The pathological complete response and secreted protein acidic and rich in cysteine expression in patients with breast cancer receiving neoadjuvant nab-paclitaxel chemotherapy

YUKO NAKAZAWA[1-3], SESHIRU NAKAZAWA[1], SASAGU KUROZUMI[1], MISATO OGINO[1,2], YUKIO KOIBUCHI[2], HIROKI ODAWARA[4], TETSUNARI OYAMA[3], JUN HORIGUCHI[5], TAKAAKI FUJII[1] and KEN SHIRABE[1]

[1]Department of General Surgical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511; [2]Department of Breast and Endocrine Surgery, National Hospital Organization Takasaki General Medical Center, Takasaki, Gunma 370-0829; [3]Department of Diagnostic Pathology, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511; [4]Department of Surgery, Toho Hospital, Midori, Gunma 376-0121; [5]Department of Breast Surgery, International University of Health and Welfare, Chiba 286-8686, Japan

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Abstract. Biomarkers that can accurately predict treatment response are required for indicating optimal neoadjuvant treatments. The current study assessed the predictive value of secreted protein acidic and rich in cysteine (SPARC) mRNA expression for the response to neoadjuvant nab-paclitaxel (nab-PTX) therapy in patients with breast cancer. It was hypothesized that SPARC expression can affect the response to albumin-bound taxanes, including nab-PTX since SPARC binds albumin with a high affinity. Pre-therapeutic specimens of core needle biopsies were analyzed from 50 patients in a phase II trial of neoadjuvant nab-PTX and the factors that were associated with a pathological complete response (pCR) were assessed. The pre-therapeutic tumor mRNA levels of chemotherapy-related proteins were quantified, including SPARC, and the correlations with post-therapeutic clinicopathological factors were assessed, including with pCR. The results demonstrated that pre-therapeutic SPARC mRNA expression was significantly higher in non-pCR patients compared with patients with pCR (92.37±55.33 vs. 56.53±30.19; P=0.027). A cutoff point of 48.5 was determined using receiver operating characteristic (ROC) curve analysis (sensitivity, 83.3%; specificity, 50.0%), and patients were classified into low and high SPARC expression groups. High SPARC expression was associated with histological grade (P=0.035), estrogen receptor expression (P=0.037), and progesterone receptor expression (P=0.002) but not with HER2 (P=0.895), and Ki-67 LI (P=0.743) expression. The results of the current study indicated that a high SPARC mRNA expression was a negative predictor of pCR following neoadjuvant nab-PTX therapy regardless of breast cancer subtype. The phase II study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the National Hospital Organization Takasaki General Medical Center (Registration nos. H23-9 and H23-33).

Correspondence to: Professor Takaaki Fujii, Department of General Surgical Science, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan
E-mail: ftakaaki@gunma-u.ac.jp

Abbreviations: SPARC, secreted protein acidic and rich in cysteine; nab-PTX, nab-paclitaxel; pCR, pathological complete response; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ER, estrogen receptor; PgR, progesterone receptor; AST, aspartate aminotransferase; ULN, upper limit of normal; ALT, alanine aminotransferase; FISH, fluorescence *in situ* hybridization; RT-qPCR, quantitative reverse-transcription PCR; LI, labeling index; FFPE, formalin-fixed, paraffin-embedded; FEC, 5-FU, epirubicin and cyclophosphamide; HER, trastuzumab

Key words: secreted protein acidic and rich in cysteine, nab-paclitaxel, breast cancer, pathological complete response, neoadjuvant therapy

Introduction

Breast cancer is the most common cancer in women and is a leading cause of mortality worldwide (1). Treatment strategies have been constantly evolving and chemotherapy has shifted from postoperative administration to preoperative therapy, or neoadjuvant therapy. Good response to neoadjuvant therapy allows patients to be treated using breast-conserving surgery rather than using mastectomy (2). Furthermore, a pathological complete response (pCR) after neoadjuvant therapy improves survival (3), particularly in subtypes such as triple-negative breast cancer (4). Breast cancer subtypes have different molecular profiles and biological behaviours and, thus, require individualized therapies (5). Patients who do not receive optimal chemotherapy suffer unnecessary toxic side effects. Therefore, pre-therapeutic biomarkers that can adequately predict treatment response, particularly of pCR, are necessary for selecting the most adequate neoadjuvant chemotherapy for each patient. So far, several biomarkers, such as thymidylate synthase (TS) (6), dihydropyrimidine

dehydrogenase (DPD) (7), ATP-binding cassette, sub-family B, member 1 (MDR1) (8), ATP-binding cassette, sub-family C, member 1 (MRP1) (9), and topoisomerase (DNA) II alpha (Topo IIα) (10,11), have attracted attention as predictive factors of treatment response to chemotherapy.

Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin or BM-40, is an albumin-binding glycoprotein that is secreted by cells to modulate their interactions with the extracellular matrix (12-18). SPARC plays a critical role in the regulation of cellular functions, such as proliferation and cell migration, and its overexpression is associated with tumor growth, metastasis, and aggressiveness (12-19). Studies have revealed the association of high SPARC expression with poor prognosis and treatment response in breast cancer (12,19-21). A high SPARC expression evaluated by IHC has been reported to be associated with a high treatment response (20), whereas a high SPARC expression assessed by mRNA levels has been reported to be associated with low treatment response in breast cancer (21). The role of SPARC in breast cancer has not yet been established and a more focused analysis between SPARC expression and response to specific treatments is necessary to use SPARC as a biomarker.

In this study, we focused on the predictive role of SPARC in response to neoadjuvant treatment with nab-paclitaxel (nab-PTX), which is a nanoparticle albumin-bound taxane drug used as neoadjuvant treatment for breast cancer. We analyzed the pre-treatment specimens of a phase II trial of neoadjuvant nab-PTX chemotherapy for breast cancer. A previous study that compared treatment with nab-PTX and docetaxel has shown a higher therapy response and prolonged progression free survival for patients treated with nab-PTX (22). Also, ongoing trials, such as the phase III GeparSepto trial, have shown that a regimen including nab-PTX achieves higher pCR rates than a regimen with solvent-based PTX (23). Since SPARC binds albumin with high affinity, we hypothesized that SPARC expression levels can affect the response to albumin-bound taxanes such as nab-PTX.

The purpose of our study was to evaluate the predictive value of SPARC mRNA expression for the response to neoadjuvant nab-PTX therapy in breast cancer patients. We analyzed patient specimens from a phase II trial involving nab-PTX and evaluated the association of pre-treatment SPARC mRNA expression with the response to neoadjuvant nab-PTX treatment. Our results suggested that SPARC mRNA expression in breast cancer is a negative predictor of treatment response to neoadjuvant nab-PTX therapy.

Materials and methods

Patients and data. We retrospectively analyzed data from a total of 50 consecutive patients who were enrolled in a single center phase II trial of neoadjuvant nab-PTX therapy (National Hospital Organization Takasaki General Medical Center, Takasaki, Japan) between May 2011 and September 2013. We collected the clinicopathological data such as age, tumor subtype, tumor staging (based on the Union for International Cancer Control TNM classification, 7th edition). Immunohistochemistry (IHC) analysis of hormone receptors [estrogen (ER) or progesterone (PgR)], HER2 score, and Ki67 expression of the primary tumor was assessed via our staining platform as previously described (24). Using quantitative reverse-transcription PCR (RT-qPCR), we evaluated the intratumoral mRNA levels of SPARC and other chemotherapy-related genes as follows; TS, DPD, MDR1, MRP1, and Topo IIα. We defined the state of pCR as the absence of any invasive cancer in the breast and in lymph nodes (ypT0/ypTis, ypN0).

Treatment protocol. All patients underwent core needle biopsy prior to receiving nab-PTX as neoadjuvant therapy and then underwent standard breast cancer surgery (Fig. 1). For HER2-negative breast cancer patients, neoadjuvant chemotherapy comprised the administration of nab-PTX, followed by the administration of 5-FU, epirubicin, and cyclophosphamide. For HER2-positive breast cancer patients, neoadjuvant chemotherapy comprised the concurrent administration of nab-PTX and trastuzumab, followed by surgery and post-operative administration of trastuzumab for one year. Surgery was performed 6 months after treatment initiation, and the operative method (mastectomy or breast-conserving surgery) was selected based on the post-treatment tumor size and patient's preference. Sentinel lymph node dissection was performed for patients who were preoperatively diagnosed as negative for lymph node metastasis, and axillary lymph node dissection was performed for all patients who were suspected or diagnosed as positive for lymph node metastases. We enrolled patients with cytologically or histologically confirmed unilateral primary breast cancer, aged between 20 and 75 years, with an ECOG performance status of grade 0 or 1, and without any prior breast cancer treatment. Further eligibility criteria were: No severe comorbidities such as uncontrollable diabetes, infection, cardiac disease, or psychological symptoms; no interstitial lung disease confirmed on chest radiography; no brain metastases; no history of severe drug allergy; no concurrent malignant disease; no history of inflammatory breast cancer; and no pregnancy. Laboratory requirements included white blood cell counts $\geq 4.0 \times 10^3$ cells per mm^3, neutrophil counts $\geq 2.0 \times 10^3$ cells per mm^3, platelets $\geq 100 \times 10^3$ cells per mm^3, hemoglobin level ≥9.0 g/dl, aspartate aminotransferase (AST) level ≤2.5x upper limit of normal (ULN), alanine aminotransferase (ALT) level ≤2.5xULN, total bilirubin level ≤1.5 mg/dl, and creatinine level ≤1.5 mg/dl. Additional requirement for HER2-positivity were 3+ HER2 by IHC or 2+ by IHC and positive by fluorescence *in situ* hybridization (FISH) and only tumors with a diameter of >1 cm were included for HER2-positive breast cancer. Informed consent was obtained from all patients prior to enrollment in the study. The phase II study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the National Hospital Organization Takasaki General Medical Center (Registration numbers: H23-9 and H23-33).

Macro-dissection and analysis of mRNA expression. We performed macro-dissection of tumor cells in core needle biopsy specimens to exclude the influence from stromal tissue contamination and quantified the expression levels of chemotherapy-related factors using RT-qPCR. A pathologist reviewed representative hematoxylin and eosin-stained slides from formalin-fixed, paraffin-embedded (FFPE) core needle biopsy specimens. Tumor tissue was selected and dissected via manual macro-dissection (Fig. S1).

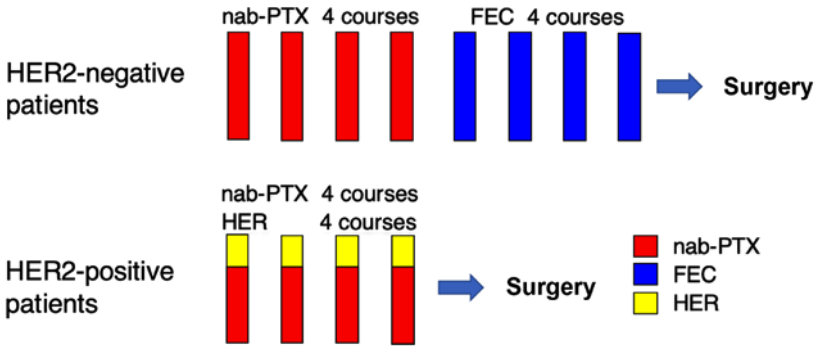

Figure 1. Treatment protocol of phase II trial of neoadjuvant nab-paclitaxel. For patients that are HER2-negative, nab-PTX (260 mg/m^2) was administered every 3 weeks for 4 courses, followed by administration of FEC (500 mg/m^2 5-FU, 100 mg/m^2 epirubicin and 500 mg/m^2 cyclophosphamide) every 3 weeks for 4 courses. For HER2-positive patients, nab-PTX (260 mg/m^2) and trastuzumab (initial dose 8 mg/kg, sequential dose 6 mg/kg) was administered every 3 weeks for 4 courses. HER2, human epidermal growth factor receptor 2; nab-PTX, nab-paclitaxel; FEC, 5-FU, epirubicin and cyclophosphamide; HER, trastuzumab.

RNA was isolated from the tumor tissues using the RNeasy FFPE Kit (Qiagen). cDNA was prepared using High Capacity Reverse Transcription kit (Life Technologies) according to the manufacturer's instructions. SPARC, TS, DPD, MDR1, MRP1, and Topo IIα expression levels were determined using TaqMan real-time PCR (TaqMan array card; Life Technologies) after TaqMan assay-based pre-amplification. Briefly, 2.5 μl cDNA was pre-amplified using the TaqMan PreAmp Master Mix (2x) and a pool of TaqMan® Gene Expression Assays (0.2x) in a 10-μl PCR reaction. The pre-amplification cycling conditions included 95˚C for 10 min followed by 14 cycles of 95˚C for 15 sec and 60˚C for 4 min. Each amplified cDNA sample was diluted 20 times in TE buffer. Amplified cDNA (25 μl) was added to 25 μl RNase-free water and 50 μL of 2x TaqMan Gene Expression Master Mix. The mixture was then transferred to a loading port for the TaqMan array card. The array card was centrifuged twice and sealed, and PCR was performed using the Applied Biosystems Prism 7900HT Sequence Detection system (Life Technologies). The thermocycler protocol included the following conditions: 50˚C for 2 min and 94.5˚C for 10 min, followed by 40 cycles of 97˚C for 30 sec and 59.7˚C for 1 min. Beta-actin was used as an internal standard for normalization. The gene expression (relative mRNA) levels were expressed as ratios (differences between the Ct values) between the gene of interest and the reference gene. The assay IDs used in the array card are shown in Table SI.

Immunohistochemical evaluation and subtype classification. IHC analysis was performed using the core needle biopsy samples. A pathologist assessed the expressions of hormone receptors (ER and PgR), HER2, and Ki67 in all the specimens. ER and PgR expression levels were scored from 0 to 8 according to the Allred score (25) and expression was classified as negative from 0 to 3 and positive from 4 to 8. HER2 expression was positive if the results were 3+ or 2+ by IHC and positive by FISH. The Ki67 score was calculated at hot spots and classified as low if ≤30% and as high if >30%. To assess the correlation of SPARC mRNA expression with its protein expression, IHC staining of SPARC was performed (n=10). The cytoplasmic expression of SPARC was classified as low, medium, or high (Fig. 2). The antibodies used were anti-ER (SP1; Ventana Medical Systems), anti-PgR (1E2; Ventana Medical Systems), anti-HER2 (4B5; Ventana Medical Systems), anti-Ki-67 (30-9; Ventana Medical Systems), and anti-SPARC (ON1-1; Thermo Fisher Scientific). Breast cancer subtypes were defined according to the IHC results as luminal type (ER-positive, HER2-negative), luminal-HER2 type (ER-positive and HER2-positive), HER2 type (ER-negative and HER2-positive), and triple-negative (ER-negative and HER2-negative) breast cancer.

Statistical analysis. Statistical analysis was performed using Mann-Whitney's U test and Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. ROC curve analysis was used to assess the cutoff point of mRNA SPARC expression between pCR and non-pCR. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using the IBM SPSS Statistics software (v24, IBM Corp.).

Results

Patient characteristics. The median age of patients was 55 years (range, 30-75 years). We found 30.0% luminal type, 18.0% luminal-HER2 type, 22.0% HER2 type, and 30.0% triple-negative breast cancer patients. All patients with luminal type breast cancer enrolled in the phase II trial had lymph node metastasis. ER, PgR, and HER2 expressions were positive in 48.0, 36.0, and 40.0% of patients, respectively. The mean score of Ki67 was 48.2±33.2%, with 36.0% of patients exhibiting low Ki-67 expression and 64.0% high Ki-67 expression. Fourteen (28.0%) patients achieved pCR after neoadjuvant therapy including nab-PTX.

Intra-tumor mRNA expression of chemotherapy-related proteins. The correlations between the intra-tumor mRNA levels of SPARC, TS, DPD, MDR1, MRP1, and Topo IIα and the treatment response were assessed (Table I). SPARC mRNA expression was significantly higher in the non-pCR group (P=0.027). Also, TS mRNA expression was significantly higher in the pCR group (P=0.030). However, other markers as DPD, MDR1, MRP1, and Topo IIα were not the significant predictive markers of pCR. The intensity of SPARC expression detected by IHC correlated with SPARC mRNA expression levels (P=0.043; Fig. 3).

Table I. Association between mRNA expression of chemotherapy-related factors and pCR.

mRNA	All cases (n=50)	Non-pCR cases (n=36)	pCR cases (n=14)	P-value
SPARC	82.34±51.89	92.37±55.33	56.53±30.19	0.027
TS	2.69±2.38	2.24±1.76	3.85±3.31	0.030
DPD	4.65±2.05	4.75±2.06	4.40±2.06	0.593
MDR1	0.46±0.43	0.51±0.46	0.32±0.31	0.179
MRP1	0.90±0.58	0.96±0.67	0.75±0.22	0.241
TopoIIα	10.01±9.01	8.91±7.57	11.72±10.37	0.411

All values are mean ± SE. pCR, pathological complete response; SPARC, secreted protein acidic and rich in cysteine; TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; MDR1, ATP-binding cassette, sub-family B, member 1; MRP1, ATP-binding cassette, sub-family C, member 1; Topo IIα, Topoisomerase (DNA) II alpha.

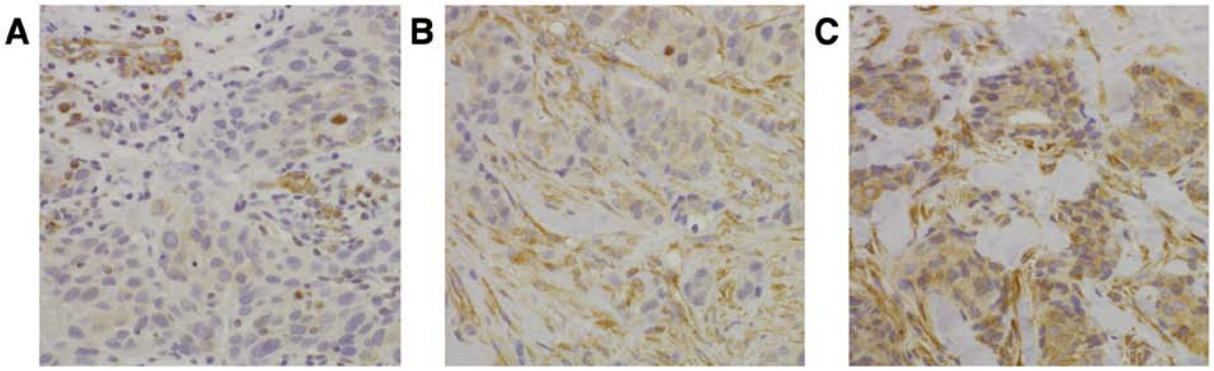

Figure 2. Immunohistochemistry analysis of cytoplasmic SPARC expression in breast cancer specimens. (A) Low SPARC expression (magnification, x200). (B) Medium SPARC expression (magnification, x200). (C) High SPARC expression (magnification, x200). SPARC, secreted protein acidic and rich in cysteine.

Analysis according to SPARC expression. The ROC curve for the relative mRNA SPARC expression between pCR and non-pCR is shown in Fig. S2. The area under the curve was 0.700, and the cutoff point was set at 48.5 (sensitivity, 83.3%; specificity, 50.0%). Patients were classified into low and high SPARC expression groups (Table II). Patients in the low SPARC expression group had significantly higher pCR rates (P=0.029). We found no differences in the mean age (P=0.467) and tumor staging (P=0.507) between patients in the two groups. However, patients with low SPARC mRNA expression had a significantly higher histological grade (P=0.035), lower ER expression (P=0.037), and lower PgR expression (P=0.002) in core needle biopsy specimen. In contrast, there were no significant differences in the HER2 (P=0.895), and Ki-67 LI (P=0.285) expressions.

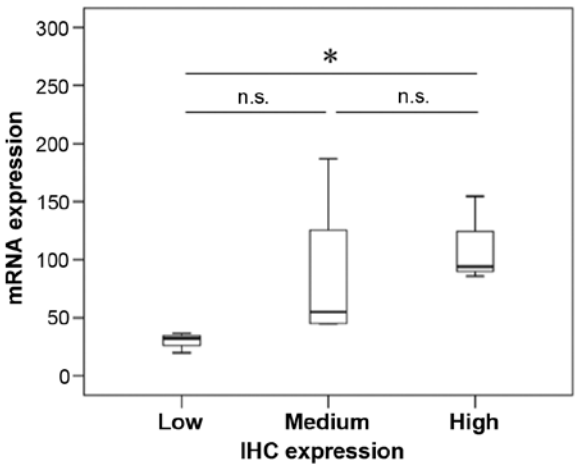

Figure 3. Correlation between SPARC mRNA values and IHC expression. Expression of SPARC mRNA indicated a significant difference between low (n=3), medium (n=4) and high SPARC expression groups (n=3) detected using IHC (P=0.043). The x-axis represents expression of SPARC classified according to IHC and the y-axis represents the mRNA expression assessed using RT-qPCR. *P<0.05; SPARC, secreted protein acidic and rich in cysteine; IHC, immunohistochemistry.

Discussion

Our study revealed that the pre-therapeutic SPARC mRNA expression was significantly higher in the non-pCR patients than in the pCR patients after neoadjuvant nab-PTX therapy. Conclusively, our results suggested that the relative SPARC mRNA expression level predicts the treatment response to neoadjuvant nab-PTX therapy in breast cancer patients.

SPARC is a multifunctional matricellular glycoprotein that controls physiological and pathological processes, such as cellular differentiation, development, remodeling, cell turnover, and tissue repair (12-18). It is highly expressed in several types of tumors, such as melanoma (26), glioblastoma (27), prostate (28), colorectal (29), pancreatic (30), and gastric (31) cancers. This overexpression in tumors suggests

Table II. Association between SPARC mRNA expression and clinicopathological features.

Characteristics	SPARC expression		P-value
	Low expression (n=13)	High expression (n=37)	
Age (years)			0.467
Mean ± SE	57.5±12.4	54.7±12.2	
Range	36-72	30-75	
Stage			0.507
I	2	6	
II	10	23	
III	1	8	
Tumor size (cm)			0.545
Mean ± SE	3.0±1.1	2.8±1.4	
Range	1.8-5.7	1.1-7.8	
Histological grade			0.035
Grade 1-2	2	18	
Grade 3	11	19	
Nodal status			0.191
Negative	8	15	
Positive	5	22	
ER			0.037
Negative	10	16	
Positive	3	21	
PgR			0.002
Negative	13	19	
Positive	0	18	
HER2			0.895
Negative	8	22	
Positive	5	15	
Ki-67 labeling index (%)			0.285
Mean ± SE	56.69±34.69	44.53±33.00	
Range	9-99	3-98	
Ki-67			0.743
Low (≦30%)	4	14	
High (30%<)	9	22	
Missing	0	1	
IHC based subtypes			0.219
Luminal	2	13	
Luminal-HER2	1	8	
HER2	4	7	
Triple-negative	6	9	
pCR			0.029
No	6	30	
Yes	7	7	

SPARC, secreted protein acidic and rich in cysteine; NG, nuclear grade; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; pCR, pathological complete response.

that SPARC promotes tumor development and is a potential treatment target. Although the association of high SPARC expression with some cancers remains controversial (32), several studies have reported high SPARC expressions in breast cancers (19-21). Moreover, SPARC is reportedly expressed in the juxta-tumoral stromal cells, indicating its possible role in breast cancer invasion (33). Yet, its prognostic role in breast cancer remains indeterminate, and the reports have been contradictory. Some studies have found that high SPARC expression is associated with low overall survival (19-21), whereas others have reported that low SPARC expression is associated with low disease-free and overall survival (34). Moreover, the association of SPARC expression with breast cancer subtypes also varies between studies. It has also been frequently expressed in triple-negative breast cancer (35) or has shown an inverse correlation with ER expression, thereby associating with less differentiated and more aggressive tumors (36).

An important advance resulting from our study is the finding that low SPARC mRNA expression is associated with higher pCR rates after neoadjuvant nab-PTX therapy. Thus far, the prognostic value of SPARC expression as a marker of treatment response remains controversial. For example, a previous study has reported an association of high SPARC expression with low pCR rates in HER2-type breast cancer patients (21), whereas another study has reported no association of SPARC expression with the response to nab-PTX therapy in metastatic breast cancer patients (35). In addition, high SPARC expression has been reported to be associated with a high pCR rate after treatment including docetaxel, doxorubicin, and cyclophosphamide (36). These conflicting results may be caused by differences in treatment protocol, ratio of breast cancer subtypes enrolled in the study, and methods used for sample analysis (19-21,34-36). Thus, for overcoming the difference in treatment protocol, our study focused on patients who were enrolled in a study on phase II neoadjuvant nab-PTX therapy study within a single institute. In theory, because SPARC is an albumin-binding protein, its high expression in cancer cells and the surrounding stroma would increase the accumulation of albumin-bound drugs in the tumor, thereby leading to a higher efficiency and less side effects (37). Therefore, the initial hypothesis was that tumors with high SPARC expression would show better treatment response to nab-PTX therapy (38). However, our results were contrary to this hypothesis, and the low SPARC expression group showed higher pCR rates to nab-PTX therapy.

Perou *et al* initially suggested a molecular classification as the intrinsic subtypes for breast cancer (39,40). Response to specific treatments may vary according to breast cancer subtype. For example, triple-negative breast cancer patients showed an increased pCR rate in response to neoadjuvant chemotherapy with nab-PTX in the GeparSepto-GBG 69 study (23). The relation between SPARC expression and breast cancer subtypes is inconsistent between studies (21,23,36,41). We showed here that SPARC expression was associated with high PgR and ER expression. PgR is known to be induced by ER and acts as a key factor in induction, progression and maintenance of the neoplastic phenotype of ER-positive breast cancer (42,43). Also, recent clinical findings demonstrated that the PgR status is associated with low response to neoadjuvant

chemotherapy (44). Therefore, SPARC mRNA expression level might directly affect the ER/PgR signaling and thus treatment response. However, our results suggest that SPARC mRNA expression might predict pCR after neoadjuvant nab-PTX therapy not only in ER-positive breast cancer, but in all breast cancer subtypes. Further research is necessary to elucidate the biological mechanisms underlying the relationship between ER/PgR and SPARC expressions.

The diversity in the methods used for sample analysis to evaluate protein expression may also lead to differing results. For example, a high SPARC expression evaluated by IHC has been reported to be associated with a high pCR rate (20), whereas a high SPARC expression assessed by mRNA levels has been reported to be associated with low pCR rate (21). In the present study, we focused on tumor-specific expression using macro-dissection to extract tumor mRNA. Also, we evaluated expression of target proteins by RT-qPCR. SPARC is a secreted protein and, extracellularly secreted proteins cannot be intracellularly detected by IHC unless secretion is inhibited (45). Moreover, SPARC expression also exists in the stromal tissues and inclusion of stromal components can falsely elevate true SPARC expression levels in tumor cells. Indeed, a study on colorectal cancers has shown a decrease in SPARC expression after the microdissection of tumor components compared with the initial expression analyzed in the bulk undissected tumor (46). Previously, a study on ovarian cancers has reported that the use of different SPARC antibodies can result in inconsistencies in the SPARC expression patterns (32). We confirmed the positive correlation between mRNA and protein expressions of SPARC in a small cohort. To be reliable and representable for SPARC-IHC scoring, further analysis regarding the inter-observer and inter-institutional variability with a larger cohort is warrant.

In our patient cohort, ER, PgR, and HER2 expressions were positive in 48.0, 36.0, and 40.0% of patients, respectively, and triple-negative breast cancer patients were 30.0%. Our present translational research is based on a phase II trial of neoadjuvant nab-PTX chemotherapy including all breast cancer subtypes. The evaluation of the pathological response of neoadjuvant chemotherapy have mainly been determined based on the results of NSABP protocol B-18 (47) and B-27 (48). These studies confirmed the utility of pCR as a prognositic surrogator for breast cancer patient with neoadjuvant chemotherapy. However, von Minckwitz G *et al* (4) suggested that pCR is a potent surrogate marker to predict the prognosis in most patients with breast cancer, but not in patients with ER-positive tumors. However, they also demonstrated that pCR was predictive of good survival rate in ER-positive tumors with high tumour proliferation (49). Therefore, ER-positive early-stage breast cancer patients with low tumour proliferation usually undergo surgery at first and thus do not meet the eligibility criteria of our phase II trial. This might be a reason why our study population had low rate of breast cancer with hormone receptor expression and a high rate of triple-negative breast cancer patients.

We recognize several limitations to our study. First, this study is a part of a phase II trial conducted at a single institution, and its small sample size may have influenced the results. Further large-scale studies will be necessary to validate our findings of the relationship between SPARC expression and pCR rates based on the breast cancer subtype. Second, we did not assess the predictive value of SPARC expression in stromal cells, which may also affect the treatment response. Indeed, pancreatic and ovarian cancer cells have shown increased growth when implanted in SPARC-null mice (50,51), suggesting that SPARC expression in the surrounding tissues may affect tumor growth and drug delivery. However, recent reports have shown only a minimal correlation between nab-PTX delivery and SPARC expression in the hosts (52). Further studies are needed to explore the effects of SPARC mRNA expression in stromal cells in response to nab-PTX therapy.

In conclusion, we found that high SPARC mRNA expression was a negative predictor of pCR after neoadjuvant nab-PTX therapy. The pre-therapeutic analysis of SPARC mRNA expression in core needle biopsy specimens may be valuable for selecting the optimal patients for neoadjuvant nab-PTX therapy regardless of their breast cancer subtype. Our results suggest that a high SPARC expression in tumor cells indicates that regimens other than nab-PTX should be selected. A preoperative panel of tumor-specific mRNAs including SPARC may lead to a more tailored selection of neoadjuvant treatment regimen for each patient.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YN and SN analyzed data and wrote the initial draft of the manuscript. YN, SN, MO, HO and YK collected data and were involved in the initial study conception and design. TO contributed to the analysis and assessment of pathological data. SN, SK, MO, YK, TO, TF, JH and KS interpreted the results and were involved in drafting the manuscript and revising the manuscript critically for important intellectual content. TF gave final approval of the version to be published. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the National Hospital Organization Takasaki General Medical Center (registration nos. H23-9 and H23-33). Written informed consent was obtained from all patients prior to enrollment in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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