic factor for patients with various types of tumors, its prognostic value in patients with CRC remains controversial. The presence of s-p53Ab did not significantly correlate with OS, whereas Cox regression analysis revealed that a high CEA level served as an independent prognostic factor for our patients with CRC. Kaplan–Meier analysis revealed significant differences in survival between patients with elevated levels of both s-p53Ab and CEA and those with elevated levels of either one or neither (Figure 1). We did not find a significant correlation between the presence of s-p53Ab and prognosis. However, combining CEA with s-p53Ab was useful for detecting CRC

and allowed clear allocation of patients with CRC into two independent groups, and positivity for s-p53Ab and CEA was associated with poor prognosis of CRC. Therefore, it may be possible to improve the prognosis of patients with CRC after treatment by determining CEA and s-p53Ab routinely at the time of admission or on an outpatient basis. A potential limitation of this study is that is a retrospective, single-centre study. Therefore, a large-scale prospective validation study is needed to confirm these results.

Conclusion

Our study demonstrated that combination of s-p53Ab and CEA can serve as a useful biomarker of CRC and permit more accurate stratification of patients with CRC. This in turn should improve clinical decision-making, and perhaps contribute to more rational study design and analysis.

Conflicts of Interest

No financial or other potential conflicts of interest exist for any of the Authors.

References

1   Tominaga T, Takeshita H, Arai J, Takagi K, Kunizaki M, To K, Abo T, Hidaka S, Nanashima A, Nagayasu T and Sawai T: Short-Term Outcomes of Laparoscopic Surgery for Colorectal Cancer in Oldest-Old Patients. Dig Surg *2*: 32-38, 2015.

2   Lubin R, Schlichtholz B, Teillaud JL, Garay E, Bussel A and Wild C: p53 antibodies in patients with various types of cancer: assay, identification and characterization. Clin Cancer Res *1*: 1463-1469, 1995.

3   Shimada H, Ochiai T and Nomura F: Titration of serum p53 antibodies in 1085 patients with various types of malignant tumors. Cancer *97(3)*: 682-689, 2003.

4   Yamaguchi T, Takii Y and Maruyama S: Usefulness of serum p53 antibody measurement in colorectal cancer: an examination of 1384 primary colorectal cancer patients. Surg Today *44(8)*: 1529-1535, 2014.

5   Kawahara H, Watanabe K, Enomoto H, Toyama Y, Akiba T and Yanaga K: Normalization of serum p53 antibody levels in patients after curative resection for colorectal cancer. Anticancer Res *33(5)*: 2221-2225, 2013.

6   Mattioni M, Chinzari P, Soddu S, Strigari L, Cilenti V and Mastropasqua E: Serum p53 antibody detection in patients with impaired lung function. BMC Cancer Feb 13: 62, 2013.

7   Mattioni M, Soddu S, Porrello A, D'Alessandro R, Spila A and Guadagni F: Serum anti-p53 antibodies as a useful marker for prognosis of gastric carcinoma. Int J Biol Markers *22(4)*: 302-306, 2007.

8   Breveren von MC, Hollstein MC, Cawley HM, De Benedetti VGM, Bennett WP, Liang L, He AG, Zhu SM, Tursz T, Janin N and Trivers GE: Circulating anti-p53 antibodies in oesophageal cancer patients are found predominantly in individuals with p53 core domain mutations in their tumors. Cancer Res *56*: 4917-4921, 1996.

9   Kanda M, Fujii T, Takami H, Suenaga M, Inokawa Y, Yamada S, Nakayama G, Sugimoto H, Koike M, Nomoto S and Kodera Y: Combination of the serum carbohydrate antigen 19-9 and carcinoembryonic antigen is a simple and accurate predictor of mortality in pancreatic cancer patients. Surg Today *44*: 1692-1701, 2014.

10   Freydanck MK, Laubender RP, Rack B, Schuhmacher L, Jeschke U and Scholz C: Two-marker combinations for preperative discrimination of benign and malignant ovarian masses. Anticancer Res *32(5)*: 2003-2008, 2012.

11   Sobin LH and Wittekind C eds: UICC TNM Classification of Malignant Tumors. 7th ed. New York: John Wiley & Sons, Inc. 2007.

12   Ishizuka M, Nagata H, Takagi K, Iwasaki Y and Kubota K: Inflammation-based prognostic system predicts postoperative survival of colorectal cancer patients with a normal preoperative serum level of carcinoembryonic antigen. Ann Surg Oncol *19*: 3422-3431, 2012.

13   Kato A, Tsuji T, Sakao Y, Ohashi N, Yasuda H, Fujimoto T, Takita T, Furuhashi M and Kumagai H: A comparison of systemic inflammation-based prognostic scores in patients on regular hemodialysis. Nephron Extra *3*: 91-100, 2013.

14   Al-Shuneigat JM, Mahgoub SS and Huq F: Colorectal carcinoma: nucleosomes, carcinoembryonic antigen and ca 19-9 as apoptotic markers; a comparative study. J Biomed Sci *18*: 50, 2011.

15   Swiderska M, Choromanska B, Dabrowska E, Konarzewska-Duchnowska E, Choromanska K, Szczurko G, Mysliwiec P, Dadan J, Ladny JR and Zwierz K: The diagnostic of colorectal cancer. Contemp Oncol (Pozn) *18*: 1-6, 2014.

16   Takahashi Y, Mai M, Watanabe M, Tokiwa M and Nishioka K: Relationship between serum ELAM-1 (endothelial leukocyte adhesion molecule-1) and metastasis among colon cancer patients. Dis Colon Rectum *41*: 770-774, 1998.

17   Yonesaka K, Satoh T, Ueda S, Yoshida T, Takeda M, Shimizu T, Okamoto I, Nishio K, Tamura T and Nakagawa K: Circulating hepatocyte growth factor is correlated with resistance to cetuximab in metastatic colorectal cancer. Anticancer Res *35(3)*: 1683-1689, 2015.

18   Lubin R, Schlichtholz B, Teillaud JL, Garay E, Bussel A and Wild CP: p53 antibodies in patients with various types of cancer: assay, identification and characterization. Clin Cancer Res *1*: 1463-1469, 1995.

19   Angelopoulou K, Diamandis EP, Sutherland DJA, Kellen JA and Bunting PS: Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. Int J Cancer *58*: 480-487, 1994.

20   Shimizu K, Ueda Y and Yamagishi H: Titration of serum p53 antibodies in patients with gastric cancer: a single-institute of 40 patients. Gastric Cancer *8*: 214-219, 2005.

21   Lubin R, Zalcman G, Bouchet L, Tredaniel J, Legros Y, Cazals D, Hirsch A and Soussi T: Serum p53 antibodies as early markers of lung cancer. Nature Med *1(7)*: 701-702, 1995.