

SOX2 Autoantibodies As Noninvasive Serum Biomarker for Breast Carcinoma

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Abstract

Background: A clear association has been established between antibodies to the transcription factor sex-determining region Y (SRY)-box 2 (SOX2) and small cell lung cancer. In light of the pathologic role of SOX2 and its aberrant expression in breast cancer, we measured serum SOX2 autoantibodies (SOX2-Abs) in breast cancer patients.

Methods: The presence of SOX2-Abs was determined by an indirect enzyme-linked immunosorbent assay (ELISA) in sera from 282 patients with breast cancer, 78 patients with benign breast disease, and 194 healthy women.

Results: SOX2-Abs were more prevalent in patients with breast cancer (18.4%) compared with healthy women (2.6%, $P < 0.0001$), and patients with benign breast disease (6.4%, $P = 0.011$). The concentrations of circulating SOX2-Abs were found to discriminate between breast cancer patients and healthy controls ($P < 0.001$) and between breast cancer patients and those with benign breast disease ($P < 0.001$). In addition, measurement of SOX2-Abs was more effective than assays of serum tissue polypeptide-specific antigen, carcinoembryonic antigen, carbohydrate antigen (CA) 125, and CA 15-3 in distinguishing between malignant and benign breast disease. In breast cancer patients, the prevalence of SOX2-Abs was associated with a higher tumor grade ($P = 0.021$) and positive nodal status ($P = 0.021$).

Conclusion: The presence of SOX2-Abs in breast cancer may be of clinical value.

Impact: This study provides the first evidence for the presence of circulating SOX2-Abs in breast cancer and shows their potential clinical application. *Cancer Epidemiol Biomarkers Prev*; 21(11); 2043–7. ©2012 AACR.

Introduction

Breast cancer remains the primary cause of cancer death among females worldwide. To date, no effective blood-based marker is available for breast cancer detection, particularly for patients with early-stage disease (1). As part of the effort to develop additional blood-based tests for breast cancer, attention has recently focused on the host response to tumor-associated antigens (TAA).

Evidence collected in the last few years suggests that SOX2, one of the embryonic stem cell markers, plays a

role in breast carcinogenesis (2, 3). Correspondingly, SOX2 has been shown to be aberrantly expressed in breast cancer tissues (3–5). To date, the immunogenicity of SOX2 had mainly been investigated in individuals with small cell lung carcinoma (SCLC; refs. 6–10). Initially identified by serological analysis of expression cDNA libraries (SEREX; ref. 7), the presence of SOX2 autoantibodies (SOX2-Abs) in SCLC patients has been confirmed by several subsequent studies (6–10). The 22% to 33% frequency of SOX2-Abs has promising potential for clinical use. Moreover, SOX2-Abs have also been detected in meningioma (11), monoclonal gammopathy of undetermined significance (MGUS; ref. 12), and myeloma (13). The aforementioned findings raised the possibility that the host response to SOX2 may be spontaneously primed in breast cancer and the presence of SOX2-Abs may be of clinical significance.

Therefore, we developed an in house ELISA (i) to analyze the prevalence of serum SOX2-Abs in patients with benign and malignant breast disease and in healthy controls, (ii) to determine the clinical significance of SOX2-Abs in breast cancer screening, and (iii) to compare the performance of the SOX2-Abs assay with assays for commonly used breast cancer markers such as serum tissue polypeptide-specific antigen (TPS), CEA, CA 15-3, and CA 125.

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Materials and Methods

Patients and samples

The majority of the total breast cancer patient cohort ($n = 282$) comprised patients with early-stage breast cancer ($n = 233$). The control populations consisted of 194 healthy controls and 78 patients with benign breast disease (Table S1 in Supplementary Data). None of the patients with benign breast disease or the healthy controls had a past or concomitant history of any malignancy. The clinicopathologic data for the patient population were collected. All participants were ethnic Chinese women. Cases and controls did not differ significantly with respect to age ($P = 0.074$). Sera from patients with breast cancer or benign breast disease were obtained at the time of diagnosis at Cancer Hospital, Chinese Academy of Medical Sciences. Sera from healthy women were obtained at Beijing Hospital. All collection procedures followed a standardized protocol. Samples were processed within 2 hours after blood withdrawal and were stored at -80°C until use. Informed consent was obtained from each subject, and the study protocol was approved by the Ethics Committee of the National Center for Clinical Laboratories, and adhered to the tenets of the Declaration of Helsinki.

ELISA for detection of SOX2-Abs

Recombinant His₆-tagged SOX2 protein was purified and prepared for antibody detection, and the measurement of SOX2-Abs was conducted as described (Supplementary Data).

All the assays were conducted in duplicate. To correct for interassay variation, the results were expressed as arbitrary units (AU), as described previously (14). To verify the ELISA results, serum samples with AU values near the assay cut-off value (see below) and a group of randomly selected serum samples were further tested for SOX2 reactivity by western blotting, as described (Supplementary Data).

CEA, CA 125, CA 15-3, and TPS assays

Serum levels of TPS, CEA, CA 125, and CA 15-3 were measured using commercially available kits (Elecsys CEA, CA 125, and CA 15-3 Immunoassays from Roche Diagnostics; TPS ELISA kit from IDL Biotech AB), according to the manufacturer's recommendations. The cut-off values described in the protocol for positive and negative reactivity were 110 U/L for TPS, 5 ng/mL for CEA, 35 U/mL for CA 125, and 25 U/mL for CA 15-3.

Statistical analysis

We used Pearson's two-tailed χ^2 test to compare proportions. The differences in the concentrations of SOX-Abs among groups were assessed by the Mann-Whitney test or Kruskal-Wallis test, as appropriate. P values less than 0.05 were considered statistically significant. Statistical analyses were carried out using SPSS v. 16.0.

To assess the diagnostic performance of the SOX2-Ab ELISA in breast cancer screening, we used receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) was estimated to evaluate the diagnostic power.

We used Venn diagrams to exam whether CA 15-3, CA 125, CEA, TPS, and SOX2-Abs pinpoint overlapping group of patients.

Results

Prevalence of SOX2-Abs

Positivity was defined as 3 SDs above the mean AU for normal controls. SOX2-Abs were observed to be more prevalent in patients with breast cancer (18.4%) compared with healthy women (2.6%, $P < 0.0001$), and patients with benign breast disease (6.4%, $P = 0.011$). The distribution of SOX2-Abs in the 3 study groups is presented in Fig. 1A. The circulating levels of SOX2-Abs in patients with breast cancer were significantly higher than the levels in healthy

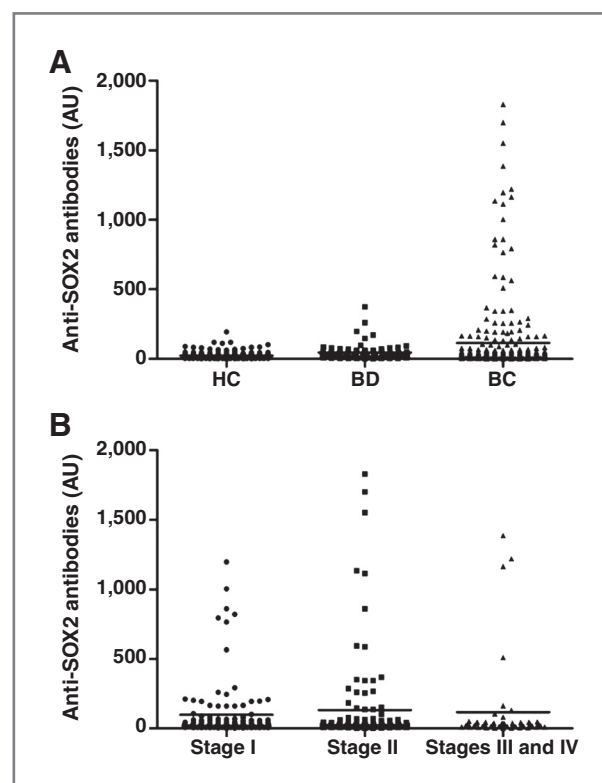


Figure 1. A, distribution of SOX2-Abs in sera from patient and control groups. Scatter plots of arbitrary unit (AU) values from 194 healthy controls (HC), 78 patients with benign disease (BD), and 282 patients with breast cancer (BC). The SOX2-Ab levels in patients with breast cancer were significantly higher than the levels in healthy donors ($P < 0.001$) and in patients with benign disease ($P < 0.001$). The cut-off value was set at mean + 3 SDs above the mean AU of normal sera. B, distribution of SOX2-Abs in sera from patients with different stages of breast cancer. Scatter plots of absorbance values of autoantibodies from patients with stage I ($n = 118$), stage II ($n = 115$), and stage III + IV ($n = 49$) cancer. No significant differences were found among the groups. Antibody titers were standardized as AUs.

donors ($P < 0.001$) or in patients with benign disease ($P < 0.001$). These results were confirmed by western blot analysis of patient sera (Supplementary Fig. S1). We next examined the levels of SOX2-Abs in breast cancer patients according to clinical disease stage. For this analysis, the results for patients with stage III and stage IV disease were combined because of the limited number of patients with stage IV disease ($n = 5$). However, we found no significant differences in SOX2-Ab levels among the clinical stages ($P = 0.920$; Fig. 1B).

Circulating SOX2-Abs and clinicopathologic characteristics

Table 1 presents the associations between SOX2-Ab levels and clinicopathological variables in the 282 breast cancer patients. The presence of SOX2-Abs correlated with higher tumor grade ($P = 0.021$) and nodal status ($P = 0.021$). No significant association was observed between SOX2-Ab expression and patient age, T classification, Ki-67 proliferative rate, HER2 overexpression, or hormone receptor positivity.

Comparison of serum SOX2-Abs with TPS, CEA, CA 125, and CA 15-3 assays

The serum levels of TPS, CEA, CA 125, and CA 15-3 were measured for patients with benign ($n = 78$) and malignant ($n = 282$) breast disease (Supplementary Fig. S2). The sensitivity and specificity were 2.1% and 100% for CA 15-3, 2.8% and 100% for CEA, 22.3% and 75.6% for TPS, and 3.6% and 92.4% for CA 125, respectively; while the sensitivity and specificity were 18.4% and 93.6% for SOX2-Abs to discriminate malignant from benign breast disease. In the Venn diagrams, we did not observe significant overlapping group of patients among these markers, except that SOX2-Abs and TPS were both positive in 10 patients (Supplementary Fig. S3).

Diagnostic utility of SOX2-Abs

Using ROC curves, we determined the diagnostic capability of the SOX2-Ab assay. The sensitivity of the assay was 18.4% and the specificity was 97.4% for differentiating between breast cancer patients and healthy subjects, whereas the sensitivity was 18.4% and the specificity was 93.6% for differentiating between breast cancer patients and patients with benign disease. The former had an AUC of 0.671 [95% confidence interval (CI), 0.623–0.719; $P < 0.001$] (Fig. 2A) whereas the latter had an AUC of 0.630 (95% CI, 0.584–0.676; $P < 0.001$; Fig. 2B). Similar results were obtained when analyzing the ability of SOX2-Abs to discriminate between stage I and II breast cancer patients and the control groups (data not shown).

Discussion

Recently, SOX2 has drawn increasing attention for the frequency with which it elicits a spontaneous host response in cancer patients (6, 15). The 18.4% frequency of SOX2-Abs obtained in our study on breast cancer

Table 1. Association of SOX2-Abs with clinicopathologic features and molecular characteristics in breast cancer patients

Variable	SOX2-Ab negative N (%) <i>n</i> = 230	SOX2-Ab positive N (%) <i>n</i> = 52	<i>P</i> value
Age			
<50	122 (53.0)	26 (50.0)	
≥50	108 (47.0)	26 (50.0)	0.913
Stage			
I and II	188 (81.7)	45 (86.5)	
III and IV	42 (18.3)	7 (13.5)	0.409
Tumor size			
<2 cm	135 (58.7)	34 (65.4)	
2–5 cm	89 (38.7)	15 (28.8)	
>5 cm	6 (2.6)	3 (5.8)	0.252
Nodal status			
Negative	142 (61.7)	23 (44.2)	
Positive	88 (38.3)	29 (55.8)	0.021
Grading			
G1	40 (18.0)	6 (13.6)	
G2	117 (52.7)	19 (43.2)	
G3	65 (29.3)	19 (43.2)	0.021
GX	8	8	
ER			
Negative	84 (37.2)	17 (34)	
Positive	142 (62.8)	33 (66)	0.854
Unclassified	4	2	
PR			
Negative	39 (20.3)	8 (22.9)	
Positive	153 (79.7)	27 (77.1)	0.265
Unclassified	38	17	
HER2			
Negative	171 (76.3)	36 (72)	
Positive	53 (23.7)	14 (28)	0.474
Unclassified	6	2	
Subtype			
Luminal A/B	158 (82.3)	36 (83.7)	
HER2 subtype	12 (6.3)	3 (7.0)	
Triple negative	22 (11.4)	4 (9.3)	0.911
Unclassified	38	9	
Ki-67			
Low (<25%)	83 (36.1)	20 (48.8)	
High (≥25%)	147 (63.9)	21 (51.2)	0.123
Unclassified	31	11	

patients is comparable to the published frequencies for SCLC and MGUS, despite the highly heterogeneous nature of breast cancer.

It is perhaps not surprising that autoimmune responses to SOX2 were detected in breast cancer patients. SOX2 has been shown to promote the proliferation of tumor cells by facilitating G₁ to S transition (2). Additional studies have revealed the molecular and biologic basis of SOX2 involvement in early events in breast carcinogenesis by

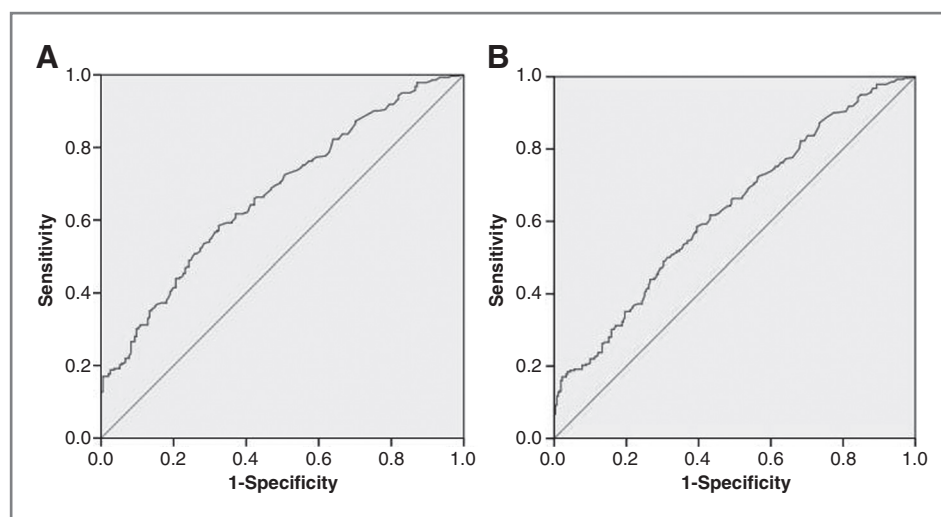


Figure 2. Diagnostic performance of SOX2-Ab measurement conducted by ROC analysis. A, the AUC of serum SOX2-Abs was 0.671 (95% CI, 0.623–0.719) for differentiation between patients with breast cancer and healthy controls. B, the AUC of serum SOX2-Abs for differentiation between patients with breast cancer and controls (healthy controls plus patients with benign breast disease) was 0.630 (95% CI, 0.584–0.676).

confirming its potential to induce stem cell-like cancer cells (3, 4). In addition, SOX2 has been shown to be aberrantly expressed in breast cancer tissues, particularly in early-stage cancers (3–5), which has the potential to support a sustained memory response through ongoing antigenic exposure. Apart from the aberrant expression of SOX2, certain therapies may be responsible for the development of SOX2-Abs. It was reported that patients with multiple myeloma who undergone allogeneic stem cell transplantation presented SOX2-Abs (13), as allogeneic stem cell transplantation was shown to promote immunogenicity of tumor-associated antigens. It is of interest that most of the seropositive patients showed no immune response against SOX2 before treatment (13), suggesting a different underlying mechanism for the development of SOX2-Abs compared with solid tumors. In the same study, the amino acid region from 211 to 230 in SOX2 was identified as the immunodominant epitope (13).

To date, the pathogenetic role of SOX2-Abs in tumors has not been well characterized in the literature. That is partially because the lack of correlation between SOX2 seropositivity and known clinical parameters. A large-scale screening conducted in SCLC patients did not observe a significant effect of SOX2-Abs on survival (10). The presence of SOX2-Abs was reported to be not related to disease burden in multiple myeloma (13).

Our study further revealed the clinical significance of the presence of SOX2-Abs. We found that SOX2-Abs were more prevalent in sera from patients with a higher tumor grade and positive nodal status, but there was no association between the presence of SOX2-Abs and other clinicopathological variables. Intriguingly, these results revealed a positive correlation between the presence of SOX2-Abs and previous immunohistochemical findings for breast cancer. These immunohistochemical studies showed that SOX2-Abs was more prevalent in triple negative breast cancer (3, 5). A lack of correlation between SOX2-Abs status and estrogen receptor (ER)/progesterone receptor (PR) or HER2 overexpression was observed

as well (4). Although we did not observe SOX2-Abs to be more prevalent in triple negative breast cancer, we did find a positive association with the less differentiated phenotypes. SOX2-Ab status was not correlated with ER/PR or HER2 overexpression, which was consistent with the results of another study on early-stage breast carcinoma (4).

To determine whether the presence of serum SOX2-Abs might be useful in breast cancer detection, we compared the sensitivity and specificity of SOX2-Ab detection with assays for serum TPS, CEA, CA 15-3, and CA 125 in the cohort of patients with benign or malignant breast disease. Using the recommended assay cut-off values, the sensitivity of the SOX2-Ab assay (18.4%) was considerable higher than that of the CEA, CA 15-3, and CA 125 assays (all < 5%). Moreover, the SOX2-Ab ELISA showed specificity that was comparable to those of the CEA, CA 15-3, and CA 125 assays and superior to that of the TPS assay. In our study, therefore, measurement of SOX2-Abs was a more powerful diagnostic tool than these clinically "accepted" diagnostic markers. Although the diagnostic efficiency of SOX2-Abs was lower than expected, it was as effective as other well-studied biomarkers, such as autoantibodies against p53 and HER2. Lu and colleagues (16) reported that the detection of p53-Abs had an ROC-AUC of 0.48 ($P = 0.538$) whereas the combination of p53-Abs and HER2-Abs had an ROC-AUC of 0.61 ($P = 0.006$), in discriminating advanced-stage breast cancer patients from nonmalignant disease and healthy controls. To meet the stringent sensitivity and accuracy requirements for a diagnostic assay, we would recommend that SOX2-Abs are assayed in combination with a panel of other serum biomarkers, such as antibodies to p53, c-myc, and survivin (17–19).

In conclusion, the preliminary results presented here indicate that SOX2-Abs are not confined to SCLCs but are also present in breast cancer patients, raising the possibility that SOX2-Abs may serve as serum biomarkers for breast cancer. This study raises further questions about (i)

whether the presence of SOX2-Abs in breast cancer and SCLC indicates that the diseases share a common pathogenic mechanism and (ii) whether SOX2-Abs could play a role as a prognostic indicator in breast cancer. These questions should be addressed in future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Sun, R. Zhang, J. Qi
Development of methodology: Y. Sun, R. Zhang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Sun, R. Zhang, M. Wang, Y. Zhang
Writing, review, and/or revision of the manuscript: Y. Sun, M. Wang, J. Qi, J. Li

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Study supervision: J. Li

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