Ca^{2+}-binding protein S100A11: A novel diagnostic marker for breast carcinoma

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Abstract. To evaluate whether S100A11 could be considered to be a novel diagnostic marker in breast carcinoma, the method of differential proteomics, Western blotting, and immunohistochemistry were used to detect the expression pattern and subcellular localization of S100A11. Statistical analyses indicated that specific up-regulated of A100A11 did not correlate with other prognostic factors such as age, tumor size, grade and stage, ER, PR, HER-2 and nodal status. Our data support that S100A11 is a novel diagnostic marker in breast carcinoma. Analysis of S100A11 expression in breast cancer may be an effective tool help in detection of early-stage breast cancer.

Introduction

Breast carcinoma is one of the most malignant diseases and causes death in women worldwide, although it is highly curable if diagnosed at an early stage (1). Therefore, to improve the prognosis of patients with breast cancer, novel diagnostic biomarkers for early detection of the disease are needed (2). Over the last several years, despite the fact that a large number of molecules have been proposed to be valuable as prognostic or predictive factors, the National Institute of Health Consensus Development Conference has continually stressed the need for validation and appropriate quality control for most of the markers studied to date (3).

The S100 family of proteins consists of small Ca^{2+}-binding proteins of the EF-hand type, which have been implicated in the regulation of a variety of intracellular and extracellular processes (4-6). Recently, it has been implicated that the function of S100 family proteins is related to the development of metastases in several cancer types, and therefore this family of proteins has emerged as potentially useful diagnostic and prognostic biomarkers (7,8). In breast carcinoma, several members of the S100 gene family, such as S100A2 and S100A4, have been associated with cancer progression and are therefore suggested to be potential prognostic markers (9,10).

Another member of the S100 family, S100A11/S100C, was originally discovered as a homolog of rabbit calgizzarin in a cloning study of colorectal cancer cell lines (11). In detailed functional studies, S100A11 was shown to increase transcription of p21CIP1/WAF1 and was suggested to be a negative regulator of cell growth. Consequently, S100A11 is now considered a strong tumor suppressor gene candidate (12-14). To test whether S100A11 has the potential to be a novel diagnostic biomarker, expression studies of the molecule have been performed in multiple human cancers such as gastric cancer (15), prostate cancer (16), esophageal carcinoma (17), pancreatic cancer (18,19), uterine leiomyoma (20) and colorectal cancer (21). These results have shown that the progression of cancer is related to high level expression of S100A11 in the tumor tissue, suggesting that S100A11 may be a novel diagnostic marker.

However, these studies also created controversy regarding the expression pattern, subcellular localization and functions of S100A11 in breast tumors. The first report to demonstrate the expression patterns of S100A11 in breast carcinoma was by Kondo *et al* in 2002 (22). They found that the expression of S100A11 is decreased in tumor cells and that there is cytoplasmic staining in normal tissues. This result was very

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Abbreviations: IDCA, infiltrating ductal breast carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2/neu ErbB2, human epidermal growth factor receptor-2; 2-DE, two-dimensional gel electrophoresis; IHC, immunohistochemistry; EGFR, epidermal growth factor receptor

Key words: S100A11, breast carcinoma, tumor marker, differential proteomics

similar to the expression of S100A2. The authors therefore speculated that like S100A2, with whom it shares a similar structure (~50% identity at the amino acid level), S100A11 functions as a growth inhibitor. Additionally, Cross *et al* (23) surveyed the expression of members of the S100 family of proteins in normal human tissues and common cancers. They obtained different results regarding the expression pattern of S100A11 in breast carcinoma. In their study, S100A11 displayed increased expression in breast carcinoma tumors, and the subcellular localization of S100A11 changed from a strictly nuclear-localization in normal tissues to a more cytoplasmic-localization in tumor tissues. They speculated that this movement correlates with the probable function of S100A11. In normal tissue, S100A11 can translocate to the nucleus, which leads to increased transcription of negative regulators of cell growth. In breast carcinomas, the loss of nuclear translocation may lead to an inability to control or suppress cell growth.

Published reports on S100A11 in human breast carcinoma are sparse, and therefore it is difficult to develop a clear understanding of the expression pattern and function of S100A11 in breast carcinoma. Further experimentation is therefore necessary to confirm the expression pattern and function of S100A11, and to further evaluate whether S100A11 could serve as a new diagnostic marker in breast carcinoma.

In this study, two-dimensional gel electrophoresis (2-DE) was used to identify the deferential proteome profiles between five subtypes of IDCA (infiltrating ductal breast carcinoma) and their corresponding adjacent normal tissue. To exclude possible inter-sample variations, the resulting five differential profiles were further compared, which led to the identification of three proteins. Expression of these proteins was at least 3-fold up-regulated in the tumor tissues. Among these up-regulated proteins, one was identified as calgizzarin (S100A11; S100C) by MALDI-TOF/TOF analysis. Western blot analysis was used to further validate the results from the 2-DE analysis. Furthermore, to explore subcellular localization of S100A11 and to evaluate whether S100A11 could be a novel diagnostic marker in breast carcinoma, we performed immunohistochemistry on the tumor tissue of 50 breast cancer patients and on breast adenosis tissue of 13 patients.

Materials and methods

Patients and sample collection. Breast tumors histologically classified by a licensed pathologist as invasive breast carcinoma of ductal origin were obtained with written consent during 2007 and 2008, from female patients who underwent partial breast resection or radical mastectomy at the Department of Breast Surgery, First Hospital of Jilin University. The size of the tumors ranged from 1.2 to 6.2 cm, tumor stages ranged from stage I to III and age of the donors at diagnosis ranged from 32 to 74 years. All specimens with known histological stage were used in this study. For differential proteomic analysis, the samples were from patients between 51 and 58 years of age. All of the corresponding matched sets used in this comparative proteomic study were obtained from the tissue that was located ~3 cm away from the tumors. This tissue was histologically normal breast tissue, without signs of malignant transformation of the ductal epithelium. Tissues were rinsed thoroughly in phosphate-buffered saline (PBS; pH7.4), and 1 mm sections were shaved off. The tissues were then placed in sterile 1.5 ml microcentrifuge tubes, snap-frozen within 10 min after harvesting, and stored at -80˚C until use. For immunohistochemical analysis, 50 tumor tissues and 26 corresponding adjacent normal tissues of patients with invasive ductal breast carcinoma were selected. Moreover, 13 cases of breast adenosis tissue were selected in our study as controls. Samples were routinely fixed in 10% neutral formalin and embedded in paraffin. The clinicopathological characteristics of the tissues are shown in Table III.

Sample preparation for 2-DE. Frozen tissues of breast tumors were powdered under liquid nitrogen, suspended and homogenized in 500 μl of 2-DE lysis buffer containing 8 M urea, 40 mM Tris, 100 mM DTT, 4% w/v CHAPS, 2% v/v IPG buffer (pH 4-7) and a complete protease inhibitor cocktail. After sonication, complete solubilization of the proteins was performed for 1 h at room temperature (RT). The homogenate was centrifuged at 14000 x g for 30 min at 4˚C to precipitate cell debris, and the supernatant was transferred to a sterile microcentrifuge tube. Then, the sample was treated by acetone/methanol delipidation with the standard process of Hopkinson *et al* (36). The protein concentration was determined using the Bradford method (37) prior to electrophoretic analysis. All samples were stored at -80˚C until use.

Differential 2-DE analysis and image analysis. 2-DE was performed as previously described by Xi *et al* (38). The sample was diluted with rehydration buffer (RB) (8 M urea, 4% CHAPS, 20 mM DTT and 0.5% v/v IPG-buffer (Amersham Biosciences, Sweden) to a concentration of 0.7 μg/μl in a final volume of 400 μl, and an IPG strip of 18 cm (pH 4-7) (Amersham Biosciences) was rehydrated for 16 h to allow proteins to be taken up. Isoelectric focusing (IEF) was performed using the Investigator-5000 (Genomic Solutions, USA) at 20˚C with a current limit of 50 μA per strip. The total focusing time was 46000 Vh in 12.5 h. The strips were equilibrated by two steps: i) 15 min in a 10 ml solution containing 6 M urea, 30% (w/v) glycerol, 2% SDS, 0.002% bromophenol blue, 50 mM Tris, pH 8.8; and ii) 15 min in a similar solution containing 4.5% iodoacetamide rather than DTT. After equilibration, the second dimension electrophoresis was performed in an ETTAN Dalt 6 (Amersham Biosciences) using 12.5% SDS-PAGE. After 2-DE separation, the proteins on the gel were visualized by silver staining as described previously by Yan *et al* (39). For preparative 2-DE, 1 mg of total proteins were separated as described above. Proteins were detected by Coomassie Brilliant Blue staining method compatible with MS analysis.

After 2-DE, the gel profiles of protein spots were scanned using an ImageScanner (Amersham Biosciences) followed by the image analysis in the Image Master-2D Platinum software (Amersham Biosciences). By utilizing the software according to the manufacturer's instructions, individual protein spots of normal and malignant tumor were quantified and matched, and the individual spot volume values were calculated.

Table I. Proteins identified in solid tumor tissue and corresponding adjacent normal breast tissue samples.

Case no.	Subtype	Total no.		Correspondent spots[c]	Changes 3-folds no.	Up-regulated no.	Down-regulated no.	Correspondence rated (%)[d]
		N[a]	C[b]					
1	($ER^+/PR^+/HER\text{-}2^+$)	1283	1453	771	108	76	32	60.1
2	($ER^-/PR^+/HER\text{-}2^+$)	916	1168	310	92	60	32	33.8
3	($ER^-/PR^+/HER\text{-}2^-$)	1328	1260	774	124	69	55	58.3
4	($ER^-/PR^-/HER\text{-}2^+$)	884	998	573	112	49	63	64.8
5	($ER^-/PR^-/HER\text{-}2^-$)	779	991	358	101	42	59	46.0

[a]The number of protein spots detected in the whole protein gels of normal breast tissue. [b]The number of protein spots detected in the whole protein gels of breast carcinoma tissue. [c]Corresponding protein spots were identified by comparing the gels of breast carcinoma tissue and the gels of normal breast tissue, which was taken as the reference gel. [d]Correspondence efficiency of protein spots in all pairs of gels.

Identification of proteins using mass spectrometry. Protein spots of interest were excised from gels and in-gel digested with modified sequencing grade trypsin (Promega). Peptides were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) peptide mass fingerprint analysis on a Bruker-Daltonics AutoFlex TOF-TOF LIFT (Bruker) mass spectrometer. The peptide mass fingerprints and protein searches were performed against the National Center for Biotechnology Information (NCBI) non-redundant human protein database by using the MASCOT software (http://www.matrixscience.com).

Western blotting. The proteins separated by 2-DE and SDS-PAGE were electrotransferred onto nitrocellulose (NC) membranes (Millipore) according to the standard protocol. After blocking with TBS containing 5% milk powder and 0.1% Tween-20 for 1 h at RT, the membranes were incubated with the anti-S100A11 antibody (ProteinTech Group, Inc.) at the concentration ratio of 1:1000 for 1 h at RT followed by treatment with horseradish peroxidase-conjugated secondary antibody for 1 h at RT. Target proteins were detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech). Each sample was again examined by SDS-PAGE and Western blot analysis. GAPDH was used as an internal positive control.

Immunohistochemistry. Immunohistochemical staining was carried out to validate the differentially expressed proteins in human spontaneous metastatic breast carcinoma. Paraffin blocks were cut into 4-μm-thick sections. Then, the sections were deparaffinized in xylene and rehydrated by alcohol with a concentration gradient. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in 50% methanol for 30 min at RT. The sections were washed with TBS, and then non-specific binding was blocked by preincubation with blocking solution for 30 min at RT. Next, a rabbit anti-human S100A11 antibody (ProteinTech Group, Inc.; diluted at 1:1000) made up in TBS was applied and the mixture was incubated for 1 h at RT. After incubation, the sections were rinsed with TBST and incubated with secondary biotinylated goat anti-mouse IgG for 1 h at RT. The slides were then incubated with chromogen (DAB) for 5-10 min at RT and washed with distilled water. Finally, sections were slightly counterstained with hematoxylin for 1 min followed by dehydration and coverslip mounting. TBS was used in place of the primary antibodies for the negative control.

Scoring of immunoreactivity and statistical analysis. To quantify the expression of S100A11, the number of positive immunostained cells from 300-400 cells in three randomly chosen fields of each tumor and adjacent normal tissue were counted. We then calculated the mean percentage of positive cells and the labeling intensity was also calculated. Considering the preciseness of the experiment, a stringent standard was established to determine the positivity and negativity of samples. Score for intensity labeling was defined as follows: no staining, 1; light yellow, 1; brown-yellow, 2; deep brown, 3. The score for the proportion of positive cells in the total cells was defined as follows: <5% positive cells, 0; 5-25% positive cells, 1; 26-50%, 2; 51-75%, 3; and >75%, 4. The total score was multiplied by two scores: <4 points mean a negative expression (-), 4-8 points mean a weak positive (+) and >8 points mean a strong positive (++). All of the data were generated from independent observations by three of the authors (X.L., W.L. and X.Z.). Differences were resolved by joint examination of the slides, and the final values were used in all statistical analyses. Associations between categorical variables were assessed by χ^2 tests. All the analyses were performed by SPSS software (Version 13.0), and a P-value of <0.05 was set as statistical significance.

Results

2-D gel electrophoresis of IDCA tissue and adjacent normal tissue. Tumors and corresponding adjacent normal tissue from 15 patients, which were divided into 5 comparison groups according to different immunophenotyping ($ER^+/PR^+/HER\text{-}2^+$; $ER^-/PR^-/HER\text{-}2^-$; $ER^-/PR^+/HER\text{-}2^-$; $ER^-/PR^-/HER\text{-}2^+$; $ER^-/PR^+/HER\text{-}2^+$), were used in this study for total protein extraction. In each comparison group, three samples of the same subtype were pooled to eliminate experimental error in the 2-DE experiment. Each subpool contained normalized equal amounts of protein from three tumors or normal tissues. Five pairs of 2-DE maps are displayed, and each pair of 2-DE maps includes a protein profile of breast carcinoma and a protein profile of adjacent normal tissue (Table I). A pair of

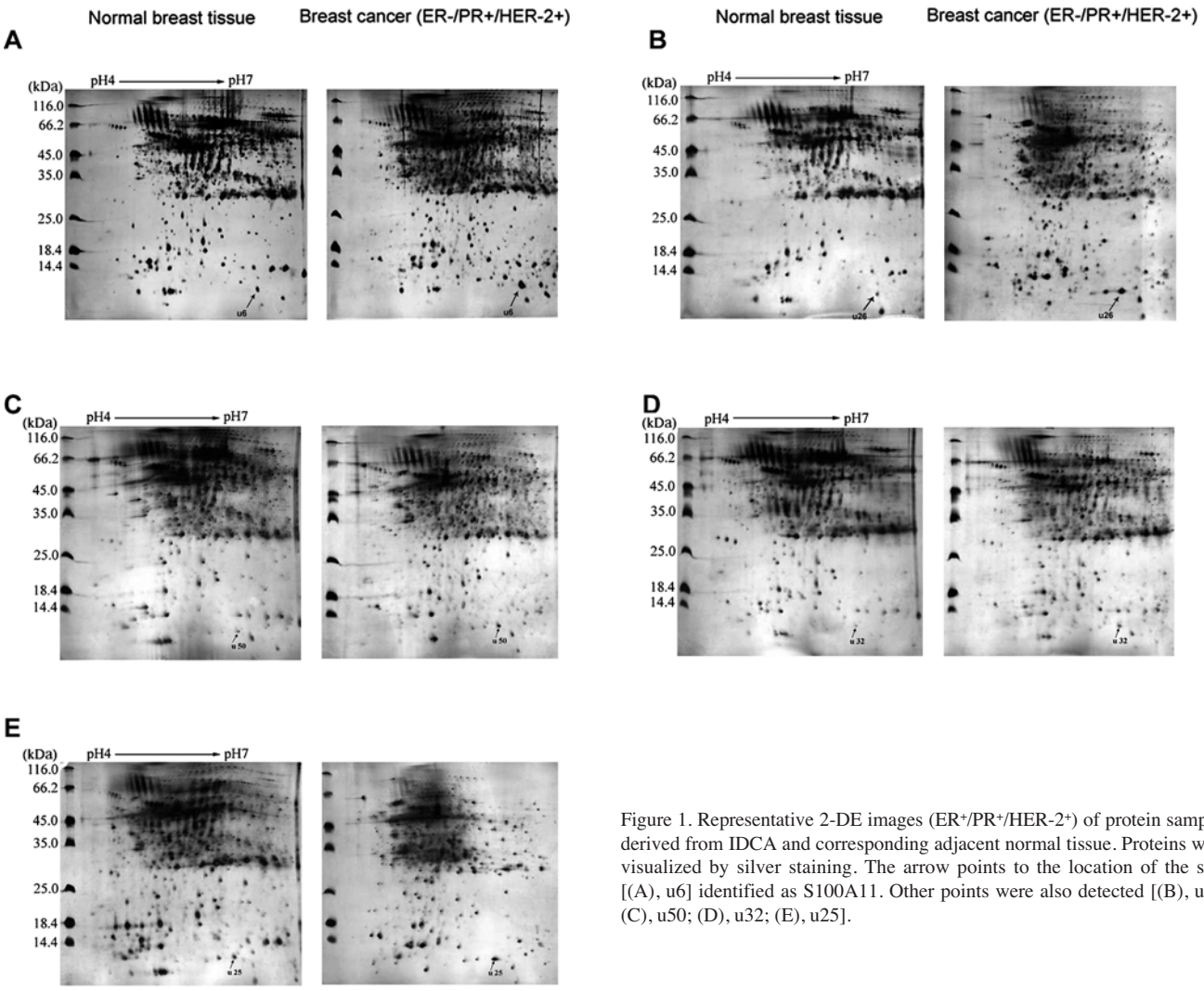

Figure 1. Representative 2-DE images ($ER^+/PR^+/HER\text{-}2^+$) of protein samples derived from IDCA and corresponding adjacent normal tissue. Proteins were visualized by silver staining. The arrow points to the location of the spot [(A), u6] identified as S100A11. Other points were also detected [(B), u26; (C), u50; (D), u32; (E), u25].

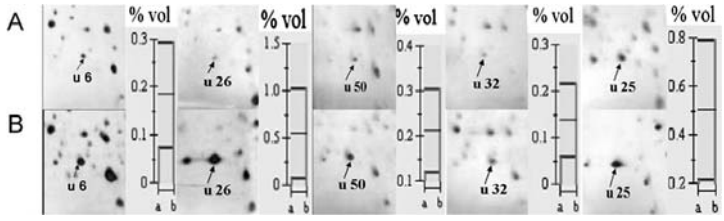

Figure 2. Comparison of the expression of S100A11 between IDCA (B) and corresponding adjacent normal tissue (A). The arrow points to the location of the spots representing S100A11 and proteins that were up-regulated (>3-fold) in all of the comparison groups under stringent selection conditions. Visualization is by silver staining. The letter u before the number means 'up-regulated' and the number is the corresponding marker in each group of differential comparisons between cancer and adjacent normal tissue.

representative proteome maps derived from breast carcinoma and corresponding adjacent normal tissue is displayed in Fig. 1.

Identification of differentially expressed proteins by differential comparison. In this study, a special method of differential comparison was used to investigate the natural variation in the protein profiles among breast carcinomas and adjacent normal tissues in different subtypes of IDCA. At first, the 2-DE maps of each pair were compared to identify the differentially expressed protein spots with a 3-fold difference or more. As shown in Table I, a total of 537 differential proteins spots were found in all the five pairs, among which 296 spots were up-regulated and 241 spots were down-regulated. Then, these differential protein spots were inter-compared among the five groups. Under stringent selection conditions, only three spots shared a common expression pattern in all the groups. One of the spots (u6) (Fig. 1) was picked up from preparative gels stained by Coomassie Brilliant Blue and was identified as S100A11 by MALDI-TOF/TOF (Fig. 3). The apparent molecular weight and isoelectric point of the protein were in agreement with the theoretical data of S100A11 at ~11.8 and 6.56 kDa, respectively. Fig. 2 shows that the expression of S100A11

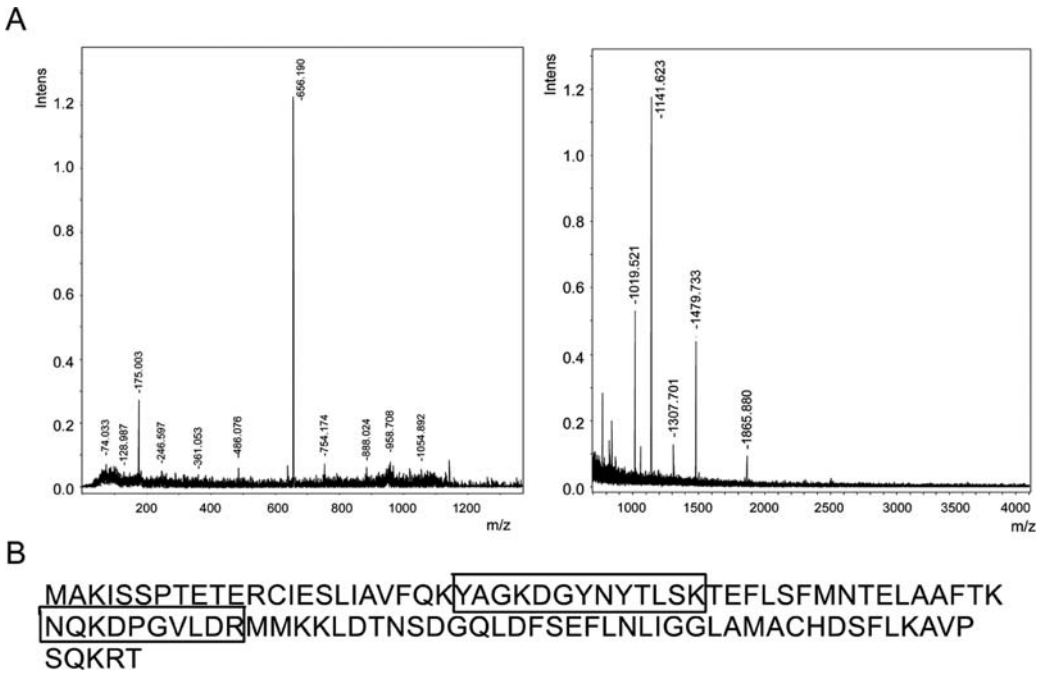

Figure 3. S100A11 protein identified by MALDI-TOF/TOF. (A) A full scan MS-MS spectrum of protein S100A11. (B) Matched peptides are shown in panes (sequence coverage, 21%).

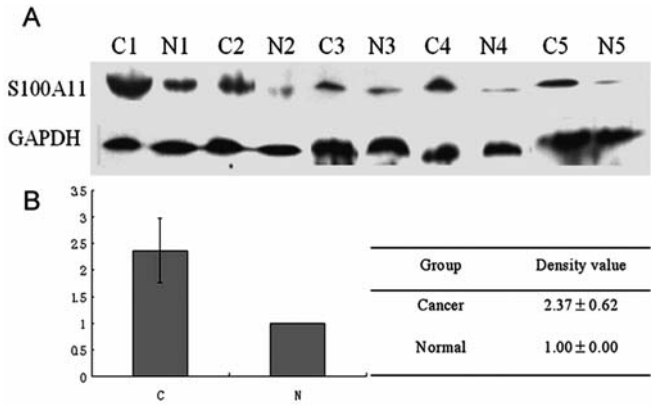

Figure 4. Western blot analysis confirmed the expression of S100A11 in breast tumor tissues and adjacent normal tissues. The letter C means 'group of cancer tissue' and the letter N means 'group of normal tissue'. Numbers 1-5 correspond to different subtypes (Table I). (A) Western blot for human tissues. Comparison between breast carcinoma and adjacent normal tissue. (B) Integrated density value (IDV).

was significantly higher (>3-fold) in tumors as compared to normal tissues.

Western blot analysis for verification of S100A11. Western blot analysis with a specific antibody for S100A11 was used to analyze breast carcinoma and adjacent normal tissues for expression of S100A11 (GADPH was used as an internal control). Fig. 4 shows a group of representative Western blot experiments. The S100A11 protein was predominantly expressed in tumor tissues as compared to corresponding normal tissues. These results confirmed the up-regulation of S100A11 in different subtypes of IDCA.

Further confirmation of S100A11 overexpression by immunohistochemistry. To further confirm the results from 2-DE and Western blot, 26 pairs of cancer and adjacent normal tissue and another 24 breast tumors and 13 breast adenosis tissues were tested by immunohistochemical staining. S100A11 was expressed in 72% of breast cancer tissues and in 42.31% of adjacent normal tissues (Table II). The results in Fig. 5A and B show that predominant S100A11 expression was observed in the cytoplasm of cells. The expression of S100A11 increased significantly in cancer tissues. Moreover, in breast adenosis tissues a predominant cytoplasmic staining was also observed (Fig. 5C). In conclusion, S100A11 is predominantly expressed in the cytoplasm; there were no cases with a predominant nuclear staining pattern.

Relationship between S100A11 and other prognostic factors. The clinicopathological features of the 50 women were all of invasive ductal breast carcinoma with no special type (Table II). Among these patients, the average age was 50 years and the median tumor size was 3.2 cm. Univariate analysis was performed using χ^2 tests, and the results show that the expression of S100A11 was significantly higher (χ^2=6.391, P<0.05) in breast cancer than in adjacent normal tissues (Table II). The multivariate analysis of the relationship between S100A11 expression and age, tumor size, grade and stage, ER, PR, HER-2 and nodal status was also performed by χ^2 analysis. Table III shows that the increased expression of S100A11 did not correlate with other prognostic factors in this study.

Discussion

Reports regarding the expression and the subcellular localization of S100A11 have been controversial (22,23). In this

Table II. Expression of S100A11 in breast cancer and corresponding adjacent normal tissue.

		Expression of S100A11 n (%)			
	Case	Positive	Negative	χ^2	P-value
Tumor tissue	50	36 (72.00)	14 (28.00)	6.391	0.011
Adjacent normal tissue	26	11 (42.31)	15 (57.69)		

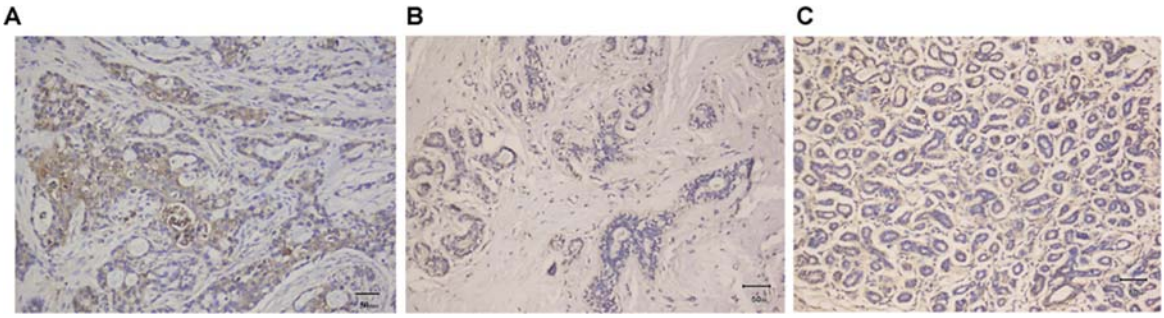

Figure 5. Representative images of S100A11 immunostaining in breast tissues. Scale bar, 50 μm. (A) S100A11 positivity in breast tumor. The staining pattern is predominantly cytoplasmic (magnification, x200). (B) S100A11 positivity in corresponding adjacent normal tissue. The staining pattern is predominantly cytoplasmic (magnification, x200). (C) S100A11 positivity in breast adenosis tissue. The staining pattern is predominantly cytoplasmic (magnification, x200).

Table III. The relationship between the expression of S100A11 and clinical parameters [n (%)].

Clinical parameters	Case	S100A11 n (%) Positive	Negative	P-value
Age				
<50	30	20 (66.67)	10 (33.33)	0.304
≥50	20	16 (80.00)	4 (20.00)	
Size (cm)				
<5	43	31 (72.09)	12 (27.91)	1.000
≥5	7	5 (71.43)	2 (28.57)	
TNM stages				
I, II	40	28 (70.00)	12 (30.00)	0.813
III	10	8 (80.00)	2 (20.00)	
ER				
+	25	17 (68.00)	8 (32.00)	0.529
-	25	19 (76.00)	6 (24.00)	
PR				
+	41	30 (73.17)	11 (26.83)	1.000
-	9	6 (66.67)	3 (33.33)	
HER-2				
+	17	15 (88.23)	2 (11.77)	0.133
-	33	21 (63.64)	12 (36.36)	
Lymph node metastasis				
Negative	26	17 (65.38)	9 (34.62)	0.278
Positive	24	19 (79.17)	5 (20.83)	
Grade				
Pre-menopausal	29	21 (72.41)	8 (27.59)	0.939
Post-menopausal	21	15 (71.43)	6 (28.57)	

study, S100A11 displayed increased expression in breast carcinoma, and this result is consistent with the report of Cross *et al* (23), although different results were reported by Kondo *et al* (22). As far as the subcellular localization of S100A11 is concerned, our study and all other reports have demonstrated a clear, predominantly cytoplasmic expression of S100A11. Interestingly, in normal breast tissue, our results suggest that S100A11 is also expressed predominantly in the cytoplasm, which is identical to the results of the study from Kondo *et al* (22), but different from Cross *et al* (23).

There are some potential reasons that might lead to such disparate results. Among these are differences in methods used in the study, number of tests, and sample selection. The immunohistochemistry analysis applied in both of the studies of Kondo *et al* (22) and Cross *et al* (23) was a semi-quantitative method and tissue sampling, fixation and staining of samples, antibody dilution, the specificity of the antibody, and even the microscope settings may have had a significant impact on the quantitative analysis (24,25). In this study, we applied the method of differential proteomics based on IEF and two-dimensional (2-D) gel electrophoresis. By displaying the protein profiles from different tissues and quantitatively analyzing the abundance of the differentially expressed protein spots, the expression level of proteins could be determined directly on the gel. In comparison to the semi-quantitative method of immunohistochemistry, this method is more objective and quantitative. In its simplest and most widely used form, it has been successfully applied to explore the expression level of proteins between cancerous and normal tissues and to identify numerous candidate biomarkers (26,27).

Additionally, another reason that may have led to such different results is that the number of test samples was not large enough. The study conducted in 2002 (22) covered

three cases of breast carcinoma and normal breast tissue. Even though 51 breast carcinomas were used in the study of 2005 (23), only 1 normal tissue case was used in this study. In the present study, immunohistochemical analysis was applied not only to 26 normal tissues obtained from the cancer patients, but also to 13 breast adenosis tissues, thereby improving the stringency of the experiments. All of the results indicated a predominant cytoplasmic staining of S100A11 in all positive samples; localization in nucleus was not observed.

Additionally, another reason for the controversial results to date may have been the selection of the normal tissues. The research conducted previously (22,23) did not present the nature of the normal tissues. In our research, the normal tissue was obtained from tissue located >3 cm away from the tumor site from the same patient. Comparison of the expression of S100A11 between tumor and corresponding adjacent normal tissue in the same patient is necessary to identify the status of overexpression of S100A11. This will potentially allow for further development of this protein for clinical diagnosis.

The relationship between the expression pattern of S100A11 and breast carcinoma. Breast carcinoma is a highly heterogeneous malignant tumor, which shows significant variation in organization, shape, immunophenotype and treatment response (28). Clinically, breast carcinoma is classified into different subtypes by IHC according to the status of ER, PR and HER-2 (29). Molecular analysis of breast carcinoma based on microarray analysis revealed that a large number of genes are differentially expressed at different stages within different subtypes of breast carcinoma (30,31). In this study, our results showed that the majority of differentially expressed proteins were selectively up- or down-regulated in all five cases, while only a slight minority of proteins shared the same expression pattern under the stringent selection conditions. This result was similar to previous studies (32-34), suggesting that the expression profiles amongst different subtypes of breast carcinoma have significant discrepancy, and some proteins might be specifically expressed in certain histological subtypes of breast carcinoma.

Interestingly, S100A11 expression was commonly up-regulated in all five groups, even though most proteins shared differential expression patterns. Subsequent statistical analyses showed that the up-regulation of S100A11 was independent of the expression status of ER/PR/HER-2, and did not correlate with other prognostic factors (including age, size, grade, stage and nodal status). Based on these results, we suggest that S100A11 may be a protein marker of breast carcinoma, which has ubiquitously increased expression in different subtypes of IDCA. It is suggested that S100 may be helpful for clinical diagnosis because its expression is distinct between cancer and normal breast tissue.

The function of S100A11 in breast carcinoma. There have been various speculations about the specific function of S100A11 in cancer. One of the most widely accepted theories was proposed by Sakaguchi *et al* (35). In their studies, S100A11 was identified as a key mediator of calcium-induced growth inhibition in cultured keratinocytes. An increase in extra-cellular calcium may cause phosphorylation of S100A11, with subsequent binding to nucleolin and translocation to the nucleus. S100A11 may then liberate Sp1/3 from nucleolin, leading to increased transcription of p21CIP1/WAF1 and p16INK4a, which are negative regulators of cell growth. Therefore, S100A11 is considered a candidate tumor suppressor gene (12-14). In contrast, in cancer research, the widely observed up-regulation of S100A11 indicates that S100A11 may be involved in growth enhancement and/or malignant progression of cancer cells (15-21). This seems paradoxical and no reasonable interpretation has been provided in these studies (35). Cross *et al* (23) attempted to explain this discrepancy on basis of their experimental results: the finding that there appeared to be a translocation of S100A11 from the nucleus into the cytoplasm in breast tumors. They speculated that the function of S100A11 in breast cancer may correlate with this change in localization. In normal tissue, S100A11 can translocate to the nucleus and lead to increased transcription of negative regulators of cell growth. In breast carcinomas, although the expression of S100A11 is increased, the loss of S100A11 nuclear translocation may lead to the inability to control cell growth. This way, tumor cell proliferation is not suppressed. However, in our study, the expression of S100A11 was predominantly cytoplasmic both in normal breast tissue and breast carcinoma; we did not observe an obvious migration of the molecule. We presume therefore, that it is more likely that S100A11 is involved in different, unknown mechanisms in cancer, and the functional mechanism of S100A11 in different cancers may also be different. Interestingly, this was partially confirmed by Wang *et al* (21), who identified that S100A11 locates predominantly in the nucleus in colorectal carcinoma, whereas it is detected predominantly in the cytoplasm in normal tissues.

Recently published studies have revealed that in addition to being an essential mediator for growth suppression, S100A11 could also enhance growth of human keratinocytes through the induction of EGF, or other ligands of the EGF receptor, functioning as a dual mediator for growth regulation of epithelial cells (35). These results may provide new insight into the specific function of S100A11 in breast cancer.

In conclusion, our data indicate that the ubiquitous expression of S100A11 is increased in different subtypes of IDCA. S100A11 protein displayed a predominant cytoplasmic localization in both breast carcinoma and normal breast tissues. Statistical analysis showed that the expression characteristics of S100A11 are independent of the status of ER, PR, HER-2 and other prognostic factors. It may therefore be used as a protein marker of breast cancer, potentially assisting the diagnosis of breast carcinoma.

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