Table 2. Differentially expressed ABC transporters ordered by significance

Gene symbol	Genbank	Parametric *p*-value*	% CV support	RD[a]	pCR[b]	Fold difference[c]	Description
ABCC5	AF146074	0.000368	100	6009.1	2427.5	2.48	ABC, sub-family C (CFTR/MRP), member 5
ABCC5	BE550362	0.000463	100	3571.5	1234.4	2.89	ABC, sub-family C (CFTR/MRP), member 5
ABCA12	AL080207	0.000795	100	711.7	93.1	7.64	ABC, sub-family A (ABC1), member 12
ABCA1	AL833227	0.000859	100	166.8	50.5	3.3	ABC, sub-family A (ABC1), member 1
CFTR	NM_000492	0.007030	100	27.7	104.4	0.27	cystic fibrosis transmembrane conductance regulator, ABC (sub-family C, member 7)
ABCF2	NM_005692	0.015901	100	49.4	154.1	0.32	ABC, sub-family F (GCN20), member 2
TAP2	M74447	0.019345	89	543.4	1008.5	0.54	Transporter 2, ABC, sub-family B (MDR/TAP)
ABCC13	NM_172025	0.019377	100	157.5	20.9	7.54	ABC, sub-family C (CFTR/MRP), member 13
ABCB6	NM_005689	0.027077	89	1471.9	677.5	2.17	ABC, sub-family B (MDR/TAP), member 6
TAP2	AA573502	0.042069	58	1740.5	2802	0.62	Transporter 2, ABC, sub-family B (MDR/TAP)
ABCC11	AF352582	0.048626	42	160.9	59.4	2.71	ABC, sub-family C (CFTR/MRP), member 11

Table sorted by *p*-value. * *p* by random variance *t*-test.
[a]Geometric mean of intensities in the RD group.
[b]Geometric mean of intensities in the pCR group.
[c]Fold difference of geometric means RD: pCR.

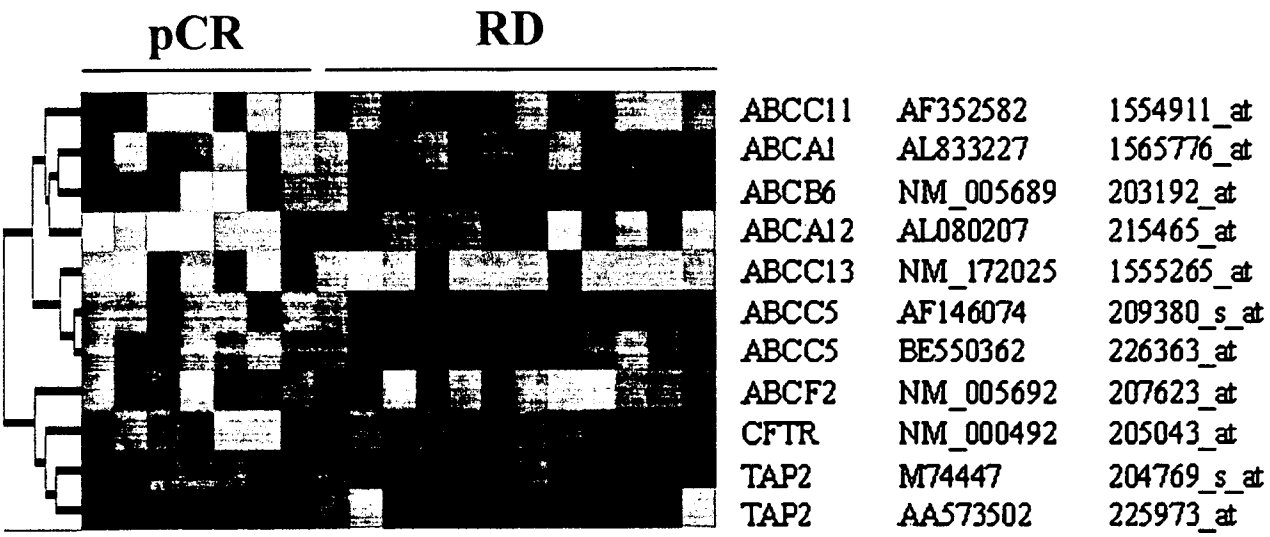

Figure 1. Hierarchical clustering of differentially expressed ABC transporters associated with the response to neoadjuvant chemotherapy in breast cancer patients. The cluster image map shows patterns of differential ABC transporter gene expression in breast cancer patients in respect to the response to neoadjuvant chemotherapy. The hierarchical clustering on each axis was performed using the complete linkage algorithm. Relatively highly expressed genes are shown in red, low expressed genes are shown in green.

an individual basis, there is a real need to develop an appropriate predictor to identify those cancer patients most likely to require or benefit from particular therapies. Resistance to chemotherapy is significant obstacle to appropriate treatment of cancer patients and affects the treatment outcome. Numerous cellular mechanisms exist which are responsible for the treatment failure due to chemoresistance. ABC transporters are the one of the major factors leading to drug resistance. Extensive study has been conducted on the ABC transporters, and ABCB1 (MDR1-P-gp) [1,2], ABCC1-MRP1 [3], and ABCG2-MXR [4] are particularly well known for their role in resistance to several chemotherapeutic agents. Because the members of the ABC transporters are grouped by sequence homology, the remained members may play roles in absorption, distribution, and excretion of chemotherapeutic agent and probably be related to drug resistance although little has been known about most of the functions of these genes. Characterization of the expression of the genes related to chemoresistance is an interesting subject and may lead to clinically useful predictors of response to chemotherapy. The profiling of ABC transporter genes in relation to the clinical response to chemotherapy may also be useful to determine the patient's underlying risk and choose the optimal therapeutic regimen to which the individual cancer patient is most likely to respond.

Focusing on the ABC transporters, we analyzed the gene expression profile in breast cancer patients using microarray data that contain the transcripts of all the

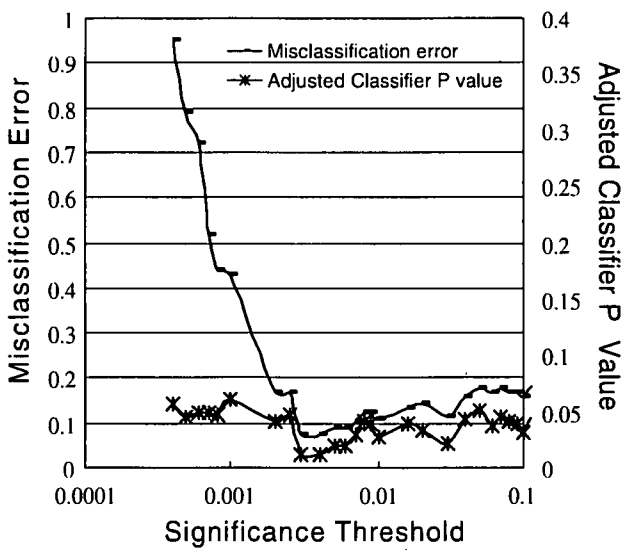

Figure 2. Multivariate predictive classification models in leave-one-out cross-validation and permutation test with an increasing significance threshold at which genes were selected as a classifier. The *x*-axis represents the significance threshold p value used to select the discriminate genes as classifiers. The *y*-axis shows the average of the misclassification error rate determined by leave-one-out cross-validation and the average classifier *p*-value, the probability that a similar low error rate could happen by chance calculated after 2000 permutations. Classifier genes selected as differentials between the 2 classes at a significance threshold $p=0.003$ level showed the highest discriminate value.

members of ABC transporter family. We compared the expression pattern of the ABC transporters between two classes of pretreatment tumor samples divided by the pathologic response to neoadjuvant chemotherapy (RD versus pCR).

On microarray analysis, several ABC transporters showed differential expression between the two groups of tumors. Of interest, several ABC transporters showed increased expression in the pCR group, including CFTR (NM_000492, ABCC7, fold ratio 0.27, $p=0.007030$), ABCF2 (NM_005692, fold ratio 0.32, $p=0.015901$) and ABCB3 (M74447, TAP2, fold ratio 0.54, $p=0.019345$). ABCB3 is known to be involved in antigen presenting by transporting peptides necessary for the assembly of major histocompatibility complex (MHC) class I molecules from the cytoplasm to the endoplasmic reticulum [18]. It is also known that its reduced expression is associated with HLA class I deficient human tumor cell lines [19] and it has been suggested that it is related to the aggressive features of some kinds of tumors [20–22]. Its increased expression has been found to be associated with pathological complete response in our clinical samples, but any clinical significance in the treatment of in breast cancer remains to be elucidated.

Five ABC transporters ABCC5 (AF146074, fold ratio 2.48, $p=0.000368$), ABCA12 (AL080207, fold ratio 7.64,

Table 3. Performance of the multivariate classifier; the sensitivity, specificity, PPV and NPV for the pCR group of each predictor model at a significance threshold of $p=0.003$

	CCV[a]	1NNC[b]	3NNC[c]	NCC[d]	SVM[e]	LDD[f]	Average[g]
Sensitivity	100	85.7	85.7	85.7	71.4	100	88.1
Specificity	100	91.7	91.7	100	100	91.7	95.9
PPV	100	85.7	85.7	100	100	87.5	93.2
NPV	100	91.7	91.7	92.3	85.7	100	93.6
Misclassification error	0	0.05	0.11	0.11	0.05	0.11	0.072
Percent correctly classified	100	95	89	89	95	89	92.8
Classifier P	5.00E-04	0.014	0.025	0.006	0.023	0.005	0.01225

[a]Compound covariate predictor classifier.
[b]1-Nearest neighbor classifier.
[c]3-Nearest neighbor classifier.
[d]Nearest centroid classifier.
[e]Support vector machine classifier.
[f]Linear diagonal discriminant analysis classifier.
[g]Average value of six multivariate classifier models.

Table 4. ABC transporters selected as best classifiers at a significance threshold of 0.003

Gene symbol	Genbank	*t*-Value	Parametric *p*-value*	% CV support	RD[a]	pCR[b]	§Fold difference	Description
ABCC5	AF146074	4.43	0.000368	100	6009.1	2427.5	2.48	ABC, sub-family C (CFTR/MRP), member 5
ABCC5	BE550362	4.32	0.000463	100	3571.5	1234.4	2.89	ABC, sub-family C (CFTR/MRP), member 5
ABCA12	AL080207	4.07	0.000795	100	711.7	93.1	7.64	ABC, sub-family A (ABC1), member 12
ABCA1	AL833227	4.04	0.000859	100	166.8	50.5	3.30	ABC, sub-family A (ABC1), member 1

Table sorted by *p* value.
*Parametric *p*-value by random variance *t*-test.
[a]Geometric mean of intensities in the RD group.
[b]Geometric mean of intensities in the pCR group. §Fold difference of geometric means; RD: pCR.

$p = 0.000795$), ABCA1 (AL833227, fold ratio 3.30, $p = 0.000859$), ABCC13 (NM_172025, fold ratio 7.54, $p = 0.0194$), ABCB6 (NM_005689, fold ratio 2.17, $p = 0.0271$) and ABCC11 (AF352582, fold ratio 2.71, $p = 0.0486$) showed significantly increased expression in the RD group associated with a decreased responsiveness to sequential weekly paclitaxel/FEC (5-fluorouracil, epirubicin and cyclophosphamide) neoadjuvant chemotherapy. Of these, ABCC5 was selected with the highest significance ($p = 0.000368$) and the highest expression level (RD: pCR 6009.1: 2427.5) although correlation between the gene expression level and the functional protein level remains to be seen. The ABCC5 (MRP5) transporter on human chromosome 3q27 has been known to be involved in the transport of nucleoside analogs [23] and has been reported to confer resistance to several drugs including methotrexate, GW1843 and ZD1694 (raltitrexed) [24]. Recently, Pratt et al. demonstrated that ABCC5 confers resistance against 5-fluorouracil [17] that was used in our neoadjuvant chemotherapy regimen. These results suggest that ABCC5 mediates transport of several chemotherapeutic agents and may contribute to resistance against 5-fluorouracil which is presently used in neoadjuvant chemotherapy.

In our clinical trial setting, ABCB1, known to confer resistance to several chemotherapeutic agents including paclitaxel, did not significantly increase in tumors with decreased response to neoadjuvant chemotherapy. Samples used in this study were all from chemotherapy-naïve patients and the time of exposure to the drug may not have been sufficient to induce the gene expression of this transporter. Although several ABC transporters showed high expression levels in the pretreatment samples, ABCB1 did not show significantly high expression. ABCB1 may thus play a greater role in resistance to chemotherapy in a secondary chemotherapy clinical setting than in first line chemotherapy when the exposure time is sufficiently long to induce the gene expression of the transporters known to be inducible by exposure to that chemotherapeutic agent [25,26].

But, some ABC transporters may also play significant role in chemoresistance in early breast cancer. Recently, it was reported that ABCC1 expression predict shorter relapse free survival and overall survival and play important role in resistance to chemotherapy in early breast cancer who underwent CMF (cyclophosphamide, methotrexate, and fluorouracil) adjuvant chemotherapy [27].

A variety of compounds are transported by ABC transporters through the lipid bilayer and still little has been known about the function of individual transporters in transport of chemotherapeutic agents. ABCA1 has been implicated in the control of the extrusion of membrane phospholipids and cholesterol toward specific extracellular acceptors [28] and macrophage interleukin-1 beta secretion and apoptosis [29]. ABCC13, highly expressed in the RD group mapped to chromosome 21q11.2 has been suggested that it might be associated with hematopoiesis. It has also been reported that ABCC13 shows decreased expression during cell differentiates [30]. ABCC11, called MRP8 is known to be a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2′,3′-dideoxycytidine and 9′-(2′-phosphonylmethoxyethyl) adenine [31]. Szakacs et al. [10] suggested ABCC11 mediated resistance may not be confined to nucleoside analog, demonstrating that the ABCC11 transfected cell confers resistance to NSC 671136 by 2–3 fold. ABCB6 is a mitochondrial half transporter that is known to be involved in the transport of a precursor of the Fe/S cluster from mitochondria to the cytosol [32]. A recent report showed that several ABC transporters including ABCB6 amplified drug resistance in a non small cell lung cancer cell line (A549/CPT) in comparison with its parental cell [33].

Although the role in chemoresistant of individual transporters selected in our study to discriminate between the pCR and RD groups remains to be revealed, the transporters may also play roles in response to chemotherapy by influencing absorption, distribution, and excretion of chemotherapeutic agents.

To evaluate the predictive signature of ABC transporters, we examined multigene predictor model of response to neoadjuvant chemotherapy using differentially expressed ABC transporters. Six different multivariate classification models were examined. When the ABC transporters differentially expressed between the two classes at a significance threshold level of 0.003 were used for class prediction, an average 92.8% of predictive accuracy was observed, with a 93.2% positive predictive value for the pCR group, 93.6% negative predictive value, sensitivity for the pCR group of 88.1%, and 95.9% specificity. The classifier p-value, the probability that a similar low error rate could happen by chance, was also low ($p = 0.012$). The optimum classifier model included ABCC5, ABCA1, and ABCA12. These genes all showed high expression in tumors in the RD group.

Of interest, although we developed the class prediction model from a small subset of genes, i.e., genes belonging only to the ABC transporter family, the predictive accuracy reached above 90% with quite a low classifier p-value although these prediction models based on ABC transporter genes need to be validated in future studies by comparing the classification model with all subsets of genes and with larger numbers of samples.

Our result suggest that several ABC transporters in human breast cancer cells may contribute to the clinical response to neoadjuvant chemotherapy and gene expression profiling of these ABC transporters may be useful in prediction of the pathologic response to sequential weekly paclitaxel/FEC in breast cancer patients.

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ORIGINAL ARTICLE

Correlation of p53 and MIB-1 expression with both the systemic recurrence and survival in cases of phyllodes tumors of the breast

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Abstract

Phyllodes tumors are rare primary tumors of the breast. The study aimed at evaluating the immunohistochemical features of phyllodes tumors of the breast that may be useful for predicting the clinical outcome. We examined the immunohistochemical expression of the epidermal growth factor receptor (EGFR), HER2/neu, CD117/c-kit, p53, and MIB-1, and analyzed correlations between the immunohistochemical findings and the clinical outcome. The study included 41 patients with phyllodes tumor (20 benign, 5 borderline, and 16 malignant). Systemic recurrence occurred in 9 patients. The 2-year survival rate was 84%, and the 2-year recurrence-free survival rate was 77%. Six patients developed systemic recurrence within the first year after surgery. None of the phyllodes tumors was positive for HER2/neu or CD117/c-kit. Positive staining for p53 was seen in 10 phyllodes tumors (24%), and the median MIB-1 index was 10%. Both p53 expression and the MIB-1 index, but not the expression status of EGFR, were significantly correlated with the recurrence-free and overall survival. p53 expression status and MIB-1 index may be significant prognostic factors in patients with phyllodes tumors, and careful postoperative follow-up may be important in those cases showing positive expression of p53 and/or MIB-1 index.

Keywords: Phyllodes tumor; p53; MIB-1; Systemic recurrence; Survival

Introduction

Phyllodes tumors of the breast are rare, accounting for less than 1% of all breast tumors. [16]. Phyllodes tumors occur predominantly in middle-aged women, and the average tumor size is 4–5 cm. Histopathologically, these tumors are distinguished from true sarcomas by the presence of epithelial elements within the cellular connective tissue stroma. At present, phyllodes tumors are classified into benign, borderline, and malignant subtypes based on a combination of histological features, stromal cellular atypia, mitotic activity, stromal overgrowth, and tumor margins [15]. Approximately 50% of phyllodes tumors are benign, while the

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incidence of the malignant subtype has been reported to range from 26% to 35% [16,17]. While local recurrence can occur in all phyllodes tumors, systemic recurrence may also develop in cases of borderline or malignant phyllodes tumors [8].

Several studies have been carried out in which different pathologists have evaluated the same histological slides, resulting in a discordance of up to 25% in the final histopathological typing [5,9]. It remains difficult to predict the clinical outcome of the patients based solely on the histological features, and no effective treatment strategies have been developed for systemic involvement in these cases. Previous studies have been conducted to investigate the usefulness of immunohistochemical analyses of the tumors for various tumor markers to predict the clinical outcome. Immunohistochemical detection of p53 expression, commonly used as an identification for tumor-suppressor gene mutation, has been correlated with tumor grade [6,13,19]. Several studies on MIB-1 immunostaining, cell proliferation, have also shown a correlation between MIB-1 positivity and the histological grade [6,11,12,24]. Furthermore, several studies have also investigated the expression of other tumor markers in phyllodes tumors, including actin, epidermal growth factor receptor (EGFR), HER2/neu, BM28/cdc, CD34, CD117/c-kit, platelet-derived growth factor, and vascular endothelial growth factor [4,7,18,19,21–23], but these previously conducted studies did not add substantially to the information already provided by standard histopathological analysis.

The aim of the present study was to conduct an immunohistochemical analysis to determine the expression status of EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 in phyllodes tumors. We assessed the correlation between the results of the immunohistochemical analysis and the clinical outcome in an attempt to identify factors predictive of the prognosis in cases of phyllodes tumors of the breast.

Patients and methods

The study group consisted of all patients with phyllodes tumor of the breast diagnosed at the National Cancer Center Hospital, Tokyo, between 1994 and 2004. The histological sections were re-reviewed by a single pathologist (T.H.) for diagnosis. Patient history and follow-up data were obtained by a review of the medical records. Recurrence-free survival (RFS) time was measured from the time of surgery until the appearance of systemic recurrence or until the last day of follow-up without evidence of systemic recurrence, and the overall survival time (OS) was measured from the time of surgery until the last day of follow-up or death, whichever came earlier.

Immunohistochemical analysis of tissue samples

Immunohistochemical staining of the tissue sections obtained from formalin-fixed, paraffin-embedded blocks was performed for EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 using the labeled streptavidin–biotin method. The antibodies used for the immunohistochemical staining were as follows: EGFR (EGFR pharmDx Kit, 2-18C9, DakoCytomation, Glostrup, Denmark), HER2/neu (CB11, BioGenex, San Ramon, USA), CD117/c-kit (A4502, DakoCytomation, Glostrup, Denmark), p53 (DO7, DakoCytomation, Glostrup, Denmark), and MIB-1 (Immunotech, Marseille, France). The anti-EGFR monoclonal antibody, clone 2-18C9, which binds to an epitope located near the ligand-binding domain on the extracellular domain of EGFR, has been shown to be specific for EGFR and not to cross-react with HER2 or other receptors of the HER family [20].

The immunohistochemical analysis of the primary tumor in all patients was conducted by the same investigator (T.H.), blinded to the clinical status of the patients. The intensity of the immunochistochemical staining for p53, EGFR, HER2/neu, and CD117/c-kit was also similarly scored as 0, negative; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. Negative controls, in which the primary antibody was omitted, were also included in each run. As for positive controls, invasive breast cancers showing strong staining (3+) were used as the positive controls for EGFR and HER2/neu staining, and tissue mast cells showing strong staining (3+) were used as the internal positive controls for CD117/c-kit staining. The proportion of positive cells was categorized as sporadic (positive cells $<10\%$); focal ($11\% <$ positive cells $<50\%$); and diffuse (positive cells $\geqslant 50\%$). The immunohistochemical scores of 2+ and 3+ with focal to diffuse distribution were considered to represent positive expression of the respective markers.

The MIB-1 index was defined as the percentage of nuclei showing positive staining calculated after counting 1,000 neoplastic cells per slide. The cut-off point for the value of the MIB-1 index ($>11.2\%$ vs. $\leqslant 11.2\%$) was defined based on the results of a previous study [13].

Statistical analysis

The Kaplan–Meier method was used to describe the distribution of the RFS and the median OS. The prognostic factors of primary tumor size ($\leqslant 10$ cm vs >10 cm) and histological types (benign vs. borderline vs. malignant) were analyzed statistically. The relationships between the expression of the biomarkers (EGFR, p53, and MIB-1 index) and the clinical outcomes of the patients were compared with the log-rank test, and the

hazard ratios and confidence intervals were estimated using the Cox proportional hazards model. p Values <0.05 were considered statistically significant. All the statistical analyses were performed with the SAS software (Release 8.2, SAS Institute Inc., Cary, NC, USA).

Results

Of the 41 patients with phyllodes tumors included in this study, 20 (48%) were benign, 5 (12%) were borderline, and 16 (39%) were malignant. The median tumor size was 6 cm (range 2–30 cm), and the median age of the patients was 47 years (range 22–65 years). Thirty patients underwent wide excision, and the remaining patients underwent mastectomy. Five patients underwent axillary dissection, and none of them was found to have axillary node metastases. The median follow-up duration was 42 months (range 1–90 months). Although the surgical margins were adequate in all the patients as assessed at the time of wide excision, local recurrence occurred in 4 patients. Three of these patients underwent mastectomy, and the other underwent repeated wide excision by preference. Two patients with local recurrence developed systemic recurrence. The 2-year OS and RFS rates were 84% and 77%, respectively. All of the 9 instances of systemic recurrence occurred in patients with malignant phyllodes tumor, and in 6 of these 9 patients, recurrence occurred within the first year after surgery on average (median duration 11.5 months, range 1–66 months). The median number of organs involved was 3 (1–4), and all patients had multiple lung metastases.

The immunohistochemical staining profiles of the phyllodes tumors are summarized in Table 1. None of the phyllodes tumors showed positive staining for HER2/neu or CD117/c-kit. Most of them (85%, $N = 35/41$) were positive for EGFR, which was seen more frequently in the benign and borderline phyllodes tumors than in the malignant tumors. Positive p53 expression was seen in 10 phyllodes tumors (24%), and approximately half of the malignant phyllodes tumors showed positive p53 expression. The overall median MIB-1 index was 10%; however, whereas it was 5% in both the benign and borderline phyllodes tumors (range 1–30% and 1–10%, respectively), the index in the malignant phyllodes tumors was 30% (range 10–90%). In 3 of the 9 patients with systemic recurrence, we analyzed the differences in the immunohistochemical staining profiles between the primary and the metastatic phyllodes tumor (two lung metastases and one skin metastasis). There were no significant differences in the p53 expression scores or the MIB-1 index between the primary and secondary tumors in these patients.

The size of the primary tumor ($\leqslant 10$ cm vs. >10 cm) was not associated with RFS and OS ($p = 0.96$, $p = 0.62$, log-rank test). There was no correlation between EGFR expression and RFS and OS, p53 expression status, and MIB-1 index was significantly associated with RFS and OS ($p = 0.009$, $p = 0.014$, Fig. 1A. and B for p53; $p = 0.01$, 0.017, Fig. 2A and B for the MIB-1 index, log-rank test). Table 2 shows the hazard ratios as estimated by Cox regression analysis. Both p53 expression and MIB-1 index, but not EGFR expression, were found to be significant prognostic factors in terms of RFS and OS in patients with phyllodes tumors (Figs. 3–6).

Discussion

Metastatic phyllodes tumors of the breast have a poor prognosis, and the average interval from diagnosis to death in patients with metastasis has been reported to be

Table 1. Immunohistochemical findings in phyllodes tumors of the breast

	Benign ($N = 20$)	Borderline ($N = 5$)	Malignant ($n = 16$)
HER2/neu positivity[a]	0	0	0
CD117/c-kit positivity[a]	0	0	0
EGFR			
Negative	1	—	5
Positive	19	5	11
p53			
Negative	19	5	7
Positive	1	—	9
MIB-1 index			
0%	—	—	—
1–9%	15	4	—
10–29%	4	1	7
$\geqslant 30\%$	1	—	9

[a]None of the tumors showed positive staining for HER2/neu or CD117/c-kit.

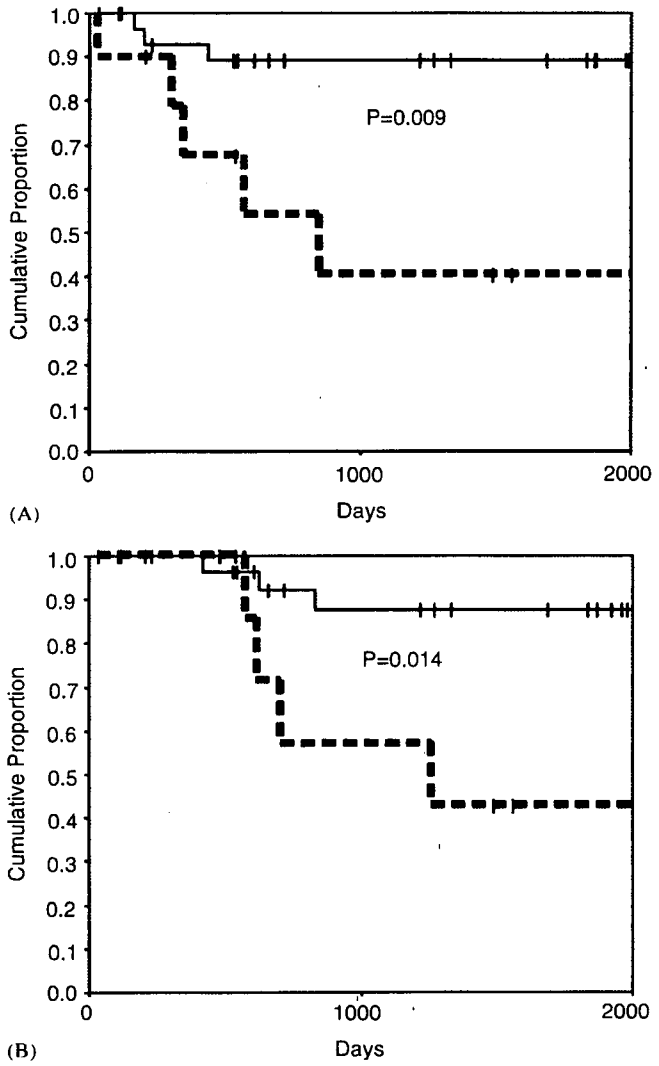

Fig. 1. (A) Correlation of p53 expression with systemic recurrence-free survival. (B) Correlation of p53 expression with overall survival. The dotted line represents patients positive for p53 expression.

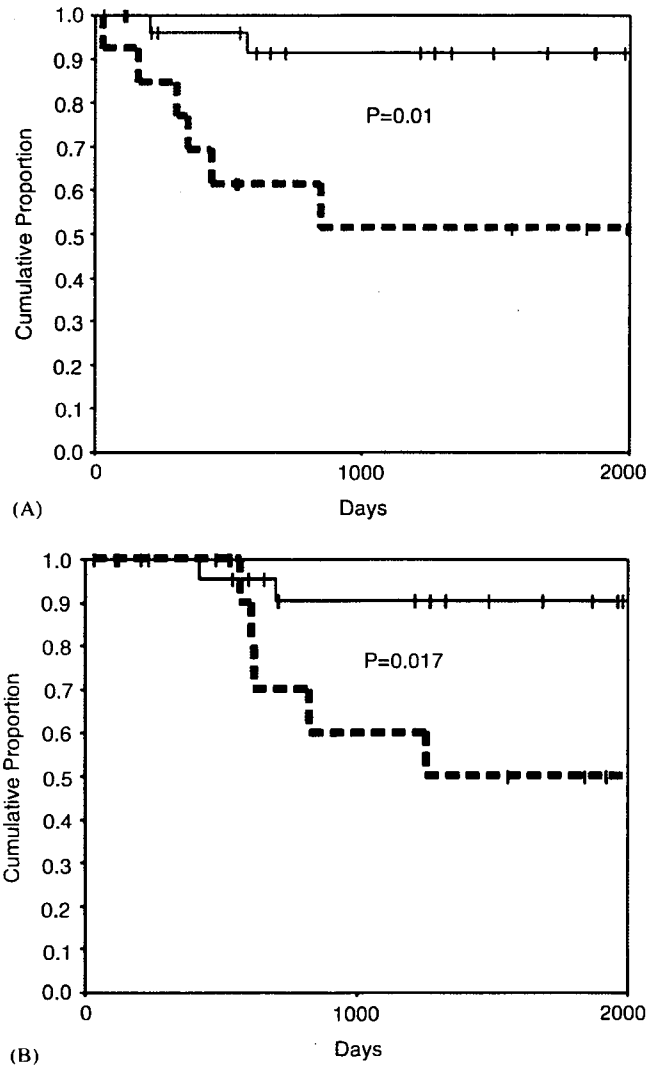

Fig. 2. (A) Correlation of MIB-1 index with systemic recurrence-free survival. (B) Correlation of MIB-1 index with overall survival. The cut-off point for value of the MIB-1 index ($>11.2\%$ vs. $\leqslant 11.2\%$) was defined based on the results of a previous study [8]. The dotted line represents patients with high MIB-1 index ($>11.2\%$).

30 months [2]. The present study demonstrated that both p53 expression and the MIB-1 index were significantly associated with RFS and OS in patients with phyllodes tumors. Numerous studies have attempted to determine whether immunohistochemical testing of phyllodes tumors might be useful to predict the clinical outcome of the patients, but without success. Many studies have suggested that p53 and MIB-1 expression status may be correlated with the histological grade of the tumors [6,11,12,19,24] but only one study investigating 118 cases has demonstrated a correlation between the expression of these two tumor markers and the rates of recurrence and survival [13]. In that study, the results of multivariate analysis suggest that p53 expression might be a prognostic factor in terms of the disease-free survival, and the MIB-1 index might be a prognostic factor in terms of OS [13]. These results are confirmed in our study, which indicates that immunohistochemical analysis might be useful for identifying patients at high risk of systemic recurrence and death from disease. The discrepancy between the results of most of the previous studies and our study might be attributable to differences in the proportions of patients with different subtypes of phyllodes tumors and in the limited rates of recurrence or death.

Although most studies define recurrence to include both local and systemic recurrence, our RFS analysis excluded local recurrence in this study. Local recurrence appears to be related to an inadequate surgical excision margin. In most patients, local recurrence was isolated

Table 2. Cox regression analysis of the immunohistochemical staining profile of PTs of breast

	Recurrent-free survival			Overall survival		
	Hazard ratio	95% CI	*p* Value	Hazard ratio	95% CI	*p* Value
EGFR						
Negative	1.0			1.0		
Positive	0.34	0.1–1.4	0.1	0.27	0.06–1.2	0.09
p53						
Negative	1.0			1.0	—	—
Positive	5.0	1.3–19	0.02	5.4	1.2–24	0.03
MIB-1 index						
⩽11.2%	1.0			1.0		
>11.2%	5.2	1.3–21	0.02	5.8	1.1–30	0.04

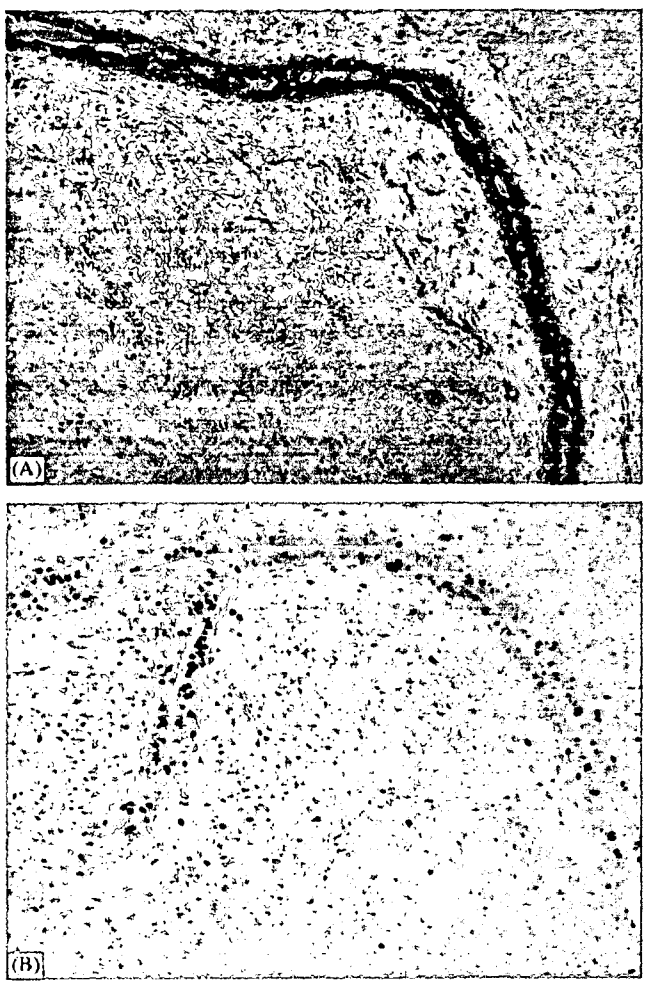

Fig. 3. Benign phyllodes tumor. Nuclei of stromal cells positive for Ki-67 antigen are rare (hematoxylin–eosin stain, original magnification × 100 (A); MIB-1 labeling index 1%, original magnification × 100 (B)).

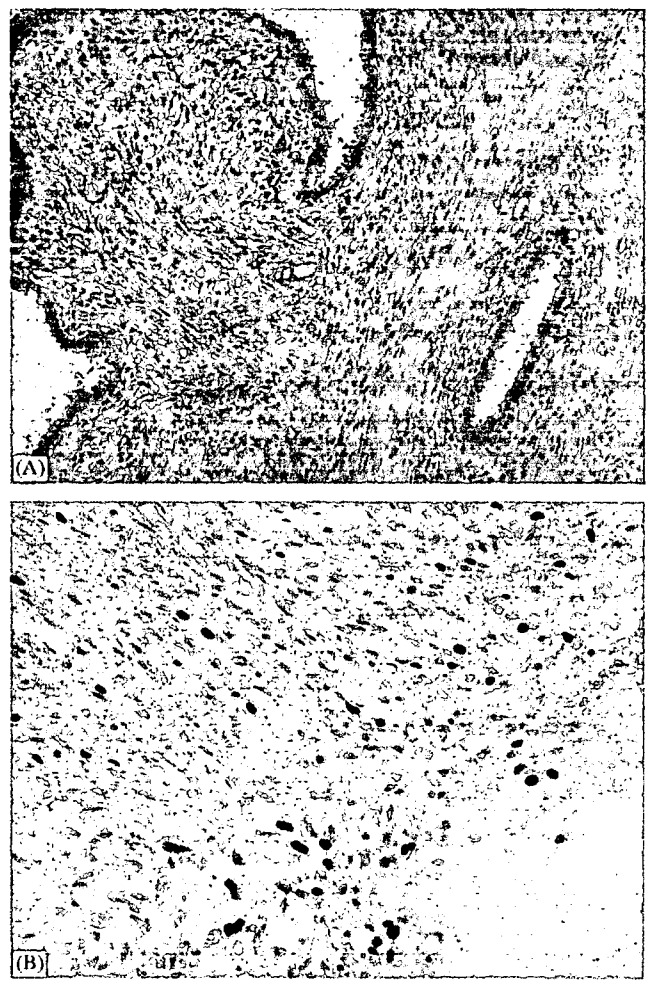

Fig. 4. Borderline phyllodes tumor. Nuclei of stromal cells positive for Ki-67 antigen are rare (hematoxylin–eosin stain, original magnification × 200 (A); MIB-1 labeling index 5%, original magnification × 200 (B)).

and not associated with distant metastases [14]. It has been reported that local recurrence can usually be controlled by repeated wide excision, without any survival disadvantage [15]. This might be the reason why most of the previous studies have failed to predict recurrence or survival.

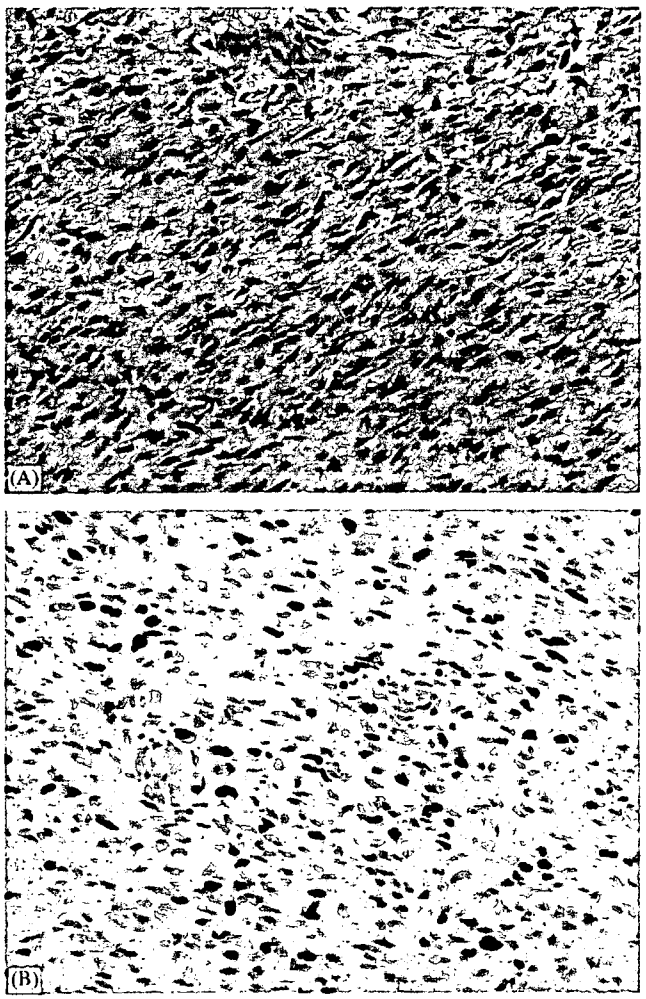

Fig. 5. Malignant phyllodes tumor. Many nuclei of stromal cells positive for Ki-67 antigen are present (hematoxylin–eosin stain, original magnification × 200 (A); MIB-1 labeling index 30%, original magnification × 200 (B)).

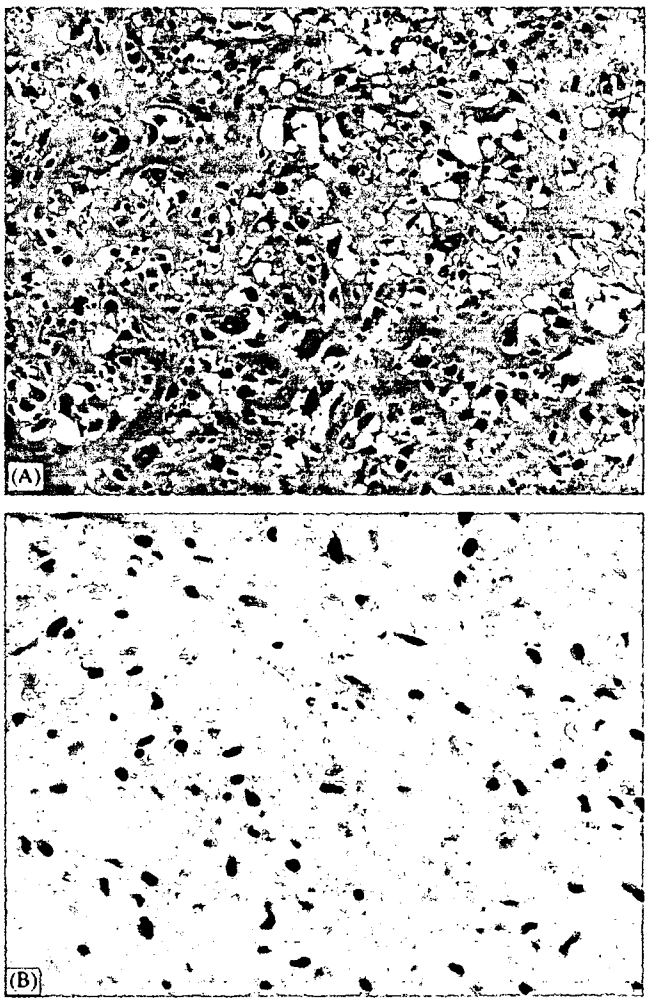

Fig. 6. Malignant phyllodes tumor. Increased nuclear atypia and mitotic activity are noted. p53 immunohistochemical staining shows strong nuclear expression (hematoxylin–eosin stain, original magnification × 200 (A); p53 immunostaining, original magnification × 200 (B)).

The frequency of systemic recurrence in cases of phyllodes tumors has been reported to range between 7% and 30% [7,13,14,16]. In the present study, systemic recurrence occurred in 22% of the patients, predominantly during the first year after surgery. Previous studies have reported that systemic recurrence mainly occurs during the first 3 years after surgery [13,16,19]. The lung is the most common site of distant metastasis in cases of phyllodes tumors [10,13,16]. August et al. suggest physical examination and breast imaging studies twice a year for the first 5 years, chest and abdominal computed tomographic scanning annually for 2–5 years in cases with high-risk lesions [1]. In addition, one study reported that three benign phyllodes tumor patients with an MIB-1 index > 10% had recurred and progressed to malignant phyllodes tumors [3]. Although the benefits of routine imaging of the lung and liver have not been proven yet, routine chest imaging might be considered in patients with positive p53 expression and/or an MIB-1 index > 11.5% for the first 3 years after surgery.

In this study, previously reported prognostic factors, such as tumor size, were not significantly associated with clinical outcome. Since this study investigated only a small number of patients regarding each histopathologic type, and recurrence was not observed in patients with borderline or benign phyllodes tumor, it is uncertain whether these immunohistochemical markers could provide independent prognostic information beyond histopathological typing, which frequently involved discordance. Although phyllodes tumor is a rare neoplasm, further investigations are necessary to resolve this question. These markers might help identify the high-risk group for a poor clinical outcome, with potential indication for adjuvant systemic therapy.

The present study investigated the expression of EGFR family members in phyllodes tumors, including EGFR and HER2/neu. While no distinct HER2/neu

expression was observed in the tumors, most of the tumors expressed EGFR. The expression of EGFR family members in phyllodes tumors has not been widely investigated. Suo et al. reported that EGFR, c-erbB-3, and c-erbB-4, but not c-erB-2 proteins, were expressed in most phyllodes tumors [21]. From these results, trastuzumab, a therapeutic monoclonal antibody used for the treatment of HER2/neu-positive breast cancer, probably would not be useful for the treatment of phyllodes tumors.

While CD117/c-kit was not expressed in the present study, previous studies have reported CD117/c-kit expression in phyllodes tumors in the range of 20–50% [4,18,23]. On the other hand, whereas in most of these studies, CD117/c-kit expression was observed only in malignant phyllodes tumors [4,18], one study reported CD117/c-kit expression in all histological types of phyllodes tumors [23]. The differences in the results of CD117/c-kit expression might be attributable to differences in the antibodies used for CD117/c-kit detection or to technical differences between the studies. The result of our immunohistochemical study in relation to CD117/c-kit expression suggests the need for further evaluation of CD117/c-kit using anti-CD117/c-kit antibodies, which have been widely used for the assessment of gastrointestinal stromal tumors. If CD117/c-kit expression was recognized at a high frequency in future studies, a trial of the new therapeutic agent, STI571 (Glivec, Novartis), for tumor recurrences should be conducted.

The present study demonstrated a correlation between the results of immunohistochemical examination and clinical outcome, and suggested that more careful postoperative follow-up may be important for patients showing expression of p53 and/or MIB-1.

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The safety and efficacy of the weekly dosing of irinotecan for platinum- and taxanes-resistant epithelial ovarian cancer

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Abstract

Objective. Irinotecan is one of the drugs that might be effective against platinum- and taxanes-resistant epithelial ovarian cancer. We investigated efficacy and safety of the weekly dosing schedule of irinotecan.

Methods. From September 2001 to March 2003, 28 eligible patients who have histologically confirmed epithelial ovarian cancer, which was resistant or refractory to both platinum and taxanes, were consecutively treated at the National Cancer Center Hospital. Irinotecan (100 mg/m^2) was administered intravenously over 90 min on days 1, 8, and 15. The chemotherapy was repeated every 4 weeks, up to 6 cycles.

Results. A total of 107 treatment cycles of irinotecan were administered to 28 patients. The median number of prior chemotherapy regimen was 3. Among 28 patients, 8 (29%) responded to irinotecan (2 complete responses and 6 partial responses). The median time to progression was 17 weeks. Three patients experienced hematological toxicities of Grade 4. Five patients experienced non-hematological toxicities Grade 3 or 4. No treatment-related death occurred.

Conclusion. The weekly dosing schedule of irinotecan seems to be effective and safe salvage chemotherapy regimen for platinum- and taxanes-resistant or refractory epithelial ovarian cancer. Gastrointestinal toxicities, especially diarrhea, were moderate and manageable in an outpatient setting.

Keywords: Irinotecan; Ovarian cancer; Platinum-resistant; Second line treatment; Taxanes-resistant; Weekly schedule

Introduction

Ovarian cancer is one of the most lethal gynecologic cancers in Japan. The first-line standard chemotherapy regimen for patients with advanced disease is combination chemotherapy with platinum and taxane [1,2]. Although the majority of women show an initial response to treatment consisting of cytoreductive surgery followed by platinum and taxane chemotherapy, more than 60% will die from recurrent disease. At relapse, the probability of response to re-treatment with platinum-based chemotherapy depends on the platinum-free interval [3]. When the interval is longer than 6 months (platinum-sensitive), many clinicians re-treat patients with platinum or platinum plus paclitaxel [4]. Women whose disease progresses while receiving the initial platinum-based treatment or who develop recurrent disease within 6 months of the platinum-based treatment (platinum-resistant) rarely respond to re-treatment with platinum. Relapse during or within 3 months of platinum-based treatment is regarded as an especially poor prognostic factor (platinum-refractory). A standard therapy for patients with platinum-refractory or resistant relapses has not been established. For platinum-refractory or resistant disease, chemotherapy regimens utilizing other agents, like paclitaxel [5], docetaxel [6], topotecan [7], irinotecan [8,9], liposomal doxorubicine [10], oral etoposide [11], gemcitabine [12], vinorelbine [13], ifosfamide [14], or oxaliplatin [15] have been utilized, yielding response rate of between 10% and 30%, however, a survival benefit has not been shown.

Since taxanes are often used as a part of first-line treatments, the development of salvage chemotherapy regimens that do not utilize taxanes is needed. Irinotecan, which is

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a semi-synthetic derivative of camptothecin, is a prodrug with little inherent topoisomerase inhibitory activity and is converted by carboxylesterases to its more active metabolite, SN-38(7-ethyl-10-hydroxycamptothecin). In vitro, SN-38 is 250 to 1000 times more potent than irinotecan as an inhibitor of topoisomerase activity [16]. Several phase trials have shown this drug to be effective for the treatment of platinum-resistant or refractory epithelial ovarian cancer or primary peritoneal cancer [8,9,17]. To date, several dosing schedules have been developed for this drug: a 5-day infusion regimen [18], a weekly regimen [19,20], a bi-weekly regimen, and an every 3 weeks regimen [21,22]. The rationale for the weekly administration of irinotecan was based on preclinical data in multiple human tumor xenograft models, indicating that a repeated intermittent treatment schedule might be superior to single injections of the same total drug dose [23]. We evaluated the efficacy and safety of weekly irinotecan as salvage chemotherapy for patients with advanced ovarian cancer, which was resistant to both platinum and taxanes. To our knowledge, this is the first report of a weekly dosing schedule for irinotecan in this clinical setting.

Patients and method

Patient selection

The present study is a retrospective chart review using the National Cancer Center Hospital database. The aim of study is to describe safety and efficacy of the weekly dosing of irinotecan to the patients with both platinum- and taxane-resistant or refractory ovarian, peritoneal, or fallopian tube cancer. Between September 2001 and March 2003, 33 patients were consecutively treated with irinotecan in our hospital. One patient was excluded from the report because she received irinotecan as phase I study of combination chemotherapy, irinotecan with oral etoposide. Four patients were excluded from the report because they did not meet the eligibility of "both platinum- and taxane-resistant or refractory". Three of these four patients were platinum-resistant but not taxane-resistant. Another patient was taxane-resistant but not platinum-resistant. Finally, 28 patients were selected to be eligible for this report. Platinum or taxane-refractory diseases were defined as tumor progression during treatment or within 3 months after the completion of therapy. Platinum or taxane-resistant disease was defined as tumor progression between 3 and 6 months of the completion of the most recent therapy. Any regimen that contained a platinum or taxane drug was counted as one regimen for the purpose of this study. For example, a patient received cisplatin with paclitaxel as first-line therapy, and after recurrence, if she received weekly carboplatin and paclitaxel, the number of regimen is counted two ("cisplatin with paclitaxel" and "weekly carboplatin with paclitaxel"). If she received carboplatin monotherapy after progression, the number of regimen is counted three ("cisplatin with paclitaxel" and "weekly carboplatin with paclitaxel" and "carboplatin"). None of the patients had severe infections or other complications. None of the patients was treated with irinotecan, topotecan, or other camptothecin analogs before.

Treatment plan, response evaluation, and toxicity grading

One treatment cycle consisted of 28 days. Irinotecan (100 mg/m^2) was administered intravenously over 90 min on days 1, 8, and 15. The administration of irinotecan was omitted if the patient exhibited hematological toxicity of Grade 4 or a non-hematological toxicity Grades 3 or 4 on the day of the scheduled administration. The treatment was repeated for up to 6 cycles, provided that disease progression or intolerable toxicity did not occur.

Response was evaluated according to the following criteria: (1) complete response was defined as the total disappearance of all clinically evident malignant disease for at least 4 weeks. (2) Partial response was defined as equal to or greater than a 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions lasting for at least 4 weeks. A partial response also required that no new lesion appeared and that no progression of evaluable disease occurred. (3) Progressive disease was defined as a 25% or greater increase in the sum of the products of the measurable lesions compared with the smallest sum observed or with the baseline value if no decrease occurred, the reappearance of any lesion that had disappeared, or the appearance of any new lesion at any site. (4) Stable disease was defined as any disease that did not meet the above criteria for complete response, partial response, or progressive disease [24]. With regard to changes in the CA125 level, we defined a 50% decrease in the CA125 in four sequential samples or a 75% decrease in two samples compared to the pretreatment value, as a response [25].

All the adverse effects were classified according to the NCI-CTC version 2 criteria [26].

Results

The patient characteristics are presented in Table 1. The median age of the patients was 55 years. Of the 28 patients entered in this study, 25 had epithelial ovarian cancer and 3 had primary peritoneal cancer. The histological types were distributed as follows: 18 serous carcinomas, 5 clear cell carcinomas, 1 mucinous carcinoma, and 4 others (3 adenocarcinomas and 1 poorly differentiated carcinoma). Prior chemotherapy regimens varied, but all of the patients were treated with platinum and taxanes and had been refractory or resistant to both of these agents. The median number of prior chemotherapy regimens was 3. Fifty-seven percent of the patients were platinum-refractory, and 43% were platinum-resistant. Similarly, 54% of the patients were taxane-refractory, and 46% were taxane-resistant.

A total of 107 treatment cycles of irinotecan were administered to 28 evaluable patients, with a median of 4 cycles per

Table 1
Patient characteristics

Characteristics	Number of patients
Age, years	
Median	55 (range 32–76)
ECOG performance status	
Median	1 (range 0–4)
Primary tumor site	
Ovary	25
Peritoneum	3
Tumor histology	
Serous	18
Mucinous	1
Clear cell	5
Unclassified	4
Prior chemotherapy regimens	
1	7
2	6
3	10
4	5
Platinum-resistant status	
Resistant (TFI <3 months)	16
Refractory (TFI 3–6 months)	12
Taxane refractoriness	
Resistant (TFI <3 months)	15
Refractory (TFI 3–6 months)	13

Abbreviations: TFI, treatment-free interval.

Table 2
Overall response, separated by CAT scan or CA125

	CAT scan	CA125
CR	2	–
PR	6	8
NC	9	14
PD	11	4
NE	0	2
Total	28	28

patient (range, 0 to 6 cycles). All treatments were administered in an outpatient setting.

Responses

Eight patients (response rate 29%; 95% CI 16–45%) demonstrated an objective response to irinotecan (Table 2). Two patients achieved complete responses lasting 8 and 2 months, and six patients demonstrated partial responses lasting from 2 to 13 months (median of 7 months). According to histology, of the 15 patients with serous carcinoma, 1 had a complete response, 2 had partial responses, 5 had stable diseases, and 7 had disease progression. Of the 5 patients with clear cell carcinoma, 1 had a partial response, 2 had stable diseases, and 2 had progressive diseases. Two patients achieved response among 13 patients with platinum- and taxane-refractory diseases. Five patients achieved response among 10 patients with platinum- and taxane-resistant diseases (Table 3). According to the CA125 response criteria, 26 patients were evaluable. Of these, eight patients experienced a reduction in their CA125 levels by more than 50%. Of these eight patients, one had a complete response, four had partial responses, and three had stable diseases according to imaging study. The median time to progression was 17 weeks. The median overall survival was 8 months at a median follow up of 35 months.

Toxicity

The adverse events reported by the 28 patients treated with irinotecan are presented in Tables 4 and 5. Thirteen of the 28 patients (46%) experienced Grade 3 or 4 hematological toxicities. None of the patients experienced febrile neutropenia. We did not use granulocyte colony stimulating factor. Five patients experienced Grade 3 or 4 non-hematological toxicities. Two patients experienced dyspnea because of pleural effusion due to disease progression, and three patients experienced diarrhea during chemotherapy. Patients were not given prophylactic loperamide but were instructed to take a 1 mg tablet of loperamide if they experienced diarrhea. Most patients who experienced diarrhea required only 2 or 3 tablets of loperamide during one cycle of therapy.

Table 3
Response according to resistance status

		Taxane	
		Refractory	Resistant
Platinum	Refractory	2/13	1/2
	Resistant	0/3	5/10

Overall response rate: 29% (95% CI 11–45%).

Table 4
Hematologic toxicities experienced by patients given irinotecan

Toxicity	No. of patients experiencing toxicities			
	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	13	3	5	0
Neutropenia	6	9	2	3
Anemia	9	14	1	0
Thrombocytopenia	0	0	0	0

Discussion

In this study, we observed a 29% response rate to irinotecan in patients with platinum- and taxane-resistant or refractory epithelial ovarian cancer or primary peritoneal cancer. Although most of the patients were heavily pretreated, this result is consistent with other earlier phase II studies [8,9,17]. Bodurka et al. reported a 17% response rate to irinotecan at a dose of 300 mg/m^2 every 3 weeks. In their study, 74% of the patients were categorized as platinum-resistant, and 26% of were categorized as platinum-refractory. All patients received paclitaxel, but the treatment-free interval for paclitaxel was not reported in their study. In our study, all the patients were resistant or refractory to both platinum and taxanes. Among them, 46% of the patients were refractory to both platinum and taxane chemotherapy yet still responded to irinotecan (response rate 15%). Although the dose and the schedule were different from those of previous reports [8], irinotecan is considered to be active in the present reported clinical setting. Another promising finding regarding the efficacy of weekly irinotecan was the response for the clear cell-type tumors, which are regarded to be relatively chemo-resistant [27]. Salvage chemotherapy is always challenging for both patients and their physicians since substantial toxicity and limited efficacy are inevitable. Unlike other study on irinotecan, the gastrointestinal toxicities, especially diarrhea, were moderate in the present study. In particular, Grade 4 diarrhea was not observed in the present study. This outcome might be due to the weekly dosing schedule. In the Bodurka study, more than half of the patients required a dose reduction (16 out of 31 patients). They concluded that an every 3 weeks dosing schedule for irinotecan

Table 5
Non-hematologic toxicities experienced by patients given irinotecan

Toxicities	No. of patients experiencing toxicities			
	Grade 1	Grade 2	Grade 3	Grade 4
Diarrhea	10	10	3	0
Nausea	16	10	0	0
Vomiting	13	5	0	0
Anorexia	4	2	0	0
Asthenia	6	1	0	0
Constipation	6	1	0	0
Dyspnea	–	0	2	0
Alopecia	9	5	–	–
Cramp	3	3	0	0
Edema	1	1	0	0

should only be considered in a clinical trial because it was too toxic.

Fuchs et al. conducted a phase III trial comparing weekly irinotecan (125 mg/m^2, once a week for 4 weeks followed by a 2-week rest period) with a once every 3 weeks schedule (350 mg/m^2, or 300 mg/m^2 in patients over the age of 70 years, with an ECOG PS of 2, or a history of pelvic irradiation) in patients with fluorouracil refractory metastatic colorectal cancer. No significant differences in survival nor quality of life were observed, however, the once-every-three-weeks regimen was associated with a significantly lower incidence of severe diarrhea and the need for dose reduction [28]. During the first treatment cycle, the full dose of irinotecan was received by 51% of the patients at week 3 and only 35% of the patients at week 4 in the weekly arm. In contrast, 69% of the patients in the every-three-weeks arm received the full dose of irinotecan at week 4 (the beginning of the second cycle). In the current study, only two patients experienced dose reduction because of toxicity. Thus, 93% of the patients received the full dose of irinotecan at week 5 (the beginning of the second cycle). They recommended another weekly administration schedule rather than 4 consecutive weeks followed by a 2-week rest, for the further investigation. The schedule in the current study, 3 consecutive weeks followed by 1 week of rest, could be regarded as a variation on the above recommendation.

The present finding also needs careful interpretation because of the possibility of bias. For both efficacy and safety, we need prospective evaluation for irinotecan with weekly schedule. To enhance the efficacy without compromising safety, we developed two combination chemotherapy regimens with irinotecan plus another drugs. For patients with platinum-sensitive relapse, we have developed irinotecan plus carboplatin regimen [29]. For patients with platinum-resistant relapse, we developed irinotecan plus oral etoposide. We have already completed phase I study [30]. This regimen was evaluated by another group in phase II study, the result was promising [31]. Now, we are planning a nationwide multi-institutional phase II study.

Moreover, considering irinotecan is effective for clear cell subtype, a cooperative group in Japan, Japan Gynecologic Oncology Group, conducted randomized phase III trial comparing cisplatin plus irinotecan regimen with carboplatin plus paclitaxel regimen as the first-line treatment for patients with clear cell carcinoma of the ovary. This regimen was chosen as the test arm for the international phase III trial for clear cell carcinoma of the ovary, which will be conducted by Gynecologic Cancer Inter Group.

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ORIGINAL ARTICLE

Reference profiling of the genomic response induced by an antimicrotubule agent, TZT-1027 (Soblidotin), *in vitro*

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TZT-1027 is an antimicrotubule agent targeting beta-tubulin that is undergoing clinical development. The genomic response of cancer cells to TZT-1027 was profiled to evaluate its biochemical activity. A lung cancer cell line, PC-14, was exposed to antimicrotubule agents including dolastatins, *Vinca* alkaloids and taxanes at an equivalent toxicity level. Alterations in the TZT-1027-induced gene expression of ~600 genes were then examined using microarray technology and the resulting gene profiles were compared with those for cells exposed to the other antimicrotubule agents. A principle component analysis using the whole gene set demonstrated that TZT-1027 produced similar gene profiles to those produced by dolastatin 10, but that these gene profiles differed from those produced by other agents. The agents were classified according to their induced genomic response in a molecular structure-dependent manner. Genes whose expression profiles differed according to drug class included intermediate filaments, extracellular matrix protein and Rho regulatory genes that may be involved in cytoskeletal and angiogenesis processes that are regulated by microtubule dynamics. TZT-1027 produces a unique genomic response profile distinct from that of *Vinca* alkaloids and taxanes, suggesting that this agent has a different mechanism of action. The selected genes may act as pharmacodynamic biomarkers allowing the unique mode of action of TZT-1027 to be discriminated from those of other antimicrotubule agents.
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Keywords: TZT-1027; dolastatin 10; cDNA microarray; taxanes; *Vinca* alkaloids; antimicrotubule agent

Introduction

Dolastatin 10 (D10) is small peptide inhibiting microtubule assembly and tubulin polymerization that was isolated in 1987 from an Indian Ocean mollusc, the sea hare (*Dolabella auricularia*).[1] Although D10 displayed significant antitumor activity in preclinical models and demonstrated good tolerability in clinical settings, recent phase II clinical trials suggested that D10, as a single agent, lacked significant activity.[2–5] TZT-1027 is a synthesized derivative of D10 with superior preclinical activity.[6] TZT-1027 showed notable antitumor activity against a broad range of human malignancies *in vivo*, including those resistant to conventional chemotherapeutic agents.[7] Superior antivascular activity resulting in the collapse

of the tumor vasculature was demonstrated, compared with the activities of taxanes and *Vinca* alkaloids.[8–10] The first clinical phase I study of TZT-1027 was initiated in 1994, and another four studies have since been completed; a phase II study is currently ongoing.[11–13] The major toxicity was hematological, in the form of neutropenia and leukopenia. Reversible peripheral neurotoxic syndrome, alopecia, fatigue and anorexia were the principal nonhematological toxicities.

TZT-1027 is a mitotic spindle poison that interacts with tubulin at the *Vinca* alkaloids binding site,[14] but the spectra of antitumor activity of TZT-1027 and the *Vinca* alkaloids are different *in vivo*.[7,10,15] TZT-1027 also showed potent antitumor activity against a vincristine-resistant cell line.[7] A recent investigation of the mode of action reported that TZT-1027-induced apoptosis and cell arrest in the G_2/M phase that was independent of caspase-3 or bcl-2.[16] According to *in vitro* studies performed with tumor tissue obtained from patients with lung and renal cell cancers, the activity of TZT-1027 is influenced less by the p53 mutation status than by DNA-damaging agents.[17] Despite these investigations, the mode of action and therapeutic class of TZT-1027 as an antimicrotubule agent remains unclear. To characterize the mode of action of this compound, microarray-based transcriptional profiles have been performed.[18,19]

Cell-based high-throughput screening technologies provide information about cellular pathways that control drug sensitivity.[20,21] Drug-to-drug comparative approaches using microarray analyses are useful for identifying drug targets; the cellular effects caused by a novel drug of incompletely characterized specificity can be matched to 'reference profiles' of the cellular effects elicited by the specific inhibition of candidate analog-sensitive drugs.[22,23] Thus, it has been proposed that phenotypic information generated by drug-induced alterations in gene expression can be matched to discrete interactions between the compound and the relevant protein targets. Using the drug-to-drug comparative approach of the microarray analysis, we obtained reference profiles of genomic expression data from cellular responses in a lung cancer cell line to antimicrotubule drugs, including five conventional agents and the mother compound D10. In the present study, we aimed to characterize TZT-1027 using these reference profiles.

Results

Growth inhibition

The growth inhibitory effect of TZT-1027 and the other six antimicrotubule agents was determined using an MTT assay. The PC-14 cell line was exposed to each drug for 72 h. The 50% growth inhibitory concentrations (IC_{50} values) were as follows: TZT-1027, 0.02 nM; D10, 0.1 nM; VDS, 4 nM; VBL, 2 nM; VCR, 7 nM; TXL, 3 nM and TXT, 3 nM. Based on these results, TZT-1027 was over a hundred times more cytotoxic than the other taxanes and *Vinca* alkaloids.

Microarray data mining

The expression intensity of the array was normalized using a global normalization method. The change in gene expression was calculated as the intensity ratio between treated and untreated samples. The complete cDNA microarray data can be found in the Supplementary Tables. This supporting information contains the raw data, normalized data and a summary of the selected genes.

Common genes regulated by all antimicrotubule agents

Of the 588 genes that were surveyed in the microarray analysis, 118 genes were upregulated by all seven antimicrotubules

Table 1 Gene ontology analysis of biological process of antimicrotubule agents

Upregulated		*Downregulated*	
Gene category	P	*Gene category*	P
G2/M transition of mitotic cell cycle	<0.0001	Cell–cell signaling	<0.0001
Cytokinesis	<0.0001	Morphogenesis	<0.0001
Regulation of cell cycle	0.0002	Organogenesis	0.0001
Obsolete biological process	0.0003	Development	0.0001
G1/S transition of mitotic cell cycle	0.0004	Cell communication	0.0001
Mitotic cell cycle	0.0008	Positive regulation of cell proliferation	0.0042
Apoptosis	0.0009	Growth	0.0074
Cell cycle	0.0010	Immune response	0.0098
Programmed cell death	0.0012	Defense response	0.0098
Cell death	0.0012		
Death	0.0012		
Regulation of CDK activity	0.0012		
Positive regulation of programmed cell death	0.0077		
Positive regulation of apoptosis	0.0077		
Induction of apoptosis	0.0077		
Regulation of programmed cell death	0.0077		
Induction of programmed cell death	0.0077		