

RESEARCH ARTICLE

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Carbonic anhydrase 9 is associated with chemosensitivity and prognosis in breast cancer patients treated with taxane and anthracycline

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Abstract

Background: Neoadjuvant chemotherapy (NAC) is one of the standard care regimens for patients with resectable early-stage breast cancer. It would be advantageous to determine the chemosensitivity of tumors before initiating NAC. One of the parameters potentially compromising such chemosensitivity would be a hypoxic microenvironment of cancer cells. The aim of this study was thus to clarify the correlation between expression of the hypoxic marker carbonic anhydrase-9 (CA9) and chemosensitivity to NAC as well as prognosis of breast cancer patients.

Methods: A total of 102 patients with resectable early-stage breast cancer was treated with NAC consisting of FEC (5-fluorouracil, epirubicin, and cyclophosphamide) followed by weekly paclitaxel before surgery. Core needle biopsy (CNB) specimens and resected tumors were obtained from all patients before and after NAC, respectively. Chemosensitivity to NAC and the prognostic potential of CA9 expression were evaluated by immunohistochemistry.

Results: CA9 positivity was detected in the CNB specimens from 47 (46%) of 102 patients. The CA9 expression in CNB specimens was significantly correlated with pathological response, lymph node metastasis, and lymph-vascular invasion. Multivariate analysis revealed that the CA9 expression in CNB specimens was an independent predictive factor for pathological response. The Kaplan-Meier survival curve revealed a significant negative correlation ($p = 0.013$) between the disease-free survival (DFS) and the CA 9 expression in resected tissues after NAC. Multivariate regression analyses indicated that the CA9 expression in resected tissues was an independent prognostic factor for DFS.

Conclusions: CA9 expression in CNB specimens is a useful marker for predicting chemosensitivity, and CA9 expression in resected tissue is prognostic of DFS in patients with resectable early-stage breast cancer treated by sequential FEC and weekly paclitaxel prior to resection.

Keywords: Breast cancer, Carbonic anhydrase 9, Neoadjuvant chemotherapy, Predictive marker, Chemosensitivity

Background

Neoadjuvant chemotherapy (NAC) increases the rate of breast-conserving surgery and decreases the risk of post-operative recurrence as effectively as adjuvant chemotherapy; thus, it might be considered to enhance survival [1,2]. For this reason, NAC has been one of the standard care regimens for patients with various types of carcinomas,

including resectable early-stage breast cancer [3]. The optimal regimen for NAC in breast cancer involves a sequential or concomitant anthracycline-containing regimen and taxane [4,5]. The aim of NAC for breast cancer is to reduce the size of the primary tumor, thereby increasing the likelihood of breast conservation [6], and might allow evaluation of the therapeutic effects that facilitate the strategies of post-operative chemotherapy [7]. Recent studies have demonstrated that the response status after NAC is correlated with improved disease-free survival (DFS) and overall survival (OS) in breast tumors [5,8]. NAC for breast cancer has a pathologic complete response (pCR) rate of approximately 30% [6,9,10] and a clinical

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complete response (cCR) rate of approximately 60% [10]. In contrast, NAC is ineffective in approximately half of all patients, and many experience toxicity. Therefore, it would be advantageous to identify patients with chemosensitive tumors before initiating NAC, to avoid potential therapy-related complications and an inappropriate delay of surgical treatment.

NAC has numerous advantages, including the provision of pathological response data that can be used as a surrogate marker for long-term clinical outcomes [11,12]. Also, the assessment of responsiveness to NAC allows the evaluation of potential predictive molecular markers for chemosensitivity. Several biological markers, including the estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki-67, p21, p53, Bcl, multi-drug-resistant P-glycoprotein, and topoisomerase 2A, have recently been investigated; however, there exists no clear correlation between the expression of these markers and chemosensitivity after sequential taxane- and anthracycline-based chemotherapies [13-17].

Carbonic anhydrase 9 (CA9) is a cell surface enzyme that catalyzes the reversible hydration of carbon dioxide to bicarbonate and a proton [18] and maintains pericellular pH homeostasis [19,20]. CA 9 is overexpressed in response to tumor hypoxia in many common tumor types [[21-24] and plays a critical role in hypoxia-associated tumor acidosis [[25-27]. Hypoxia-inducible factor-1 α (HIF-1 α) binds to the hypoxia-responsive element present in the promoter regions of CA9 and up-regulates CA9 expression [24,28]. Hypoxia plays an important role in tumor progression and chemoresistance in various types of cancer [29-32]. CA9 has been implicated in the regulation of the micro-environmental pH in tumor hypoxia. In this retrospective study, we examined the correlation between CA9 expression and chemosensitivity to NAC in breast cancer as well as the prognosis of patients.

Methods

Patients

A total of 102 patients with resectable early-stage breast cancer, which was considered to be stage IIA (T2 N0 M0), IIB (T2 N1 M0 or T3 N0 M0), or IIIA (T3 N1 M0), were treated with NAC from 2004 to 2009. Breast cancers were confirmed histopathologically by core needle biopsy (CNB) and were staged by computed tomography and bone scan. The clinicopathologic features of the 102 breast cancers are shown in Additional file 1: Table S1. The clinical stage was based on the TNM Classification of Malignant Tumors, 6th Edition [33]. No patients had evidence of distant metastasis at the time of surgery. All of the cases received neoadjuvant chemotherapy consisting of 4[cycles of 5-fluorouracil (5FU) 500 mg/m², epirubicin 75 or 100 mg/m², and cyclophosphamide 500 mg/m²

(FEC) followed by 12 cycles of weekly paclitaxel 80 mg/m² (wPTX). Sixteen of 102 patients showed HER2-positive breast cancer, and were administered weekly trastuzumab with wPTX. Patients underwent mastectomy or breast-conserving surgery after NAC. All patients who underwent breast-conserving surgery were administered postoperative radiotherapy. Overall survival time was set in days as the period from the initiation of NAC. DFS (disease-free survival) was defined as freedom from all local, regional, or distant recurrence. All patients were followed by physical examination, ultrasonography, computed tomography and bone scan. The median follow-up period was 6.2 months. This study was conducted with the approval of the ethical committee of Osaka City University, and written informed consent was obtained from all patients.

Assessment of clinical and pathological responses to NAC

Clinical response of the primary tumor was assessed by ultrasonography, computed tomography, and physical examination after NAC. Clinical responses were classified according to the WHO criteria [34]. After NAC, patients underwent appropriate surgery. The clinical response to preoperative chemotherapy was determined from the two diameters measurable in two dimensions by multiplying the longest diameter by the greatest perpendicular diameter and was classified as follows. Clinical complete response (cCR) was judged as the disappearance of all known disease determined by two observations not less than four weeks apart. Clinical partial response (cPR) was a 50% or greater decrease in total tumor lesions. Clinical no change (cNC) was a less than 50% decrease in total tumor size, without a 25% increase in tumor size. Clinical progressive disease (cPD) was defined as a 25% or greater increase in the tumor size, or the appearance of new lesions. The first two categories, cCR and cPR, were judged as effective. Pathological responses of the tumor and dissected lymph nodes were classified according to the evaluation criteria of the Japanese Breast Cancer Society (JBCS) [35], using a 5 histological-grade scale (Grades 0, 1a, 1b, 2, and 3) as follows: Grade 0, no response or almost no change in cancer cells after treatment; Grade 1, slight response; Grade 1a, mild response, mild change in cancer cells regardless of the area, or marked changes in cancer cells in less than one-third of total cancer cells; Grade 1b, moderate response, marked changes in one-third or more but less than two-thirds of tumor cells; Grade 2, marked response or marked changes in two-thirds or more of tumor cells; and Grade 3, no residual tumor cells, necrosis or disappearance of all tumor cells, or replacement of all cancer cells by granuloma-like and/or fibrous tissue. pCR (pathological complete response) was defined as the complete disappearance of infiltrates, including lymph node infiltrates. Tumors with residual

ductal carcinoma in situ were included in the pCR group. Marked changes approaching a complete response with only a few remaining cancer cells were classified as near pCR [36,37]. The others were classified in the non-pCR group.

Immunohistochemical examinations

All patients underwent a CNB before NAC, and an operation consisting of mastectomy or conserving surgery with axillary lymph node dissection after NAC at Osaka City University. Tissues from each patient were fixed in buffered formalin and embedded in paraffin. Serial tissue sections of 4 μ m thickness were stained with hematoxylin-eosin and used for immunohistochemical staining. Expressions of CA9, estrogen receptor (ER), progesterone receptor (PgR), and HER2 were assessed by immunohistochemistry. After the paraffin sections were deparaffinized, they were heated for 20 min at 105°C by autoclave in Target Retrieval Solution (Dako, Carpinteria, CA). After blocking with 10% goat serum, the slides were incubated with the primary monoclonal antibodies against each of CA9 (clone M75, 1:1000; Novus Biologicals), ER (clone 1D5, dilution 1:80; Dako, Cambridge, UK), PgR (clone PgR636, dilution 1:100; Dako), and HER2 (Hercep Test, Dako) overnight at 4 °C. Peroxidase was introduced using a streptavidin conjugate and then peroxidase reactivity was visualized using a DAB solution, followed by counterstaining with haematoxylin.

Immunohistochemical assessment

Immunohistochemical scoring was graded by trained pathologists (Masahiko Ohsawa and Kenichi Wakasa, Department of Diagnostic Pathology). The stroma was excluded from the staining evaluation. All staining was scored by counting the number of positive-stained cells, and was expressed as a percentage of the 1000 tumor cells counted across several representative fields of the section using a standard light microscope equipped with a \times 100 square graticule. The reproducibility of counting was assessed by a second investigator. The cut-off for ER positivity and PgR positivity was \geq 1% positive tumor cells with nuclear staining. HER2 was graded in four steps according to the accepted scheme: 0, 1+, 2+, 3+. HER2 was considered to be positive if immunostaining was 3+ or if a 2+ result showed gene amplification by fluorescent in situ hybridization. The ER, PR, and HER2 stainings were evaluated as described in previous reports [38]. The CA9 antibody intensely stained the membranes of cancer cells. Scores were applied as follows: score 0, negative staining in all cells; score 1+, weakly positive or focally positive staining in <10% of the cells; score 2+, moderately positive staining covering >10% of the cells; and score 3+, strongly positive staining in >10% of the cells (Figure 1). CA9 expression was considered positive for scores of 2+ or 3+.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL). The association between the expression of CA9 and clinicopathological parameters was analyzed with the chi-square test. Binary logistic regression was used for multivariate analyses to identify independent prognostic factors for a pathological complete response. The Kaplan-Meier method was used to estimate the values of DFS. DFS was compared using a log-rank test. The Cox regression model was used for multivariate analysis of prognostic factors. In all of the tests, a *p* value less than 0.05 was considered to be statistically significant.

Results

Clinicopathological responses of breast cancers to NAC

The cCR rate was 17% (18/102), cPR was 61% (62/102), cNC was 20% (20/102), and cPD was 2% (2/102). Therefore, the clinical responders (cCR + cPR) made up 78% (80/102) of the patients. The pathological response was evaluated using resected tissue after NAC. Of the tumors investigated, 12% (12/102) were histological response grade 1a, 33% (34/102) were grade 1b, 20% (20/102) were grade 2a, 16% (16/102) were grade 2b, and 20% (20/102) were grade 3. Patients were classified into pathologic responders (grade 2 and 3; 55% of all patients) and non-responders (grade 1; 45%) according to the grade of the tumor. The pCR rate was 29% (30/102). The DFS of pathologic non-responders was significantly (*p* = 0.01) shorter than that of pathologic responders, while no significant difference in DFS was found between clinical non-responders and clinical responders (Figure 2).

Association between clinicopathological parameters and CA9 expression in CNB specimens

The CA9 expression of primary breast tumors before NAC was analyzed using CNB specimens. Of the 102 breast cancer patients, 47 patients (46%) had CA9-positive breast tumors, while 55 (54%) had CA9-negative tumors. Table 1 shows the correlation between clinicopathological parameters and CA9 expression in breast cancers. The CA9 expression in CNB specimens was significantly correlated with lymph node metastasis (70%, *p* = 0.001) and lymphatic invasion (69%, *p* = 0.003). The pCR rate of CA9-positive tumors (23%, 7/30) was significantly lower (*p* = 0.003) than that of CA9-negative tumors (77%, 23/30). The pathological non-responder tumors showed significantly more frequent CA9 expression than the pathological responder tumors (*p* < 0.001). Clinical response (cCR + cPR) was not associated with CA9 expression (*p* = 0.062). Recurrent tumors were observed in 28 of 102 patients. CA9 expression was significantly more frequent (*p* < 0.001) in patients with recurrent tumors (79%, 22/28) than in those with non-recurrent tumors (34%, 25/74).

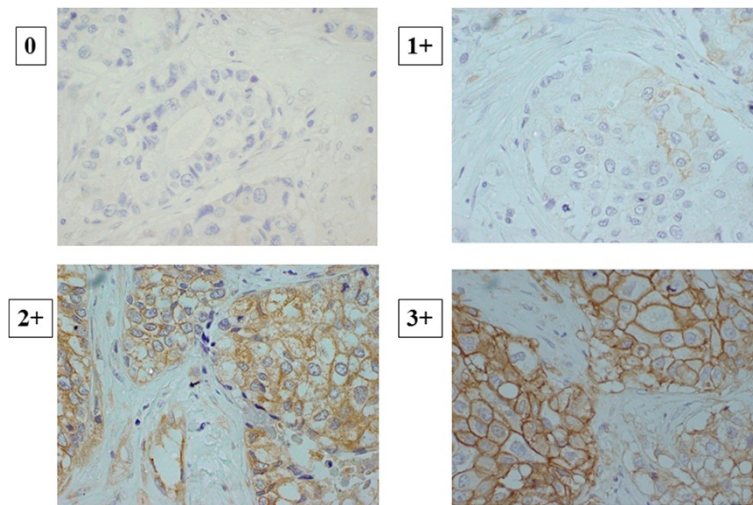


Figure 1 Immunohistochemical determination of CA9 expression. The positivity of a tumor for CA-9 was semi-quantitatively analyzed according to the percentage of cells showing membrane positivity. Score 0, negative staining in all cells; score 1+, weakly positive or focally positive staining in <10% of the cells; score 2+, moderately positive staining covering >10% of the cells; and score 3+, strongly positive staining, including >10% of the cells.

There was no significant association between CA9 expression and other clinicopathological factors.

The correlation between the pCR and the pathological or clinical response.

We examined the correlation between the pathological response (pCR vs non-pCR) and pathological or clinical response (Table 2). A pathological response was significantly ($p < 0.001$) associated with pCR, and a clinical response was also significantly ($p = 0.018$) associated with pCR.

Association between CA9 and pathological complete response in CNB specimens

Univariate analysis revealed that the expressions of CA9, ER, and PgR in CNB specimens were significantly

associated with pCR. There was no significant association between pCR and the other clinicopathological factors. Multivariate analysis revealed that only CA9 expression was significantly associated with pCR (Table 3).

Correlation between clinicopathological parameters and disease-free survival

CA9 expression of breast tumors was analyzed using both CNB specimens and resected tissues. Since 30 of the 102 breast tumors showed a pathological complete response, these cases were excluded from the evaluation of CA9 expression. CA9 expression was therefore examined in 72 resected tissues after NAC. DFS in patients with CA9-positive tumors was significantly shorter than that in those with CA9-negative tumors in both samples (CNB specimens and resected tissues) (Figure 3). Univariate analysis

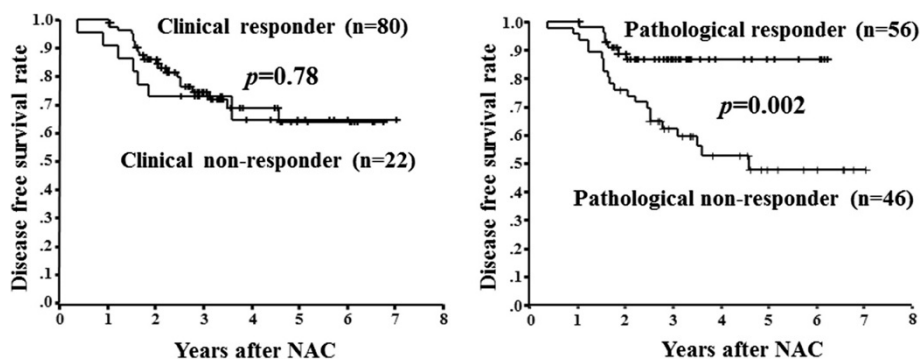


Figure 2 Association between clinicopathologic response and disease-free survival. Disease-free survival in pathologic non-responders was significantly ($p = 0.002$) shorter than that in pathologic responders, while the clinical response was not associated with disease-free survival ($p = 0.78$).

Table 1 Correlations between CA9 expression and clinicopathological parameters in CNB of 102 primary breast cancers

Parameter	CA9 expression		p-value
	Positive (n = 47)	Negative (n = 55)	
Age			
	≥55	21 (42%)	29 (58%)
	<55	26 (50%)	26 (50%)
Menopause			0.418
	Positive	31 (44%)	40 (56%)
	Negative	16 (52%)	15 (48%)
Intrinsic subtype			0.459
	Luminal	22 (48%)	24 (52%)
	Luminal-HER	4 (50%)	4 (50%)
	HER2	4 (24%)	13 (76%)
	Triple-negative	17 (55%)	14 (45%)
Tumor size			0.209
	≥ 4 cm	12 (67%)	6 (33%)
	< 4 cm	35 (42%)	49 (58%)
Lymph node status			0.053
	Positive	23 (70%)	10 (30%)
	Negative	24 (35%)	45 (65%)
Lymph-vascular invasion			0.001
	Positive	20 (69%)	9 (31%)
	Negative	27 (37%)	46 (63%)
Nuclear grade (NG)			0.003
	NG1	32 (43%)	42 (57%)
	NG 2&3	15 (54%)	13 (46%)
Pathological response			0.350
	pCR	7 (23%)	23 (77%)
	Non-pCR	40 (56%)	32 (44%)
Responder (histological grade 2&3)			0.003
non-Responder (histological grade 1)			<0.001
Clinical response			<0.001
	Responder (cCR + cPR)	16 (29%)	40 (71%)
	non-Responder (cNC + cPD)	31 (67%)	15 (33%)
Recurrence			0.062
	Responder (cCR + cPR)	33 (41%)	47 (59%)
	non-Responder (cNC + cPD)	14 (64%)	8 (36%)
Recurrence			0.062
	Yes	22 (79%)	6 (21%)
	No	25 (34%)	49 (66%)

revealed that CA9 expression in CNB specimens, CA9 expression in resected tissues, tumor size, lymph node status, and pathological response were significantly associated with DFS. There was no significant association between DFS and clinical response. Multivariate regression analyses indicated that CA9 expression in resected tissues after NAC was an independent prognostic factor for DFS (Table 4).

Discussion

In recent years, NAC has been adopted as one of the standard care regimens for primary resectable early-stage breast cancer. In such cases, the NAC generally consists of an anthracycline-containing regimen and taxane [4,5]. The evaluation of the tumor response to NAC is important to determine the appropriate post-operative chemotherapeutic regimen for patients with recurrent

Table 2 Correlations between the pathological and clinical response and the pCR

	Pathological response		<i>p</i> -value
	pCR	non-pCR	
Pathological response			
Responder (histological grade 2&3)	30	26	
non-Responder (histological grade 1)	0	46	<i>p</i> < 0.001
Clinical response			
Responder (cCR + cPR)	28	52	
non-Responder (cNC + cPD)	2	20	<i>p</i> = 0.018

tumors. There are various systems for classifying the survival response and pathological response in neoadjuvant trials—i.e., the cTMN, Fisher's, Chevailler's, and JBCS systems—and all of these have been shown to yield basically comparable results [39]. In this study, we used the WHO and JBCS classifications as the therapeutic response criteria. The pCR rate was 29% (30/102), and the response rate was 78% (80/102). These response rates were similar to those previously reported [6,9,10].

The correlation between chemosensitivity and survival remains controversial. Some papers have reported that the prognostic factors included the clinical and pathological response to primary chemotherapy. On the other

hand, at least one paper has reported that response classifications were inadequate as prognostic markers of the long-term outcome after NAC [39]. Our data indicated that clinical response was not a significant predictor of DFS. Although clinical examination provides approximate indicators of chemotherapy responses, histopathologic examination of specimens after chemotherapy is important to evaluate the accurate response or the prognosis [40-43]. A tumor diagnosed as showing a complete clinical response sometimes retains residual carcinoma cells by microscopic examination; conversely, a palpable residual mass may show fibrosis without cancer cells [42,43]. These findings might explain why the association

Table 3 Univariate and multivariate analyses of the pathological complete response in 102 breast cancers

Parameter	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	<i>p</i> value	Odds ratio	95% CI	<i>p</i> value
CA9 expression in CNB						
positive vs negative	0.24	0.09-0.63	0.041	0.21	0.07-0.59	0.003
ER						
positive vs negative	0.24	0.10-0.61	0.002	0.23	0.02-2.37	0.219
PgR						
positive vs negative	0.22	0.08-0.61	0.004	0.36	0.10-1.30	0.120
HER2						
positive vs negative	1.75	0.68-4.48	0.244			
Molecular subtypes						
HR+/HER2- vs others	0.33	0.13-0.83	0.018	1.78	0.18-17.44	0.622
HR-/HER2+ vs others	0.02	0.01-2999894	0.728			
HR-/HER2+ vs others	1.75	0.683-4.483	0.244			
HR-/HER2- vs others	1.17	0.93-1.46	0.176			
Age						
≥55 vs <55	0.88	0.37-2.05	0.759			
Menopause						
positive vs negative	0.82	0.33-2.05	0.677			
Tumor size						
≥4 cm vs <4 cm	0.64	0.19-2.12	0.463			
Lymph node status						
positive vs negative	4.33	0.93-20.1	0.061			

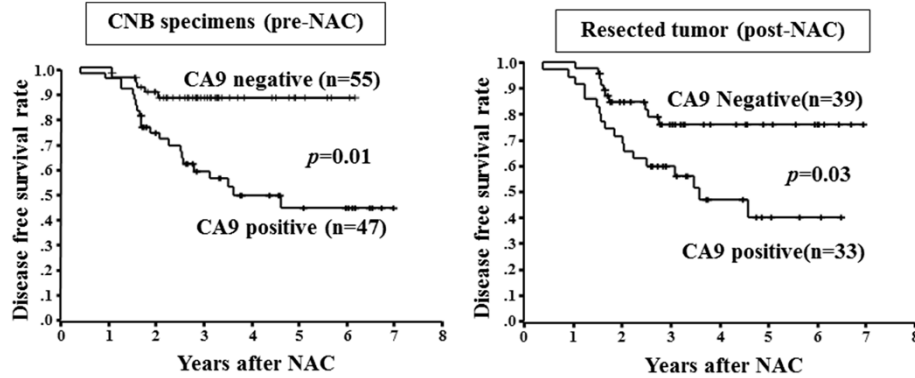


Figure 3 Disease-free survival of patients based on CA-9 expression. The Kaplan-Meier survival curve shows the disease-free survival in relation to the CA-9 expression. A statistically significant difference in the survival was observed between the CA-9-positive and CA-9-negative groups in both CNB specimens and resected tissues (log-rank, $p=0.01$ and $p=0.03$, respectively).

between clinical response and DFS was more less statistically significant than that between pathological response and DFS in our study.

Our data indicated that pathological response was a significant predictor of the DFS. In this study, FEC followed by wPTX was the only NAC regimen used for patients with resectable early-stage breast cancer. However, the variety of chemotherapy regimens used as NAC

in previous reports might have been a factor in producing these inconsistent results.

CA9, a hypoxia-associated endogenous protein, has been implicated in the regulation of the hypoxic micro-environment [44,45]. CA9 is considered to be one of the cellular biomarkers of hypoxic regions in solid tumors. In the present analysis, CA9 was positive in CNB specimens from 47 (46%) of 102 patients, similar to the ratio

Table 4 Univariate and multivariate analysis of disease-free survival

Parameter	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	p value	Odds ratio	95% CI	p value
CA9 expression in CNB specimens						
positive vs negative	4.44	1.80-10.9	0.001	2.32	0.88-6.08	0.089
CA9 expression in resected tissues						
positive vs negative	2.63	1.21-5.70	0.014	2.39	1.04-5.49	0.041
Tumor size						
≥ 4 cm vs < 4 cm	2.46	1.11-5.45	0.026	1.61	0.65-3.97	0.302
Lymph node status						
N1-3 vs N0	3.00	1.42-6.35	0.004	0.97	0.38-2.47	0.955
Lymph-vascular invasion						
positive vs negative	4.22	1.99-8.92	<0.001	2.75	1.11-6.62	0.028
ER						
positive vs negative	0.91	0.43-1.94	0.807			
PgR						
positive vs negative	0.95	0.45-2.02	0.905			
HER2						
positive vs negative	1.36	0.59-3.11	0.46			
Clinical response						
Responder vs non-responder	0.88	0.37-2.08	0.78			
Pathological response						
Responder vs non-responder	0.29	0.12-0.67	0.004	1.03	0.38-2.78	0.953
pCR vs non-pCR	0.19	0.45-0.81	0.024	1.34	0.27-6.62	0.722

in a previous study [46]. CA9 expression was significantly associated with lymph node status and lymphovascular invasion. CA9 has been shown to maintain the survival of breast tumor cells under hypoxic conditions [47]. Breast cancer cells under hypoxic conditions might be associated with aggressive tumor phenotypes, which may indicate a poor prognosis for patients with CA9-positive breast cancer, as suggested in previous studies [29,30,48-50].

Tan et al. reported that CA9 in basal-like breast tumors was associated with resistance to chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil (CMF) or adriamycin and cyclophosphamide (AC)) and poor prognosis [30]. In our present analysis of 31 triple-negative breast cancers, the DFS of patients with CA9-positive tumors was significantly shorter ($p = 0.015$) than that of patients with CA9-negative tumors (Additional file 2: Figure S1). The chemosensitivity of triple-negative breast cancer patients with CA9 was significantly lower than that of the CA9-negative cases (Additional file 1: Table S2). CA9 might be a useful biomarker for chemotherapy in triple-negative breast cancer. Supuran and colleagues found that selective CA9 inhibitors inhibited cell migration and spreading of breast cancer cells in the absence of oxygen, suggesting that CA9 is a pivotal target for antitumor therapy in patients with breast carcinoma [25,51]. These findings suggest that CA9 inhibitors followed by wPTX chemotherapy might be useful in cases of breast carcinoma with resistance to FEC.

Biological markers predicting chemosensitivity have been evaluated in several studies, but there is still no clinically useful marker. ER- or PR-positive patients showed lower pCR rates after NAC than ER- or PR-negative patients. The pCR rate of CA9-positive tumors in CNB specimens was significantly lower than that of CA9-negative tumors. Multivariate analysis revealed that CA9 expression before NAC was an independent predictive factor for pCR. An extensive hypoxic microenvironment as determined by CA9 expression in breast cancer might play a significant role in the resistance to chemotherapy. These results may indicate that CA9 expression in CNB specimens is a useful marker for predicting chemosensitivity to NAC.

We also examined the correlation of CA9 expression between CNB tissues and resected tissues in the 72 patients. Although no significant correlation of CA9 staining was observed between the two groups, CA9 expression in resected tissues showed a tendency ($p = 0.081$) toward association with that in CNB tissues (Additional file 1: Table S3). CA9 expression after NAC (67%) was higher than that before NAC (46%). CA9-positive cells were observed more frequently in tumor specimens than in CNB specimens. Eleven of 32 patients with CA9-negative tumors before NAC were found to have CA9-positive tumors after

NAC. NAC was thus effective in reducing CA9-negative cells, and resulted in an increase in hypoxic CA9-positive cells. Twenty-three of 55 patients with CA9-negative tumors before NAC achieved pCR, and could not be enrolled in the CA9 expression analysis because there was no tumor involvement detected in the resected tissues. These changes in CA9 expression before and after NAC might be one of the reasons for the lack of a significant correlation between the CA9 expression in CNB tissues and that in resected tissues.

CA9 expression in resected tissues after NAC was correlated with both prognosis and recurrence. In addition, multivariate regression analyses indicated that the CA9 expression level after NAC was an independent prognostic factor for DFS. Thus, CA9 expression after NAC may be a clinically informative prognostic marker for breast cancer patients treated with NAC. On the other hand, CA9 expression before NAC in CNB specimens may be a useful surrogate marker for predicting chemosensitivity. Our results indicate that the hypoxic marker CA9 in CNB specimens could be used to predict chemosensitivity, and that high expression of CA9 in resected tissue is correlated with worse outcomes in patients treated with FEC followed by wPTX chemotherapy.

Conclusion

Hypoxic microenvironment as determined by CA9 expression in breast cancer might play a significant role in the resistance to chemotherapy, which indicates that CA9 expression in CNB specimens is a useful marker for predicting chemosensitivity to NAC. CA9 expression in resected tissue is prognostic of DFS in patients with resectable early-stage breast cancer treated by sequential FEC and weekly paclitaxel prior to resection.

Additional files

Additional file 1: Table S1. Clinicopathologic features of 102 breast cancers. **Table S2.** Correlations between CA9 expression and chemosensitivity in 31 triple-negative breast cancers. **Table S3.** Correlation of CA9 expression before NAC to that after NAC in the 72 patients who did not achieve pCR.

Additional file 2: Figure S1. Disease-free survival of patients based on CA-9 expression in 31 cases of triple-negative breast cancer. Among the cases of triple-negative breast cancer, the DFS of patients with CA9-positive tumors was significantly shorter ($p = 0.015$) than that of patients with CA9-negative tumors. (TIFF 25 kb)

Abbreviations

CR: Complete response; CNB: Core needle biopsy; CSCs: Cancer stem cells; DFS: Disease-free survival; ER: Estrogen receptor; FEC: 5-fluorouracil + epirubicin + cyclophosphamide; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; NAC: Neoadjuvant chemotherapy; NC: No change; OS: Overall survival; PgR: Progesterone receptor; PR: Partial response; PD: Progressive disease; pCR: Pathologic complete response.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MY: study design, data analysis, paper preparation. NA: performance of experiments, data analysis, paper preparation. SK, HK, and TT: material sampling. MO, KW: pathological diagnosis. KH: manuscript review. All authors read and approved the final manuscript.

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