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S100A7 (psoriasin) expression is associated with aggressive features and alteration of Jab1 in ductal carcinoma *in situ* of the breast

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Abstract

Introduction The S100A7 (psoriasin) gene is highly expressed in ductal carcinoma *in situ* (DCIS) of the breast and can be downregulated in invasive carcinoma. Persistent S100A7 expression in invasive carcinoma is associated with a worse prognosis, and this effect may be mediated in part through interaction with the multifunctional cell signaling protein Jab1.

Methods In order to investigate the relationship between S100A7 and progression from DCIS to invasive carcinoma, we studied S100A7 expression in 136 patients with DCIS (including 46 patients with associated invasive carcinoma) by immunohistochemistry.

Results S100A7 expression was present in 63 out of 136 (46%) of DCIS lesions and was associated with estrogen receptor negative status ($P = 0.0002$), higher nuclear grade ($P < 0.0001$), necrosis ($P < 0.0001$) and inflammation ($P < 0.0001$). S100A7 status was no different between DCIS with and DCIS without an invasive component, but higher levels of S100A7 were present in DCIS associated with invasive carcinoma ($P < 0.004$). Analysis of a subset of cases showed that S100A7 expression was also associated with an increase in nuclear Jab1 ($n = 43$; $P = 0.0019$) and reduced $p27^{kip1}$ ($n = 47$; $P = 0.0168$). In cases of DCIS associated with invasive carcinoma, there was also a significant reduction in S100A7 between *in situ* and invasive components ($n = 46$; $P < 0.0001$). In pure DCIS cases treated by local excision, there was no difference in frequency of S100A7 expression between patients with recurrence of DCIS ($n = 9$) and those without ($n = 36$).

Conclusion The findings reported here suggest that, although S100A7 may not be a marker for recurrence of DCIS, it is associated with poor prognostic markers in DCIS and may influence progression of breast carcinoma through its interaction with and influence on Jab1.

Keywords: breast cancer, Jab1, p27, progression

Introduction

We previously identified S100A7 (psoriasin) as a gene that is expressed within preinvasive ductal carcinoma *in situ* (DCIS) and that can be downregulated in invasive carcinoma [1]. Later studies confirmed this observation and revealed that S100A7 is among the most highly expressed genes in DCIS [2,3] relative to both normal tissue and invasive carcinoma. A similar pattern of expression is also seen in relation to skin tumorigenesis, in which S100A7 is highly expressed in preinvasive squamous cell carcinoma and is often downregulated in the adjacent invasive component [4].

We have now identified a potential functional relationship between S100A7 and tumor progression, and a mechanism of action through an interaction with the multifunctional intracellular signaling protein Jab1 (c-jun activation domain binding protein 1) [5,6]. Overexpression of S100A7 in a breast cancer cell line is associated with increased malignancy and with several changes in gene expression that are compatible with an alteration in Jab1 activity [7]. The latter include a relative increase in nuclear Jab1 and a decrease in the levels of the cell cycle inhibitor $p27^{kip1}$ [7,8].

DCIS = ductal carcinoma *in situ*; ER = oestrogen receptor; IHC = immunohistochemistry; Jab2 = c-jun activation domain binding protein 1.

Table 1

Clinical/pathological features and frequency of S100A7 expression in ductal carcinoma in situ

Clinical/pathological features		DCIS$^-$			DCIS$^+$			All DCIS		
		n	S100A7$^+$	*P*	*n*	S100A7$^+$	*P*	*n*	S100A7$^+$	*P*
ER	Negative	23	83%	<0.0001	29	52%	0.76 (NS)	52	65%	0.0005
	Positive	67	30%		17	53%		84	35%	
Grade	Low	34	15%	<0.0001	9	11%	0.0041	43	14%	<0.0001
	Moderate	32	47%		19	47%		51	47%	
	High	24	79%		18	78%		42	79%	
Necrosis	Negative	47	23%	<0.0001	15	20%	0.0024	q	23%	<0.0001
	Positive	43	65%		31	68%		74	66%	
Inflammation	Low	47	26%	0.0017	15	27%	0.054 (NS)	62	26%	<0.0001
	Moderate	26	62%		16	63%		42	62%	
	High	17	65%		15	67%		32	66%	

P values were calculated using χ^2 tests. DCIS$^-$, pure ductal carcinoma *in situ*; DCIS$^+$, DCIS associated with invasive carcinoma; ER, estrogen receptor; NS, not significant; S100A7$^+$, proportion of S100A7 positive cases.

Expression of S100A7 has been found to be highest in DCIS but only small numbers of cases have been examined [1-3]. S100A7 is also expressed in some invasive breast tumors [2,9], in which S100A7 is associated with poor prognostic factors and with poor outcome [7]. In the present study, our aim was to determine the frequency of expression of S100A7 in a large series of DCIS patients, and its relationships to Jab1 and p27^{kip1} expression and to progression and clinical outcome in DCIS.

Methods

Tissue specimens

Cases of pure DCIS of the breast were selected by review of breast surgical resections that were done from 1981 to 1999 at the Health Sciences Centre and St. Boniface Hospitals, Winnipeg, Manitoba. Cases of DCIS associated with invasive carcinoma were selected from the Manitoba Breast Tumor Bank [10], which operates with approval from the Research Ethics Board of the Faculty of Medicine, University of Manitoba. A total of 90 patients with pure DCIS and 46 patients with DCIS associated with invasive carcinoma were identified. The histologic nuclear grade was determined according to the criteria for the Van Nuys grading system [11]. The presence of intraductal necrosis, defined as nuclear and eosinophilic debris, was evaluated in hematoxylin–eosin stained sections by light microscopy, and the percentage of ducts exhibiting necrosis was estimated semiquantitatively. Periductal inflammation was assessed semiquantitatively as low (absent or very sparse inflammatory cells), high (marked inflammation surrounding over 50% of the ducts), or intermediate. Estrogen receptor (ER) status was determined by immunohistochemistry (IHC) analysis, as described below.

The clinical/pathologic features of the entire series are shown in Table 1. Among the cases of DCIS that had no invasive carcinoma, the primary surgical treatment was local excision in 45 patients and mastectomy in the other 45 patients. Amongst the former group, 27 patients had surgery alone, 18 patients received adjuvant local radiation therapy, and 11 received adjuvant hormone therapy. Clinical follow-up data for the 45 patients treated by local excision were obtained from chart review and search of the Manitoba Cancer Registry. Recurrence in the same breast occurred in nine patients (in eight as DCIS and in one as DCIS with microinvasive carcinoma) 9–50 months after initial diagnosis. For those cases with no recurrence the median follow-up period was 66 months (range 23–184 months).

Immunohistochemistry

Serial sections (5 μm thick) were cut from a representative formalin-fixed, paraffin-embedded archival tissue block from each tumor. Immunohistochemical staining for S100A7, ER, Jab1, and p27^{kip1} was performed as previously described [7] but using an automated tissue immunostainer (Ventana Medical Systems, Phoenix, AZ, USA) and DAB immunohistochemistry kit (ABC method; Ventana Medical Systems). Briefly, the staining protocol was set to the 'extended cell conditioning' procedure, followed by 12 hours of incubation with primary antibody before incubation with secondary antibody and detection. Concentrations and sources of the primary antibodies were 1:1000 for S100A7 [12], 1:50 for ER (6F11; Novocastra Laboratories Ltd, distributed by Vector Laboratories (Canada) Inc., Burlington, Ontario, Canada), 1:200 for Jab1 (FL-334; Santa Cruz Biotechnologies Inc., Santa Cruz, CA., USA), and 1:1000 for p27^{kip1} (Clone

57; Becton-Dickinson, BD Biosciences Canada, Mississauga, Ontario). Known positive control breast tissues were included with each staining batch to confirm that comparable staining intensities existed between batches, and normal breast tissue within the sections served as additional controls for Jab1, ER, and $p27^{kip1}$.

Assessment of S100A7, ER, Jab1, and $p27^{kip1}$ staining

IHC results for all genes were assessed by light microscopy performed by a pathologist (PHW) independently of the pathologic assessment... The intraobserver variability in IHC scoring for S100A7 was tested by comparison of IHC scores from two independent sessions, and confirmed a high degree of reproducibility (Spearman test $r = 0.93$; $P < 0.0001$). Levels of expression of S100A7, $p27^{kip1}$, and Jab1 proteins (both nuclear and cytoplasmic staining was assessed separately for each) and ER protein (nuclear staining only was assessed) were all determined by scoring the intensity (0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining) and the percentage of neoplastic epithelial cells exhibiting staining within the tissue section. The product of the intensity and the percentage was determined to provide a final, semiquantitative immunostaining score (IHC scores, ranging from 0 to 300).

For S100A7 an IHC score greater than 0 was regarded as positive (because we previously showed that this cut-point can divide invasive carcinomas into good and poor outcome subgroups [12], and S100A7 protein can be secreted and so it is theoretically possible that expression in a single cell might influence a wider group of tumor cells). Among S100A7-positive cases, the lower 25th percentile of expression corresponded to an IHC score of 1–9 and the upper 75th percentile corresponded to an IHC score of 100 or greater. On this basis, IHC scores of 1–9, 10–99, and 100 or geater were regarded as low, intermediate, and high expression. For ER an IHC score greater than 10 was regarded as positive (equivalent to the clinically validated cut-point in similar IHC scoring systems).

Statistical analysis

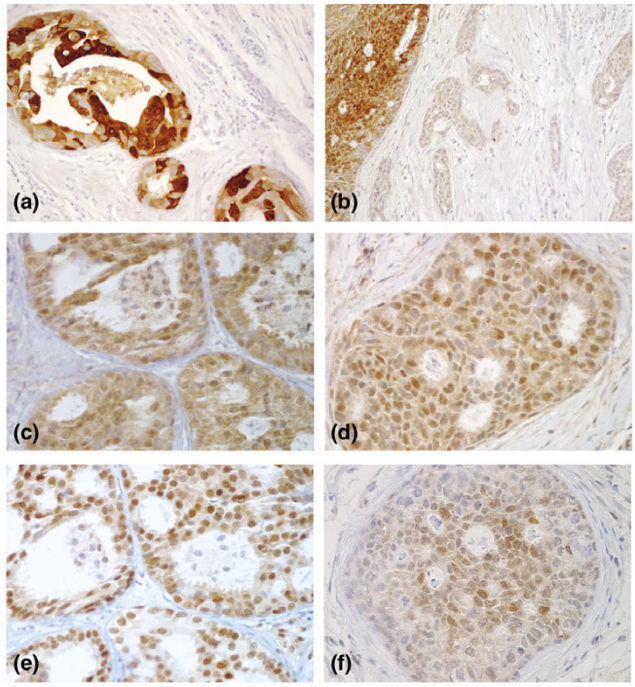
Statistical analysis was performed with Prism Graphpad software (Graphpad Prism v4.00, Graphpad software, San Diego, CA, USA) and using Spearman correlation, χ^2 test, t-test and Wilcoxon rank sum tests as appropriate. $P < 0.05$ was considered statistically significant.

Results

Evaluation of S100A7 and pathologic features within ductal carcinoma in situ

In the entire series of 136 cases of DCIS studied by IHC, 63 out of 136 (46%) of DCIS lesions expressed S100A7. The S100A7 IHC scores ranged from 0 to 270 (mean ± standard deviation 28 ± 62; median 0). S100A7 expression was high in 16 cases, intermediate in 34 cases, and low in 13 cases. In all positive cases the predominant cellular localization for expression was cytoplasmic. S100A7 nuclear expression was infrequent, being present only in 13 out of 63 (21%) of S100A7-positive cases, and was not significantly associated with any specific clinical/pathologic factors. Expression was restricted to epithelial tumor cells and was not observed in adjacent normal ducts or lobules, as we described previously [9]. Representative examples of S100A7 staining are illustrated in Fig. 1.

Figure 1



S100A7, Jab1, and $p27^{kip1}$ expression in DCIS detected by immunohistochemistry. The upper panels show high levels of S100A7 expression in **(a)** pure ductal carcinoma *in situ* (DCIS) and in **(b)** DCIS associated with invasive carcinoma. Note the reduced expression in the invasive component (panel b, right) relative to the *in situ* component (panel b, left). The middle panels show Jab1 expression in **(c)** S100A7-negative and **(d)** S100A7-positive DCIS cases. The lower panels show **(e)** high and **(f)** low $p27^{kip1}$ expression in the same two DCIS cases.

S100A7 expression in the entire series correlated with high nuclear grade ($r = 0.57$; $P < 0.0001$, Spearman test), low ER levels ($r = -0.38$; $P < 0.0001$), extent of necrosis ($r = 0.48$; $P < 0.0001$), and the presence of inflammation ($r = 0.39$; $P < 0.0001$). Similarly, when S100A7 and prognostic factors were considered as categorical variables, S100A7 expression was also significantly associated with ER-negative status ($P = 0.0005$), high grade, and presence of necrosis and inflammation ($P < 0.0001$, χ^2 test; Table 1). In subgroup analysis S100A7 was expressed in 25 out of 31 (81%) high-grade/ER-negative/necrosis-positive DCIS cases, as compared with six out of 40 (15%) low-grade/

ER-positive/necrosis-negative DCIS cases ($P < 0.0001$, χ^2 test).

Relationship between S100A7, Jab1, and p27^{kip1}

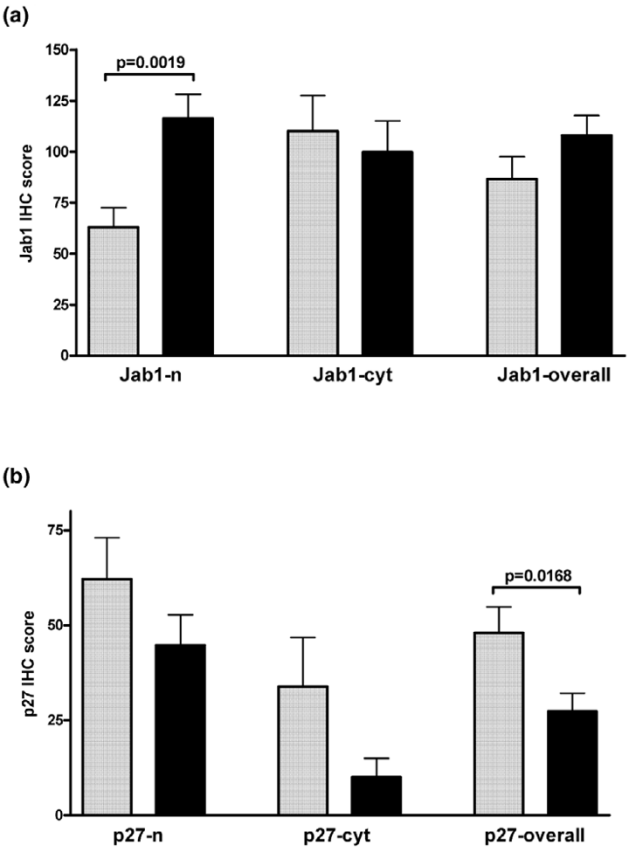
Expression of Jab1 and p27^{kip1} was also assessed in DCIS in a subgroup of 47 cases in which there was sufficient material available. These included 26 cases of pure DCIS and 21 cases of DCIS associated with invasive carcinoma. Reduced expression of p27^{kip1} was associated with high grade ($P = 0.0002$, t-test), ER-negative status ($P = 0.0008$), and presence of necrosis ($P = 0.025$). Nuclear Jab1 expression was higher in lesions with necrosis ($P = 0.026$) and exhibited a trend toward higher levels in ER-negative and high-grade lesions, whereas cytoplasmic Jab1 was lower in high-grade lesions, but these differences were not significant and overall Jab1 levels were similar in all subgroups.

S100A7 expression levels in DCIS correlated positively with nuclear Jab1 ($r = 0.44$; $P = 0.029$, Spearman test) and inversely with cytoplasmic p27^{kip1} ($r = -0.32$; $P = 0.028$). When nuclear, cytoplasmic, and total combined expression of Jab1 was considered relative to S100A7 status, S100A7-positive DCIS was associated with higher relative localization of nuclear Jab1 ($P = 0.0019$, t-test), but there was no difference in cytoplasmic or overall Jab1 expression between S100A7-positive and S100A7-negative DCIS. Conversely, S100A7-positive DCIS was associated with significantly lower overall expression of p27^{kip1} ($P = 0.0168$, t-test), and similar but nonsignificant trends toward lower nuclear and cytoplasmic localization (Figs 1 and 2).

Relationship between S100A7 and progression to invasive carcinoma

The entire DCIS series included two categories of DCIS: cases with and those without associated invasive carcinoma. When compared with pure DCIS, among DCIS cases with associated invasive carcinoma there was an increased proportion that were ER negative, necrosis positive, and high grade DCIS; this was statistically significant for the former two factors ($P < 0.0001$ and $P = 0.039$, respectively, χ^2 test) but not for grade ($P = 0.08$). S100A7-positive cases were also more frequent in DCIS associated with invasive carcinoma (24/46 [52%]) than in pure DCIS (39/90 [43%]), but this was not statistically significant. However, when only high levels of S100A7 were considered as positive, there was a significantly higher proportion of S100A7-positive cases among cases of DCIS with associated invasive carcinoma (10/46 [22%]) than among pure DCIS cases (5/90 [6%]; $p = 0.0037$, χ^2 test). Furthermore, the overall S100A7 IHC scores were also higher in the subgroup of DCIS associated with invasive components (mean ± standard deviation 51 ± 84) than in the pure DCIS subgroup (17 ± 45; $P = 0.0026$, t-test).

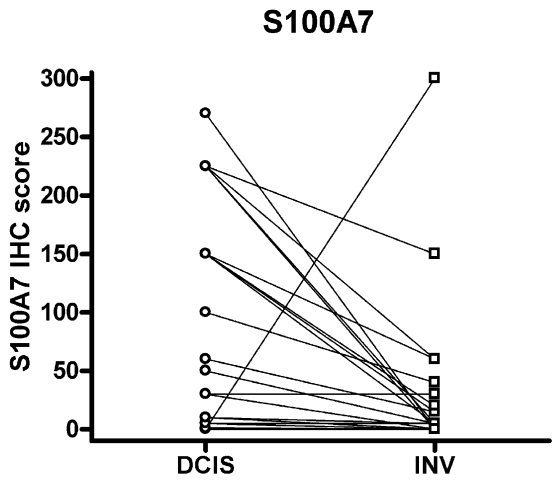
Figure 2



Jab1 and p27^{kip1} expression relative to S100A7 status. **(a)** The upper graph illustrates higher Jab1 nuclear expression (Jab-n) in S100A7-positive (black columns) than in S100A7-negative (grey columns) cases, but similar cytoplasmic (Jab-cyt) and overall expression. **(b)** The lower graph illustrates lower overall p27^{kip1} expression in S100A7-positive cases. The columns represent mean immunohistochemistry (IHC) scores and bars indicate standard deviations. Significant *P* values, as determined by t-test, are shown.

In the 46 DCIS cases associated with concurrent invasive carcinoma, differential S100A7 expression was observed between *in situ* and invasive components. S100A7 was expressed in 25 out of 46 cases. Although it was present in both the DCIS and invasive components in 14 out of 46 cases, it was limited to the DCIS component in 10 out of 46 cases and to the invasive component in one case. However, even when expressed in both components, S100A7 expression levels were consistently lower in the invasive component than in the matching *in situ* component ($P < 0.0001$, Wilcoxon test; Fig. 3). In those cases with high levels of S100A7 expression in DCIS, 10 out of 11 cases exhibited a 50% or greater reduction in IHC score in the invasive component. There was no significant change in Jab1 expression but there was a decrease in p27^{kip1} cytoplasmic expression ($P = 0.047$) between DCIS and invasive components.

Figure 3



The graph shows levels of S100A7 in matched ductal carcinoma *in situ* (DCIS) and invasive (INV) components within the 24 out of 46 cases of DCIS associated with invasive carcinoma that showed expression in either component.

S100A7 and recurrence in ductal carcinoma in situ cases treated by local excision

Among the 90 patients with pure DCIS, 45 had been treated by local excision. In this subset, 36 (80%) patients did not develop a recurrence whereas nine (20%) patients did develop a recurrence (DCIS in eight and DCIS with an invasive component in one). There was no significant difference in patient age, excision margin status, or frequency of additional radiation or hormonal treatment between the subgroups with or without recurrence. Similarly, there was no significant difference between the clinical/pathologic characteristics of these DCIS cases associated with different outcomes, although lesion size could not be accurately determined and was not included in the analysis. The subset with recurrence exhibited more frequent necrosis (66% versus 51%) and ER-negative status (44% versus 20%), and a lower proportion of cases that were treated by radiotherapy (13% versus 27%); however, none of these differences was statistically significant. There was also no significant difference in the frequency of S100A7 expression between the cases with (4/9 [44%]) and those without (21/36 [58%]) recurrence.

In seven DCIS cases with recurrence, the tissue blocks from the recurrence were also available for assessment for S100A7 expression. Although both nuclear grade and the presence of necrosis were concordant in only four out of seven (57%) cases, ER status was concordant in five out of six (83%) and S100A7 status was concordant in seven out of seven (100%) cases.

Discussion

The S100 gene family encodes small proteins that share EF-hand helix-loop-helix domains that are important for their function as calcium-binding proteins [13]. Several S100 genes are altered in neoplasia and specifically in breast cancer [14] including S100A4, which can influence the metastatic phenotype in mammary cell lines and is associated with poor outcome in invasive breast tumors [15-17].

S100A7 (psoriasin) was first identified as a highly abundant cytoplasmic and secreted protein that is induced in abnormally differentiating squamous epithelial cells derived from epidermis of skin affected by psoriasis [18]. This association with psoriasis has suggested a role for S100A7 either in keratinocyte differentiation or as a chemotactic factor [19-21].

A possible role for S100A7 in breast cancer first emerged when it was identified as a cDNA that is downregulated in breast nodal metastasis [22]. We also identified S100A7 as a differentially expressed gene between DCIS and invasive carcinoma [1]. Although previous studies have been limited to small numbers of DCIS lesions, S100A7 has also emerged from subtraction hybridization [1], SAGE (serial analysis of gene expression) [2,23], and proteomic analyses [3] as a highly expressed gene in DCIS. By comparison S100A7 expression is mostly absent in normal breast tissue, benign, and atypical proliferative ductal lesions [1]. S100A7 is also expressed at a lower frequency in invasive carcinoma [2,23], where it is associated with markers of poor prognosis [9] and is an independent prognostic factor associated with poor outcome in ER-negative tumors [12]. We also recently showed that expression of S100A7 can promote several features of malignancy in an already invasive ER-negative breast cell line, both *in vitro* and *in vivo* [7].

Taken together, these previous observations support the hypothesis that S100A7 plays a functional role in breast tumor progression. In support of a direct role in the development of the invasive phenotype, the present study confirms that S100A7 is frequently expressed in preinvasive DCIS and predominantly in DCIS with poor prognostic factors associated with progression and recurrence [11,24]. As previously shown in other tumors [4] and smaller case cohorts, S100A7 is also frequently downregulated between *in situ* and invasive carcinoma within the same tissue, and high levels of S100A7 expression are more frequent in DCIS associated with invasive carcinoma than in DCIS without invasion. We also observed that nuclear S100A7 localization is infrequent in DCIS (21% of positive cases), which is in contrast to our previous studies of invasive carcinomas in which nuclear S100A7 localization was more frequent (50% of S100A7-positive cases). The mechanism that underlies this downregulation and possible

alteration of expression is unknown, but it is unlikely to be attributable to genomic changes and may reflect changes in cellular differentiation or response to extracellular stress signals [2]. However, in the present study we observed that the overall incidence, as opposed to the level of S100A7 expression, does not necessarily differ significantly between pure DCIS and DCIS that is associated with invasive carcinoma. This observation supports an alternative view, namely that S100A7 expression may be more closely related to specific phenotypic and biologic characteristics within tumor cells, irrespective of the tumor stage. In support of this view, although S100A7 has been shown to be highly expressed in some DCIS lesions [1,23,25] and is expressed in over 85% of DCIS associated with ER-negative status and high nuclear grade, it is evident that invasive tumors within the subset that exhibit these same characteristics may also express S100A7 at a relatively high frequency (S100A7 was positive in 58% of ER-negative/high-grade invasive cases in our previous study [12]).

Consideration of these patterns of expression *in vivo*, combined with a better understanding of the mechanism of action of S100A7, will help in resolving the role played by S100A7 in tumor progression. S100A7 may exert an influence on inflammatory and immune responses, and was significantly associated with inflammation in this series of DCIS cases. However, some S100A7-positive DCIS cases were not associated with inflammation, and the association with inflammation is uncertain in skin and invasive breast tumors [4,12]. Another potential mechanism for a direct effect of S100A7 within breast cancer cells may involve an interaction between S100A7 and the multifunctional Jab1 protein [7]. Jab1 was originally identified in mammalian cells as a factor influencing c-jun mediated transcription of AP-1 regulated genes [5,26]. It is now known that Jab1 is a component both of a multimeric protein complex (CSN5 within the CSN/COP9 signalosome) and of the lid unit of the Ub-26S proteasome [6,27]. Studies in cell lines suggest that Jab1 is involved in diverse interactions with components of cell signaling pathways [26,28-34]. Jab1 therefore has the potential to facilitate several aspects of early tumor progression directly through alteration in multiple cellular properties, which include promoting degradation of $p27^{kip1}$ [33].

In studies of human tumors, increased nuclear Jab1 is consistently associated with reduced $p27^{kip1}$ and has been associated with poor outcome in some studies of invasive carcinomas in both breast and ovary, and in lymphoma [35-37]. However, in a larger cohort of node-negative invasive breast carcinomas, Jab1 was not related to disease-free survival [36,38]. This may be consistent with the observation that Jab1 can affect diverse signaling pathways and may therefore exert a different influence in subsets of tumors that depends on the sum and status of these pathways. Many of the interactions between Jab1 and signaling molecules appear to result in translocation of Jab1 from the cytoplasm to the nucleus in cell lines [7]. We previously observed that expression of S100A7 induces a relative increase in nuclear Jab1 in a breast cell line, and a concurrent reduction in $p27^{kip1}$ protein [7]. In the present study we show that S100A7 expression in DCIS is also associated with higher nuclear localization of Jab1, without a significant increase in overall expression, and with reduced $p27^{kip1}$. These findings are consistent with studies that also found that high nuclear Jab1 localization is inversely correlated with expression of $p27^{kip1}$ in invasive breast carcinoma [36,38]. However, unlike S100A7, the frequency of nuclear Jab1 localization in DCIS was not different between DCIS and invasive components in this study, and the expression of Jab1 in DCIS appears to be similar to that reported in invasive carcinomas, with over 81% of DCIS exhibiting Jab1 expression in more than 50% of tumor cells [36]. It is known that many factors apart from S100A7 can influence Jab1 cellular localization, and future studies will be needed to determine whether alterations in Jab1 localization are inherent to any specific stage in breast tumor progression.

Progression of DCIS may result in recurrence in the form of DCIS or invasive disease [39]. In this series the majority of recurrence events were DCIS lesions and we found no relationship with S100A7 expression. Recurrence in the form of DCIS may be derived from residual disease attributable to incomplete surgery of the original focus of disease, from emergence of distant lesions in an originally multifocal disease, or *de novo* disease arising in previously normal remaining tissue. Molecular studies suggest that the former may often occur [39,40]. Our finding that the S100A7 phenotype is reliably conserved between primary lesions and recurrences is also consistent with this view.

Conclusion

Although the number of cases that were treated by local resection or that developed invasive recurrence is small and limits firm conclusions, our findings suggest that S100A7 does not influence recurrence of DCIS. Similarly, the finding of comparable incidence of S100A7 expression in both pure DCIS and DCIS associated with invasive carcinoma does not support an essential role in invasion. However, the association of S100A7 with established poor prognostic factors and higher levels of expression in DCIS associated with invasive components are consistent with a role in facilitating progression. Our findings also further support an association between S100A7 and Jab1, and the view that this interaction is potentially important in influencing cell signaling and mediating tumor progression.

Competing interests

None declared.

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