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Expression and clinical significance of SATB1 and TLR4 in breast cancer

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Abstract. This study investigated the expression of special AT-rich sequence-binding protein 1 (SATB1) and toll-like receptor 4 (TLR4) protein in breast cancer and its clinical significance. We collected breast cancer tissues from 120 patients and adjacent non-cancerous tissue from 53 patients. SATB1 was expressed in 89 cases of breast cancer (74.17%) and in 7 cases of adjacent non-cancerous tissue (13.21%). TLR4 was expressed in 70 cases of breast cancer tissues (58.33%) and in 48 cases of adjacent non-cancerous tissue (90.57%). The differences of SATB and TLR4 in breast cancer and adjacent non-cancerous tissue were statistically significant. We found a negative correlation between the expression of SATB1 and TLR4 ($r=-0.624$, $P<0.05$). The expression of SATB1 and TLR4 were not significantly correlated with age, menopause, and PR and HER-2 protein expression, but were significantly correlated with tumor size, local lymphatic metastasis, histopathological grade, tumor stage, and ER protein expression ($P<0.05$). Overall, SATB1 and TLR4 proteins are involved in the development of breast cancer, a finding of great significance to identify therapeutic targets and prognosis markers for breast cancer.

Introduction

Breast cancer is one of the most common female malignant tumors with high morbidity and mortality rate caused by its strong metastatic ability (1,2). There are no consensus biomarkers for early diagnosis and prognosis assessment of breast cancer with applications in clinical practice. Therefore, the development of breast cancer biomarkers has attracted increased attention recently. Special AT-rich sequence-binding protein 1 (SATB1) binds to T-rich sequences in chromosomes to regulate the expression of downstream genes (3,4). The expression level of SATB1 is low in normal tissue, but is elevated in a variety of tumors (5-7). Toll-like receptor 4 (TLR4) is mainly expressed in immune cells, but can also be expressed in tumor cells (8-10). In this study, we detected the expression of SATB1 and RLR4 in 120 cases of cancer and 53 cases of adjacent non-cancerous tissue by immunohistochemistry. The correlation between the expression of these two proteins and the clinical characteristics of patients were analyzed.

Patients and methods

Patient information. A total of 120 patients diagnosed with breast cancer in Yuhuangding Hospital of Yantai from October 2014 to October 2016 was enrolled in the study. Cancer tissue was collected after surgical resection. At the same time, adjacent non-cancerous tissue was collected from 53 patients. All the patients were females, and their ages ranged from 28 to 65 years, with a mean age of 46.5±11.7 years. No patient had been treated with chemotherapy before the study. Specimens were collected from necrotic cancer tissue and the adjacent non-cancerous tissue within 3 cm, fixed, and embedded in paraffin. Cancerous samples were diagnosed as breast cancer by pathological examination. The study was approved by the Ethics Committee of Yuhuangding Hospital of Yantai. All the patients signed an informed consent before being enrolled in the study.

Reagents and methods. Anti-human SATB1 monoclonal antibody and rabbit anti-human TLR4 monoclonal antibody were purchased from Abcam (Cambridge, UK). The DAB kit and hematoxylin were purchased from ZSBG-Bio (Beijing, China). Horseradish peroxidase-conjugated secondary antibody was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Paraffin-embedded breast cancer samples were cut into 4 μm sections and transferred onto glass slides. After baking for 2 h at 90˚C, tissue sections were dewaxed and rehydrated. After that, antigen retrieval was performed by incubating with 0.01 M sodium citrate buffer for 15 min. Endogenous peroxidase blocker was then added and incubated at 37˚C for

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Table I. Expression of SATB1 and TLR4 in breast cancer and adjacent non-cancerous tissue.

		SATB1			TLR4		
Groups	Cases (n)	-	+	Positive rate (%)	-	+	Positive rate (%)
Breast cancer	120	31	89	74.17	50	70	58.33
Adjacent non-cancerous tissue	53	46	7	13.21	5	48	90.57
χ^2 value		37.413		26.481			
P-value		0.006		0.011			

SATB1, special AT-rich sequence-binding protein 1; TLR4, toll-like receptor 4.

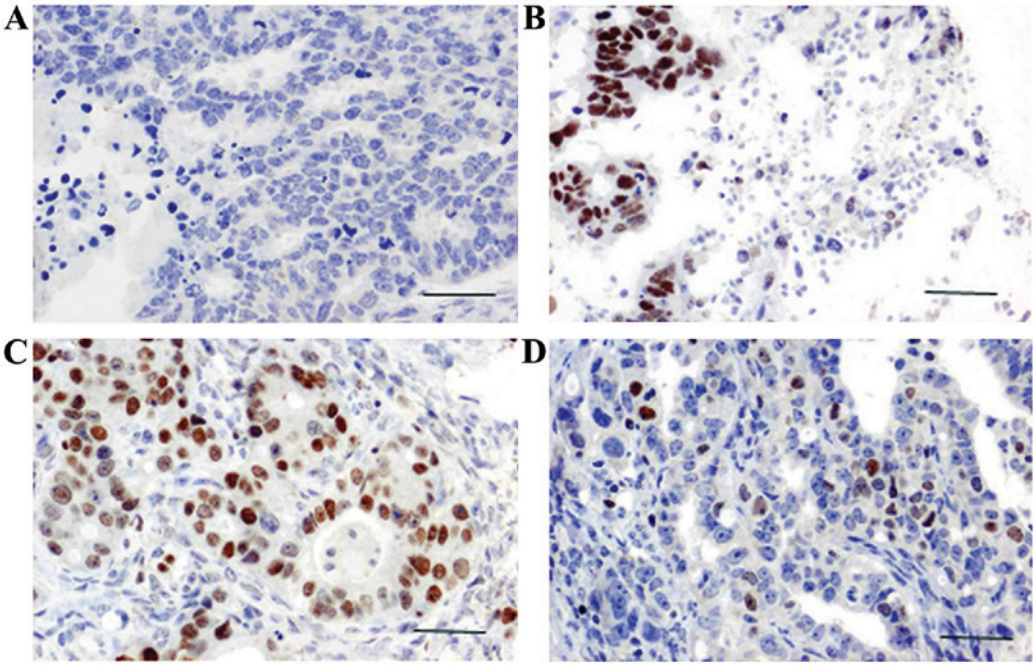

Figure 1. Expression of SATB1 and TLR4 in breast cancer and adjacent non-cancerous tissue. (A) Representative result of SATB1 expression in adjacent non-cancerous tissue (x400). (B) Representative result of SATB1 expression in breast cancer (x400). (C) Representative result of TLR4 expression in adjacent non-cancerous tissue (x400). (D) Representative result of TLR4 expression in breast cancer (x400). The positive expression rate of SATB1 in breast cancer tissues was significantly higher than that in adjacent non-cancerous tissue. The positive expression rate of TLR4 in adjacent non-cancerous tissues was significantly higher than that in breast cancer. SATB1, special AT-rich sequence-binding protein 1; TLR4, toll-like receptor 4.

10 min. After blocking with goat serum at room temperature for 20 min, the primary antibodies of SATB1 (1:300) and TLR4 (1:300) were incubated with the slides at 4˚C overnight. After washing, secondary antibody was added and incubated at 37˚C for 1 h. DAB staining was then performed and tissue sections were examined under the microscope to observe the staining. After hematoxylin staining, the slides were dehydrated, cleared, and sealed. All the operations were performed in accordance with the manufacturer's instructions.

Determination of experimental results. The brown or yellow granules on the slides showed the positive expression of SATB1 and TLR4. SATB1 mainly accumulated in the nucleus and TLR4 mainly accumulated in the cytoplasm. Using x400 magnification in a bright field microscope (Leica, Wetzlar, Germany), 10 distinct visual fields were selected to count the positive cells and record the degree of staining. We also calculated the percentage of positive cells. Scoring was performed according to the degree of staining: no staining, 0 points; light yellow, 1 point; yellowish-brown, 2 points; chocolate brown, 3 points. Scoring was also performed according to the

Table II. Correlation between the expression of SATB4 and TLR4 in breast cancer tissues.

	SATB1			
TLR4	+	-	r value	P-value
+	43	27	-0.624	0.003
-	46	4		

SATB1, special AT-rich sequence-binding protein 1; TLR4, toll-like receptor 4.

Table III. Correlation of the expression of SATB1 and TLR4 with the clinical and pathological features of patients.

		SATB1			TLR4		
Items	Cases (n)	+	χ^2 value	P-value	+	χ^2 value	P-value
Age (years)							
<50	46	35	0.25	0.416	28	0.74	0.352
≥50	74	54			42		
Menopause							
Yes	52	38	1.36	0.129	30	0.62	0.391
No	68	51			40		
Tumor size							
<2 cm	44	29	5.24	0.017	23	8.49	0.013
≥2 cm	76	60			47		
Lymph node metastasis							
Yes	56	31	9.15	0.013	26	6.37	0.015
No	64	57			44		
Histopathological grade							
I	38	19	11.48	0.007	18	7.14	0.015
II	53	44			36		
III	29	26			16		
Tumor stage							
I	32	18	12.53	0.007	16	9.61	0.011
II	57	43			33		
III, IV	31	28			21		
PR							
(-)	48	35	0.94	0.172	28	1.74	0.114
(+)	72	54			42		
ER							
(-)	53	29	6.74	0.015	24	7.93	0.013
(+)	67	60			46		
HER-2							
(-)	52	38	0.87	0.181	30	0.96	0.172
(+)	68	51			40		

SATB1, special AT-rich sequence-binding protein 1; TLR4, toll-like receptor 4.

percentage of positive cells: 0-25%, 1 point; 25-65%, 2 points; 65-100%, 3 points. The product of the 2 scores greater than 3 was taken as positive expression; values below 3 were considered negative expression (11).

Statistical analysis. SPSS 19.0 statistical software (IBM SPSS, Armonk, NY, USA) was used to analyze the data. The count data were analyzed by Chi-square test. Correlation analysis was performed by Spearman's rank correlation analysis. P<0.05 was considered to be statistically significant.

Results

Expression of SATB1 and TLR4 in breast cancer and adjacent non-cancerous tissue. SATB1 expression was observed in the nucleus under a microscope. SATB1 was positively expressed in 89 cases of breast cancer, and the positive expression rate of SATB1 was 74.1% (Fig. 1). Positive expression of SATB1 was only detected in 7 cases of adjacent non-cancerous tissue, and the positive expression rate of SATB1 was 13.21%. A statistically significant difference in the expression of SATB1 was found between breast cancer and adjacent non-cancerous tissue (P<0.05; Table I).

TLR4 expression was detected in the cytoplasm. TLR4 was positively expressed in 70 cases of breast cancer, and the positive rate was 58.33% (Fig. 1). Positive expression of SATB1 was detected in 48 cases of adjacent non-cancerous tissues, and the positive rate was 90.57%. A statistically significant difference in the expression of TLR4 was found between breast cancer and adjacent non-cancerous tissues (P<0.05; Table I).

Correlation between expression of SATB1 and TLR4. Following the immunohistochemistry results, we next analyzed the correlation between SATB1 and TLR4. As shown

in Table II, the expression of SATB1 was negatively correlated with the expression of TLR4 (r=-0.624, P<0.05).

Correlation of SATB1 and TLR4 with the clinical and pathological features of patients. We last examined the correlation of the expression of SATB1 and TLR4, and the clinical and pathological features of patients. We found that the expression levels of SATB1 and TLR4 were not significantly correlated with the age, menopause, PR protein, and HER-2 protein expression (P>0.05). However, the expression levels of SATB1 and TLR4 were significantly correlated with tumor size, local lymphatic metastasis, histopathological grade, tumor stage, and the expression of ER protein (P<0.05; Table III).

Discussion

SATB1 is a nuclear matrix attachment-binding protein with tissue-specific expression. The *SATB1* gene is located on chromosome 3p23 and encodes for a 763-amino acid protein (12). SATB1 is highly expressed in thymus where it regulates the development and maturation of T cells (13,14). Previous studies showed that *SATB1* gene knockout in mice can inhibit the production of $CD4^+$ and $CD8^+$ double positive T cells, leading to disorders of thymus cell maturation (15). SATB1 plays a role as a 'gene organizer' in the genome. SATB1 can interact with more than 1,000 proteins to specifically regulate the expression of its target genes by chromatin remodeling and protein modification (16). SATB1 can bind the BUR region of target genes and anchor the BUR region on the nuclear matrix to alter the higher-order structure of the chromatin, and regulate gene expression (17). SATB1 can also regulate DNA binding capacity and the subcellular localization of proteins through phosphorylation, ubiquitination, and acetylation (18). Liu *et al* found that high expression of SATB1 in breast cancer cells significantly increased cell invasion ability (19). Clinical data from 1,318 breast cancer patients showed that the expression level of SATB1 was negatively correlated with survival time (20). Our study shows that SATB1 is strongly expressed in breast cancer and weakly expressed in adjacent non-cancerous tissue. The expression of SATB1 was not significantly correlated with age, menopause, and the expression of the PR and HER-2 proteins, but was significantly correlated with tumor size, local lymphatic metastasis, histopathological grade, tumor stage, and ER protein expression. Our findings are consistent with previous studies (21,22).

Toll-like receptors were first found in *Drosophila*. In 1997, TLR4 homologue was identified in humans, and so far, there are 12 members of the TLRs family (23). The *TLR4* gene is located on chromosome 9q32-q33, and encodes for a 224-amino acid protein. TLR4 is widely distributed on the cell surface to sense pathogens (24). TLR4 is widely distributed in human monocytes (25), neutrophils (26), and epithelial cells (27). TLR4 can recognize a variety of pathogen-associated molecular patterns (e.g., LPS of Gram-negative bacteria) to induce different immune responses (28). Through binding to the corresponding ligands and mediating intracellular signal transduction, TLR4 plays a role as a transcription factor to activate the expression of a variety of cell growth and apoptosis-related factors (29,30). TLR4 can mediate MyD88-dependent pathways through the interaction with a series of cytokines to promote tumorigenesis (31). Clinical studies also found that TLR4 was correlated with the growth and metastasis of gastric cancer (32), ovarian cancer (33,34), cervical cancer (35), and other types of tumor cells. In our study, we found that TLR4 was positively expressed in 58.33% cases of breast cancer tissues and in 90.57% cases of adjacent non-cancerous tissue. The positive expression rate of TLR4 in this study is consistent with previous studies (35,36). We also found that TLR4 expression was not significantly correlated with age, menopause, or the expression of the PR and HER-2 proteins. However, TLR4 was significantly correlated with tumor size, local lymphatic metastasis, histopathological grade, tumor stage, and ER protein expression. In addition, correlation analysis indicated that the expression level of SATB1 was negatively correlated with the expression level of TLR4. In conclusion, SATB1 and TLR4 are involved in the development of breast cancer, which is of great significance for the identification of potential therapeutic targets and prognosis of breast cancer.

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