Serum Autoantibody Signature of Ductal Carcinoma In Situ Progression to Invasive Breast Cancer

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Abstract

Purpose: The identification of markers associated with progression to invasive breast cancer (IBC) is a major factor that can guide physicians in the initial therapeutic decision and the management of ductal carcinoma in situ (DCIS).

Experimental Design: We examined autoantibody targets in 20 DCIS and 20 IBC patients using protein microarrays and identified humoral responses that can be used to distinguish the two groups. The five most differentially targeted antigens were selected to generate an autoantibody signature for the in situ to invasive breast cancer transition. This signature was next tested on 120 independent samples (61 DCIS and 59 IBC) using specific ELISA assays. The prognosis value of the autoantibody signature was finally evaluated in a cohort of DCIS patients followed for 5 years.

Results: A set of five autoantibody targets (RBP-Jk, HMGN1, PSRC1, CIRBP, and ECHDC1) with the highest differential signal intensity found in the protein microarrays experiment was used to establish an autoantibody signature of the DCIS to IBC transition. Using ELISA, this signature significantly discriminated DCIS from IBC [area under the ROC curve (AUC) = 0.794, 95% confidence interval (CI): 0.674–0.877]. Interestingly, our panel could highly distinguish low-grade DCIS from high-grade DCIS exhibiting an AUC of 0.749 (95% CI: 0.581–0.866). Finally, using a Kaplan–Meier analysis, the autoantibody signature could significantly divide the DCIS patients into a poor prognosis group and a good prognosis group (P = 0.01).

Conclusion: These results indicate the potential of autoantibody detection as a new prognostic test with possible clinical implications for the management of DCIS. Clin Cancer Res; 18(7): 1992–2000. ©2012 AACR.

Introduction

In contrast to the dramatic improvement in our ability to detect ductal carcinoma in situ (DCIS), our understanding of the pathophysiology of this disease and the factors involved in its progression to invasive breast carcinoma (IBC) is limited (1). Clinicohistologic features, such as a younger age, positive surgical margins, tumor size, comedo necrosis and, notably, tumor grade have consistently been correlated with DCIS recurrence. However, none of these parameters can accurately predict the risk of progression from DCIS to IBC (2). Tissue markers, including estrogen receptor, progesterone receptor, HER2/neu, Ki67, and COX-2 expression, as well as comprehensive molecular profiling of gene expression have also been evaluated as prognostic indicators of progression, but the results remain controversial (3). The serum markers currently used in clinical settings, including ACE and CA15-3, are mostly used to monitor treatment effectiveness in patients with metastatic breast cancer (4). Finally, as there are no available biomarkers that can effectively distinguish aggressive DCIS from indolent DCIS, selecting an appropriate initial treatment plan can be a challenge for physicians.

The presence of a humoral response against a number of intracellular and surface tumor-associated antigens (TAA) has been clearly established in cancer patients (5). Because this response appears months to years before the clinical diagnosis of the tumor, most of the current emphasis has focused on the use of tumor-associated autoantibodies as relevant diagnostic biomarkers with very promising results (6). However, autoantibody detection could also be an effective strategy for the early identification of highly aggressive lesions, thereby improving cancer outcomes. Few studies have focused on the prognostic significance of serum autoantibodies in metastatic cancer, in particular in non–small cell lung cancer patients (7), but none have...
investigated early-stage cancer. Therefore, we hypothesized that autoantibody detection could provide an effective approach to identify DCIS patients with a high risk of progression to IBC.

Recent advances in proteomics have unlocked novel avenues for the discovery of cancer-related biomarkers. Protein microarrays, such as ProtoArray, which contain more than 8,000 nonredundant proteins translated from genes randomly selected from throughout the human genome, are a powerful screening method for the identification of candidate autoantigens (8). The feasibility of this approach has been shown by the determination of diagnostic autoantibody profiles in a limited number of cancers, including colorectal, ovarian, and pancreatic cancer (9–11).

In this study, we describe significant and consistent differences in the level of autoantibodies targeting specific antigens in a population set of 40 patients with DCIS or early-stage IBC, as detected using protein microarrays. We then evaluated the performance of a humoral signature–based predictor in an independent cohort of 120 samples by ELISA, as a blood-screening signature for the in situ to invasive breast cancer transition determination. Given the noninvasive characteristics of serum sampling and the easy detection of autoantibodies, autoantibody signature may help to avoid the overtreatment of low-risk patients and dictate more intensive treatment of aggressive DCIS, thereby reducing the mortality of patients diagnosed at this very early step of the disease.

**Protein microarray experiments**

The ProtoArray microarrays (v4.0; Invitrogen) were used according to the manufacturer’s instructions. Each microarray contains 8,000 separate proteins printed in duplicate. Briefly, microarrays were blocked for 1 hour at 4°C with 50 mmol/L HEPES, 200 mmol/L NaCl, 0.08% Triton X-100, 25% glycerol, 20 mmol/L reduced glutathione, 1 mmol/L DTT, 40 mmol/L NaOH, and 1% bovine serum albumin (BSA) at pH 7.5. The microarrays were then incubated for 90 minutes at 4°C with the patient serum samples diluted 1:500 in 1× PBS, 1% BSA, and 0.1% Tween at pH 7.5 (PBST-1% BSA). The microarrays were washed 5 times for 5 minutes with PBST-1% BSA and incubated for 90 minutes at 4°C with goat Alexa Fluor 647–conjugated anti-human IgG diluted 1:2,000 (Invitrogen). The microarrays were washed again as described above, rinsed briefly in deionized water and dried by centrifugation. Microarray data were acquired using an Innoscan700 Fluorescent MicroArray scanner (Innopsys). Mapix software (v2.8.2; Innopsys) was used to grid the images of the scanned arrays and to automatically align images for spot detection and protein identification. Pixel intensities for each spot on the microarray were determined from the software and saved to a text and gpr (genepix) file. The quantitated spot files were processed to determine which proteins were recognized by the serum samples.

**Data analysis**

The goal of the microarray analysis was to identify specific antibodies that can be used to differentiate between DCIS and IBC. For each protein on the microarray, a Z-score was calculated on the basis of the signal from the protein spots on the microarray compared with all or local protein features. Because the Z-score, which is calculated from the global mean of all of the proteins signals, produces a bias that favors the selection of proteins with high protein amount values, we narrowed the mean, SD, and Z-score calculation to a local value. To reduce the influence of a few

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**Translational Relevance**

Although protein microarrays have been successfully used for the identification of autoantibodies in the serum of cancer patients, especially in early-stage cancer detection, the use of protein microarrays to identify autoantibodies correlated with invasion has not been reported. This study shows the clinical relevance of a combination of 5 antigens, identified by protein microarrays and validated in an independent cohort of 120 samples by ELISA, as a blood-screening signature for the in situ to invasive breast cancer transition determination. Given the noninvasive characteristics of serum sampling and the easy detection of autoantibodies, autoantibody signature may help to avoid the overtreatment of low-risk patients and dictate more intensive treatment of aggressive DCIS, thereby reducing the mortality of patients diagnosed at this very early step of the disease.
highly reactive spots on the local mean, we set the range of
calculations to 2,000 values (growing order values of pro-
tein amount). For each spot value, a local mean and a local
SD were calculated using 2,000 neighboring spots. The local
Z-score was calculated as (net signal value – local mean)/
local SD. For each protein duplicate, the average Z-score
and the CV from the net signal values of the duplicates were
calculated. The background cut-off value was also calculated
by adding the local mean and 3 times the local SD. How-
ever, even with these parameters, the influence of some high
values on the local cut-off calculation was strong (Supple-
mental Data S1). To overcome this issue, we considered
values over these local cut-off outliers, and we calculated a
second pass without considering these values for the local
mean calculation. This calculation allowed the generation
of a cut-off curve that better fit the distribution of the values
(Supplementary Data S1). The following criteria were used
to select a target for further analysis: a local Z-score differ-
ence greater than a 2-fold relative to the DCIS group, a CV
between duplicate values less than 0.5, and P < 0.05.

Autoantibody detection
Autoantibodies targeting RBP-Jκ, HMGN1, PSRC1,
ECHDC1, and CIRBP were detected as previously described,
with minor modifications (12). Briefly, MaxiSorp High
Protein-Binding plates (Nunc) were coated with 50 ng of
recombinant protein (RBP-Jκ, #H00003516; HMGN1,
#H00001153; PSRC1, #H00084722; CIRBP, #H00001153;
and ECHDC1, #H10055862 from Abnova Corporation) in
100 μL PBS at 4°C overnight. The plates were washed twice
with PBST and blocked with 50 mmol/L HEPES, 200 mmol/L
NaCl, 0.08% Triton X-100, 25% glycerol, 0.1 mmol/L DTT, 40
mmol/L NaOH, and 1% BSA at pH 7.5 for 2 hours at room
temperature. The plates were then washed twice and incu-
bated with the serum samples (diluted 1:50) for 2 hours at
room temperature. Appropriate control antibodies (goat
anti-RBP-Jκ; SC-8213 from Santa Cruz Biotechnology; rabbit
anti-HMGN1, #A302-363A from Bethyl Laboratories; mouse
anti-PSRC1, #H00084722-B01 from Abnova Corporation;
rabbit anti-ECHDC1, AV48841 from Sigma-Aldrich; and
rabbit anti-CIRBP, 10209-2-AP from Proteintech Group)
were also included. After 4 washes, the plates were incu-
bated with a horseradish peroxidase–conjugated polyclonal
antibody specific for human IgG (Jackson Immunoresearch) for
1 hour at room temperature with agitation. After 4 washes,
the plates were incubated with TMB substrate solution (Stress-
gen) for 15 minutes, and absorbance values were
determined at 450 nm after addition of H2SO4 to stop the
reaction. Each serum sample was assayed in triplicate.

Immunohistochemical staining
Tissue sections were treated for 10 minutes with 10% H2O2
to inhibit endogenous peroxidase activity and then
incubated in antigen retrieval solution (1 mmol/L EDTA,
ph 8.0) for 45 minutes at 95°C. The Vectastain ABC Kit
(Vector laboratories) was used for blocking and detection,
as recommended by the manufacturer. The sections were
incubated in diluted normal serum for 20 minutes to block
nonspecific binding and then in primary antibody over-
night at 4°C. The sections were then incubated with a
biotinylated secondary antibody for 30 minutes at room
temperature and then with the peroxidase-conjugated bio-
tin–avidin complex (Vectastain ABC Kit) for 30 minutes.
Finally, peroxidase activity was detected using the AEC
Staining Kit (Sigma-Aldrich). The sections were then coun-
tained with hematoxylin, except for RBP-Jκ slides in
which the nuclei were stained using Hoechst 33258 (Sigma-
Aldrich). Scores were obtained by estimating average signal
intensity (scale of 0–3) and the proportion of cells showing
a positive signal (0–100%). The intensity and proportion
scores were then multiplied to give an overall IHC score.

Statistical analysis
Data about the different seroreactivities were summa-
rized by median and range and compared using the
Wilcoxon test. Differences were considered statistically sig-
nificant when P < 0.05. Individual and combined autoan-
tibody performance from the ELISA data are based on
receiver operating characteristic (ROC) curves, which allow
the characterization of the discrimination between 2 well-
deﬁned populations. The generalized ROC criterion (13)
determines the best linear combination (virtual marker) of tumor
markers such that the area under the ROC curve (AUC) is
maximized. The accuracy, sensitivity, and specificity for
individual and combined antibody performance were evalu-
ated using the optimal threshold value calculated to max-
imize the Youden’s index. The performance of the 5 auto-
antibodies based predictor was also analyzed using SVM
analysis. To determine how accurately the learning algo-
rithm was able to predict data, a leave-one-out cross-
validation was used.

Relapse-free survival (RFS) was calculated from the date
of inclusion until local recurrence or last follow-up. Local
recurrence was defined as a locoregional relapse of DCIS
and/or the presence of IBC. Nonbreast cancer deaths were
considered as nonevents. Patients who were alive without
relapse or died without relapse were censored at the time of
last contact or the time of death, respectively. Patients who
were alive at the last follow-up were censored at the time of
last contact. The Kaplan–Meier method was used to esti-
mate RFS rates. The log-rank test was used to test the
differences between groups. Differences were considered
statistically significant at P < 0.05.

Statistical analyses were carried out using InStat (v3.06,
GraphPad), STATA 11.0 (StataCorp. 2009. Stata: Release
11) and mROC (14). SVM was implemented using Multi-
Experiment Viewer (Mev, version 4.6.2).

Results
Protein microarray–based detection of autoantibodies
in the DCIS and early-stage IBC populations
To identify the targets of autoantibodies present in the
serum of patients with breast cancer, serum samples from
40 age-matched breast cancer patients (DCIS, n = 20, and
early-stage IBC, n = 20) were profiled using protein
microarrays containing 8,000 human recombinant proteins spotted in duplicate. The clinicopathologic characteristics of patients of serum set 1 (TAAs identification set) are shown in Supplementary Data S2. All serum samples were tested as described in the Material and Methods, and the presence of autoantibodies bound to each protein spot was detected using fluorophore-conjugated anti-human IgG (Fig. 1A). As expected, all controls (Alexa Fluor 647–conjugated antibody, anti-human IgG antibody and human IgG) were highly reactive for all of the tested samples (Fig. 1A). Of the 8,000 proteins present on the microarray, 1,896 proteins were recognized by autoantibodies present in the serum. Of these, 480 reacted only with serum samples from DCIS patients, and 730 reacted only with serum samples from IBC patients. Using our selection criteria (a differential expression of at least 2-fold relative to the DCIS samples and \( P < 0.05 \)), 20 antigens elicited a significant different humoral response. The complete list of proteins defined by these criteria is shown in Supplementary Data S3, and the distribution and strength of the autoantibody seroreactivities that differentiate noninvasive breast cancer from invasive breast cancer are shown in Fig. 1B. On the basis of this initial microarray experiment, a set of 5 autoantibody targets with the highest differential signal intensity between the 2 groups was used to establish an autoantibody signature for the DCIS to IBC transition (Supplementary Data S3). The serum level of each 5 proteins was also quantified in the same population using ELISA. The results were similar with those obtained by microarray, and there were significant differences between DCIS and IBC groups for the 5 autoantibodies (Fig. 2B).

**Expression of RBP-Jκ, HMGN1, PSRC, CIRBP, and ECHDC1 in breast cancer tissue**

Because the presence of specific autoantibodies is often associated with overexpression of the corresponding autoantigens, we examined the expression levels of RBP-Jκ, HMGN1, PSRC, CIRBP, and ECHDC1 in breast tissue. IHC was done on 20 tissue samples with normal, DCIS, and early-stage IBC components. For each autoantigen, the same patients were used, thereby allowing a direct comparison of the results for each protein. Although the expression of HMGN1, PSRC, CIRBP, and ECHDC1 did not differ between the groups, RBP-Jκ, which was predominantly localized to the nucleus, was increased in the DCIS and IBC samples (score 2–3) compared with normal breast epithelium (score 0–1; Fig. 3).

**Evaluation of an autoantibody signature for the DCIS to early-stage IBC transition**

We next quantified the average levels of the autoantibodies targeting RBP-Jκ, HMGN1, PSRC, CIRBP, and ECHDC1 using ELISAs in 120 independent serum samples (DCIS, \( n = 59 \); and IBC, \( n = 61 \)). The clinicopathologic
characteristics of cancer patients of serum set 2 (TAAs evaluation set) are shown in Supplementary Data S2. We could not find significant difference in optical density signal for individual autoantibodies between DCIS and IBC populations (Fig. 2C). However, when the discrimination between the DCIS and IBC populations was maximized, for each autoantibody target, by calculating the optimal threshold from ROC curves using mROC multivariate analysis, individual autoantibody overall accuracies ranged from 53% to 60%, with individual sensitivities from 17% to 81%, and individual specificity from 32% to 93% (Supplementary Data S4). To assess the ability of the combined antibody measurements to differentiate between preinvasive and invasive tumors, the associations of 2 or more antigens were randomly permuted, the corresponding AUC was estimated, and the best linear combination was determined. The best antigen combination for classification performance included all 5 proteins with an overall AUC of 0.794 [95% confidence interval (CI): 0.674–0.877; Fig. 4A]. When the optimal threshold was defined for this combination, the overall classification accuracy of the autoantibody signature was 80.0%, with an overall sensitivity of 86.1% and an overall specificity of 75.0%, which are greatly improved compared with the performance of the individual autoantibodies (Table 1). We then used a SVM classification algorithm to confirm the robustness of our predictor. Interestingly, the overall classification accuracy was maintained at 77.5%, with an overall sensitivity of 83.3% and an overall specificity of 72.7% (Table 1). To further validate our results, a leave-one-out cross-validation algorithm was used with the same data used above. Interestingly, the accuracies were similar to the values calculated by the mROC criterion or the SVM algorithm, proving the robustness of the predictor (76.2 and 73.7, respectively).

Relationship between the autoantibody signature and the clinicohistologic features of DCIS progression

We first analyzed the correlation between both the 5 autoantibodies and the clinical, histologic, and biologic characteristics of both groups. We did not find a correlation with any of the parameters, except for the histotype grade of DCIS. Indeed, a significant correlation between the histotype grade (low grade vs. high grade) and the RBP-Jκ-specific autoantibody level was found for the DCIS specimens (P < 0.006). We therefore optimized the threshold value of the autoantibody panel to better distinguish low-grade DCIS (grade 1 and 2) from high-grade DCIS (grade 3) using mROC analysis. The autoantibody panel exhibited an AUC of 0.749 (95% CI: 0.581–0.866; Fig. 4B, Supplementary Data S4). To optimize the classification performance of our 5-autoantibody signature, we determined a new threshold using mROC and observed an overall accuracy, sensitivity, and specificity of 73.0%, 69.6%, and 81.0%, respectively (Table 1). Interestingly, using the SVM classification algorithm, the overall performance of our predictor was maintained (Table 1).

Autoantibody signature and local recurrence in the DCIS population

To address whether our autoantibody signature can accurately predict clinical outcome, we conducted a Kaplan–Meier analysis comparing local recurrence and absence of recurrence in a population of DCIS patients followed for 5
years (Fig. 5). The local recurrence was 9.1% with 4 recurrences; 1 recurrence occurred in the second year, 1 in the third year, and 2 in the fourth and fifth years. No deaths were observed from the date of inclusion until last follow-up, and consequently, no patient was censored for this reason in our analysis. Importantly, the autoantibody signature could be used to divide the DCIS patients into a poor-prognosis group (local recurrence) and a good-prognosis group (recurrence free; predictor cut-off 0.0756; and \( P = 0.011, \) Log-rank test).

**Discussion**

A major challenge in human DCIS research has been the identification of markers associated with the transition from DCIS to invasive breast carcinoma. Progress toward achieving this goal could be enhanced by using advanced microarray techniques to measure the humoral response against the cancer. In this study, we successfully used protein microarray technology to identify an autoantibody signature in patients with preinvasive and early invasive breast cancer. In doing so, we identified a subset of 5 autoantibodies that can accurately distinguish between the groups. These data suggest that autoantibody detection could serve as a new prognostic test.

Surprisingly, the autoantigens identified in this study were different from previously identified DCIS-associated TAAs (12, 15–17). Substantial differences exist between protein microarrays and other techniques used to identify TAAs. The ProtoArray protein microarrays contain proteins that are expressed in insect cells and, therefore, contain proteins expected to have undergone physiologic posttranslational modifications. Moreover, all of the proteins are purified under native conditions, and the immobilized proteins are expected to maintain their native conformation. All of these technical aspects could increase the likelihood of identifying new breast cancer–specific TAAs. In addition, to identify specific proteins that can be used to differentiate our populations, we optimized the data analysis and, notably, the calculations for threshold determination. To reduce the influence of a few highly reactive spots on a specific spot value, we determined a local cut-off value using the 2,000 neighboring spots rather than a global cut-off. This local threshold was then corrected in a second pass to better fit with the distribution of values by removing specific local high values for the local cut-off calculation. The comparison of the seroreactivity, achieved from the local Z-score calculation, allowed us to select a larger number of relevant target proteins.

Most of the functions of the TAAs identified in this study, and the 5 proteins that were included in our predictor in particular, are associated with cancer invasion. The mechanism by which immunogenicity appears remains unknown. A prerequisite for an immune response against a cellular protein is its presentation as an antigen. It is not clear why only a subset of patients with a specific tumor type develop a humoral response to a particular antigen. One of the first explanations for this humoral response is that antigens are encoded by overexpressed genes in tumors. We did not observe any change in HMGN1, PSRC1, CIRBP, and ECHDC1 expression in the malignant breast tissue, suggesting that other mechanisms may be involved during breast cancer carcinogenesis. Among these mechanisms, those resulting in modification of the processing, the function, or the routing of antigens could be proposed, as observed for previously identified autoantigens (18–21). Further studies will be needed to investigate more specifically the origin of the humoral response for these 4 autoantibodies. However, we found that RBP-Jk expression is upregulated in the nucleus of DCIS and early-stage IBC tumor cells relative to normal epithelium. RBP-Jk is a transcriptional repressor that regulates Notch target genes.

![Figure 3.](image-url)
The Notch signaling pathway is a highly conserved cell-cell signaling pathway that regulates cell proliferation, differentiation, and apoptosis and stem cell maintenance (23). The increase observed in our study is consistent with Notch1 expression studies that detected Notch1 activation very early in breast cancer progression, specifically in pre-invasive lesions and early-stage IBC, but not in normal breast tissue (24). In addition, the overexpression of Notch1 is sufficient to transform normal breast epithelial cells (25) and is significantly correlated with poor survival of patients with IBC (26) and DCIS (24). We also provide evidence of an apparent link between RBP-J and the DCIS histologic grade. These results are important because patients with high-grade DCIS are believed to be at high risk of developing invasive and aggressive tumors (27). Altogether, these results indicate that the identification of autoantigens provides a powerful approach to identify proteins associated with cancer progression. Although the direct or indirect role of these proteins in DCIS transformation and progression remains to be established, they may be relevant candidate targets for understanding the mechanism of breast cancer progression.

Notch1 expression studies that detected Notch1 activation very early in breast cancer progression, specifically in pre-invasive lesions and early-stage IBC, but not in normal breast tissue (24). In addition, the overexpression of Notch1 is sufficient to transform normal breast epithelial cells (25) and is significantly correlated with poor survival of patients with IBC (26) and DCIS (24). We also provide evidence of an apparent link between RBP-J and the DCIS histologic grade. These results are important because patients with high-grade DCIS are believed to be at high risk of developing invasive and aggressive tumors (27). Altogether, these results indicate that the identification of autoantigens provides a powerful approach to identify proteins associated with cancer progression. Although the direct or indirect role of these proteins in DCIS transformation and progression remains to be established, they may be relevant candidate targets for understanding the mechanism of breast cancer progression.

In situ carcinomas are clinically complex. To date, it is not possible to discriminate with absolute certainty the lesions that will recur or progress to invasive carcinoma from those that will remain quiescent. The treatment guidelines for DCIS have changed over the last 2 decades, and most patients are now treated with breast-conserving surgery. However, 16% to 22% of women experience local relapse within 10 years following lumpectomy alone, and

![Figure 4. ROC curve analysis of the 5 autoantibodies–based predictor. Value obtained when discriminating DCIS patients from IBC patients (A) and low-grade DCIS patients from high-grade DCIS patients (B).](image)

![Figure 5. Kaplan–Meier curves for RFS stratifying DCIS patients by autoantibody predictor (n = 44; P = 0.011, Log-rank test). With a prediction cut-off of 0.0756, the DCIS patients could be split into 2 groups on the basis of their autoantibody signature: a poor prognosis group with high risk of recurrence (black line) and a good prognosis group with low risk of recurrence (grey line). Censored patients are indicated by vertical bars.](image)

| Table 1. Performance of the predictor in the validation set using AUC linear combination and SVM |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Statistic model                  | DCIS vs. IBC                     | Low-grade DCIS vs. high-grade DCIS |
| AUC linear combination           | Accuracy (%) | Sensitivity (%) | Specificity (%) | Accuracy (%) | Sensitivity (%) | Specificity (%) |
| AUC linear combination           | 80.0          | 86.1           | 75.0             | 73.0          | 69.6           | 81.0             |
| SVM classification               | 77.5          | 83.3           | 72.7             | 75.0          | 65.2           | 85.7             |

![Figure 6. Kaplan–Meier curves for RFS stratifying DCIS patients by autoantibody predictor (n = 44; P = 0.011, Log-rank test). With a prediction cut-off of 0.0756, the DCIS patients could be split into 2 groups on the basis of their autoantibody signature: a poor prognosis group with high risk of recurrence (black line) and a good prognosis group with low risk of recurrence (grey line). Censored patients are indicated by vertical bars.](image)
approximately half of these relapses are invasive breast cancer (28). Adjuvant breast irradiation has been proposed as an additional therapy and results in a 50% decrease in the number of ipsilateral breast recurrences (29, 30). However, it is unlikely that all patients with DCIS require radiation following breast-conserving therapy. Conventional prognostic clinicopathologic factors, such as a young patient age, a high nuclear grade, comedo necrosis, a large tumor size, and involved excision margins have been reported as factors associated with a high risk of local recurrence. However, the reported effect of these factors on the risk of local recurrence has varied (2, 31). Biologic markers such as estrogen receptor, progesterone receptor, HER2/neu, or Ki67 expression have also been tested, but no clear results have emerged. Several combinations of risk factors for local recurrence, including the St. Gallen criteria (32, 33), gene expression signatures (34), the Van Nuys Prognostic Index (VNPI), and its more recent variation, including age [University of Southern California (USC)-VNPI; ref. 35], have been assessed, but no consensus has been made. Together, these studies have shown that advances are needed, such as the identification of new markers of breast cancer invasion, to improve decision making following resection of DCIS. Therefore, we propose that our predictor could help predict those DCIS patients with a higher risk of invasion. However, the potential pitfall in our analysis is the low frequency of local recurrence in our followed DCIS population. Although the rate of local recurrence observed in our study corresponds to the predicted percentage in this surgically and radiation-treated population, this low frequency clearly limits statistical evaluations. Therefore, our preliminary results about RFS must be confirmed in a larger cohort of patients before this autoantibody signature can be considered a relevant prognostic feature of DCIS progression.

This study provides the first integrated analysis of serum autoantibody expression profiling in patients with preinvasive or invasive breast cancer, and novel candidate markers associated with an increased risk of preinvasive breast cancer recurrence have been identified. By carrying out several statistical analyses, we showed that an autoantibody signature based on the presence of several autoantibodies can accurately predict local recurrence in DCIS. Given the noninvasive characteristics of serum sampling and the easy detection of autoantibodies, we envision that an autoantibody signature may help to avoid the overtreatment of low-risk patients and dictate more intensive treatment of aggressive DCIS, thereby reducing the mortality of patients diagnosed at this very early step of the disease. Finally, because nipple aspirate fluid and ductal lavage represent a relevant source of immunoglobulins (36) and biomarkers specifically related to breast cancer (37), this signature could also be evaluated in breast fluids. Also, studies that link these 5 immunogenic proteins to the in situ to invasive breast carcinoma transition may provide key insights into the molecular mechanisms that drive early breast cancer tumorigenesis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
The authors thank E. Laurenceau for helpful discussions and for review of the manuscript and J.P. Mols for technical help with the immunohistology experiments.

Grant Support
This work was supported by grants of Ligue Contre le Cancer and a grant from the GEFLUC Languedoc-Roussillon.

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Received September 30, 2011, revised January 20, 2012; accepted January 23, 2012; published OnlineFirst February 9, 2012.

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