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Original article

SATB1 gene expression and breast cancer prognosis

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ABSTRACT

Recently it has been shown that the genome organizer SATB1 plays an important role in breast cancer progression and predicts a poor prognosis. However its prognostic value compared to markers as the estrogen receptor is currently unclear. The expression levels of SATB1 mRNA from Affymetrix microarray in a cohort of 2058 breast cancer samples and its prognostic impact were analyzed. There was no significant difference in disease-free survival among ER negative cancers but instead a benefit for high SATB1 expression among ER positive tumors ($p = 0.042$). However, even in ER positive cancer no independent prognostic value in multivariate analysis with standard parameters was observed. Thus the use of SATB1 as target or prognostic marker for breast cancer should be viewed with caution and a possible confounding effect of the estrogen receptor status of the tumor should be taken into account when analysing new markers as SATB1.

Introduction

The Special AT-rich Sequence-Binding protein 1 (SATB1) is a protein that binds AT-rich DNA sequences. It is a cell-type-specific organizer of the genome, regulating both, gene expression, as well as cellular delineation.[1–3] Furthermore, genes associated with proliferation mechanisms, such as c-myc, are directly regulated by SATB1.[4] It acts as a transcriptional activator,[2] although the activity of certain genes can also be suppressed.[4]

Han et al.[5] reported that SATB1 is necessary for breast cancer cells to achieve the potential for metastasis. Both high levels of SATB1 mRNA and protein were detected in aggressive breast cancer cell lines as well as in tumor cells of poorly differentiated carcinoma samples. Moreover the authors demonstrated that SATB1 expression is highly prognostic among primary breast cancers independent of tumor size, lymph node status and histological grade. Ectopic expression of SATB1 in non-metastatic SKBR3 cells resulted in an induction of invasive tumors in mice.[5] In contrast, the silencing of SATB1 in MDA-MB-231 breast cancer cells resulted in restoration of cell polarity and reduced invasive capacity.[5] Patani et al. examined mRNA expression in $n = 110$ breast cancer samples by real-time PCR.[6] They demonstrated only a weak trend of a shorter overall survival in patients with high SATB1 expression. In this study the transcript levels were associated with estrogen receptor (ER) positivity.

Numerous publications have shown that the expression of specific genes in breast cancer depends on the expression of the estrogen receptor and presence or absence of proliferative activity.[7–11] This should be taken into account in a special way when analyzing and evaluating new predictive and prognostic markers.[12] However, previous analyses did not always consider the estrogen receptor status of the tumor which is still one of the most important prognostic and predictive factors for clinical stratification of breast cancers and treatment decision. For this reason, we conducted a large-scale study in which we have determined the expression of SATB1 by gene expression analysis and correlated it with the estrogen receptor status. Corresponding survival data were analyzed for the prognostic value of SATB1 expression.

Material and methods

We analyzed SATB1 gene expression using Affymetrix microarray data and compared it to clinico-pathological characteristics as well as disease-free survival. A database of 3030 Affymetrix microarrays and clinical characteristics from primary breast cancer patients was established including 238 samples from our own institution[9,10,13,14] as well as 2792 samples from publicly available datasets[15–23] as described elsewhere.[24] Only Affymetrix HG U133A arrays were included in the analyses to allow full comparibility of the expression data. All expression data were analyzed using the MAS5.0 algorithm[25] of the *affy* package[26] of the Bioconductor

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software project.[27] Subsequently data from each array were log_2-transformed, median-centered, and the expression values of all the probesets from the U133A array were multiplied by a scale factor S so that the magnitude (sum of the squares of the values) equals one. Since there is no cutoff definition for SATB1 we first looked at the distribution of SATB1 expression values in the complete dataset. As shown in Fig. 1 the distribution is not bimodal even if samples were stratified according to ER status (data not shown). In a second step we splitted the complete dataset by deciles since in the previous study by Han et al. it was demonstrated that approximately 6% of all breast cancer samples displayed a high SATB1 expression in immunohistochemistry. In addition we used a quartile split of the dataset to avoid a confounding effect caused by small numbers of events in each strata. Moreover, to avoid bias effects between different datasets on SATB1 expression we also used an alternative approach: To avoid confounding effects caused by higher gene expression in individual datasets which might influence further analysis we defined highest decile and quartile separately for each dataset. These alternative stratification methods led to similar results. Previous studies have also shown the reliability of conclusions drawn from analysis of this type of data.[26,28]

Chi-square test was used to test for associations between SATB1 expression of tumors and categorical parameters. For those patients with available follow up data ($n = 2058$, median follow up 72 months) Kaplan–Meier curves were constructed and the log rank test used to determine the univariate significance of the variables. Cox regression analysis was performed to determine hazard ratios. TaqMan assays corresponding to the region of the Affymetrix SATB1 probe set were applied in real-time PCR to validate microarray expression results of selected samples.

Fishers exact test was applied for associations between categorical parameters. All reported *P* values are two sided and *P* values of less than 0.05 were considered to indicate a significant result. All analyses were performed using the R software environment (http://www.r-project.org/) and SPSS version 17.0 (SPSS Inc, Chicago, Illinois).

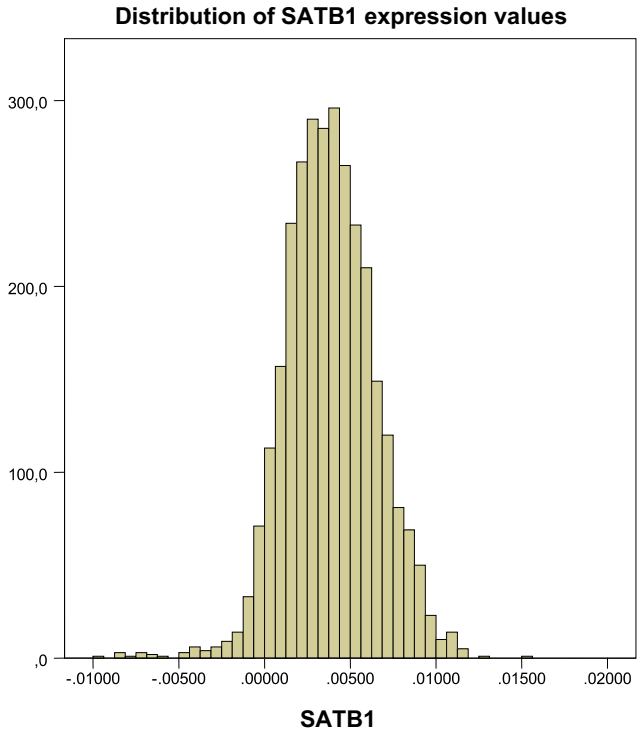

Fig. 1. Distribution of SATB1 expression values in the dataset of $n = 3030$ breast cancer patients.

Results

The clinical characteristics of breast cancer patients stratified by the highest decile of SATB1 mRNA expression vs. the remaining 90% as well as ER status are given in Table 1. In a cohort of 3030 patients no significant difference of SATB1 expression according to lymph node status, pathohistological grading and PgR-status was observed for both ER positive and ER negative tumors. Additionally in the group of ER positive tumors no correlation of SATB1 expression to HER2-status was found, whereas in the group of ER negative tumors no difference of SATB1 expression was found according to age, tumor size and Ki67 expression. However in ER positive tumors high SATB1 expression was associated with a lower proliferation rate with respect to Ki67 expression ($p < 0.001$).

Next we analyzed the survival of patients according to the expression of SATB1. Previous studies reported a worse survival for patients with high SATB1 expression.[5] In contrast to these data the survival analysis of our cohort revealed no significant difference according to SATB1 mRNA expression among ER negative cancers ($p = 0.72$; Fig. 2B). Moreover, we observed a trend for an improved prognosis for high SATB1 expression among ER positive tumors

Table 1
Clinical characteristics of ER positive and ER negative breast cancers stratified according to high and low SATB1 expression using deciles separately in each dataset.

	Clinical variable	SATB1 high	SATB1 low	*p*-value total $n = 3030$
Tumor size[a]				
ER positive	<2 cm	64 (11.9%)	473 (88.1%)	0.018
	>2 cm	64 (7.9%)	743 (92.1%)	
ER negative	<2 cm	17 (12.4%)	120 (87.6%)	n.s.
	>2 cm	26 (7.4%)	327 (92.6%)	
Lymph node status[b]				
ER positive	Node negative	119 (10.1%)	1059 (89.9%)	n.s.
	Node positive	42 (8.6%)	445 (91.4%)	
ER negative	Node negative	39 (9.1%)	390 (90.9%)	n.s.
	Node positive	17 (8.9%)	173 (91.1%)	
Histological Grading[c]				
ER positive	Grade 1 and 2	102 (10.1%)	903 (89.9%)	n.s.
	Grade 3	27 (8.0%)	311 (92.0%)	
ER negative	Grade 1 and 2	15 (10.5%)	128 (89.5%)	n.s.
	Grade 3	28 (8.0%)	323 (92.0%)	
Age[d]				
ER positive	<50	47 (12.6%)	326 (87.4%)	0.02
	>50	69 (8.2%)	770 (91.8%)	
ER negative	<50	18 (8.6%)	192 (91.4%)	n.s.
	>50	24 (9.6%)	226 (90.4%)	
HER2-status				
ER positive	HER2 negative	192 (9.6%)	1812 (90.4%)	n.s.
	HER2 positive	13 (8.4%)	141 (91.6%)	
ER negative	HER2 negative	71 (11.4%)	550 (88.6%)	< 0.001
	HER2 positive	7 (2.8%)	244 (97.2%)	
Ki67				
ER positive	Ki67low	134 (12.5%)	940 (87.5%)	< 0.001
	Ki67 high	71 (6.5%)	1013 (93.5%)	
ER negative	Ki67low	37 (8.6%)	394 (91.4%)	n.s.
	Ki67 high	41 (9.3%)	400 (90.7%)	
PgR				
ER positive	PgR negative	54 (8.6%)	574 (91.4%)	n.s.
	PgR positive	151 (9.9%)	1379 (90.1%)	
ER negative	PgR negative	59 (8.8%)	609 (91.2%)	n.s.
	PgR positive	19 (9.3%)	185 (90.7%)	

[a] Information on tumor size was not available for $n = 1196$ patients.
[b] Information on nodal status was not available for $n = 746$ patients.
[c] Information on tumour grade was not available for $n = 1193$ patients.
[d] Information on age was not available for $n = 1358$.

($p = 0.14$, Fig. 2A). Assuming that a split by deciles would obviously confounded by a small number of events in SATB1 high expressing samples we performed an alternative splitting using quartiles. As depicted in Fig. 3, the increase of the number of SATB1 positive samples when using the upper quartile did further improve its prognostic relevance. Patients with a high SATB1 expression among ER positive tumors show a significant better prognosis ($p = 0.042$, Fig. 3A) when the quartile stratification was used.

However, multivariate analysis of standard clinical parameters and SATB1 expression in ER positive and negative breast cancers separately revealed that SATB1 is not an independent prognostic marker in either subgroup (Table 2). In ER positive cancers only tumor size, grading and HER2-status remained an independent prognostic factor in multivariate analysis, whereas for ER negative cancers only lymph node status and tumor size remained significant.

Discussion

In previous studies a negative prognostic effect of high SATB1 expression in breast cancer has been demonstrated. Han et al.[5] showed that this protein when nuclear localized is an

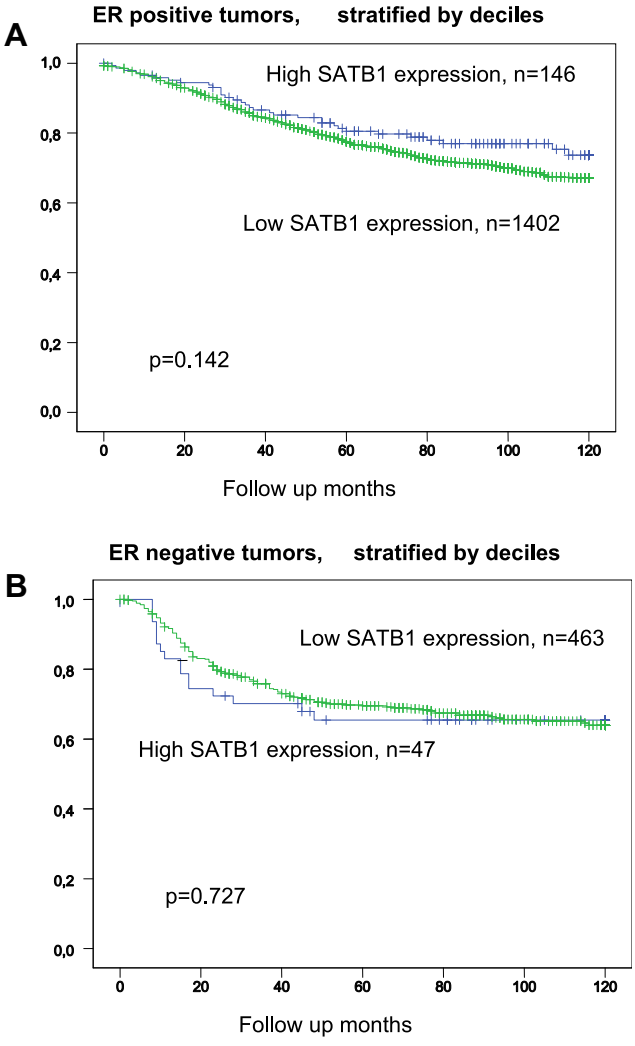

Fig. 2. Prognostic value of SATB1 expression in ER positive (A) and ER negative (B) subgroups of breast cancer patients. Kaplan–Meier analyses of disease-free survival were performed in different tumor subgroups according to a decile split of SATB1 expression.

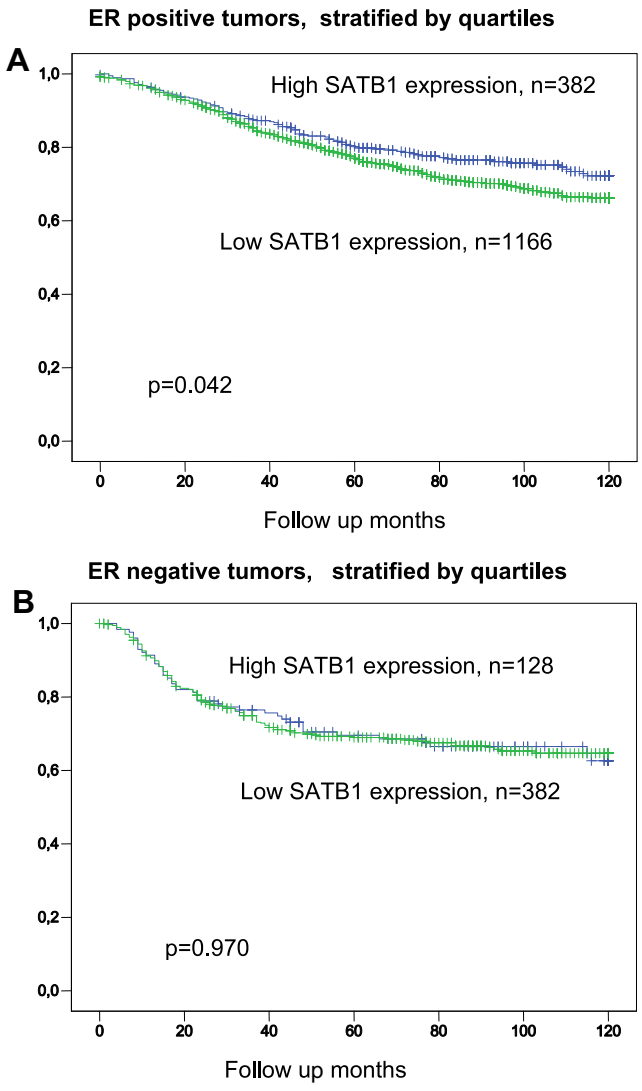

Fig. 3. Prognostic value of SATB1 expression in ER positive (A) and ER negative (B) subgroups of breast cancer patients. Kaplan–Meier analyses of disease-free survival were performed in different tumor subgroups according to a quartile split of SATB1 expression.

independent prognostic factor beside tumor size, lymph node status, and pathological grading. It was expressed most intensively in poorly differentiated ductal carcinomas whereas a significant expression was missing in adjacent normal tissue. However, in multivariate analysis ER status was not taken into account.

Patani et al. have demonstrated that SATB1 mRNA is overexpressed in breast cancer compared to normal breast tissue ($p = 0.0167$), but they were not able to demonstrate that high expression of SATB1 is a negative prognostic factor.[7] These authors only detected a non-significant trend for an association of higher SATB1 expression with poor outcome among a cohort of $n = 110$. However, Patani et al. have shown that SATB1 transcript levels correlated significantly with estrogen receptor status (i.e. a high expression of SATB1 was more often found in ER negative tumor tissue samples ($p = 0.046$)).[7]

Our analyses revealed that within the group of ER positive breast cancers high SATB1 mRNA expression identified a subgroup of patients having an improved prognosis. In contrast when analyzing only ER negative cancers we found no difference in the prognosis for patients with higher SATB1 expression. Thus the effect of SATB1 on tumor cell behaviour seems to depend on

Table 2
Cox regression analysis of standard parameters and SATB1 expression (high vs. low by deciles) in relation to disease-free survival for ER positive (a) and ER negative (b) breast cancer patients.

		p-value	HR	95% confidence interval [CI]
(a) ER positive breast cancer ($n = 786$)				
SATB1	High ($n = 77$) vs. low ($n = 709$)	0.631	0.896	0.571–1.405
Nodal status	Negative ($n = 505$) vs. positive ($n = 281$)	0.149	1.220	0.931–1.598
Age	Age > 50 ($n = 532$) vs. <50 ($n = 254$)	0.249	0.849	0.643–1.122
Tumor size	<2 cm ($n = 340$) vs. >2 cm ($n = 446$)	<0.001	0.540	0.405–0.720
Pathological grading	Grade 3 ($n = 186$) vs. grade 1 and 2 ($n = 600$)	0.049	1.334	1.001–1.778
HER2	Positive ($n = 47$) vs. negative ($n = 739$)	0.049	1.585	1.003–2.506
PgR	Positive ($n = 597$) vs. negative ($n = 189$)	0.192	0.826	0.620–1.101
(b) ER negative breast cancer ($n = 247$)				
SATB1	High ($n = 24$) vs. low ($n = 223$)	0.394	0.695	0.300–1.606
Nodal status	Negative ($n = 174$) vs. positive ($n = 73$)	0.042	1.609	1.017–2.545
Age	Age >50 ($n = 119$) vs. <50 ($n = 128$)	0.338	1.237	0.800–1.913
Tumor size	<2 cm ($n = 84$) vs. >2 cm ($n = 163$)	0.036	0.580	0.348–0.966
Pathological grading	Grade 3 ($n = 158$) vs. grade 1 and 2 ($n = 89$)	0.852	0.958	0.609–1.507
HER2	Positive ($n = 71$) vs. negative ($n = 176$)	0.578	1.147	0.707–1.861
PgR	Positive ($n = 46$) vs. negative ($n = 201$)	0.740	0.908	0.512–1.610

the presence of the steroid hormone receptor. This point is of special interest since it might suggest that the described reprogramming of gene expression through SATB1[5] and its effect on breast tumor growth and metastasis could lead to strikingly different outcomes in ER positive and ER negative cancers. In multivariate analysis we could not confirm the observed beneficial effect of SATB1 in ER positive disease as an independent marker.

Since only nuclear localization of SATB1 has been reported to predict poor prognosis,[5] the improved survival of high SAT1B expression seen in ER-positive breast cancers might suggest an altered cellular distribution of the protein caused by the presence of the estrogen receptor. Alternatively, other ER-dependent mechanisms might exist which oppose the previously described unfavourable effect of SATB1. Supposed that the expression of the estrogen receptor could prevent the detrimental effects of nuclear SATB1 an endocrine treatment of those patients might in fact interfere with this preventive effect of the estrogen receptor. Therefore the analysis of ER positive tumors of patients who have received different antiestrogenic treatment (e.g. selective estrogen receptor modulators or downregulators as well as aromatase inhibitors) might reveal further insight with regard to the interaction of SATB1 and estrogen receptor expression and function.

In our study we conducted a large-scale microarray analysis to evaluate SATB1 mRNA expression. Therefore it has several limitations. A quantitative analysis of protein expression should be undertaken to validate these results with particular attention of ER expression, cellular and subcellular localization. It could be argued that RNA levels from tissues will not necessarily provide an accurate measure for SATB1 expression in tumor cells and expression from T-lymphocytes and fibroblasts might confound microarray results. However, when we looked for expression of T cell associated markers and stromal markers we did not find any differences between subgroups making this hypothesis unlikely (data not shown). In addition, we found no correlation of SATB1 expression with the number of lymphocytes or the amount of fibroblasts in those samples where this information was available ($n = 171$; data not shown).

Very recently Iorns et al. also analyzed the role of SATB1 in breast cancer pathogenesis. In line with our data these authors also did not find an association between SATB1 and poor outcome of breast cancer patients. Moreover numerous transfection assays using short hairpin RNAs against SATB1 and xenograft mouse models did not indicate that SATB1 expression promote breast cancer progression.[29] Therefore these authors concluded that the use of SATB1 as a therapeutic target or prognostic marker for human breast cancer should be viewed with caution.

In summary, in contrast to previous studies which described a negative prognostic value for SATB1 expression we found no difference in the prognosis for patients positive for SATB1 expression in ER negative breast cancer but instead a better prognosis in the ER positive cancers when these groups were analyzed separately. Thus a possible confounding effect of the estrogen receptor status of the tumor as the most important prognostic and predictive factor in breast cancer should be taken into account when analysing new markers as SATB1.

Conflict of interest statement

There is no conflict of interest to declare.

The work has been approved by the appropriate local ethical committee.

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