



SERUM LEVEL OF P53 ANTIBODY AND TISSUE EXPRESSION OF P53 IN BREAST CANCER

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Abstract

Mutation of the p53 tumor suppressor gene is common event in breast cancer. This alteration can result in cellular accumulation of p53 and may also found in serum p53-antibodies. The current study was established to investigate the correlation between the appearance of the serum p53-antibodies and tissue expression of p53 protein, as well as to explore the relationship between serum p53-antibodies and clinicopathological features in patients. Serum p53-antibodies levels were investigated in 40 breast cancer patients, 20 adenofibrosis patients control and in 20 healthy controls by ELISA. Immunohistochemical assay for tissue expression of the p53 mutant protein was also undertaken in the same patients with breast cancer. The median serum levels of p53-antibodies in breast cancer patients was significantly higher than those in patients control and healthy individuals ($P < 0.05$). Antibody against p53 was detected in the sera of 8 patients (20%) whereas the mutant p53 protein was detected in 16 (40%) of the breast cancer tissue. Moreover significant correlation was found between serum p53-antibody status and tissue expression of the mutant p53 protein ($P < 0.05$). Interestingly, the positive rate of p53-antibodies in breast cancer were related to the absence of steroid hormone receptors ($P < 0.05$), but it was not related to age, tumor stage, histologic grade and the size of tumor ($P > 0.05$). These results indicated that the presence of p53-antibodies is probably triggered by the accumulation of tumor p53 protein, and it could be a useful marker to complement routine prognostic factors in breast cancer patients.

المستوى المصلّي للجسام المضادة لـ p53 والتعبير النسيجي لـ p53 في سرطان الثدي

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الخلاصة

أن حدوث الطفرة في p53 يعد من أكثر التغيرات شيوعاً في سرطان الثدي كما أن هذا التغيير أو التحول يؤدي إلى التراكم الخلوي لبروتين p53 وربما يؤدي إلى وجود أضداد في المصل ضد p53. أن هذه الدراسة جاءت للبحث عن الارتباط بين ظهور الأضداد في المصل ضد p53 والتعبير النسيجي لبروتين p53 كذلك البحث عن العلاقة بين وجود الأضداد في المصل والصور السريرية للأمراض لدى المرضى. تم قياس المستوى المصلّي لـ p53 باستخدام تقنية ELISA لـ 40 مريضة مصابة بسرطان الثدي مقارنة بمجموعة السيطرة والتي شملت 20 مريضة مصابة بتليف غدي و 20 امرأة سليمة. كما تم استخدام طريقة التصبيغ

الكيميائي النسيجي المناعي لغرض فحص التعبير النسيجي لبروتين الطافر p53 . أن متوسط المستويات المصلية للأضداد ضد p53 في مرضى السرطان كانت مرتفعة معنويًا عند مقارنتها بمجموعتي السيطرة ($P<0.05$) كما تم تحديد الأضداد ضد p53 في مصل ٨ من المرضى (٢٠%)، بينما وجد البروتين الطافر p53 في نسيج ١٦ (٤٠%) بالإضافة إلى ذلك لوحظ ارتباط قوي بين مستوى الأضداد في المصل وبين مستوى البروتين الطافر p53 في النسيج ($P<0.05$) . ومن جهة أخرى وجد أن هناك ارتباط معنوي بين النسبة الموجبة للأضداد لل p53 وغياب مستقبلات هرمون الأستروجين و البروجسترون، في حين وجد أن هذه الأضداد ليست لها علاقة بعمر المريض و لا بمرحلة الورم و التميز النسيجي للورم ولا حتى بحجم الورم. النتائج الحالية تشير إلى أن الأضداد ضد p53 في مصل المرضى ربما يعود إلى تراكم البروتين الطافر في النسيج كما أن هذه الأضداد يمكن أن تكون كعلامة مفيدة و مكتملة للعوامل الأندارية في سرطان الثدي.

Introduction

The p53 gene is localized on the short arm of chromosome 17 and encodes a 393-amino acid phosphoprotein, which is present at very low levels in normal cells. This molecule appears to play a major role in the maintenance of genomic integrity (1).

Mutation of p53 gene are common gene alterations in most malignant tumors, including breast cancer. 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 (IHC) staining for p53 (the use of IHC was based on the fact the missense mutations normally results in an increased half-life of the protein product and a consequence accumulation of the mutant p53 protein in the nucleus) (2).

The anti-p53 antibody (p53-Ab) assay is based on the initial results of Crawford et al. (3) who detected p53-Abs in the sera of patients with BC. 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 (4,5). 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 (6,7,8,9). 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 (10). The presence of serum p53-Abs was reported to be an independent prognostic factor for BC (11). The current study was designed to investigate the correlation between the appearance of the serum p53-Ab and tissue expression of the

mutant p53 protein in breast cancer, as well as to explore the relationship between serum p53-Abs and clinicopathological features in patients.

Patients and Methods

Patients

A total of 40 women with breast cancer (mean age 47.3 years, ranged between 28-69 years) were included in this study. They were admitted for surgery at Al-Kadimia teaching hospital, compared with 20 women have adenofibrosis (10 with fibroadenoma and 10 with fibrocystic change) as patient's control, as well as, 20 apparently healthy women; age were matched to patients group. Blood samples were collected from three studies groups, where as tissue samples were taken only from patients with breast cancer. This study was conducted during the period from April 2006 to August 2007.

Methods

Detection of serum p53-Abs: p53-Abs were determined in serum using commercially available ELISA and performed as recommended in leaflet with kit (Euroimmune, Germany).

Detection of tissue p53 protein: Monoclonal Mouse Anti-Human p53 protein, Clone DO-7, isotype: IgG2b, kappa (DakoCytomation, Carpinteria, USA) was used for detection p53 by IHC. The antibody labels wild-type and mutant – type p53 protein. Dilution used was 1:100. The tumor was considered positive for p53, when nuclear staining was seen in over 10% of tumor nuclei (12).

Statistical analysis

It was assessed using P (Kruskall-Wallis-test) and P (Mann-Whitney-test), as well as p (chi-

square-test) and (spearman's correlation test). Values of $P < 0.05$ were considered as statistically significant.

cancer patients (147.5pg/ml) as compared to adenofibrosis patients and healthy control (100pg/ml and 0pg/ml respectively) $P < 0.05$

Results

Using ELISA, serum p53-Ab level was investigated in breast cancer patients, adenofibrosis patients and healthy control. The

Table 1: Mean and median levels of serum p53 (pg/ml) among the three studied groups.

	<i>breast cancer cases</i>	<i>adenofibrosis cases</i>	<i>Healthy control</i>	<i>P (Kruskall-Wallis)</i>
serum p53-Abs	∧	∩	∪	
Minimum	120	90	0	
Maximum	320	120	80	
mean	160.6	103.3	80	
Median	147.0	100	0	$P < 0.05$
NO.	40	20	20	
P (Mann-Whitney)				
Breast cancer X H C*				
$P < 0.05$				
Breast cancer X AF**				
$P < 0.05$				

*Healthy control

**Adenofibrosis

Antibody against p53 was detected in the sera of 8 patients with breast cancer (20%), in 3 (15%) of 20 patients with adenofibrosis and in one case (5%) of 20 healthy control, whereas the mutant p53 protein was detected in 16 (40%) of the breast cancer tissue.

Moreover significant correlation was found between serum p53-antibody status and tissue expression of the mutant p53 protein in breast

cancer [spearman's correlation=0.667; ($P < 0.05$); Table-2]. Interestingly, the positive rate of p53-antibodies in breast cancer were significantly associated with the absence of estrogen and progesterone receptors ($P < 0.05$), but it was not associated with the age, tumor stage, histologic grade and the size of tumor ($P > 0.05$) as shown in table-3.

Table 2: Association between p53-Abs in serum and p53 in the tumor of breast cancer .

	<i>p53-Abs (+) No.=8</i>	<i>p53-Abs (-) No.=32</i>	<i>spearman's correlation</i>	<i>Chi-square</i>	<i>p-value</i>
Tumor p53 (+)	∨(87.5)	9(28.1)	0.667	Chi=9.401	$P < 0.05$
Tumor p53(-)	∪(12.5)	23(71.9)		D.F= 4	

results of the median serum level of p53-Abs are shown in table-1. There was a significant increase in median serum p53-Ab level in breast

Table 3: Association between serum p53-Abs and Clinicopa

	<i>Number of patients (%)</i>			
	<i>p53-Abs (+) No.=8</i>	<i>p53-Abs (-) No.=32</i>	<i>r</i>	<i>Chi-square</i>
Age				0.401

Discussion

The development of molecular marker is needed to improve the diagnosis and assessment of tumor progression in breast cancer patients. Overexpression of serum p53-Abs and of p53 protein in tumor tissue, have been encountered in variety of human malignancies(13).

P53-Ab was originally described by Crawford et al. (3) in the serum of 9% of breast cancer patients. Using ELISA more than 15 studies have been performed by Soussi and associates in breast cancer and observed that the frequency of p53-Abs in breast cancer ranges from 15 to 20% (13). Our results were in agreement with these findings, since the p53-Ab was found in 8 (20%) of 40 women with breast cancer.

On the other hand 16 (40%) had p53 accumulation in their tumor tissue, this result is corresponded with other studies investigate the accumulation of mutant p53 protein in tissue of breast cancer (14,15,16) who reported that 41% of patients revealed mutant p53 protein in tumor tissue.

Much work has been done towards identifying the clinical value of p53-Abs in serum, the molecular mechanisms that render this self protein immunogenic, leading to generation of autoantibodies in some patients still remain obscure. One hypothesis suggests that mutant p53 accumulation in the tumor is the major trigger for the development of the humoral immune response.

Accumulation of mutant p53 in tumors may induce self-immunization, which would result in the development of these antibodies. This hypothesis may explain the results obtained from present study about the significant correlation was found between serum p53-Ab status and tissue expression of the mutant p53 protein ($r=0.667$), similarly to other studies reported by (15,17) who also observed that there was strong significant correlation between serum p53-Ab and tissue expression of p53 protein, and they mentioned that accumulation of p53 protein in tumor cells may induce an immune response with appearance of p53-Abs in the sera of breast cancer patients.

In contrast Angelopoulou and colleagues (14) pointed out that no correlation was identified between p53 protein in tumor and p53-Ab titers in the serum. The presence of p53 autoantibodies with absence of the antigen from corresponding tumor may be due to a few possible explanations: (i) the immune system may be triggered by minute amounts of p53 protein; and (ii) another mechanisms that is not related to protein accumulation but rather to an effective presentation of p53 to the immune system might be operating. Binding of wild type p53 to cellular or viral proteins may elicit an immune response due to altered protein processing as shown by Dong and colleagues (18).

Interestingly, the present study failed to demonstrate a significant association between the positive rate of p53-Abs and each of age, tumor stage, histologic grade and the size of tumor, these results are in correspondent with some other studies (19,20) which found that the presence of p53-Abs were strongly correlated with these clinicopathological characteristic.

Moreover, results reported by Gao et al. and Pavel et al. (16,21) were consistent to our results, who reported that the presence of serum p53-Abs was significantly associated with negative estrogen and progesterone receptor status ($p=0.049$ and 0.042 respectively). However; Sangrajang and associates (22) noted that the presence of p53-Ab was associated with lack of estrogen receptor expression but was not related to progesterone receptor. In conclusion these results indicated that the presence of p53-Abs is probably triggered by the accumulation of tumor p53 protein, and it could be a useful marker to complement routine prognostic factors in breast cancer patients.

References

- Lane DP. **1992**. P53, Guardian of The Genome. *Nature*.**358**:15-26.
- Bourhis J, Lubin R, Roche B. **1996**. Analysis of p53 serum antibodies in patients with head and neck squamous cell carcinoma. *J.Natl.Can.Inst.* **88**:1228-33.
- Crawford LV, Piw DC, Ulbrook RD.**1982**. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int.J. Can.* **30**:403-408.
- Liu XF, Zhang H, Zhu SG, Zhou XT.**2006**.Correlation of p53 gene mutation and expression of p53 protein in cholangiocarcinoma. *World. J. Gastro.* **12**(29):4706-4709.
- Schlichtholz B, Legros Y, Gillet D, Gaillare C.**1992**. The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Can.Res.* **52**:6380-6384.
- Angelopoulou K, Diamandis EP. **1993**. Quantification of antibodies against the p53 tumor suppressor gene product in the serum of cancer patients.*Can. J.* **6**:315-321.
- Shimada H, Ochiai T, Nomura F. **2003**. Titration of serum p53 antibodies in 1,085 patients with various type of malignant tumors: a multiinstitutional analysis by the Japan p53 antibody. *Cancer.* **97**:682-689.
- Labrecque S, Naor N, Thomson D, Matlashewski G. **1993**. Analysis of the anti-p53-Ab response in cancer patients. *Can.Res.* **53**:3468-3471.
- Von Brevern MC, Hollstein MC, Cawly HM, DE Benedetti VM. **1996**. Circulating anti-p53-Abs in esophageal cancer patients are found predominantly in individuals with p53 core domain mutations in their tumors. *Can,Res.* **56**:4917-4921.
- Coomber D, Hawkins NJ, Clark M, Meagher A. **1996**. Characterization and clinicopathological correlates of serum anti-p53 antibodies in breast and colon cancer. *J.Can.Res.Clin.Onco.* **122**(12):1332-1335.
- Pyrate JP, Bonnetterre J, Lubin R, VanlemmensL.**1994**.Prognostic significance of circulating p53 in patients undergoing surgery for locoregional breast cancer. *Lancet.* **345**:621-622.
- Kerns BJ, Jordan P, Moore H, Beruhcuk A. **1992**. p53 overexpression in formalin-fixed, paraffin-embedded tissue detected by immunohistochemistry.*Bioche.* **40**(7):1047-1051.
- Balogh GA, Maili DM, Corte MM, Roncoroni P, Nardi H. **2006**. Mutant p53 protein in serum could be used as a molecular marker in human breast cancer. *Intern.J.Onco.* **28**:695-2.
- Angelopuoluo K, Yu H, Bharaj B, Gai M. **2000**. P53 gene mutation, tumor p53 protein overexpression, and serum p53 autoantibody generation in patients with breast cancer. *Cli.Bio.* **33**(1)77:53-62.
- Mudenda B, Green JA, Green B. **1994**. The relationship between serum p53 autoantibodies and characteristics of human breast cancer. *Br.J.Can.* **69**:1115-1119.

16. Gao RJ, Bao HZ, Yang Q. **2005**. The presence of serum anti-p53-Abs from patients with invasive ductal carcinoma of breast: Correlation to other clinical and biological parameters. *Brea.Can.Rese.Treat.* **93**(2):111-115.
17. Ahn SH, Moon H, Han S. **2000**. Correlation between serum p53 antibody and tissue expression of mutant p53 protein in primary breast carcinoma patients. *J. Korean. Syrg. Soc.* **59**(4):441-446.
18. Dong BX, Hamilton KJ, Satoh M, Revees WH. **1994**. Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. *J.Exp.Med.* **179**:1243-1252.
19. Cai HY, Wang XH, Tian Y, Gao L. **2008**. Changes of serum p53 antibodies and clinical significance of radiotherapy for esophageal squamous cell carcinoma. *World.J.Gastro.* **12**(25):4082-4086.
20. Balogh GA, Corte MM, Roncoroni P, Nardi H. **2005**. Detection of mutant p53 protein in serum could be use to differentiated malignant from benign breast cancer. *J.Women.Can.* **5**(1):18-23.
21. Pavel RJ, Gammon MD, Zhang YJ, Terry MB. **2008**. Mutation in p53, p53 protein overexpression and breast cancer survival. *J.Cell.Mole.Med.* **43**:67-71.
22. Sangrajrang S, Arpornwirat W, Chheirsilpa A, Thisuphakorn P. **2003**. Serum p53 antibodies in correlation to other biological parameters of breast cancer. *Can.Dete.Prev.* **27**(3):182-186