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Expression of secreted protein acidic and rich in cysteine (SPARC) in breast cancer and response to neoadjuvant chemotherapy†

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Background: Secreted protein acidic and rich in cysteine (SPARC) has been suggested as a new biomarker and therapeutic target in breast cancer, as well as other tumor types.

Patients and methods: We evaluated the frequency of SPARC expression among different molecular breast cancer subtypes and its role for therapy response after neoadjuvant chemotherapy. In this study, pretherapeutic core biopsies of 667 patients from the neoadjuvant GeparTrio trial were evaluated for SPARC expression by immunohistochemistry using a standardized immunoreactive score (IRS).

Results: An increased SPARC expression (IRS ≥6) was observed in 26% of all tumors. In triple-negative tumors, SPARC expression was increased in 37% of tumors, compared with other molecular subtypes (23% HR+/HER2–, 29% HR+/HER2+ and 22% HR–/HER2+; $P = 0.038$). Increased SPARC expression was associated with an increased pathological complete response (pCR) rate of 27%, compared with 15% in tumors with low SPARC expression ($P < 0.001$). In the triple-negative subgroup, pCR rates were 47% in tumors with high SPARC expression, compared with 26% in tumors with low SPARC expression ($P = 0.032$). In multivariable analysis, SPARC was independently predictive in the overall population ($P = 0.010$) as well as the triple-negative subgroup ($P = 0.036$).

Conclusions: SPARC is frequently expressed in breast cancer with triple-negative breast cancer revealing the highest expression rate. High SPARC expression of the primary tumor is associated with a higher chance of achieving a pathological complete remission after TAC or TAC-NX chemotherapy. As SPARC is an albumin-binding protein and might mediate intratumoral accumulation of albumin bound drugs, SPARC should be further evaluated as a predictive marker especially for response to albumin-bound drugs like nab-paclitaxel.

Clinical trial number: NCT00544765.

Key words: SPARC, breast cancer, neoadjuvant chemotherapy, pCR

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introduction

Secreted protein acidic and rich in cysteine (SPARC; alternative names osteonectin; ON or basement-membrane-40; BM40) is an albumin-binding glycoprotein. The matricellular protein

SPARC is secreted by cells and has the ability to interact with receptors on the surface of cells, growth factors, proteases and other components of the extracellular matrix (ECM) [1]. Its main function is to mediate interactions between cells and their extracellular surrounding during morphogenesis, tissue remodeling [2] and angiogenesis [3].

In vitro experiments with cultured cells supplied evidence that SPARC may play a role in development and growth of tumors and metastasis. By interacting with growth factors such as vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor-β (TGF-β) and integrins [3, 4], metalloproteases and other components of the ECM, SPARC can mediate proliferation, shape and adhesion of cells [2, 5], affect remodeling of the ECM [6], and thereby enable tumors to interact with stromal cells and the ECM [1].

A differential expression of SPARC in tumor tissue and its surrounding stroma compared with normal tissues has been reported for many different types of cancer. An upregulation of SPARC suggests a promoting role in tumor development and growth. Additionally, SPARC was repeatedly associated with poor prognosis and aggressive tumor growth in multiple cancers [7–10].

The hypothesis that an accumulation of SPARC in breast cancer cells and stroma could affect its ability to bind albumin makes SPARC of special interest as a biomarker for therapy response, especially for response prediction of treatment with albumin-bound drugs such as nab-paclitaxel, which is evaluated in ongoing clinical trials.

The aim of this study was to investigate SPARC protein expression among different molecular breast cancer subtypes and to evaluate its predictive value for therapy response after neoadjuvant anthracycline–taxane-based chemotherapy in a cohort of 667 participants of the GeparTrio trial.

patients and methods

study design

The neoadjuvant GeparTrio (NCT00544765) pilot [11] and main [12, 13] trials were prospective, randomized, multicenter trials conducted by the German Breast Group (GBG), Neu-Isenburg, Germany. Patients were recruited between 2001 and 2005. In total, 2357 women with primary uni- or bilateral previously untreated breast cancer were included (cT2-4, cN0-3, cM0). All study participants received two initial cycles of docetaxel, doxorubicin and cyclophosphamide (TAC). Subsequently response was evaluated by breast sonography. In the pilot trial, responders received four additional cycles of TAC. In the main trial, responders were randomized to receive either four or six additional cycles of TAC. Non-responders randomly either received four additional cycles of TAC or four cycles of vinorelbine and capecitabine (NX). Neoadjuvant trastuzumab treatment of patients with HER2-positive tumors was not available at the time the study was conducted. Further details on the studies are given in the main publications on these trials [11–14].

Pathological complete response (pCR) was defined as the absence of any invasive cancer in the breast and in lymph nodes (ypT0/ypTis, ypN0).

collection of samples and immunohistochemical staining of SPARC

All samples were formalin-fixed paraffin-embedded (FFPE) core biopsies collected prospectively before randomization and treatment at the participating institutions. The samples were centrally archived and stored at the GBG tumor bank located at the Institute of Pathology at the Charité Hospital, Berlin, Germany. All patients gave written informed consent for participation in the trial and collection of tumor material; ethics committee approval for the clinical study, biomarker collection and the translational investigations was obtained. Inclusion criteria for this translational investigation were available tissue samples from the FFPE core biopsy and available outcome data (pathological response to neoadjuvant chemotherapy).

Tumor tissue was identified on H&E-stained slides of the core biopsies and processed for tissue microarray (TMA) construction. Immunohistochemical staining of SPARC (Novocastra NCL-O-NECTIN; Clone: 15G12; 1 : 100; Leica, Wetzlar, Germany) was carried out on TMAs according to standard procedures. To control for the specificity of the used antibody to SPARC, a western blot using 12 different breast cancer cell lines with various levels of SPARC expression was carried out. One single band with the size of ~43 kD was observed responding to glycosylated SPARC (data not shown).

As a secondary antibody and for visualization, a peroxidase/3,3′-diaminobenzidine (DAB+) was used according to manufacturer's protocol (Dako REAL Detection System PeroxidaseDAB+ Rabbit/Mouse; K5001, Dako, Glostrup, Denmark). The stained slides were digitalized (Mirax Scan; Zeiss, Jena, Germany) and virtual slides were evaluated using the VMscope Slide Explorer (VMscope, Berlin, Germany). The staining intensity (negative = 0, weak = 1, moderate = 2, strong = 3) and percentage of positive tumor cells (0% = 0, 1%–10% = 1, 11%–50% = 2, 51%–80% = 3, 81%–100% = 4) were evaluated. An immunoreactive score (IRS) ranging from 0–12 was calculated by multiplying the numeric values of both parameters [15]. For stromal SPARC expression, only staining intensity was evaluated.

Finally, cases were divided into two groups with low or high cytoplasmic SPARC expression based on data distribution (IRS <6 versus IRS ≥6); this cut point was defined using the web-based software Cutoff Finder (http://molpath.charite.de/cutoff/). For cutoff optimization, the fit of mixture model was used [16]. Additional analysis using slightly different cut points (IRS 0–3 versus 4–12 and IRS 0–7 versus 8–12) and the IRS as a continuous factor were carried out to verify stability of our study.

Hormone receptor (HR) and HER2 status were assessed centrally at the Charité University Hospital. If central parameters were not available, data from the local pathologists was used as a substitute. Positive HR status was defined as ≥10% of tumor cells expressing estrogen receptor and/or progesterone receptor. Positive HER2 status was defined using immunohistochemistry as HER2 3+ (DAKO score) or HER2 2+ with HER2 gene amplification (*in situ* hybridization).

statistical analysis

Correlations between SPARC expression and clinicopathological parameters and pCR were analyzed by Fisher's exact test, Pearson's χ^2 test, univariable and multivariable logistic regression using IBM SPSS statistics 19 (IBM Cooperation, Armonk, NY) and GraphPad Prism 5 (GraphPad software, La Jolla, CA). The following clinicopathological parameters were included in the analysis: age, tumor size, histological type, grade, nodal status, HR and HER2 status. All *P*-values were defined as statistically significant when <0.05.

results

baseline clinical data and SPARC expression

A total of 667 patients were included in the analysis; the consort statement is shown in Figure 1. Clinicopathological characteristics are given in Table 1.

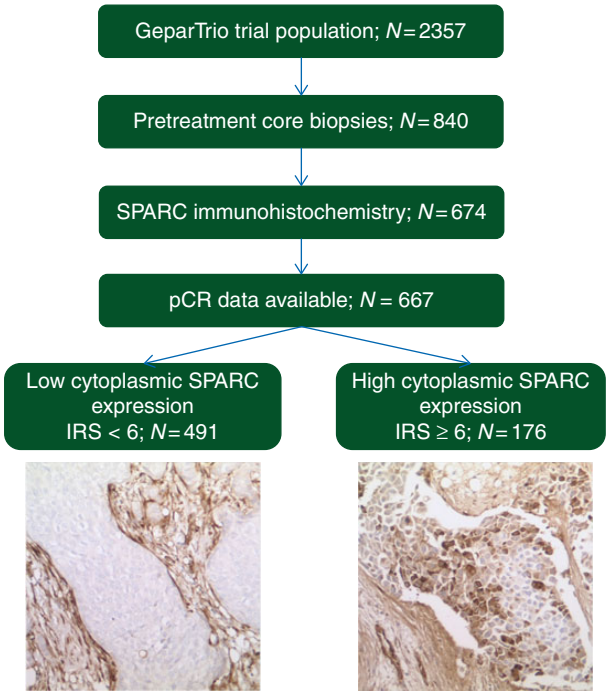

Figure 1. Consort statement and immunohistological SPARC expression in tumors from patients of the GeparTrio study.

The cytoplasmic expression of SPARC in invasive breast cancer tumor cells was high (IRS ≥6) in 176 samples (26%) and low (IRS <6) in 491 cases (74%) (Figure 1). Analyses of molecular breast cancer subtypes revealed an increased SPARC expression in patients with triple-negative breast cancer (TNBC) (37%) compared with HR (HR+/HER2− 23%) or HER2-positive subtypes (HR+/HER2+ 29%; HR−/HER2+ 22%; Figure 2A and Table 1; $P = 0.037$, Pearson's χ^2 test). No further correlations of SPARC expression in tumor cells with other clinicopathological parameters including age, tumor size, histological type, grade and nodal status could be seen (Table 1).

A stromal SPARC expression was observed in almost all samples. Only 7 (1%) were negative for stromal SPARC, 141 (21%) showed a weak, 387 (58%) a moderate and 115 (17%) a strong expression. In 17 (3%) samples, the spots contained no stromal component. There were no significant correlations between stromal SPARC expression and clinicopathological parameters.

Table 1. Patient characteristics and association of SPARC expression with clinicopathological data

Characteristic	No. of patients (%)			
	All cases	SPARC low	SPARC high	*P*-value
All patients	667 (100)	491 (73.6)	176 (26.4)	
Age				0.536
<50 years	295 (44.2)	221 (74.9)	74 (25.1)	
≥50 years	372 (55.8)	270 (72.6)	102 (27.4)	
Stage				0.128
T1–2	436 (65.4)	332 (76.1)	104 (23.9)	
T3–4	216 (32.4)	152 (70.4)	64 (29.6)	
Missing	15 (2.2)			
Histologic type				0.269
Lobular	56 (8.4)	45 (80.4)	11 (19.6)	
Ductal	611 (91.6)	446 (73.0)	165 (27.0)	
Tumor grade				0.090
G1–2	519 (77.8)	390 (75.1)	129 (24.9)	
G3	147 (22.0)	100 (68.0)	47 (32.0)	
Missing	1 (0.1)			
Nodal status				0.529
Negative	285 (42.7)	206 (72.3)	79 (27.7)	
Positive	358 (53.7)	267 (74.6)	91 (25.4)	
Missing	24 (3.6)			
HR status				0.053
Positive	512 (76.8)	388 (75.8)	124 (24.2)	
Negative	144 (21.6)	97 (67.4)	47 (32.6)	
Missing	11 (1.6)			
HER2 status				0.744
Negative	516 (77.4)	381 (73.8)	135 (26.2)	
Positive	137 (20.5)	99 (72.3)	38 (27.7)	
Missing	14 (2.1)			
Molecular subtype				0.038
HR+/HER2−	405 (60.7)	311 (76.8)	94 (23.2)	
HR+/HER2+	99 (14.8)	70 (70.7)	29 (29.3)	
HR−/HER2+	36 (5.6)	28 (77.8)	8 (22.2)	
HR−/HER2−	104 (15.6)	66 (63.5)	38 (36.5)	
Missing	23 (3.4)			
Pathological response				<0.001
No-pCR	546 (81.9)	418 (76.6)	128 (23.4)	
pCR	121 (18.1)	73 (60.3)	48 (39.7)	

pCR rate and survival analysis

A high expression of the SPARC protein in tumor cells was associated with an increased pCR rate in the overall study population ($P < 0.001$, Fisher's exact test). Tumors with a low SPARC protein expression had a pCR rate of 15%, whereas pCR rate was 27% in SPARC high expressing tumors (Figure 2B). In a subanalysis restricted to the TNBCs, the pCR rate was 26% in low SPARC expressing TNBCs and 47% in tumors with high SPARC expression ($P = 0.032$, Fisher's exact test; Figure 2C).

In univariable and multivariable logistic regression analysis of predictive factors for pCR to neoadjuvant chemotherapy adjusted for standard clinicopathological factors, SPARC remained significantly associated with response to chemotherapy, both for the complete cohort (Table 2) as well as for the triple-negative subset (Table 3).

In multivariable analysis, SPARC ($P = 0.010$), HR status ($P < 0.001$) and HER2 status ($P = 0.001$) were independently predictive for pCR in the overall study population (Table 2). In the molecular subgroup of patients with TNBC, only SPARC ($P = 0.036$) and age at diagnosis ($P = 0.044$) were independent predictive factors for pCR (Table 3).

To verify the stability of our findings, we carried out additional univariable logistic regression analysis with slightly changed cut points for SPARC (IRS 0–3 versus 4–12 and IRS 0–7 versus 8–12) and the IRS as a continuous factor, which lead to

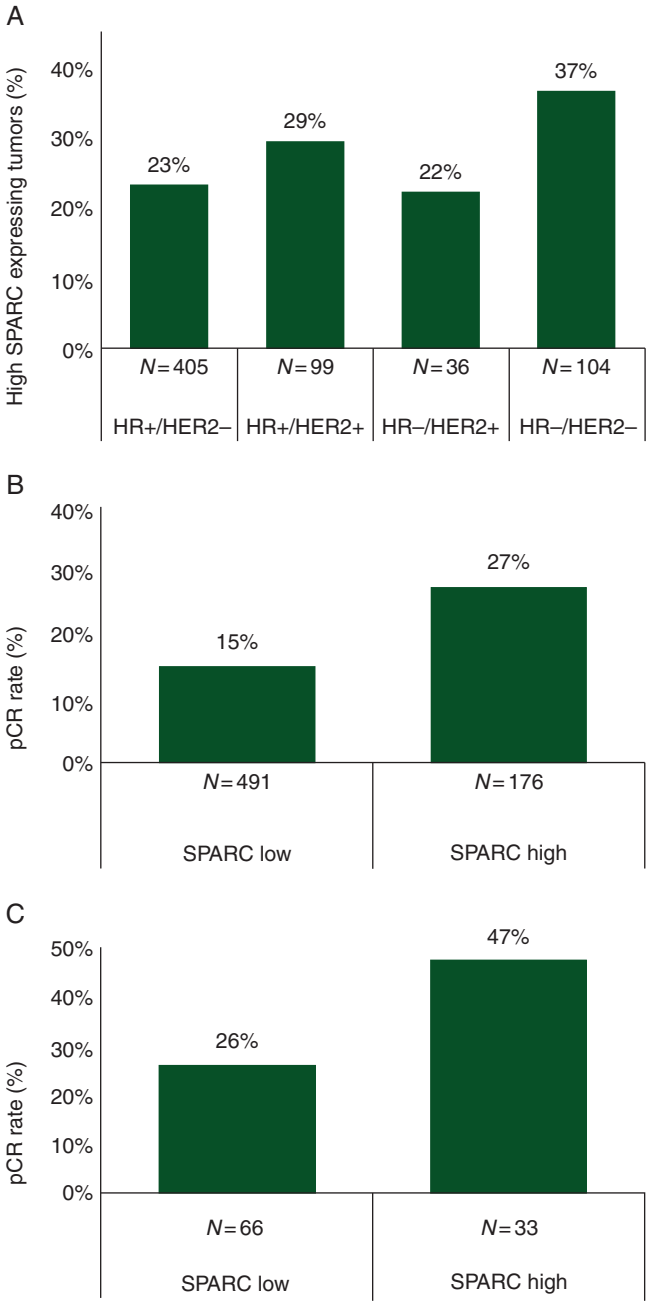

Figure 2. SPARC protein expression in different breast cancer subtypes. SPARC is increased in TNBC compared with other subtypes (A). Comparison of pCR rates in SPARC low and SPARC high expressing tumors in the whole study cohort (B) and the TNBC subgroup (C).

statistically significant results in the overall study cohort for all three approaches ($P = 0.020$, $P = 0.016$ and $P < 0.001$, respectively; data not shown). In the TNBC subgroup, the cutoff 0–3 versus 4–12 as well as the continuous analysis were significant ($P = 0.004$ and $P = 0.005$, respectively; data not shown).

In contrast to the predictive value for pCR, we did not detect a prognostic value of SPARC protein expression for overall or disease-free survival. Only within the TNBC, a nonsignificant trend towards a longer disease-free survival in patients with a high SPARC expression could be observed (data not shown).

discussion

In this study, we analyzed SPARC protein expression in human breast cancer samples. We could show that SPARC is frequently expressed in breast tumors, and especially high expression rates were seen in the subgroup of triple-negative tumors. Furthermore, patients with high SPARC expression in the primary tumor were more likely to achieve a pCR after TAC or TAC-NX chemotherapy.

For breast cancer tissue, an increased expression of SPARC compared with healthy breast tissue was described [8, 17]. Several studies have evaluated the prognostic relevance of SPARC expression in breast cancer tissue. In the majority of experimental and translational studies, SPARC expression is associated with promotion of tumor growth and metastasis, more aggressive tumor types and worse prognosis [8, 9, 18–20].

Our study demonstrates that SPARC is frequently expressed in all biological breast cancer subtypes. The highest expression rates were observed in the molecular subgroup of TNBC. However, no statistically significant correlation of SPARC expression with overall and progression-free survival could be observed in our study. In the triple-negative subset, a nonsignificant trend for better survival for SPARC positive tumors could be observed, which has to be further evaluated with longer follow-up periods.

Azim et al. evaluated SPARC mRNA expression according to molecular breast cancer subtypes and its association to response *in silico* [21]. They found SPARC to be significantly higher expressed in the luminal-A subtype. An association between high SPARC and low pCR rate was found in the HER2 subtype. The main difference to our study is the focus on mRNA expression, which has the advantage that the quantification is more straightforward. However, on the mRNA level, it is not possible to separate stromal and intratumoral SPARC expression. This may explain the differences to our findings.

We could show that SPARC is an independent predictive factor for response to TAC or TAC-NX chemotherapy in the neoadjuvant setting. High SPARC expression was correlated with a higher probability of achieving pCR after chemotherapy in the overall study population as well as the subgroup of patients with triple-negative cancer. To our knowledge, this is the first time this biologic role of SPARC could be shown for breast cancer patients treated with neoadjuvant chemotherapy. This finding is of special interest as SPARC is a potential biomarker for the response to treatment with chemotherapeutics.

A weakness of our study is that all participating patients were treated with an anthracycline–taxane-based chemotherapy, and therefore it is not possible to evaluate whether SPARC is suitable to select patients that benefit from a particular treatment. The observed higher response rate in SPARC high expressing patients could be due to a sensitivity of the primary tumor to chemotherapeutic treatment in general. A possible clinical utility for SPARC analysis is only given in the context of nab-paclitaxel treatment, in this setting, further validations are needed.

The strength of our study is a large, well-characterized cohort from a neoadjuvant clinical trial with predefined treatment as well as corresponding response and outcome data. The incomplete tissue availability from the initial trial population could be seen as a limitation. Furthermore, in this setting, it is not

Table 2. Univariable and multivariable analysis for prediction of pathological complete response (pCR) in the complete GeparTrio cohort

	% pCR	Univariable analysis		Multivariable analysis	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
SPARC					
Low	14.2	1		1	
High	27.3	2.15 (1.42–3.25)	<0.001	1.84 (1.15–2.95)	0.010
Age					
≥50 years	14.2	1		1	
<50 years	23.1	1.80 (1.21–2.68)	0.004	1.48 (0.95–2.30)	ns
Tumor stage					
T3–4	14.8	1		1	
T1–2	19.5	1.39 (0.89–2.17)	ns	1.61 (0.98–2.64)	ns
Histotype					
Lobular	3.6	1		1	
Ductal	19.5	6.53 (1.57–27.17)	0.010	3.06 (0.72–13.10)	ns
Tumor grade					
G1–2	15.6	1		1	
G3	27.2	2.02 (1.31–3.12)	0.001	1.47 (0.89–2.42)	ns
Nodal status					
Negative	17.2	1		1	
Positive	19.0	1.13 (0.75–169)	ns	1.09 (0.69–1.71)	ns
HR					
Positive	12.5	1		1	
Negative	36.1	3.96 (2.58–6.08)	<0.0005	3.10 (1.92–4.98)	<0.001
HER2					
Negative	15.3	1		1	
Positive	28.5	2.20 (1.42–3.24)	<0.0005	2.23 (1.37–3.65)	0.001

Table 3. Univariable and multivariable analysis for prediction of pathological complete response (pCR) in the TNBC subgroup of GeparTrio

	% pCR	Univariable analysis		Multivariable analysis	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
SPARC					
Low	25.8	1		1	
High	47.4	2.59 (1.12–6.02)	0.027	2.64 (1.07–6.55)	0.036
Age					
≥50 years	22.4	1		1	
<50 years	47.8	3.17 (1.36–7.39)	0.007	2.52 (1.02–6.21)	0.044
Tumor stage					
T3–4	21.2	1		1	
T1–2	39.1	2.39 (0.91–6.26)	ns	2.43 (0.84–7.03)	ns
Tumor grade					
G3	30.9	1		1	
G1–2	36.7	1.30 (0.57–2.93)	ns	1.09 (0.44–2.739	ns
Nodal status					
Positive	33.3	1		1	
Negative	34.1	1.03 (0.45–2.38)	ns	1.18 (0.47–3.00)	ns

possible to evaluate SPARC as a predictive marker to nab-paclitaxel, a nanoparticle albumin-bound taxane. SPARC has the ability to bind albumin with a high affinity [22]; therefore, patients with SPARC expressing tumors could benefit from nab-paclitaxel treatment as the drug can be transported to the tumor in a targeted way and accumulate within the tissue. It has been suggested that this might lead to a higher effectivity and a better tolerance for the drug with less side-effects [23, 24].

Studies comparing treatment with solvent-based taxane and nab-paclitaxel showed a higher therapy response and a prolonged progression-free survival for patients treated with the nanoparticle albumin-bound variant of the drug [25, 26].

Nab-paclitaxel is approved for second-line therapy in advanced breast cancer.

Our findings highlight the important biological role of SPARC in breast cancer. Based on data from basic research projects, SPARC might become a promising new predictive biomarker in diverse cancer types and especially in breast cancer. Therefore, a further investigation in the context of clinical studies should be carried out. On the basis of the results of this study and to investigate SPARC as a predictive marker to nab-paclitaxel, we have implemented SPARC evaluation as a prospective biomarker in the neoadjuvant GeparSepto (NCT01583426) trial conducted by the German Breast Group (GBG), where patients are stratified to the treatment arms according to cytoplasmic SPARC expression in tumor tissue.

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disclosure

The authors have declared no conflict of interest.

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