The role of anti-CENP-B and anti-SS-B antibodies in breast cancer

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A close relationship between autoimmunity and malignant diseases has been supposed for a long time. In clinical practice, anti-SS-B and anti-CENP-B antibodies are used as serologic markers for autoimmune diseases. In this study, anti-SS-B and anti-CENP-B autoantibodies were studied in breast cancer patients and compared to a control group surgically treated due to benign diseases. These antibodies were evaluated by enzyme linked immunoassay and serum values >10 U/ml were accepted as positive. Fifty-five patients with breast cancer and 25 patients with benign diseases were prospectively included in the study. In the breast cancer group, both anti-CENP-B (33% vs. 8%) and anti-SS-B (44% vs. 24%) autoantibodies had higher positivity compared to the control group, but this difference reached statistical significance only for anti-CENP-B antibodies (p=0.02). Besides, anti-SS-B positivity was detected more frequently in breast cancer patients with axillary involvement (63% vs. 24%) (p=0.006) and increased as the number of involved lymph nodes increased in the axilla (p=0.03). Although the clinical significance of autoantibody detection in cancer patients is still not clear, autoantibodies especially detected in individuals without proven autoimmune diseases needs to be thoroughly evaluated for early diagnosis and treatment of various cancers.

Key words: anti-SS-B, anti-CENP-B, autoimmunity, breast cancer

A possible relationship between autoimmunity and malignant diseases has been supposed for a long time. Patients with malignancies may develop autoimmune and rheumatic manifestations as primary signs of the disease [1, 12]. The prevalence of solid tumors among patients with systemic sclerosis is between 3–7% [2]. Autoantibody production has been reported in patients with various malignancies [10, 14]. Increased autoantibody production observed in cancer patients could be due to different causes such as overexpression of autoantigens, alterations in the patient's immune system or exposure of the cellular antigens during rapid cell turnover and death.

In clinical practice, autoantibodies are used as serologic markers for autoimmune diseases. Anti-SS-B and anti centromere protein (anti-CENP) antibodies are among antibodies characteristic for systemic lupus erythematosus, Sjögren's syndrome and systemic sclerosis. Previous studies have reported an increased production of anti-SS-B antibodies in cell cultures in response to estrogen stimulation [17]. Likewise, a possible role for estrogen in breast cancer development and progression cannot be denied.

On the other hand, CENPs are responsible for the kinetochore formation, alignment and proper segregation of the chromosomes, regulation of metaphase, anaphase spindle stabilization, and cytokinesis during cell proliferation [11]. CENP-A, B, and C are universally present in all cell types [7]. CENPs are increased in number of malignant diseases due to their role in controlling the cell cycle. They coud be used to depict the proliferative status of the cancer cells. Besides, CENP overexpression has been previously detected in breast cancer cell lines [16].

For these reasons, in presented study, anti-SS-B and anti-CENP-B autoantibodies were studied in breast cancer patients and compared to a control group. Thus, the role of these two antibodies in breast cancer was evaluated.

Patients and methods

Fifty-five patients with breast cancer treated between 1 January – 30 June 2003 were prospectively included in this study. In addition, a control group comprising 25 of the

patients surgically treated in the same time period due to benign diseases was formed. Patients with previous history or clinical evidence of autoimmune diseases or using drugs interfering with the immune system were excluded from the study.

Patients' clinical and histopathologic data were recorded from the files. Anti-CENP-B and anti-SS-B autoantibodies were studied from the patients' serum samples obtained preoperatively during routine blood withdrawal. Enzyme-linked immunosorbent assay was used according to the manufacturer's manual (Medizym, Selchow, Germany) to measure the antibody levels. Serum values >10 U/ml were accepted as positive for both of the antibodies.

The frequency of anti-CENP-B and anti-SS-B positivity was compared in the breast cancer and control groups. Besides, a possible relationship between autoantibody positivity and tumor size, grade, axillary status, estrogen and progesterone receptor status, c-erb-B2 and p53 positivity was evaluated in the breast cancer group. Patients were grouped according to tumor size as ≤2 cm vs. 2-5 cm vs. ≥5 cm. Similarly, axillary lymph node involvement was grouped as N1 (1–3+), N2 (4–9+), and N3 (>9+).

Chi-square and Fisher exact tests were used for statistical analysis as appropriate. Statistical analyses were performed with SPSS 9.0 statistical software package (SPSS Inc., Chicago, IL). $p<0.05$ value was accepted as significant.

Results

Fifty-five patients with breast cancer were included in the study. Control group comprised 25 patients with benign diseases. In the control group, 11 patients had inguinal hernia, 9 had chronic cholecystitis, and five had pilonidal sinus. The breast cancer and control groups were comparable in terms of sociodemographic properties except that all of the patients with breast cancer were females while the control group had 11 males ($p<0.0001$) (Tab. 1). The stages of the breast cancer patients were as follows; stage I 10 patients, IIA 17 patients, IIB 9 patients, IIIA 10 patients, and IIIB 9 patients.

The comparison of the autoantibody positivity in the breast cancer and control groups is shown in Table 2. In the breast cancer group, both anti-CENP-B (33% vs. 8%) and anti-SS-B (44% vs. 24%) autoantibodies had higher positivity compared to the control group. However, this difference between the two groups reached statistical significance only for anti-CENP-B antibodies ($p=0.02$). In addition, a similar trend was observed for anti-SS-B antibodies ($p=0.09$).

The relationship between the autoantibody positivity and the histopathologic properties of the tumor is depicted in Table 3. Anti-SS-B positivity was detected more frequently in breast cancer patients with axillary involvement (63% vs. 24%) ($p=0.006$). Besides, anti-SS-B positivity increased as the number of involved lymph nodes increased in the axilla ($p=0.03$). In contrast, anti-SS-B positivity had no significant correlation with the other histopathologic parameters. Similarly, anti-CENP-B antibodies were found to have no significant relationship with all of the tumor properties studied.

Table 1. Demographic properties of patients in breast cancer and control groups

	Breast cancer	Control
Age		
median (range)	44 (32–58)	41 (27–54)
Gender		
Male	–	11
Female	55	14

Table 2. Distribution of autoantibody positive patients in the study groups

	Breast cancer n (%)	Control n (%)	p
Anti-CENP-B			
Positive	18 (33)	2 (8)	
Negative	37 (67)	23 (92)	0.02
Anti - SS - B			
Positive	24 (44)	6 (24)	
Negative	31 (56)	19 (76)	0.09

Discussion

Previous studies have demonstrated an increased autoantibody production in various cancers including breast cancer [10, 14]. Similarly, in this study, anti-CENP-B and anti-SS-B antibodies were detected in a higher number of patients in the breast cancer group compared to patients with benign diseases. The background of this observation could be explained by stimulation of anti-SS-B antibody formation by estrogen which at the same time plays a central role in the etiology of breast cancer. An increased prevalence of autoimmune diseases in women, similar to breast cancer, supports the possibility of estrogen as a common factor in the etiology [17]. Besides, previous studies have shown an increased membranous detection of anti-SS-B autoantigens in keratinocyte and estrogen receptor positive MCF-7 cell cultures [8, 17]. Similarly, anti-SS-B autoantibody production increased in mice in response to exogenous estrogen [3]. In addition, genomic DNA analysis revealed estrogen responsive elements located near the relevant genes suggesting an estrogen control over the gene expression [17]. Overexpression of these antigens may re-

Table 3. Relationship between autoantibody positivity and histopathologic properties of the tumor

	Anti-SS-B			Anti-CENP-B		
	Positive	Negative	p	Positive	Negative	p
Tumor size						
T1	7	10		6	11	
T2	7	14		7	14	
T3	5	3		2	6	
T4	6	3	0.27	3	6	0.96
Axilla						
N0	6	19		11	14	
N+	19	11	0.006	7	23	0.15
N1(1–3)	7	4		4	9	
N2(4–6)	5	2		1	5	
N3(>9)	7	5	0.03	2	9	0.36
Grade						
I	4	7		5	6	
II	5	9		5	9	
III	16	14	0.44	8	22	0.5
ER						
Negative	14	9		6	17	
Positive	11	21	0.06	12	20	0.4
PR						
Negative	6	6		5	7	
Positive	19	24	0.75	13	30	0.5
c-erb-B2						
Negative	16	18		12	22	
Positive	9	12	0.8	6	15	0.76
p53						
Negative	12	16		7	21	
Positive	13	14	0.8	11	16	0.26

ER – estrogen receptor, PR – progesterone receptor

sult in their translocation to the cell membrane or apoptosis induced by estrogen may cause antigen translocation leading to easier accessibility to these antigens by the host immune system.

Increased cell proliferation is a well known property of cancer that leads to cell degradation as a discrepancy develops between the vascular supply and the size of the tumor. This process may cause the exposure of intracellular components and an altered immune response against autoantigens may result in autoantibody formation. Thus, CENPs increase in the cells with a high proliferation rate. This increased production of CENPs is recognized by the host immune system resulting in the formation of autoantibodies against them. There is considerable evidence supporting that immune responses detected in cancer include the production of autoantibodies against altered or overexpressed self-antigens involved in cell proliferation. The observation of autoantibodies against CENP-F in malignant diseases suggests a potential connection between overexpression of CENPs and malignancies [4, 13]. Among these, breast and lung cancers are the most frequent types with anti-CENP-F antibodies. However, overall frequency of anti-CENP-F among patients with various cancers is less than 1% [4, 13]. Similarly, overexpression of CENP-A and CENP-B antibodies were detected in colorectal cancer tissues and this was shown to be due to an increased transcription of their genes [15]. In accordance with these results, a significant 4-fold increase in anti-CENP-B autoantibody formation was detected in breast cancer group compared to the control group in this study.

The clinical significance of autoantibody detection in cancer patients is still not clear. These autotantibodies may have a diagnostic value, prognostic importance, predictive role for specific treatment modalities or be important to mark a transition period between chronic diseases and malignancy [1]. Autoantibody production can also be solely result to a self immunization process due to strong immunogenicity of these autoantigens [1]. COVINI et al have reported an increased rate of anti-nuclear antigen positivity in patients with hepatocellular cancer compared to a control group [6]. The presence of autoantibodies in cancer patients, as in this study, may enable us to diagnose the disease in early stages. In cancer types which may follow a precancerous lesion, appearance of autoantibodies may indicate the conversion to malignancy. In supporting this, IMAI et al found anti-nuclear antibody positivity in patients with chronic hepatitis or cirrhosis as 13% where as this rate increased to 31% in hepatocellular carcinoma patients [10]. Similarly, ZHANG et al detected anti-CENP-F antibodies in patients' sera at the time of conversion from chronic liver diseases to liver cancer [19]. It seems therefore that these antibodies could be used to detect occult malignancies [12]. Thus, in patients with precancerous lesions of the breast, such as atypical hyperplasia or lobular carcinoma *in situ*, both of the two studied antibodies, especially anti-CENP-B, can be used for early detection of breast cancer.

The prognostic value of autoantibodies has been mentioned in some of the previous studies. SYRIGOS et al studied anti-ds-DNA antibodies in colorectal cancer patients and reported 21% antibody positivity preoperatively and the positivity decreased to zero after surgery. In addition, antibody positive patients had no recurrence during follow-up suggesting a better response of the host immune system in these patients [14]. Similarly, prognostic importance of anti-nuclear antibodies has been reported for breast cancer [18]. In addition, anti-CENP-F antibodies were found to have prognostic significance in node-negative breast cancer patients [5]. In the previous studies, anti-CENP-F antibodies were found to correlate with nodal metastases in head and neck cancers. As the gene overexpression increased, the possibility of lymph node metastases increased as well [9]. This finding was similar to the relation between anti-SS-

B antibody and axillary involvement detected in this study for breast cancer group. Thus, autoantibody positivity may help us to predict the lymph node involvement before surgery.

As clearly recognized, cancer is a multi-step process during which expression of various genes and encoded proteins is altered and host immune response directed against these antigens is unpredictable. Although the mechanisms that provoke immune system responses against self-antigens is unclear, there is considerable amount of evidence for a relationship between the appearance of autoantibodies and carcinogenesis. Autoantibodies detected especially in individuals without proven autoimmune diseases could be considered as a possible indicator of malignancy. Thorough investigation of these individuals may lead to early diagnosis and treatment of various cancers.

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