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Impact of Expression Levels of Platinum-uptake Transporters Copper Transporter 1 and Organic Cation Transporter 2 on Resistance to Anthracycline/Taxane-based Chemotherapy in Triple-negative Breast Cancer

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ABSTRACT: Adding platinum drugs to anthracycline/taxane (ANC-Tax)-based neoadjuvant chemotherapy (NAC) improves pathological complete response (pCR) rates in triple-negative breast cancer (TNBC). Copper transporter 1 (CTR1) and organic cation transporter 2 (OCT2) critically affect the uptake and cytotoxicity of platinum drugs. We immunohistochemically determined CTR1 and OCT2 levels in pre-chemotherapy biopsies from 105 patients with HER2-negative breast cancer treated with ANC-Tax-based NAC. In the TNBC group, Ki-67^{high} [pathological good response (pGR), $P = 0.04$] was associated with response, whereas CTR1^{high} (non-pGR, $P = 0.03$), OCT2^{high} (non-pGR, $P = 0.01$; non-pCR, $P = 0.03$), and combined CTR1^{high} and/or OCT2^{high} (non-pGR, $P = 0.005$; non-pCR, $P = 0.003$) were associated with non-response. In multivariate analysis, Ki-67^{high} was an independent factor for pGR and CTR1 for non-pGR. Combined CTR1/OCT2 was a strong independent factor for non-pGR. However, no variables were associated with response in luminal BC. These results indicate that platinum uptake transporters are predominantly expressed in ANC-Tax-resistant TNBCs, which implies that advantage associated with adding platinum drugs may depend on high drug uptake.

KEYWORDS: copper transporter 1, organic cation transporter 2, platinum drug, neoadjuvant chemotherapy, triple-negative breast cancer, immunohistochemistry

Introduction

Triple-negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). TNBCs derive no benefit from targeted treatments such as endocrine therapy or anti-HER2 therapy, and thus cytotoxic chemotherapy is the only systemic therapy available for TNBC patients.[1] Anthracycline/taxane (ANC-Tax)-based chemotherapy remains the standard treatment for TNBC patients. However, some TNBC patients fail to respond to the chemotherapy and show worse prognosis due to an increased risk of recurrence.[2,3] Thus, there is a major need to identify non-cross-resistant drugs and more appropriate regimens for this aggressive type of breast cancer (BC).

Platinum drugs, including cisplatin, carboplatin, and oxaliplatin, exert their cytotoxic effect through direct damage to DNA. As TNBC may be highly susceptible to DNA damage,[4,5] the effectiveness of platinum drugs as neoadjuvant chemotherapy (NAC) for TNBC has become more widely studied; and several clinical trials have concluded that pathological complete response (pCR) rates in TNBC increased significantly with the addition of platinum drugs to ANC-Tax-based regimens.[6–11] However, the mechanisms that underlie these results have not been fully elucidated.

As platinum drugs are highly polar, they do not readily diffuse across cell membranes.[12] Influx of drug molecules via solute carrier (SLC) transporters is an important determinant of intracellular drug concentrations, and uptake mechanisms into tumor cells may greatly affect susceptibility to anticancer drugs including platinum.[13] Copper transporter 1 (CTR1), also called SLC31A1, has been shown to control intracellular accumulation and cytotoxicity of the platinum drugs cisplatin and carboplatin.[12,14,15] This is supported by several studies using clinical tissue samples. Lee et al[16] reported that high levels of *CTR1* mRNA were significantly associated with longer disease-free survival (DFS) in patients with ovarian cancer treated with adjuvant carboplatin-containing chemotherapy. Chen et al[17] found that high levels of CTR1 were

associated with better tumor response, longer DFS, and overall survival (OS) in non-small-cell lung cancer treated with cisplatin- or carboplatin-containing first-line chemotherapy. Furthermore, our recent study showed that high CTR1 expression was significantly associated with longer OS of patients with endometrial cancer treated with adjuvant paclitaxel/carboplatin chemotherapy.[18] Conversely, organic cation transporter 2 (OCT2), also known as SLC22A2, is a critical determinant in the uptake and consequent cytotoxicity of cisplatin and oxaliplatin.[19–21] Recently, we found that a high OCT2 expression was significantly correlated with longer progression-free survival in patients with metastatic colorectal cancer treated with first-line 5-fluorouracil/leucovorin/oxaliplatin chemotherapy.[22] Taken together, the expression levels of CTR1 and OCT2 may critically affect an individual's susceptibility to platinum-containing chemotherapy. Therefore, we explored the association of CTR1 and OCT2 expression with pathological response to ANC-Tax-based NAC in TNBCs and the underlying mechanisms that mediate the effectiveness of adding platinum drugs to the conventional regimens.

Materials and Methods

Patients and NAC. We studied 105 patients with primary BCs. The eligible criteria were invasive, HER2-negative BCs with stages II–III disease that were histopathologically diagnosed. Each patient received one of the following regimens of ANC-Tax-based NAC (FEC-T, EC-T, or AC-T) at the Kanagawa Cancer Center Hospital during 2004–2012: a median of four cycles of fluorouracil/epirubicin/cyclophosphamide at $500/100/500$ mg/m^2 every 3 weeks; a median of four cycles of epirubicin/cyclophosphamide at $90/600$ mg/m^2 every 3 weeks; or a median of four cycles of doxorubicin/cyclophosphamide at $60/600$ mg/m^2 every 3 weeks, followed by a median of 12 cycles of paclitaxel at 80 mg/m^2 weekly or a median of four cycles of docetaxel at 75 mg/m^2 every 3 weeks. The percentages of patients receiving FEC-T, EC-T, and AC-T were 91.4, 4.8, and 3.8, respectively.

Each patient underwent a pre-chemotherapy core needle biopsy of her tumor. The present study was approved by the ethics committees of Kobe University Graduate School of Health Sciences and Kanagawa Cancer Center Hospital. All the experiments were performed under the current laws of Japan. Written informed consent for the core needle biopsy and experimental use of tumor samples was obtained from all patients. The study was conducted according to the principles of the Declaration of Helsinki.

Tumor specimens and histopathological assessment. Biopsy specimens were fixed in 10% formalin and embedded into paraffin wax, and 3-μm-thick sections were prepared. Histological grade was assessed using the Nottingham modification of the Bloom and Richardson system.[23] HER2-negative status was defined as a score of 0 or 1+ by immunohistochemistry or *HER2* gene/centromere 17 ratio of ≤2.2

by fluorescence in situ hybridization. ER, PR, and Ki-67 status prior to NAC were determined immunohistochemically. Tumors were classified as positive for ER and PR when nuclear staining was visible in >10% of tumor cells. For Ki-67, nuclear staining in ≥14% of the tumor cells was considered to indicate Ki-67^{high} level. Of the 105 patients, 41 (39%) were identified as TNBCs, 29 (27%) as luminal A, and 35 (33%) as luminal B.

After NAC, all patients underwent breast-conserving surgery or mastectomy. Sentinel lymph node biopsy and axillary node dissection were performed in 8% and 90% of the patients, respectively. Pathological response to NAC was evaluated using the surgical specimens. A pCR was defined as the absence of residual invasive tumor cells in both the breast and lymph nodes. Minimal residual tumor was defined as invasive tumors that showed marked changes with isolated cancer cells or tumor clusters of fewer than 10 cancer cells.[24] pCR plus minimal residual tumor was defined as a pathological good response (pGR).

Expression of CTR1 and OCT2. Sections of biopsy specimens were deparaffinized in xylene and rehydrated with a graded alcohol series. Endogenous peroxidase was blocked by incubating sections in 0.3% hydrogen peroxide in methanol for 30 minutes. The antigenicities of CTR1 and OCT2 were retrieved by protease-induced antigen retrieval (PIAR) and heat-induced antigen retrieval (HIAR) methods, respectively. PIAR was performed using 0.16 U/g pronase solution (Nichirei Bioscience) for four minutes at room temperature (RT). HIAR was applied using a pressure cooker for 10 minutes at 120°C in 0.001 mol/L EDTA, pH 8.0. After pressure-cooking, the sections were allowed to cool to RT in the soaking solution for 30 minutes. Sections were then rinsed in tap water followed by 0.01 mol/L phosphate-buffered saline (PBS; pH 7.2).

After treatment with a protein-blocking solution (Dako) for 5 minutes at RT, sections were incubated overnight at RT with rabbit polyclonal anti-human CTR1 antibody (1:100 dilution; Abnova) or rabbit polyclonal anti-human OCT2 antibody (1:400 dilution; Sigma-Aldrich). Sections were then rinsed in PBS, followed by incubation with the Histofine Simple Stain MAX-PO (Nichirei Bioscience) for one hour at RT. Reaction products were developed using diaminobenzidine solution (Nichirei Bioscience). A negative control was included in each run by omitting the primary antibody. Sections of serous ovarian cancer for CTR1 and normal kidney for OCT2 were used as positive controls.

Evaluation of CTR1 and OCT2 levels. Three investigators (RT, AN, and SK) with no previous knowledge of patients' clinicopathological characteristics evaluated the immunostained sections. Differences in scores were adjudicated by consensus among the three investigators. Staining was regarded as positive when tumor cells showed membrane staining. Expression levels of CTR1 and OCT2 were semiquantitatively assessed by scoring the staining intensity and the percentage of stained tumor cells. Briefly, staining

intensity scores were: 0, no staining; 1, weakly positive; 2, moderately positive; and 3, strongly positive; and positive percentage of tumor cell scores were: 0, none; 1, 1–10%; 2, 11–50%; 3, >50%. These two scores were added for a composite score (0–6). Receiver operating characteristic curve analysis showed the best sensitivity and specificity cut-off values of 5 for CTR1 and 4 for OCT2: 0–4, CTR1^{low}; 0–3, OCT2^{low}; 5–6, CTR1^{high}; and 4–6, OCT2^{high}. Because CTR1 and OCT2 share common ground in terms of cisplatin uptake, as described in the Introduction, we also analyzed data from CTR1 and OCT2 in combination.

Statistical analysis. All statistical analyses were performed using SPSS 21.0 software (SPSS Inc.). Statistical significance was defined as $P < 0.05$. Spearman rank correlation test was used to calculate correlations between CTR1 and OCT2 expression levels. Fisher's exact test was used to assess associations of CTR1 and OCT2 levels with patient age, clinical tumor stage and node status, histological grade and type, Ki-67 level, intrinsic subtype, and pathological response. To identify variables independently associated with pathological response, multivariate analysis was performed. Variables with a P-value <0.10 in the univariate analysis were included in a logistic regression model.

Results

Association of CTR1 and OCT2 levels with clinicopathological parameters. Clinicopathological characteristics of patients are shown in Table 1. The patients' median age was 54 years (range: 32–73 years). Median tumor size was 4.3 cm (range: 1.0–16.3 cm). Eight percent of patients had T1 tumors, 70% had T2 tumors, 7% had T3 tumors, and 16% had T4 tumors. Clinically positive lymph nodes were found in 75% of the patients. Histological types were invasive ductal carcinoma in 87%, invasive lobular carcinoma in 7%, and other types (mucinous, apocrine) in 7% of tumors.

Representative staining patterns of CTR1 and OCT2 are shown in Figure 1. CTR1 and OCT2 were expressed in cell membranes and/or cytoplasm of tumor cells. We found CTR1^{high} and OCT2^{high} expression in 33 (31%) and 65 (62%) tumors, respectively, but CTR1 and OCT2 levels were not

Table 1. Correlation of CTR1 and OCT2 levels with clinicopathological characteristics in the HER2-negative BC patients.

VARIABLES	NO	CTR1		OCT2	
		HIGH (%)	*P*-VALUE	HIGH (%)	*P*-VALUE
Age (years)					
≥50	68	18 (26)	0.19	40 (59)	0.41
<50	37	15 (41)		25 (68)	
Clinical tumor stage					
T3 + T4	24	9 (38)	0.47	14 (58)	0.81
T1 + T2	81	24 (30)		51 (63)	
Clinical lymph node status					
N1–N3	79	24 (30)	0.81	48 (61)	0.82
N0	26	9 (35)		17 (65)	
Histological grade					
3	17	6 (35)	0.78	8 (47)	0.18
1 + 2	88	27 (31)		57 (65)	
Histological type					
Invasive ductal carcinoma	91	28 (31)	0.90	53 (58)	0.07
Invasive lobular carcinoma	7	2 (29)		7 (100)	
Others	7	3 (43)		5 (71)	
Ki-67					
High	64	19 (30)	0.67	33 (52)	0.01[a]
Low	41	14 (34)		32 (78)	
Intrinsic subtype					
TNBC	41	15 (37)	0.39	17 (41)	<0.001[a]
Luminal BC	64	18 (28)		48 (75)	
Luminal A	29	11 (38)		25 (86)	
Luminal B	35	7 (20)		23 (66)	

Note: [a]Statistically significant.
Abbreviations: CTR1, copper transporter 1; OCT2, organic cation transporter 2; HER2, human epidermal growth factor receptor type 2; BC, breast cancer; TNBC, triple negative BC.
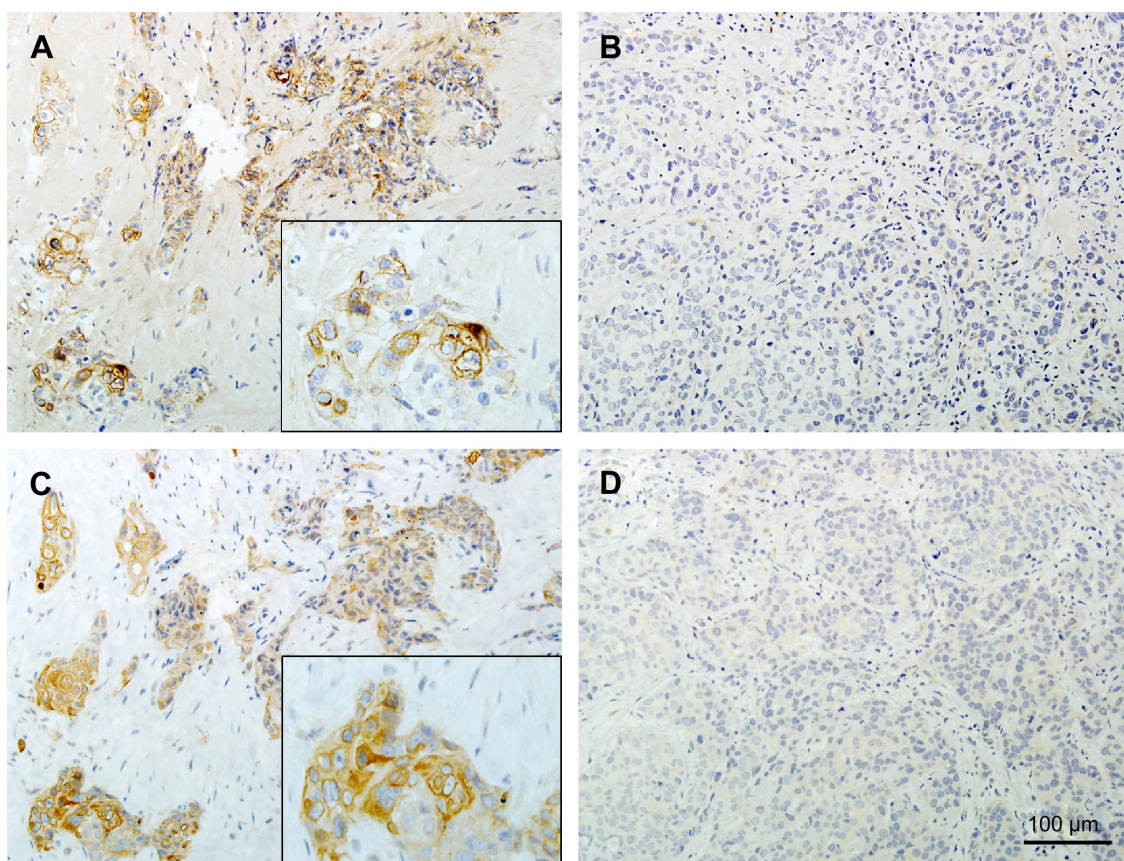
Figure 1. Representative immunostaining patterns in pre-chemotherapy biopsies of triple negative breast cancers according to pathological response. (**A**, **B**) Copper transporter 1 (CTR1). (**C**, **D**) Organic cation transporter 2 (OCT2). (**A**, **C**) Immunohistochemical results of a tumor from a patient with no pathological response, showing high expression levels of CTR1 and OCT2. Insets show high-power views of positive cells. (**B**, **D**) Immunohistochemical staining of a tumor from a patient with pathological complete response, showing low expression levels of CTR1 and OCT2.

correlated ($r = 0.11$, $P = 0.27$). Seventy-five tumors (71%) showed high levels of at least one transporter (CTR1^{high} and/or OCT2^{high}), whereas 30 tumors (29%) had low levels of both CTR1 and OCT2 (CTR1^{low} and OCT2^{low}).

Table 1 shows the association of CTR1 and OCT2 levels with clinicopathological findings. OCT2^{high} level was significantly more prevalent in Ki-67^{low} tumors than in Ki-67^{high} tumors (78% vs 52%; $P = 0.01$). Additionally, the rate of OCT2^{high} tumors was significantly higher in luminal BCs than in TNBCs (75% vs 41%; $P < 0.001$). However, OCT2 level was not associated with other clinicopathological parameters. No association was found between CTR1 level and any of the clinicopathological parameters.

Association of CTR1 and OCT2 levels with pathological response in the HER2-negative BCs. pGR and pCR were achieved in 23 (22%) and 11 (10%) patients, respectively. In the univariate analysis of the entire cohort (Table 2), patients with Ki-67^{high} tumors had significantly higher pGR rates than patients with Ki-67^{low} tumors (30% vs 10%, $P = 0.02$). The pGR rate in patients with TNBCs was significantly higher than that in patients with luminal BCs (44% vs 8%, $P < 0.001$). The pCR rate in patients with TNBCs was 27%, but no patients with luminal BCs achieved pCR ($P < 0.001$).

In contrast, pGR and pCR rates were significantly lower in patients with OCT2^{high} tumors than in patients with OCT2^{low} tumors (pGR, 12% vs 38%, $P = 0.003$; pCR, 2% vs 25%, $P < 0.001$). However, no significant association between CTR1 level and pathological response was detected. When analyzing CTR1 and OCT2 in combination, pGR and pCR rates in patients with CTR1^{high} and/or OCT2^{high} tumors were significantly lower in patients with CTR1^{low} and OCT2^{low} tumors (pGR, 13% vs 43%, $P = 0.002$; pCR, 3% vs 30%, $P < 0.001$). However, other parameters showed no significant association with pathological response.

Multivariate analysis in HER2-negative BCs. Table 3 shows the results of multivariate analysis of the entire cohort. We first included the histological grade, Ki-67 level, intrinsic subtype, and OCT2 level (Model 1). TNBC subtype was an independent factor for pGR [odds ratio (OR), 6.79; 95% confidence interval (CI), 2.03–22.74; $P = 0.002$]. Unfortunately, the OR for pCR in intrinsic subtype could not be calculated because the data contained zero: no patients with luminal BCs achieved pCR. Conversely, OCT2^{high} level was identified as an independent factor for non-pCR (OR, 0.10; 95% CI, 0.01–0.92; $P = 0.04$).

Next, we included combined CTR1 and OCT2 status instead of OCT2 level (Model 2). The TNBC subtype

Table 2. Univariate analysis of the correlation of CTR1 and OCT2 levels or clinicopathological parameters with pGR or pCR in the HER2-negative BC patients.

VARIABLES	NO.	pGR (%)	NON-pGR (%)	*P*-VALUE	pCR (%)	NON-pCR (%)	*P*-VALUE
Age (years)							
≥50	68	17 (25)	51 (75)	0.34	9 (13)	59 (87)	0.32
<50	37	6 (16)	31 (84)		2 (5)	35 (95)	
Clinical tumor stage							
T3 + T4	24	6 (25)	18 (75)	0.78	2 (8)	22 (92)	1.00
T1 + T2	81	17 (21)	64 (79)		9 (11)	72 (89)	
Clinical lymph node status							
N1–N3	79	19 (24)	60 (76)	0.42	9 (11)	70 (89)	0.73
N0	26	4 (15)	22 (85)		2 (8)	24 (92)	
Histological grade							
3	17	7 (41)	10 (59)	0.05	3 (18)	14 (82)	0.38
1 + 2	88	16 (18)	72 (82)		8 (9)	80 (91)	
Histological type							
Invasive ductal carcinoma	91	20 (22)	71 (78)	0.17	11 (12)	80 (88)	1.00
Invasive lobular carcinoma	7	3 (43)	4 (57)		0 (0)	7 (100)	
Others	7	0 (0)	7 (100)		0 (0)	7 (100)	
Ki-67							
High	64	19 (30)	45 (70)	0.02[a]	10 (16)	54 (84)	0.05
Low	41	4 (10)	37 (90)		1 (2)	40 (98)	
Intrinsic subtype							
TNBC	41	18 (44)	23 (56)	<0.001[a]	11 (27)	30 (73)	<0.001[a]
Luminal BC	64	5 (8)	59 (92)		0 (0)	64 (100)	
Luminal A	29	2 (7)	27 (93)		0 (0)	29 (100)	
Luminal B	35	3 (9)	32 (91)		0 (0)	35 (100)	
CTR1							
High	33	5 (15)	28 (85)	0.32	2 (6)	31 (94)	0.50
Low	72	18 (25)	54 (75)		9 (12)	63 (88)	
OCT2							
High	65	8 (12)	57 (88)	0.003[a]	1 (2)	64 (98)	<0.001[a]
Low	40	15 (37)	25 (63)		10 (25)	30 (75)	
Combined CTR1/OCT2							
CTR1-high and/or OCT2-high	75	10 (13)	65 (87)	0.002[a]	2 (3)	73 (97)	<0.001[a]
CTR1-low and OCT2-low	30	13 (43)	17 (57)		9 (30)	21 (70)	

Note: [a]Statistically significant.
Abbreviations: CTR1, copper transporter 1; OCT2, organic cation transporter 2; pGR, pathological good response; pCR, pathological complete response; HER2, human epidermal growth factor receptor type 2; BC, breast cancer; TNBC, triple negative BC.

remained an independent factor for pGR (OR, 7.40; 95% CI, 2.22–24.63; *P* = 0.001). Combined CTR1/OCT2 was identified as an independent factor for non-pCR (OR, 0.10; 95% CI, 0.02–0.57; *P* = 0.01). However, histological grade and Ki-67 level were not significant in Model 1 or Model 2.

Association of CTR1 and OCT2 levels with pathological response according to intrinsic subtype. To confirm the significance of CTR1 and OCT2 levels in TNBCs, the TNBC and luminal BC groups were separately analyzed (Table 4). In the TNBC group, pGR was observed in 55%

of patients with Ki-67^{high} tumors and in 17% of patients with Ki-67^{low} tumors (*P* = 0.04). In contrast, a significantly lower pGR rate was seen in patients with CTR1^{high} tumors than in patients with CTR1^{low} tumors (20% vs 58%, *P* = 0.03). However, Ki-67 and CTR1 levels were not associated with the pCR rate. pGR and pCR rates were significantly lower in patients with OCT2^{high} tumors than in patients with OCT2^{low} tumors (pGR: 19% vs 60%, *P* = 0.01; pCR: 6% vs 40%, *P* = 0.03). In the combined analysis, pGR and pCR rates in patients with CTR1^{high} and/or OCT2^{high} tumors were significantly

Table 3. Multivariate logistic regression analysis of predictive factors for pGR or pCR in the HER2-negative BC patients.

VARIABLES	pGR		pCR	
	OR (95% CI)	*P*-VALUE	OR (95% CI)	*P*-VALUE
Model 1				
Histological grade	1.13 (0.32–4.08)	0.85	NA	NA
Ki-67	2.81 (0.78–10.13)	0.11	4.49 (0.46–44.02)	0.20
Intrinsic subtype	6.79 (2.03–22.74)	0.002[a]	–	–
OCT2	0.46 (0.15–1.40)	0.17	0.10 (0.01–0.92)	0.04[a]
Model 2				
Histological grade	1.01 (0.28–3.73)	0.98	NA	NA
Ki-67	2.78 (0.76–10.16)	0.12	5.57 (0.54–57.08)	0.15
Intrinsic subtype	7.40 (2.22–24.63)	0.001[a]	–	–
Combined CTR1/OCT2	0.34 (0.11–1.01)	0.05	0.10 (0.02–0.57)	0.01[a]

Notes: [a]Statistically significant. OR for pCR in the intrinsic subtype could not be calculated because the data contained zero.
Abbreviations: pGR, pathological good response; pCR, pathological complete response; HER2, human epidermal growth factor receptor type 2; BC, breast cancer; OR, odds ratio; CI, confidence interval; NA, not applicable; OCT2, organic cation transporter 2; CTR1, copper transporter 1.

lower than in patients with CTR1^{low} and OCT2^{low} tumors (pGR: 25% vs 71%, $P = 0.005$; pCR: 8% vs 53%, $P = 0.003$; Fig. 1), which implies greater statistical significance for the combination than for OCT2 alone. However, no significant association with pathological response was detected for other parameters in the TNBC group or for any parameters in the luminal BC group.

The above-mentioned trends remained unchanged even after we eliminated from our analysis the nine patients who were not treated with FEC-T alone. In the TNBC group, the pGR rate was significantly higher in the Ki-67^{high} group than in the Ki-67^{low} group ($P = 0.01$). In contrast, the pGR rate was significantly lower in the CTR1^{high} group than in the CTR1^{low} group ($P = 0.04$), and also in the OCT2^{high} group than in the OCT2^{low} group ($P = 0.04$). The groups with CTR1^{high} and/or OCT2^{high} tumors had significantly lower pGR ($P = 0.005$) and pCR rates ($P = 0.01$) than did the CTR1^{low}/OCT2^{low} group. However, no parameters were associated with pathological response in the luminal BC group.

Multivariate analysis of TNBC. Multivariate analysis was performed only for pGR in the TNBC group (Table 5) because in the univariate analysis only OCT2 and combined CTR1/OCT2 were significant factors for pCR and no significant factors for pGR were found in the luminal BC group. In Model 1, Ki-67^{high} level was a significant independent factor for pGR (OR, 9.04; 95% CI, 1.28–63.82; $P = 0.03$), whereas CTR1^{high} level was an independent factor for non-pGR (OR, 0.14; 95% CI, 0.02–0.82; $P = 0.03$). However, OCT2 level did not reach statistical significance.

In Model 2, Ki-67 remained a significant independent factor for pGR (OR, 7.33; 95% CI, 1.10–48.78; $P = 0.04$). Combined CTR1/OCT2 was an independent factor for non-pGR, with a higher significance level than CTR1 alone (OR, 0.12; 95% CI, 0.03–0.57; $P = 0.008$).

Even when only FEC-T-treated patients in the TNBC group were analyzed, Ki-67^{high} level remained a significant independent predictor for pGR in Model 1 ($P = 0.009$) and Model 2 ($P = 0.03$), as did CTR1^{high} for non-pGR in Model 1 ($P = 0.02$) and combined CTR1/OCT2 for non-pGR in Model 2 ($P = 0.02$).

Discussion

ANC-Tax-based regimens are most commonly used to treat TNBC, but have pCR rates of only ≤30%.[25,26] Early identification of non-cross-resistant drugs could improve TNBC responsiveness. Platinum drugs are another cytotoxic drug type that is often effective for TNBC.[4,5] Torrisi et al[6] evaluated epirubicin/cisplatin/5-fluorouracil followed by paclitaxel as NAC, which resulted in a pCR rate of 40%. Frasci et al[7] assessed a weekly NAC regimen of cisplatin/epirubicin/paclitaxel supported by granulocyte colony-stimulating factor in which 62% of patients showed pCRs.

The addition of carboplatin to ANC-Tax-based NAC for TNBC improved pCR rates in two recent randomized studies. In a Phase II study by Ando et al,[9] carboplatin and weekly paclitaxel followed by cyclophosphamide/epirubicin/5-fluorouracil showed a significantly higher pCR rate than the regimen without carboplatin (61% vs 26%). Von Minckwitz et al[11] assessed adding carboplatin to paclitaxel/liposomal doxorubicin/bevacizumab and obtained a pCR rate of 53%. Unfortunately, these regimens were associated with high incidences of adverse events and increased discontinuation rates. Furthermore, Alba et al[27] found no improvement of the pCR rate by adding carboplatin to epirubicin/cyclophosphamide followed by docetaxel. Hurley et al[8] found ANC-Tax-based NAC with cisplatin to be superior to that with carboplatin, with significantly improved OS for cisplatin-treated patients.

Table 4. Univariate analysis of the correlation of CTR1 and OCT2 levels or clinicopathological parameters with pGR or pCR according to intrinsic subtype.

VARIABLES	TNBC							LUMINAL BC			
	NO.	pGR (%)	NON-pGR (%)	P-VALUE	pCR (%)	NON-pCR (%)	P-VALUE	NO.	pGR (%)	NON-pGR (%)	P-VALUE
Clinical tumor stage											
T3 + T4	14	5 (36)	9 (64)	0.52	2 (14)	12 (86)	0.28	10	1 (10)	9 (90)	1.00
T1 + T2	27	13 (48)	14 (52)		9 (33)	18 (67)		54	4 (7)	50 (93)	
Clinical lymph node status											
N1–N3	34	15 (44)	19 (56)	1.00	9 (26)	25 (74)	1.00	45	4 (9)	41 (91)	1.00
N0	7	3 (43)	4 (57)		2 (29)	5 (71)		19	1 (5)	18 (95)	
Histological grade											
3	14	7 (50)	7 (50)	0.74	3 (21)	11 (79)	0.72	3	0 (0)	3 (100)	1.00
1 + 2	27	11 (41)	16 (59)		8 (30)	19 (70)		61	5 (8)	56 (92)	
Ki-67											
High	29	16 (55)	13 (45)	0.04[a]	10 (34)	19 (66)	0.13	35	3 (9)	32 (91)	1.00
Low	12	2 (17)	10 (83)		1 (8)	11 (92)		29	2 (7)	27 (93)	
CTR1											
High	15	3 (20)	12 (80)	0.03[a]	2 (13)	13 (87)	0.17	18	2 (11)	16 (89)	0.62
Low	26	15 (58)	11 (42)		9 (35)	17 (65)		46	3 (7)	43 (93)	
OCT2											
High	16	3 (19)	13 (81)	0.01[a]	1 (6)	15 (94)	0.03[a]	48	5 (10)	43 (90)	0.32
Low	25	15 (60)	10 (40)		10 (40)	15 (60)		16	0 (0)	16 (100)	
Combined CTR1/OCT2											
CTR1-high and/or OCT2-high	24	6 (25)	18 (75)	0.005[a]	2 (8)	22 (92)	0.003[a]	52	5 (10)	47 (90)	0.57
CTR1-low and OCT2-low	17	12 (71)	5 (29)		9 (53)	8 (47)		12	0 (0)	12 (100)	

Note: [a]Statistically significant.
Abbreviations: CTR1, copper transporter 1; OCT2, organic cation transporter 2; pGR, pathological good response; pCR, pathological complete response; TNBC, triple negative breast cancer; BC, breast cancer.

Table 5. Multivariate logistic regression analysis of predictive factors for pGR or pCR in the TNBC patients.

VARIABLES	pGR	
	OR (95% CI)	*P*-VALUE
Model 1		
Ki-67	9.04 (1.28–63.82)	0.03[a]
CTR1	0.14 (0.02–0.82)	0.03[a]
OCT2	0.22 (0.04–1.20)	0.08
Model 2		
Ki-67	7.33 (1.10–48.78)	0.04[a]
Combined CTR1/OCT2	0.12 (0.03–0.57)	0.008[a]

Note: [a]Statistically significant.
Abbreviations: pGR, pathological good response; pCR, pathological complete response; TNBC, triple negative breast cancer; OR, odds ratio; CI, confidence interval; CTR1, copper transporter 1; OCT2, organic cation transporter 2.

Relatively few studies of the effectiveness of oxaliplatin-containing regimens in BC have been reported. Phase II studies have shown the combination of oxaliplatin with 5-fluorouracil/leucovorin to be effective and safe.[28,29] However, to the best of our knowledge, the effect of adding oxaliplatin to ANC-Tax-based NAC for TNBC has not been studied. We therefore considered that a better understanding of the underlying characteristics of TNBC is needed to identify any specific subgroup that might benefit from the addition of platinum drugs, particularly cisplatin.

Because intracellular uptake of platinum drugs depends on facilitated transport systems, expression levels of platinum uptake transporters may predict the sensitivity of tumors to platinum-based chemotherapy.[30] CTR1 is a critical determinant of the uptake and consequent cytotoxicity of cisplatin and carboplatin,[12,14,15] as OCT2 is of cisplatin and oxaliplatin.[19–21] In the present study, therefore, we immunohistochemically investigated the expression levels of CTR1 and OCT2 in pre-chemotherapy biopsies from HER2-negative BC patients, with or without pathological responses to ANC-Tax-based NAC. In a univariate analysis of the entire cohort, Ki-67^{high} level (pGR, *P* = 0.02) and TNBC subtype (pGR, *P* < 0.001; pCR, *P* < 0.001) were associated with response, whereas OCT2^{high} level was associated with non-response (non-pGR, *P* = 0.003; non-pCR, *P* < 0.001). Because CTR1 and OCT2 both function as cisplatin uptake transporters, we also analyzed data from CTR1 and OCT2 in combination. CTR1^{high} and/or OCT2^{high} status were significantly associated with non-response (non-pGR, *P* = 0.002; non-pCR, *P* < 0.001). Two separate multivariate analyses showed the TNBC subtype to be a powerful independent factor for pGR (*P* = 0.002 in Model 1 and *P* = 0.001 in Model 2). Several studies showed that pCR rates of patients with TNBC were significantly higher than those of patients with non-TNBC.[2,26] In contrast, OCT2 (*P* = 0.04) and combined CTR1/OCT2 (*P* = 0.01) were independent factors for non-pCR but not for pGR; this may be due to the small number of pCR patients.

Because platinum drugs are proposed to be effective for TNBC,[6–8] we further analyzed TNBC and luminal BC to confirm the significance of CTR1 and OCT2 levels in resistance to ANC-Tax-based NAC in TNBCs. In the TNBC group, Ki-67^{high} was associated with response (pGR, *P* = 0.04), whereas CTR1^{high} (non-pGR, *P* = 0.03), OCT2^{high} (non-pGR, *P* = 0.01; non-pCR, *P* = 0.03), and combined CTR1^{high} and/or OCT2^{high} status (non-pGR, *P* = 0.005; non-pCR, *P* = 0.003) were associated with non-response. Multivariate analysis showed that Ki-67^{high} status was a significant independent factor for pGR (*P* = 0.03 in Model 1 and *P* = 0.04 in Model 2). Although we could not detect a significant correlation between Ki-67 level and pCR rate, several investigators reported higher pCR rates in Ki-67^{high} TNBC than in Ki-67^{low} TNBC.[31,32] In contrast, CTR1 was identified as an independent factor for non-pGR (*P* = 0.03). Combined CTR1/OCT2 was the most powerful independent factor for non-pGR (*P* = 0.008), with greater significance than CTR1 alone. Conversely, in the luminal BC group, no variables were associated with pathological response.

Ideally, chemoresistance evaluations should focus on patient groups that received the same chemotherapy regimen. As 91.4% of patients in this study received FEC-T, we also analyzed the FEC-T-treated patients separately. We found that the major conclusion of our study remained unchanged.

These results indicate that platinum uptake transporters are predominantly expressed in ANC-Tax-resistant TNBCs, which implies that the advantage associated with adding platinum drugs, especially cisplatin, may depend on a high level of drug uptake. However, this study has several limitations: its retrospective design, the small number of patients, and the lack of molecular subtyping based on gene profiles. Our results are thus still exploratory, and larger studies are required to confirm the feasibility of our results.

Our additional findings were that the OCT2^{high} level was significantly more prevalent in Ki-67^{low} tumors than in Ki-67^{high} tumors and in luminal BCs than in TNBCs. Some of these results are most likely due to the inverse correlation between Ki-67 level and hormone receptor expression in BCs.[33] However, the possible role of OCT2 in cell cycle regulation and its hormone-dependent expression have not yet been clarified.

Conclusion

Our results suggest a possible contribution of CTR1 and OCT2 to the effect of platinum drugs for ANC-Tax-resistant TNBCs. Further studies that use TNBC cell lines or TNBC-based xenograft models are needed to verify the associations between high CTR1 and OCT2 levels and ANC-Tax-resistance, ANC-Tax dose dependency, and the effectiveness of adding platinum drugs to ANC-Tax-based regimens. Whether CTR1 and OCT2 expression levels predict the

prognosis of patients treated with platinum-containing chemotherapy also warrants further study.

Author Contributions

Performed experiments and data analysis and wrote the manuscript: RT. Performed experiments: AN; Collected clinicopathologic data and tissue samples: NO, YK, KK, SS. Designed the study, guided the experiments, and edited the manuscript: SK. All authors read and approved the final manuscript.

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