Clinical Significance of the Histoculture Drug Response Assay in Breast Cancer

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Abstract. *Background/Aim: Despite the fact that breast cancer patients are generally administered systemic chemotherapy after surgical treatment, predictive factors that allow optimization of chemotherapy are needed. The histoculture drug response assay (HDRA) is a clinically practical in vitro drug-response assay for identifying optimal anticancer agents. Patients and Methods: Thirty-eight primary breast cancer patients who underwent surgical treatment without receiving systemic neoadjuvant therapy were analyzed. We retrospectively examined the relationships between clinicopathological factors and the HDRA results of 5 anticancer agents (mitomycin C (MMC), 5-fluorouracil (5-FU), doxorubicin hydrochloride (ADM), cisplatin (CDDP) and paclitaxel (PTX)). Results: The relationships between the inhibition rates of anticancer drugs and clinicopathological factors were not significant, except for nuclear grade and venous invasion with the inhibition rate of 5-FU. We also established the threshold inhibition rate for PTX. The paclitaxel inhibition rate was significantly associated with disease-free survival (DFS). Conclusion: HDRA results were independent from the clinicopathological factors of breast cancer patients demonstrating that individualized treatment is needed.*

Breast cancer is the most common cancer in women worldwide. Postoperative adjuvant chemotherapy is widely used for the treatment of breast cancer. Nowadays, systemic

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chemotherapy for breast cancer generally includes anthracyclines and/or taxanes and/or the antimetabolite 5-fluorouracil (5-FU), which are the most active cytotoxic agents for both early and advanced breast cancer (1). Although anthracyclines have been shown to provide increased benefits for breast cancer patients with *HER2* overexpression compared to patients without *HER2* overexpression (2), other clinicopathological breast cancer markers that might allow the optimization of chemotherapy remain unverified.

The histoculture drug response assay (HDRA) was developed as an *in vitro* drug-response assay for choosing anticancer agents (3). The tumor inhibition rates (IRs) of chemotherapy agents evaluated by the HDRA were found to be predictive of response of various types of cancer to chemotherapy (4-8). However, the relationships between the clinicopathological factors of tumors and HDRA IRs of chemotherapy agents have not been clarified.

Herein, we report an investigation of HDRA used for primary breast cancer tumors, which included an analysis of the relationship between the clinicopathological characteristics of tumors and their *in vitro* response to chemotherapy agents.

Patients and Methods

Patients. Thirty-eight patients with primary breast cancer who underwent surgical treatment without receiving neoadjuvant therapy at Kagoshima University Hospital, Kagoshima, Japan, were enrolled in this study. Diagnosis of breast cancer was performed on a core needle biopsy (CNB) specimen from each patient. After histopathological diagnosis of hematoxyline-and-eosin-stained CNB sections, followed by immunohistochemical staining for estrogen receptor (ER), progesterone receptor (PgR), HER2 and Ki67 (9), the tumors were classified into the following 4 subtypes: luminal A, luminal B, HER2-enriched and triple-negative (TN). From June 2011 to March 2014, only TN specimens were included and from April 2014 to August 2015, all subtypes were included. Patients with an excised tumor with insufficient tissue for the HDRA were excluded. Adjuvant therapy was selected for each patient based on the National Comprehensive Cancer Network guidelines (10). Demographic data of the patients, clinicopathological features of the

Table I. Clinicopathological characteristics of patients.

Factors	Number
Total number of patients	38
Median age (years)	62
Median follow-up duration (months)	12
Estrogen receptor	
Positive	19
Negative	19
Progestrone receptor	
Positive	16
Negative	22
HER2	
Positive	2
Negative	36
Subtype*	
Luminal A	7
Luminal B	10
Luminal HER2	2
HER2 enriched	0
Triple negative	19
T factors**	
T1-2	26
T3-4	12
Lymph node metastasis	
Absent	17
Present	21
Nuclear Grade	
1-2	21
3	16
Not available	1
Lymphatic invasion	
Absent	10
Present	28
Venous invasion	
Absent	34
Present	4
Stage**	
Stage I-II	21
Stage III-IV	17
Adjuvant systemic therapy	
Chemotherapy	11
Anthracycline	(3)
Taxane	(1)
Anthracycline + Taxane	(6)
Fluorouracil	(1)
Chemotherapy + Endocrine therapy	6
Anthracycline	(0)
Taxane	(3)
Anthracycline + Taxane	(3)
Endocrine therapy	11
No systemic therapy	10
Reccurrence	7
Cancer death	2

*Subtypes were classified accroding to the St. Gallen International Expert Consensus (7). The cut-off of Ki-67 was set at 20%. **Clinical stage was assessed using TNM classification of malignant tumors, 7th edition (13).
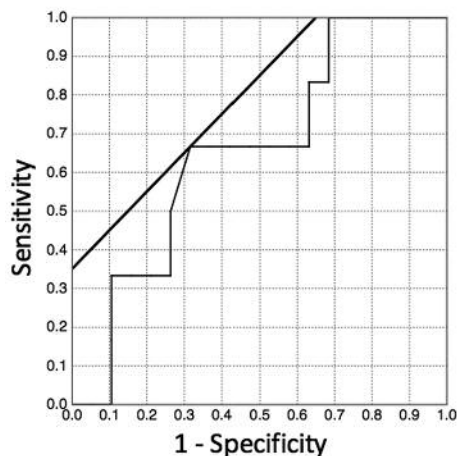
Figure 1. Receiver operating characteristic curve analysis of tumor inhibition rates associated with paclitaxel in relation to predicting the recurrence of breast cancer after treatment.
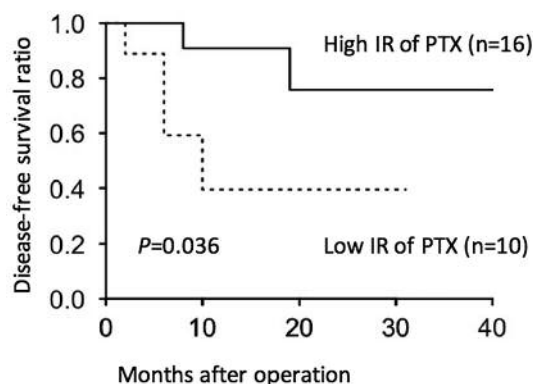

Figure 2. Kaplan-Meier disease-free survival curves of patients with breast cancer whose tumors showed high vs. low inhibition rates associated with paclitaxel.

tumors and other relevant data are shown in Table I. Each patient provided written informed consent before sample acquisition; this study was approved by the Institutional Review Board of Kagoshima University Hospital.

HDRA. Samples were immediately collected from resected primary tumors and cut into 5-mm cubes. The HDRA was performed by SRL Inc. (Tokyo, Japan). The HDRA procedures have been reported previously (6, 9). Briefly, pieces of tumor tissue, approximately 10 mg each, were placed on a collagen sponge gel. They were incubated for 7 days with 5 anticancer agents (mitomycin C (MMC), 5-fluorouracil (5-FU), doxorubicin hydrochloride (ADM), cisplatin (CDDP) and paclitaxel (PTX)) in RPMI 1640 medium containing 20% fetal calf serum at 37°C in a 5% CO_2 atmosphere. Collagenase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Na
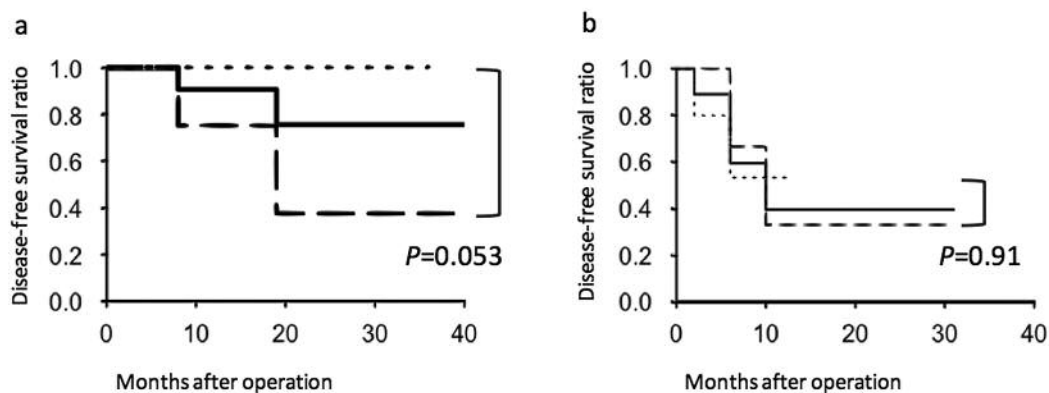
Figure 3. *Kaplan-Meier disease-free survival curves of patients with breast cancer based on whether or not they received a taxane regimen. (a) Patients whose tumors showed high inhibition rates associated with paclitaxel (PTX) are shown by a solid line. Patients who received taxane (dashed line) or did not receive taxane (dotted line) were compared. (b) Patients whose tumors showed low IRs associated with PTX are shown by a solid line. Patients who received taxane (dashed line) or did not receive taxane (dotted line) were compared.*

succinate were then added to the cultures that were incubated for an additional 16-h period. The resulting formazan crystals were extracted from the tumor cultures by dimethyl sulfoxide. The optical density of each culture was measured at 540 nm. The IR was calculated as (1-mean absorbance of treated tumor per 1 g/mean absorbance of untreated control tumor per 1g) ×100. The IR cut-off values, as previously determined by SRL Inc., have been based on the histological response rates of each drug in breast cancer tumors (11). According to the predetermined threshold values for MMC (70%), ADM (60%), 5FU (60%) and CDDP (50%), the breast cancer patients were divided into groups according to the IR values for each agent. Clinicopathological factors of the patients with high and low IR values for each agent were compared. Since the cut-off value of IR had never been determined for PTX, a receiver operating characteristic (ROC) curve of PTX IRs was plotted to predict recurrence and establish the missing cut-off value.

Statistical analysis. Differences in the clinicopathological factors between the groups were evaluated using analysis of variance for continuous variables and the Pearson Chi-square test for categorical variables. Disease-free survival (DFS) and overall survival (OS) were measured from the time of the first surgery until the date of death or last follow-up. Survival curves were constructed using the Kaplan-Meier method and statistical significance between groups was assessed using the log-rank test. Multivariate analysis was conducted using the Cox proportional hazards model. Statistical analysis was performed by JMP Pro Version 12.1.0 for Mac OS (SAS Institute Japan, Tokyo, Japan). *p*-Values less than 0.05 were considered statistically significant.

Results

Prognostic analysis based on PTX inhibition rates. To determine the cut-off value for the IR of PTX, we analyzed DFS and OS according to the IRs of PTX. To predict recurrence, the ROC curve of PTX IRs was analyzed and a cut-off value of 84% was established (Figure 1). Patients whose tumors had high PTX IR values had significantly longer DFS than the patients with low PTX IR values (Figure 2). The difference between the OS rates of the patients with high *vs*. low IRs for PTX was not significant (data not shown). Another analysis found no significant difference between the DFS rates of patients receiving PTX *vs*. those not receiving PTX (Figure 3). There was no significant difference between the DFS and OS rates of patients with high *vs*. low IRs for MMC, 5-FU, ADM and CDDP (data not shown). Multivariate analysis, using the Cox proportional hazards model, revealed that the PTX IR was an independent prognostic predictor for DFS (relative risk (RR)= 0.0097; 95% confidence interval (CI)=0.0038-0.87; *p*=0.0036) (Table II). Pathological N and nuclear grade were also found to be independent prognostic predictors (RR=16; 95% CI=1.3-660; *p*=0.03 and RR=14; 95% CI=1.4-420; *p*=0.025, respectively).

Relationship between clinicopathological factors and tumor-chemotherapy inhibition rates in the HDRA. The clinicopathological factors of patients and tumors classified based on high and low IR of the 5 anticancer agents (with the IR cut-off value of PTX as determined by us) are shown in Table III. The differences in the number of patients treated with 5-FU with high *vs*. low IR were significant for nuclear grade (*p*=0.018) and venous invasion (*p*=0.030). ER, PgR and HER2 positivity of tumors with high *vs*. low IR were not significant for any of the 5 agents.

Discussion

There are many clinicopathological factors that may predict the risk of postoperative recurrence in breast cancer; thus, the identification of risk factors is considered important for

Table II. *Results of the multivariate analysis of clinicopathological factors and disease-free survival (Cox proportional hazards model).*

Factors	Univariate *p*-Value	Multivariate *p*-Value	Relative Risk (95% CI)
T factor (Tis-2/T3-4)	0.9		
N factor (N0/N1-3)	0.01	0.03	16 (1.3-660)
Nuclear Grade (1-2/3)	0.21	0.025	14 (1.4-420)
Ki-67 (≤20%/<20%)	0.85		
ER (positive/negative)	0.54		
PgR (positive/negative)	0.45		
Chemotherapy (+/−)			
All regimen (+/−)	0.17	0.12	6.2 (0.66-200)
PTX regimen (+/−)	0.34		
Endocrine therapy (+/−)	0.76		
PTX Inhibition rate (low/high)	0.06	0.036	0.097 (0.0038-0.87)

CI, Confidence interval; PTX, paclitaxel; ER, estrogen receptor; PgR; progesterone receptor.

deciding if systemic therapy is appropriate for a patient (12). However, the clinicopathological factors that are predictive of the efficacy of systemic chemotherapy have not been adequately clarified. Although HDRA is the only clinically practical method for estimating the efficacy of a specific chemotherapeutic agent, the relationships between clinicopathological factors, patient outcome and the data from HDRA are not completely understood. Thus, we analyzed the relationships between the tumor-chemotherapy IRs as assessed by HDRA and the clinicopathological factors of patients with primary breast cancer. There was a significant relationship between high nuclear grade and/or aggressive venous invasion of tumors with high IRs in response to 5-FU, which may indicate that highly aggressive tumors are sensitive to chemotherapy. High-grade breast cancers have been reported to be highly sensitive to cytotoxic chemotherapy, including 5-FU regimens, in the setting of neoadjuvant chemotherapy (13). ER, PgR, HER2 and Ki-67 expression was not significantly different between the patients whose tumors had high *vs.* low IR to any of the drugs. These findings are consistent with a previous study by Kim *et al.* who did not find a significant relationship between drug sensitivity as determined by HDRA and subtypes of breast cancer (14).

In this study, we established a cut-off IR value for PTX of 84%, since there was no previously determined value. Patients whose tumors showed high IRs to PTX had good DFS, with the PTX IR being significantly associated with DFS by multivariate analysis (Table III). However, none of the cut-off IR values of the 4 other anticancer drugs were found to be significantly associated with DFS. The significant association between the tumors with high PTX IRs and DFS might partially account for the effectiveness of taxane regimens. The effectiveness of taxanes against breast cancer has been reported by *in vitro* and neoadjuvant studies. Higher nuclear grade (15), higher Ki-67 values (16), younger age

(17) and TN subtypes (18) have been reported to be significant factors in patients with tumors that were highly sensitive to taxanes. We could not find any significant association between these factors and tumor IRs in relation to PTX. In our study, the chemotherapy regimens were not chosen based on HDRA results but on other clinicopathological factors. Only 5 out of 16 patients with tumors showing a high PTX IR received taxane-based chemotherapy. Although postoperative chemotherapy was only administered to patients considered to have poor prognosis, the presence or absence of chemotherapy using PTX was not found to be significantly associated with DFS by multivariate analysis. In addition, we did not find a significant difference in DFS associated with whether or not the patient received PTX (Figure 3). The predictive power of the PTX IR, as determined by HDRA, might have been influenced by other tumor biological features, which may differ with respect to PTX sensitivity. Some molecules, reported to have suppressive effects on tumors, enhance paclitaxel chemosensitivity in breast cancer (19-21) and other cancers (22, 23). Therefore, we strongly believe that additional studies to examine the importance of HDRA are needed.

Neoadjuvant chemotherapy is now frequently used for patients with breast cancer. HDRA might be a useful clinical tool for the assessment of breast cancer before surgery. We evaluated HDRA using CNB samples or vacuum-assisted CNB samples from several patients before they underwent surgery or systemic therapy; however, the tissue was insufficient for performing successful HDRAs (data not shown).

The limitations of this study include the small number of patients, especially HER2-positive breast cancer patients, and the short observation period. We also could not choose the type of adjuvant chemotherapy based on the HDRA results. However, we did find a significant association between high PTX IR and outcome. We believe that the results obtained

Table III. *Relationships between clinicopathological factors and inhibition rate of each anticancer agent.*

Drug agents	MMC			5-FU			ADM			CDDP			PTX		
Inhibition rate	High (n=22)	Low (n=15)		High (n=18)	Low (n=19)		High (n=17)	Low (n=16)		High (n=18)	Low (n=12)		High (n=16)	Low (n=10)	
Factors	Number	Number	p-Value	Number	Number	p-Value	Number	Number	p-Value	Number	Number	p-Value	Number	Number	p-Value
Age (mean±SD)	64±3	55±3	0.061	62±3	60±3	0.65	62±4	59±4	0.54	60±3	63±4	0.67	63±3	57±4	0.27
Estrogen receptor															
Positive	9	10	0.12	9	10	0.87	9	7	0,.73	8	6	0,77	7	3	0.48
Negative	13	5		9	9		8	9		10	6		9	7	
Progestrone receptor															
Positive	8	8	0.31	7	9	0.6	7	6	0.83	7	5	0.88	7	1	0.07
Negative	14	7		11	10		10	10		11	7		9	9	
HER2															
Positive	1	1	0.78	1	1	0.97	1	1	0.97	0	1	0.21	0	0	NA
Negative	21	14		17	18		16	15		18	11		16	10	
Ki67															
≤20%	5	3	0.84	5	3	0.38	5	3	0.48	4	3	0.86	4	0	0.086
20%<	17	12		13	16		12	13		14	9		12	10	
T factors															
Tis-2	14	11	0.54	11	14	0.41	10	12	0.32	11	9	0.43	9	8	0.22
T3-4	8	4		7	5		7	4		7	3		7	2	
Lymph node metastasis															
Absent	11	5	0.32	8	8	0.89	9	5	0.21	8	4	0.54	7	3	0.48
Present	11	10		10	11		8	11		10	8		9	7	
Nuclear Grade															
1-2	10	11	0.12	7	14	0.018	10	10	0.65	10	9	0.37	9	6	1
3	11	4		11	4		7	5		7	3		6	4	
Lymphatic invasion															
Absent	6	3	0.61	3	6	0.29	3	4	0.61	3	4	0.29	5	1	0.21
Present	16	12		15	13		14	12		15	8		11	9	
Venous invasion															
Absent	19	14	0.5	14	19	0.030	14	15	0.32	14	12	0.079	13	9	0.55
Present	3	1		4	0		3	1		4	0		3	1	
Stage															
Stage I-II	12	8	0.94	10	10	0.86	10	8	0.61	10	6	0.77	8	5	1
Stage III-IV	10	7		8	9		7	8		8	6		8	5	
Reccurence	5	2	0.43	4	3	0.56	4	3	0.1	4	2	0.65	2	4	0.13
Non reccurent	16	13		13	16		13	12		13	10		13	6	
Cancer death	2	0	0.23	1	1	0.97	2	0	0.16	1	1	0.77	1	1	0.73
Alive	20	15		17	18		15	16		17	11		15	9	

SD, Standard deviation; MMC, mitomycin C; 5-FU, 5-fluorouracil; ADM, doxorubicin hydrochloride; CDDP, cisplatin; PTX, paclitaxel.

from patients who received standard adjuvant chemotherapy are noteworthy; however, a larger study with a longer observation period is considered necessary.

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