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Expression, Clinical significance and correlation of survivin and p53 in breast cancer

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ABSTRACT

Objective The aim of this study was to investigate the clinical significance and correlation between auto-antibodies to survivin and protein-expression to p53 in breast cancer. Methods Enzyme-linked immunosorbent assay (ELISA) was used to examine the level of auto-antibodies against survivin in the serum of 92 breast cancer patients. The expression of p53 was evaluated by SP immunohistochemical staining technique. Results The positive rates of survivin auto-antibody and p53 protein-expression in breast cancer were 15% and 34.78%. Survivin antibody and p53 overexpression in patients are closely related with lymph node metastasis of breast cancer. Conclusion Combined detection of p53 expression and survivin auto-antibody are valuable to estimation of prognosis and treatment of breast cancer.

KEYWORDS

Breast cancer;
Survivin;
P53;
ELISA;
Immunohistochemistry.

INTRODUCTION

Breast cancer is the most common malignancy in women in Western countries[1]. The incidence is not as high as western countries in China, but the growth rate was higher than some western countries. The development of breast cancer is related with a variety of genetic and molecular changes. Survivin is a member of inhibitor of apoptosis proteins (IAP) family. Many reports imply that the expression of survivin is associated with tumor development[2]. The wild-type p53 is a tumor suppressor gene, which is also closely associated with tumor development[3]. In this study, enzyme-linked immunosorbent assays (ELISA) were used to examine the level of auto-antibodies against survivin, immunohistochemical SP method were used for the determination of p53 in breast cancer patients. Moreover, the relationship between them and their association with clinical parameters was also evaluated for the early diagnosis and prognosis.

MATERIALS AND METHODS

Materials

Before treatment for cancer, we obtained paired sera and tissue samples from 92 patients with breast cancer, both were women patients, 29–87 years of age (median age 53 years), consecutively admitted to China-

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Japan Union Hospital of Jilin University, Chang Chun, Jilin Province. Sera samples were collected before radiotherapy and chemotherapy treatment. Written informed consent from patients was obtained, and the study was performed in accordance with medical ethics. Serum samples from 95 healthy individuals were selected to approximately match the median age of the breast cancer population.

Method

Elisa

The recombinant survivin fusion protein was prepared as reported previously[4]. The survivin and MUC1 solutions were dispensed at 250 μg/mL into ELISA plates (Jet Biofil) (100 μL/well) and incubated overnight at 4°C. After removal of the protein solution, plates were blocked with 5% bovine serum albumin (BSA) solution in phosphate buffered saline (PBS) for 1 h at 37°C (100 μL/well). The plates were washed five times with PBST (PBS containing 0.1% Tween 20). Serum samples diluted 1:100 in PBS were added at 100 μL/pre-coated well. After 2 h, the serum was removed, and the plates were washed five times with PBST. Each well was then incubated for 1 h with 100 μL of a 1:10,000 dilution of goat anti-human IgG1 labeled with horseradish peroxidase (Jackson Immuno Research), washed three times with PBST, and developed by adding 100 μL of TMB substrate (Tiangen). After a 25-min incubation in the dark, the reaction was stopped with 50 μL of 2 M H_2SO_4, and the absorbance at 450 nm was measured. All serum samples were run in duplicate and randomly distributed on the plates. Sera from cancer patients and sera from healthy donors were tested simultaneously. All experiments were repeated at least three times.

Immunohistochemistry

Immunohistochemistry for p53 was performed as follows. The primary antibody was a polyclonal antibody directed against p53. Secondary biotinylated antibodies (code No. E 0432), *i.e.*, goat antirabbit immunoglobulin G (IgG), were purchased from Dako, Denmark. The colour reaction was developed by 3-amino-9-ethylcarbazole in acetate buffer containing H2O2. Specificity of the immunohistochemical staining was assessed by neutralization incubation of the primary antibodies with a 5- to 10-fold (by weight) excess of blocking peptide, and incubation of preimmune serum or buffer instead of the primary antibody, all showing negative staining.

Judgment criteria

Means + 2 standard deviations (SD) taken from healthy donors' index were selected to determine the cut-off for positivity in the ELISA. The cut-off value for positivity in the anti-survivin ELISA, determined from healthy donor samples, was 0.84. The scoring assessment was carried out. In brief, randomly 10 viable tumor fields were scanned for immunoreactivity under high power objective (400×magnification). Any appreciable brown color was considered positive immunoreactivity. The percentage (s) of positive tumor cells were noted for each viable field and the staining intensity was graded as: 0-10% for negative, more than 10% for positive.

Statistical analysis

Statistical analysis (unpaired Student's t-test) of differences in the absorbance of anti-survivin antibodies and p53 expression was performed using GraphPad Prism 5.01 (GraphPad Software). Significance in a two-tailed test was defined as $P < 0.05$.

RESULT

1. A total of 92 breast cancer patients were recruited after histopathological confirmation of the tumor. The cut-off value for positivity in the anti-survivin ELISA, determined from healthy donor samples, was 0.84. Based on this criterion, sera from 13 of 92 breast cancer patients were positive by ELISA using recombinant survivin protein, indicating that the sensitivity of this assay was 14.13%. Whereas for the control group, only 1 out of 99 samples was positive, indicating the specificity was 98.9% (Figure.1). There were significant differences between breast cancer patients and healthy controls in anti-survivin auto-antibodies ($P < 0.05$), which suggested that a significant proportion of the cancer patients had generated antibodies against survivin in the context of tumorigenesis.
2. The expression of survivin auto-antibody was closely correlated with clinical staging and lymph

node metastasis in breast cancer patients (P<0.05), but it didn't significantly correlated with age or tumor size (P>0.05) (TABLE 1).

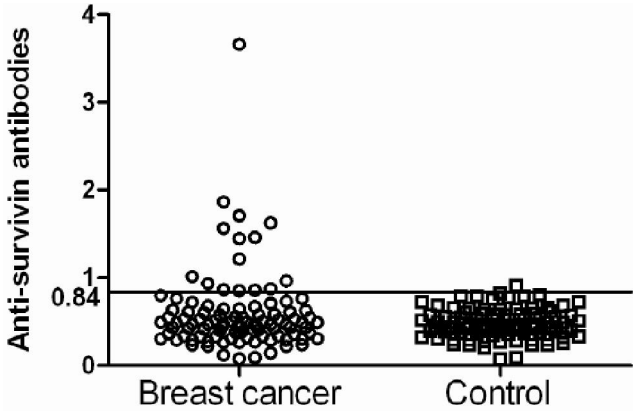

Figure 1 : ELISA Analysis of Anti-survivin in Sera from Breast Cancer Patients (n = 92) and Healthy Controls (n = 99). Data represent mean values (A450) from three determinations. The horizontal line indicates the cut-off value for seropositivity (A450 > 0.84).

TABLE 1 : Relationship between survivin antibody, p53 protein expression and clinicopathological features in breast cancer patients

Groups		P53	*P* value	survivin	*P* value
Breast cancer	92				
Age					
>60	20	26.85(33.92)	>0.05	0.60(0.09)	>0.05
<60	72	30.72(38.92)		0.58(0.05)	
Tumor size					
<5cm	50	34.52(5.669)	>0.05	0.55(0.06)	>0.05
>5cm	23	20.09(7.004)		0.55(0.05)	
Staging					
I + II	56	28.63(5.029)	<0.05	0.57(0.05)	<0.05
III	18	37.5(10.48)		0.48(0.03)	
lymph node metastasis					
No	46	26.3(5.46)	<0.05	0.51(0.03)	<0.05
Yes	29	36.86(7.654)		0.61(0.09)	

3. For the 92 cases of breast cancer patients, 32 cases were positive for p53, which accounted for 34.78%. P53 expression didn't significantly association with patient age and tumor size (P>0.05), but there was a significant difference between p53 expression with clinical stage and lymph node metastasis (P <0.05) (TABLE 1).
4. The auto-antibody level of survivin and p53 expression were analyzed for correlation and compared between the 92 breast cancer patients. It was showed that there was no significant correlation between them (P>0.05). However, the co-positive part of survivin auto-antibody and p53 protein expression showed a negative correlation with lymph node metastasis. The nine co-positive cases had no lymph node metastasis at all, which suggested that survivin auto-antibody and p53 protein expression combination could improve the prognosis of patients with breast cancer.

TABLE 2 : Correlationship between survivin auto-antibody and p53 protein

p53 protein	Survivin antibody	
	Positive	Negative
Positive	9	51
Negative	4	28

DISCUSSION

It is reported that the occurrence and development of tumors is controlled by many factors. It is closely linked not only the oncogenes activation or tumor suppressor genes inactivation, but also apoptosis regulation inhibition. Survivin and p53 are enrolled in our research. P53 mutates in 50% of all the malignant tumors, which will lead to the loss of their normal biological functions. As a member of IAP, survivin is a widely studied tumor-associated antigen. It is reported that a certain correlation exits between survivin auto-antibody level and pathologic parameters[5]. Moreover, survivin auto-antibody is closely linked with p53 expression[6].

Our results showed that the survivin auto-antibody in breast cancer patients was higher than healthy controls, and the antibody level was closely correlated with pathological stage and lymph node metastasis. This showed the possibility of survivin auto-antibody detection as biomarker for breast cancer diagnosis. The expression of p53 protein was correlated with pathological stage and lymph node metastasis, which was similar with the results of Lin Lijuan et al[7]. The intensity of anti-survivin antibody responses was not significantly correlated with intensity of p53 expression (P >0.05).

However, combined detection of p53 expression and survivin auto-antibody are valuable to estimation of prognosis and treatment of breast cancer.

Our study indicate that survivin antibody can be used as a marker for breast cancer diagnosis, and the combined detection of survivin antibody and p53 protein expression may served as a predictor of lymph node metastasis and prognostic indicator.

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