Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.14.4082

World J Gastroenterol 2008 July 7; 14(25): 4082-4086
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Changes of serum p53 antibodies and clinical significance of radiotherapy for esophageal squamous cell carcinoma

Hong-Yi Cai, Xiao-Hu Wang, Ying Tian, Li-Ying Gao, Li-Juan Zhang, Zhi-Yan Zhang

Hong-Yi Cai, Xiao-Hu Wang, Li-Ying Gao, Li-Juan Zhang, Department of Radiation Oncology, Gansu Provincial Tumor Hospital, Lanzhou 730050, Gansu Province, China
Ying Tian, Zhi-Yan Zhang, Department of Clinical Laboratory, Gansu Provincial Tumor Hospital, Lanzhou 730050, Gansu Province, China
Supported by Technology Research and Exploration Funds of Gansu Province, No. 0709TCYA030
Author contributions: Cai HY and Wang XH designed the research, performed most of the research work and wrote the paper; Gao LY and Zhang LJ performed part of the research; Tian Y and Zhang ZY did the ELISA assays.
Correspondence to: Xiao-Hu Wang, Department of Radiation Oncology, Gansu Provincial Tumor Hospital, Lanzhou 730050, Gansu Province, China. wangxiaohu@csco.org.cn
Telephone: +86-931-2614521 Fax: +86-931-2614551
Received: January 27, 2008 Revised: April 30, 2008
Accepted: May 7, 2008
Published online: July 7, 2008

Abstract

AIM: To explore the relationship between serum p53 antibodies (p53-Abs) and clinicopathological characteristics and therapeutic effect in patients with esophageal carcinoma (EC), and to investigate sequential changing regularity of serum p53-Abs after radiotherapy.

METHODS: The serum p53-Ab levels were detected in 46 EC patients and 30 healthy adults by enzyme linked immunosorbent assay (ELISA). The blood samples were collected on the day before radiotherapy and on the administration of an irradiation dose of 20 Gy/10 f/12 d, 40 Gy/20 f/24 d and 60 Gy/30 f/36 d after radiotherapy.

RESULTS: The level and positive rate of serum p53-Abs in EC patients were significantly higher than those in normal individuals ($P < 0.05$). Serum anti-p53 antibodies were positive in 18 of 46 EC patients (39.1%). The positive rate of p53-Abs in EC was related to histological grade, disease stage and lymph node metastasis ($P < 0.05$), but it was not significantly related to sex, age and to the size and site of tumor. The level and positive rate of p53-Abs had significant differences between before radiotherapy and after administration of an irradiation dose of 40 Gy/20 f/24 d and 60 Gy/30 f/36 d ($P < 0.05$ or $P < 0.01$). The positive rate of p53-Abs in EC patients with effect was significantly lower than that in those without effect after radiotherapy ($P < 0.0001$).

CONCLUSION: Detection of serum p53-Abs is helpful to the diagnosis of esophageal carcinoma. Monitoring for sequential change of serum p53-Abs before and after radiotherapy in patients with esophageal carcinoma is also useful to evaluate the response to the treatment and prognosis of the patients.

Key words: Esophageal carcinoma; Radiotherapy; Serum p53 antibodies; Enzyme linked immunosorbent assay

Peer reviewer: Jian-Zhong Zhang, Professor, Department of Pathology, Beijing 306 Hospital, 9 North Anxiang Road, PO Box 9720, Beijing 100101, China

Cai HY, Wang XH, Tian Y, Gao LY, Zhang LJ, Zhang ZY. Changes of serum p53 antibodies and clinical significance of radiotherapy for esophageal squamous cell carcinoma. *World J Gastroenterol* 2008; 14(25): 4082-4086 Available from: URL: http://www.wjgnet.com/1007-9327/14/4082.asp DOI: http://dx.doi.org/10.3748/wjg.14.4082

INTRODUCTION

Esophageal carcinoma (EC) is one of the most common malignant diseases in China. The prognosis of this disease is unfavorable in spite of advances in therapies. Mutations of the p53 gene are common gene alterations in most malignant tumors, including EC. Mutant p53 protein is accumulated in cells because of its longer half-life compared with wild-type protein. Therefore, p53 overexpression can be detected by immunohistochemical staining for p53. The anti-p53 antibody (p53-Ab) assay is based on the initial results of Crawford *et al*[1] who detected p53-Abs in the sera of patients with breast carcinomas. Because mutation in the p53 gene and the consequent overexpression of p53 are associated with tumor tissues, both wild type and mutant p53 may act as targets of tumor specific humoral and cellular immune responses[2,3]. The presence of p53-Abs in the sera of patients has been noted in various types of carcinomas. Non-carcinoma patients do not exhibit such antibodies[4-7]. Close correlations were observed between the presence of such antibodies and other factors related to a poor prognosis, such as high histological grade and

the absence of hormone receptors[3,8]. The presence of serum p53-Abs was reported to be an independent prognostic factor for breast carcinoma[9]. Studies have revealed that overexpression of p53 protein closely related to induction of drug resistance in cancer patients and it can be used as a predictor for chemosensitivity of tumor. Specific p53-Ab was found in the sera of cancer patients with p53 protein overexpression. Furthermore, the presence of serum p53-Ab is closely correlated with p53 protein overexpression[10,11]. Thus, serum p53-Ab could theoretically be useful in predicting radiosensitivity in cancer patients. However, the clinical relevance of p53-Ab and radiotherapy for EC has not been fully studied. In the present study, the serum p53-Ab level was detected in 46 EC patients and 30 healthy adults as control by enzyme linked immunosorbent assay (ELISA). We explored the relationship between serum p53-Abs and clinicopathological characteristics and therapeutic effect in EC patients, and investigated the sequential changing regularity of serum p53-Abs after radiotherapy.

MATERIALS AND METHODS

Clinical data

A total of 46 consecutive patients with primary EC were enrolled in this study. They consisted of 38 males (82.6%) and 8 females (17.4%), with a mean age of 56.4 years (range, 46-70 years), who were treated at Gansu Provincial Tumor Hospital between April 2005 and June 2007. The patients' eligibility for this study was as follows: (1) Primary lesion was squamous carcinoma in histology. There was no previous malignant disease or clinical evidence of a second primary tumor elsewhere; (2) Karnofsky performance status $\geqslant$ 70, white blood cell and hemoglobin levels within the normal range; and (3) Having not received radiotherapy, chemotherapy and operation. Tumor, nodes and metastasis (TNM) classification was established on the basis of pathologic examinations of biopsied specimens. Lymph node status in the patients was determined by computed tomography (CT). According to the UICC (2002) classification, 10 patients were in stage Ⅰ, 17 in stage Ⅱ, 14 in stage Ⅲ and 5 in stage Ⅳ. Twenty-eight patients had no signs of lymph node metastasis, and the other 18 patients had metastasis of mediastinal and/or supraclavicular lymph nodes. Thirty-two had primary lesions < 6 cm and 14 were $\geqslant$ 6 cm in size. Five tumors were located in the upper thorax, 21 in the middle thorax and 20 in the lower thorax. To compare the reliability of seronegative testing, a total of 30 healthy volunteers, 19 males and 11 females, with a mean age of 36 years (range, 21-62 years) from our hospital staff and medical students served as controls. Informed consent was obtained from all patients. The blood samples were collected on the day before radiotherapy and on the administration of an irradiation dose of 20 Gy/10 f/12 d, 40 Gy/20 f/24 d and 60 Gy/30 f/36 after radiotherapy, and stored at -80℃ until Assay.

Radiotherapy

Radiation source was a 6MV linear accelerator. The design of the radiation fields was based on the diagnosis by CT and barium examination. For all patients, a three-field approach was administered: one anterior and two posterior oblique portals. The width of the fields was adjusted to cover gross tumors with 3-4 cm extended margins so as to include subclinical lesions. The length of the field covered clinical tumors with a 3-5 cm extended margin at both ends of the lesion. All patients received conventional fractions, 2.0 Gy per fraction, five fractions per week. The total dose given to the tumor was 60-70 Gy/6-7 W.

Assay of serum anti-p53 antibodies

Anti-p53 antibodies in sera of patients was detected using a commercially available sandwich enzyme-linked immunosorbent assay kit (Immunotech, France). The assay was done according to the manufacturer's instructions, with the following specifications: patient serum was added for 60 min at 37℃ to microtiter wells coated with recombinant wild type human p53 protein to detect specific anti-p53 antibodies or with a control protein to detect nonspecific interactions. After washing, goat antihuman immunoglobulin G (IgG) antibody conjugated with peroxidase was added and stayed for 60 min at 37℃. Next, the substrate 3,3,5,5-tetramethyl benzidine (TMB) was added for 10 min. The enzymatic process was halted by adding 2N hydrogen chloride. Light absorption was measured at 450 nm using a photospectrometer. Because human serum may give rise to variable background signals, an internal control was used to measure the nonspecific background of each sample. This background reflects nonspecific interactions of serum components either with the plastic or with the components used in the ELISA. In the assay, the nonspecific background corresponds to the absorbance measured on wells coated with control protein. The presence of anti-p53 antibodies in a sample is determined by different parameters, i.e., for the specific signal of the sample, index = specific signal of the positive control (low); for p53 net absorbance of the sample, ratio of net absorbance = control protein net absorbance of the sample. An index of 1.1 and a ratio of net absorbance of 1.6 were determined to be positive for the presence of anti-p53 antibodies.

Clinical evaluation of radiation response

At the end of RT, all patients received esophageal barium examination and the clinical radiation response was evaluated according to standard X-ray diagnosis of EC after radiotherapy[12]. A complete response (CR) was defined as the disappearance of the mass shadow, no narrowing observed in the esophageal lumen, and none or slight rigidity of the esophageal wall without residual ulceration. Partial response (PR) was > 50% reduction in tumor bulk, but < 100% resolution of the disease and a residual shallow ulcer with a diameter < 1.5 cm, despite the disappearance of the mass shadow. No response (NR) was defined as no improvement in the X-ray findings, with a deep and large residual ulcer or complete

Table 1 Sequential change of positive rate of serum p53-Abs in EC patients before and after radiotherapy

Groups	*n*	-	+	%	χ^2	*P*
Before RT	46	28	18	39.1	0.000	1.000[1]
20 Gy	46	28	18	39.1	0.187	0.666[2]
40 Gy	46	30	16	34.8	4.696	0.030[3]
60 Gy	46	39	7	15.2	6.646	0.010[4]

[1]Before RT *vs* 20 Gy; [2]Before RT *vs* 40 Gy; [3]40 Gy *vs* 60 Gy; [4]Before RT *vs* 60 Gy.

Table 2 Relationship between positive rate of serum p53-Abs and clinical pathophysiological characteristics in EC patients

	n	-	+	Positive (%)	χ^2	*P*
Age (yr)					0.27	0.870
< 50	16	10	6	37.5		
⩾ 50	30	18	12	40.0		
Sex					0.11	0.918
Male	38	23	15	39.5		
Female	8	5	3	37.5		
TNM stage					6.09	0.014
Ⅰ + Ⅱ	27	21	6	22.2		
Ⅲ + Ⅳ	19	8	11	57.9		
Histological grade					10.43	0.002
Ⅰ	15	13	2	13.3		
Ⅱ	17	11	6	35.3		
Ⅲ	14	4	10	71.4		
Lymph node metastasis					6.00	0.015
Positive	18	7	11	61.1		
Negative	28	21	7	25.0		
Size of tumor					2.74	0.102
< 6 cm	32	22	10	31.3		
⩾ 6 cm	14	6	8	57.1		
Site of the tumor					0.02	0.953
Upper thorax	5	3	2	40.0		
Middle thorax	21	13	8	38.1		
Lower thorax	20	12	8	40.0		

TNM: Tumor, nodes and metastasis.

obstruction of the esophageal lumen, regardless of the residual state of the mass shadow.

Statistical analysis

The Statistical Package for Social Sciences, version 10.0 was used for statistical analysis. Serum p53-Abs levels were expressed as means ± SD. Student's *t* test, Pearson Chi-square and logistic analysis were used to determine the significance of difference between the two groups. A P value < 0.05 was considered significant.

RESULTS

Comparison of index and ratio of serum p53-Abs

The index and ratio of serum p53-Abs for EC patients were 1.5847 ± 0.5133 and 3.0293 ± 0.7013, and those for healthy controls were 0.2418 ± 0.1438 and 1.0361 ± 0.2175, the former was higher than the latter ($P < 0.0001$).

Comparison of positive rate of serum p53-Abs

The positive rate of serum p53-Ab was 39.1% (18 of 46) for EC patients and 0% (0 of 30) for healthy controls, with a significant difference ($P < 0.0001$). The sensitivity, specificity, accurate rate, positive predictive value and negative predictive value of p53-Ab detection in EC were 39.1%, 100%, 63.2%, 100% and 52%, respectively.

Table 3 Logistic analysis on the relationship between positive rate of serum p53-Abs and clinical pathophysiological characteristics in EC patients

Variables	B	OR	95% CI	*P*
Age	0.0241	1.0237	0.9583-1.0816	0.4659
Sex	-0.6230	0.5363	0.0863-3.3401	0.5165
TNM stage	1.8389	6.2899	2.4658-3.8954	0.0248
Histological grade	-4.1430	0.0159	0.0039-0.5867	0.0156
Lymph node metastasis	-0.4891	0.5973	0.3327-1.1406	0.1314
Size of tumor	0.6954	1.8924	0.7859-4.5968	0.1602
Site of tumor	-0.0695	0.9530	0.3240-2.6575	0.7986

Comparison of before and after radiotherapy sequential change

The level and positive rate of serum p53-Abs had significant differences between before radiotherapy, after administration of an irradiation dose of 40 Gy/20 f/24 d and after administration of an irradiation dose of 60 Gy/30 f/36 d (Table 1).

Relationship between positive rate of serum p53-Abs and clinical pathophysiological characteristics

The positive rate of serum p53-Abs in EC was related to histological grade, disease stage and lymph node metastasis, but it was not significantly related to sex, age and to the size and site of tumor (Table 2).

Logistic analysis

Logistic analysis showed that the positive rate of serum p53-Abs in EC had positive correlation with disease stage, negative correlation with histological grade and independence with sex, age and with the size and site of tumor (Table 3).

Clinical response to RT and serum p53-Abs

At the end of RT, CR was achieved in 21 patients, RR in 18 and NR in 7. In patients with CR plus PR, 61.1% (11/39) were positive for serum p53-Abs, but 100% (7/7) of patients with NR were positive for serum p53-Abs. The positive rate of serum p53-Abs in EC patients with effect was significantly lower than those without effect after radiotherapy ($P < 0.0001$).

DISCUSSION

p53 protein plays a crucial role in the regulation of the cell cycle and has been implicated in cell differentiation, apoptosis, DNA synthesis, and repair[13,14]. This nuclear protein, because of its critical function in triggering cell death *via* apoptosis in cells affected by irreparable genomic damage, has been designated as the "guardian of the genome" by Lane[15].

Mutations in the p53 tumor suppressor gene are

among the most common genetic alterations in human malignancies. In ovarian carcinoma, alterations in this tumor suppressor gene occur in approximately 50% of cases[16-18]. The commonly termed overexpression of p53 corresponds to a cellular accumulation of a biologically inactive protein stabilized either by a decreased rate of degradation of mutated gene product or by complex formation with certain proteins, such as viral oncoproteins or heat-shock protein 70[14,19,20]. In normal cells, p53 protein, through its short half-life, is present at such low levels that it is undetectable by conventional immunohistochemical methods. However, the results obtained in a number of studies on the prognostic impact of overexpression of p53 protein in ovarian carcinoma tissue were controversial. Whereas some investigators reported impaired clinical outcome in patients with p53 overexpressing tumors[21-23], others failed to demonstrate a relation between the course of the disease and p53 expression[16,24].

Recently, circulating p53-Ab has been detected in the serum or plasma of patients with various carcinomas. Detection is made by a simple and rapid ELISA procedure. The positive rate for p53-Ab was reported to be 24% for lung cancer, 19% for pancreatic cancer and 25% for colorectal cancer. The frequency of positive p53-Ab in patients with EC ranges from 25% to 53% in the literature. Our study detected p53-Ab in 18 (39.1%) of the 46 patients with EC, while it was not detected in any of the 30 healthy subjects. This positive rate for p53-Ab in esophageal cancer patients is thus similar to that in the published literatures[25,26]. The index and ratio of serum p53-Abs for patients with EC before radiotherapy were obviously higher than those for healthy controls ($P < 0.05$). This suggests that the detection of serum p53-Ab in patients with EC is helpful to its diagnosis. We also found that the positive rate of serum p53-Abs in EC was related to the histological grade, disease stage and lymph node metastasis ($P < 0.05$), but it was not significantly related to sex, age and the size and site of tumor ($P > 0.05$). The lower the histological grade and clinical stage, the higher the level and positive rate of serum p53-Abs in EC, indicating that the positive serum p53-Abs is a poor characteristic and identifying marker of unfavorable prognosis for patients with EC.

At the end of RT, CR was achieved in 21 patients, RR in 18 and NR in 7. In patients with CR plus PR, 61.1% (11/39) were positive for serum p53-Abs, but 100% (7/7) of patients with NR were positive for serum p53-Abs. The positive rate of serum p53-Abs in EC patients with effect was significantly lower than those without effect after radiotherapy. This suggests that serum p53-Ab overexpression can be one of the reference indicators for predicting radiosensitivity in patients EC. Future studies will be required to reveal and confirm the correlation between radiosensitivity and serum p53-Abs.

We also found that the serum p53-Abs level was useful for the monitoring of treatment. In the present study, the level and positive rate of serum p53-Abs had significant differences between before radiotherapy, after administration of an irradiation dose of 40 Gy/20 f/24 d and after administration of an irradiation dose of 60 Gy/30 f/36 d. It was reported that the presence of p53-Ab correlates closely with p53 overexpression and/or mutation[2,27]. This suggests that the p53 immune response in the patients can be attributed to accumulation of mutant p53 in the nucleus of tumor cells. The p53 protein was decreased and the tumor cell was destroyed through effective radiotherapy, the source of mutation of the p53 gene was eliminated, and then the immune response was weakened. Thus, monitoring of sequential change of serum p53-Abs before and after radiotherapy in patients with EC is helpful to evaluating the response to treatment and prognosis. A larger series of studies will be required to reveal and confirm the correlation between radiosensitivity and serum p53-Abs.

ACKNOWLEDGMENTS

The authors thank Professor Wei-Zhi Yang of the Department of Radiation Oncology, Cancer Hospital (Institute), Chinese Academy of Medical Sciences, Beijing Union Medical College for her assistance with data management and review of this manuscript.

COMMENTS

Background

Mutations of the p53 gene are common gene alterations in most malignant tumors, including esophageal carcinoma. Mutant p53 protein is accumulated in the cell because of its longer half-life compared with wild-type protein. p53 protein plays a crucial role in the regulation of the cell cycle and has been implicated in cell differentiation, apoptosis, DNA synthesis and repairment. In addition, it has been shown that patients with various types of neoplasias have p53 antibodies in their sera. ELISA was used to detect anti-p53 antibodies in the sera. The authors explored the relationship between serum p53-Abs and clinicopathological characteristics and therapeutic effect in patients with esophageal carcinoma, and investigated the sequential changing regularity of serum p53-Abs after radiotherapy.

Research frontiers

Studies have revealed that overexpression of p53 protein is closely related to induction of drug resistance in cancer patients and it can be used as a predictor for chemosensitivity to tumor treatment. Specific p53-Ab was found in the sera of cancer patients with p53 protein overexpression. Furthermore, the presence of serum p53-Ab is closely correlated with p53 protein overexpression. Thus, serum p53-Ab could theoretically be useful in predicting radiosensitivity in cancer patients. However, the clinical relevance of p53-Ab and radiotherapy for esophageal carcinoma has not been fully studied.

Innovations and breakthroughs

From the results of this study, the following conclusions can be drawn that the positive rate of serum p53-Abs in esophageal carcinoma is related to histological grade, disease stage and lymph node metastasis, but it is not significantly related to sex, age and the size and site of tumor. The level and positive rate of serum p53-Abs had significant differences between before radiotherapy, after administration of an irradiation dose of 40 Gy/20 f/24 d and after administration of an irradiation dose of 60 Gy/30 f/36 d. The positive rate of serum p53-Abs in esophageal carcinoma patients with effect is significantly lower than those without effect after radiotherapy.

Applications

Detection of serum p53-Abs is helpful to diagnosis of esophageal carcinoma. Monitoring of sequential change of serum p53-Abs before and after radiotherapy in patients with esophageal carcinoma is also useful in evaluating the response to the treatment and prognosis of the patients.

Peer review

The authors detected the serum p53 antibody level in patients with esophageal

squamous cell carcinoma and its sequential changes before and after radiotherapy. The results showed that changes of serum p53-Abs are helpful to the diagnosis of esophageal carcinoma. Monitoring of sequential change of serum p53-Abs before and after radiotherapy in patients with esophageal carcinoma is helpful to evaluating the response to the treatment and prognosis of the patients. Therefore, this study has values in clinical management of the patients.

REFERENCES

1 **Crawford LV**, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int J Cancer* 1982; **30**: 403-408
2 **Winter SF**, Minna JD, Johnson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. *Cancer Res* 1992; **52**: 4168-4174
3 **Schlichtholz B**, Legros Y, Gillet D, Gaillard C, Marty M, Lane D, Calvo F, Soussi T. The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res* 1992; **52**: 6380-6384
4 **Angelopoulou K**, Diamandis EP, Sutherland DJ, Kellen JA, Bunting PS. Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. *Int J Cancer* 1994; **58**: 480-487
5 **Shimada H**, Ochiai T, Nomura F. Titration of serum p53 antibodies in 1,085 patients with various types of malignant tumors: a multiinstitutional analysis by the Japan p53 Antibody Research Group. *Cancer* 2003; **97**: 682-689
6 **Labrecque S**, Naor N, Thomson D, Matlashewski G. Analysis of the anti-p53 antibody response in cancer patients. *Cancer Res* 1993; **53**: 3468-3471
7 **von Brevern MC**, Hollstein MC, Cawley HM, De Benedetti VM, Bennett WP, Liang L, He AG, Zhu SM, Tursz T, Janin N, Trivers GE. Circulating anti-p53 antibodies in esophageal cancer patients are found predominantly in individuals with p53 core domain mutations in their tumors. *Cancer Res* 1996; **56**: 4917-4921
8 **Mudenda B**, Green JA, Green B, Jenkins JR, Robertson L, Tarunina M, Leinster SJ. The relationship between serum p53 autoantibodies and characteristics of human breast cancer. *Br J Cancer* 1994; **69**: 1115-1119
9 **Peyrat JP**, Bonneterre J, Lubin R, Vanlemmens L, Fournier J, Soussi T. Prognostic significance of circulating p53 antibodies in patients undergoing surgery for locoregional breast cancer. *Lancet* 1995; **345**: 621-622
10 **Sato S**, Kigawa J, Minagawa Y, Okada M, Shimada M, Takahashi M, Kamazawa S, Terakawa N. Chemosensitivity and p53-dependent apoptosis in epithelial ovarian carcinoma. *Cancer* 1999; **86**: 1307-1313
11 **Nasierowska-Guttmejer A**, Szawlowski A, Jastrzebska M, Jeziorski K, Radziszewski J. p53 Protein accumulation as a prognostic marker of preoperative radiotherapy and/or chemotherapy in advanced squamous cell esophageal carcinoma--preliminary report. *Dis Esophagus* 1999; **12**: 128-131
12 **Zhao KL**, Wang Y, Shi XH. Late course accelerated hyperfractionated radiotherapy for clinical T1-2 esophageal carcinoma. *World J Gastroenterol* 2003; **9**: 1374-1376
13 **Linzer DI**, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 1979; **17**: 43-52
14 **Levine AJ**. The p53 protein and its interactions with the oncogene products of the small DNA tumor viruses. *Virology* 1990; **177**: 419-426
15 **Lane DP**. Cancer. p53, guardian of the genome. *Nature* 1992; **358**: 15-16
16 **Marks JR**, Davidoff AM, Kerns BJ, Humphrey PA, Pence JC, Dodge RK, Clarke-Pearson DL, Iglehart JD, Bast RC Jr, Berchuck A. Overexpression and mutation of p53 in epithelial ovarian cancer. *Cancer Res* 1991; **51**: 2979-2984
17 **Goff BA**, Shy K, Greer BE, Muntz HG, Skelly M, Gown AM. Overexpression and relationships of HER-2/neu, epidermal growth factor receptor, p53, Ki-67, and tumor necrosis factor alpha in epithelial ovarian cancer. *Eur J Gynaecol Oncol* 1996; **17**: 487-492
18 **Gadducci A**, Ferdeghini M, Buttitta F, Cosio S, Fanucchi A, Annicchiarico C, Gagetti O, Bevilacqua G, Genazzani AR. Assessment of the prognostic relevance of serum anti-p53 antibodies in epithelial ovarian cancer. *Gynecol Oncol* 1999; **72**: 76-81
19 **Kupryjanczyk J**, Thor AD, Beauchamp R, Merritt V, Edgerton SM, Bell DA, Yandell DW. p53 gene mutations and protein accumulation in human ovarian cancer. *Proc Natl Acad Sci USA* 1993; **90**: 4961-4965
20 **Momand J**, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992; **69**: 1237-1245
21 **Diebold J**, Baretton G, Felchner M, Meier W, Dopfer K, Schmidt M, Lohrs U. bcl-2 expression, p53 accumulation, and apoptosis in ovarian carcinomas. *Am J Clin Pathol* 1996; **105**: 341-349
22 **Klemi PJ**, Pylkkanen L, Kiilholma P, Kurvinen K, Joensuu H. p53 protein detected by immunohistochemistry as a prognostic factor in patients with epithelial ovarian carcinoma. *Cancer* 1995; **76**: 1201-1208
23 **Henriksen R**, Strang P, Wilander E, Backstrom T, Tribukait B, Oberg K. p53 expression in epithelial ovarian neoplasms: relationship to clinical and pathological parameters, Ki-67 expression and flow cytometry. *Gynecol Oncol* 1994; **53**: 301-306
24 **Green JA**, Robertson LJ, Campbell IR, Jenkins J. Expression of the p53 gene and presence of serum autoantibodies in ovarian cancer: correlation with differentiation. *Cancer Detect Prev* 1995; **19**: 151-155
25 **Shimada H**, Nabeya Y, Okazumi S, Matsubara H, Funami Y, Shiratori T, Hayashi H, Takeda A, Ochiai T. Prognostic significance of serum p53 antibody in patients with esophageal squamous cell carcinoma. *Surgery* 2002; **132**: 41-47
26 **Kressner U**, Glimelius B, Bergstrom R, Pahlman L, Larsson A, Lindmark G. Increased serum p53 antibody levels indicate poor prognosis in patients with colorectal cancer. *Br J Cancer* 1998; **77**: 1848-1851
27 **Davidoff AM**, Iglehart JD, Marks JR. Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. *Proc Natl Acad Sci USA* 1992; **89**: 3439-3442

S- Editor Zhong XY **L- Editor** Ma JY **E- Editor** Lin YP