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Amplicon profiling reveals cytoplasmic overexpression of MUC1 protein as an indicator of resistance to platinum-based chemotherapy in patients with ovarian cancer

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Abstract. Chromosomal gains of 1q21-q22 and 13q12-q14 were closely related with the chemoresistance of patients with ovarian cancer in our previous CGH (comparative genomic hybridization) study. We conducted the present study to determine the amplified genes on chromosome 1q. Comparisons of relative copy numbers of clinically platinum-sensitive ovarian tumors (CR-group, n=14) and platinum-resistant tumors (PD-group, n=14) were carried out using real-time PCR from ten different gene loci on chromosome 1q. Increased copy numbers were frequently observed in PD-group tumors, especially in the region between WI-8123 and *MUC1*. Relative copy number of MUC1 over 1.5 was observed in 13 (92%) of 14 PD-group tumors and 3 (21%) of 14 CR-group tumors ($p<0.05$). Moreover, cytoplasmic expressions of MUC1 protein were significantly higher in PD-group than those in CR-group ($p<0.01$). We concluded that the cytoplasmic overexpression of MUC1 might be an indicator of resistance to platinum-based chemotherapy and a prognostic marker in ovarian cancer.

Introduction

Ovarian cancer is the third most common malignancy of the female genital tract, but survival rates of affected patients are the lowest in the patients with genital tract carcinomas. It is the fifth most common cause of death among women (1). The mechanisms of chemoresistance is so complicated that the clinical response to chemotherapy is not predictable. *In vitro* studies suggested that acquired resistance to cisplatin is associated with increased levels of glutathione and glutathione-S-transferase activity, increased metallothionein and decreased accumulation of cisplatin (2).

In our previous study, gains of 1q21-q22 and 13q12-q14 in PD-group tumors were observed in significantly high abundance, compared with those in CR-group tumors (3). Cisplatin-resistance related genes seemed to be located on chromosomal regions of 1q21-q22 and 13q12-q14. Many candidate genes which might be involved in chemoresistance are located on the chromosomal region of 1q21-q22, including *BCL-2*-related myeloid leukemia sequence (*MCL-1*), the polymorphic mucinous tumor-associated gene (*MUC-1*), and the papillary renal cell carcinoma gene (*PRCC*).

We conducted the present study to determine whether highly amplified genes exist on 1q21-q22 by real-time PCR with the primers located on this region. Moreover, MUC1 the highly amplified gene located on this region, was immunohistochemically examined to see if there is a relationship with cisplatin-resistance in ovarian cancer patients.

Material and methods

Patients, chemotherapy, and response evaluation. Of 71 patients with primary epithelial ovarian cancer treated at the National Defense Medical College Hospital (Saitama, Japan) between 1993 and 1997, the following patients were selected: a) patients who received no prior chemotherapy prior to the any surgical therapy; b) patients who harbored any residual tumors after initial debulking surgery; and c) patients treated with six courses of cisplatin-containing chemotherapy (described below) after the initial surgery. The patients were then grouped into the following four categories according to their response to chemotherapy: a) CR, complete response; b) partial response; c) stable disease; and d) PD, progressive disease (4). Only patients in the CR (n=14) and PD (n=14) were included in the study. The chemotherapy regimen for the patients was as follows: one cycle consisted of a drip infusion of 50 mg/m^2 cisplatin for 3 h accompanied by an i.v.

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Abbreviations: CGH, comparative genomic hybridization; CR, complete response; PD, progressive disease

Key words: real-time PCR, comparative genomic hybridization, chemoresistance, ovarian cancer, MUC1 protein

Table I. Patient characteristics.

	CR-group	PD-group	p-value
Age			
median	50.3	51.2	
range	20-69	42-74	0.381[b]
Stage			
II	3	1	
III	10	10	
IV	1	3	0.368[c]
Histology[a]			
S,E	9	6	
C,M	5	8	0.256[c]
Grade			
1	2	1	
2	6	5	
3	6	8	0.157[c]
Residual tumor			
<2 cm	11	7	
>2 cm	3	7	0.298[c]
1q21-q22 gain			
yes	2	9	
no	12	5	0.0068[c]

[a]S, serous cystadenocarcinoma; E, endometrioid adenocarcinoma; C, clear cell adenocarcinoma; M, mucinous cystadenocarcinoma. [b]Exact score test. [c]Chi-squared test or Fisher's exact method.

Table II. Primer sequences used in real-time PCR.

Name of primers	Sequence
D1S442	5'-AACAAAGCTGGACTGGTAATC (sense) 5'-CAGTGTCACACAACTGGTTG (antisense)
WI-8123	5'-TTCTCTGGGAAAACCCCTG (sense) 5'-ATTGCCCATCATAGACTTTTTACA (antisense)
MUC1 (exon2)	5'-TACTCCTACCACCCTTGCCA (sense) 5'-GAGAAGTGCTGTGATTGGAGG (antisense)
H59801	5'-TAGGCCCGGCTGTGGCT (sense) 5'-AGCCCCTCACAGGCATCACT (antisense)
NTRK1	5'-TAGGCCCGGCTGTGCTGGCT (sense) 5'-AGCCCCTCACAGGCATCACT (antisense)
WI-8997	5'-GTGGCCATCGATCTGGAC (sense) 5'-ACCATGAGACACACAGTTCTGG (antisense)
WI-9108	5'-CTCTTCCCCCTGACTCCC (sense) 5'GCAGAAAGAGAAACAATTTAAATGG (antisense)
WI-9232	5'-GAATTGATGCCCTTCGATGT (sense) 5'-GTATCAATTTTCCTCGACTGTGC (antisense)
WI-9272	5'-CAAAGATCTGCTCCTCGCTC (sense) 5'-AGTGGTGGCTCCCACCTAG (antisense)
WI-9317	5'-GCTGTTAGTGAGATGGTGAAGC (sense) 5'-AAAAAGTTCAAGAGTCAGCAGTAGA (antisense)

injection of 50 mg/m^2 doxorubicin and 500 mg/m^2 cyclophosphamide and 6 cycles were given every 4 weeks (4). CR-group consisted of six serous, two mucinous, four endometrioid, and two clear cell adenocarcinomas. PD-group contained five serous, two mucinous, one endometrioid, and six clear cell adenocarcinomas. The patient characteristics are shown in Table I. In the previous CGH analysis, the numbers of patients harboring chromosomal gains of 1q21-q22 were 2 (14%) of 14 patients with complete response (CR) and 9 (64%) of 14 patients with progressive disease (PD), respectively (3).

DNA extraction. High molecular weight genomic DNAs were isolated from primary tumors of the first debulking operation using phenol-chloroform methods (5). All samples were examined by 1% agarose gel electrophoresis for conservation of high molecular weight.

Real-time PCR. The real-time PCR was used to validate differential expression of genes located on chromosomal region of 1q21-q22. All samples of ovarian cancer patients showed chromosomal gains of 1q21-q22 in our previous study with CGH. Analysis was carried out using the ABI Prism 7700 Sequencer Detector System according to the manufacturer's recommended protocol (ABI/Perkin Elmer, Foster City, CA, USA). Each reaction was run in duplicate. For the need to measure precisely the concentration of the sample DNA, ß2-microglobulin (chromosome 15q21-22.2) was used as an internal control gene. Chromosome 15 is rarely involved in the karyotyping of ovarian cancers (6-8). The comparative threshold cycle (C_T) method was used for the calculation of amplification fold as specified by the manufacturer. The standard curves used to measure copy number in this study were constructed from DNA of normal female placental tissue. DNAs of placental tissue were diluted in five concentrations; 50, 25, 12.5, 6.25, and 3.13 ng/µl. To avoid the increased and decreased validity, test samples were

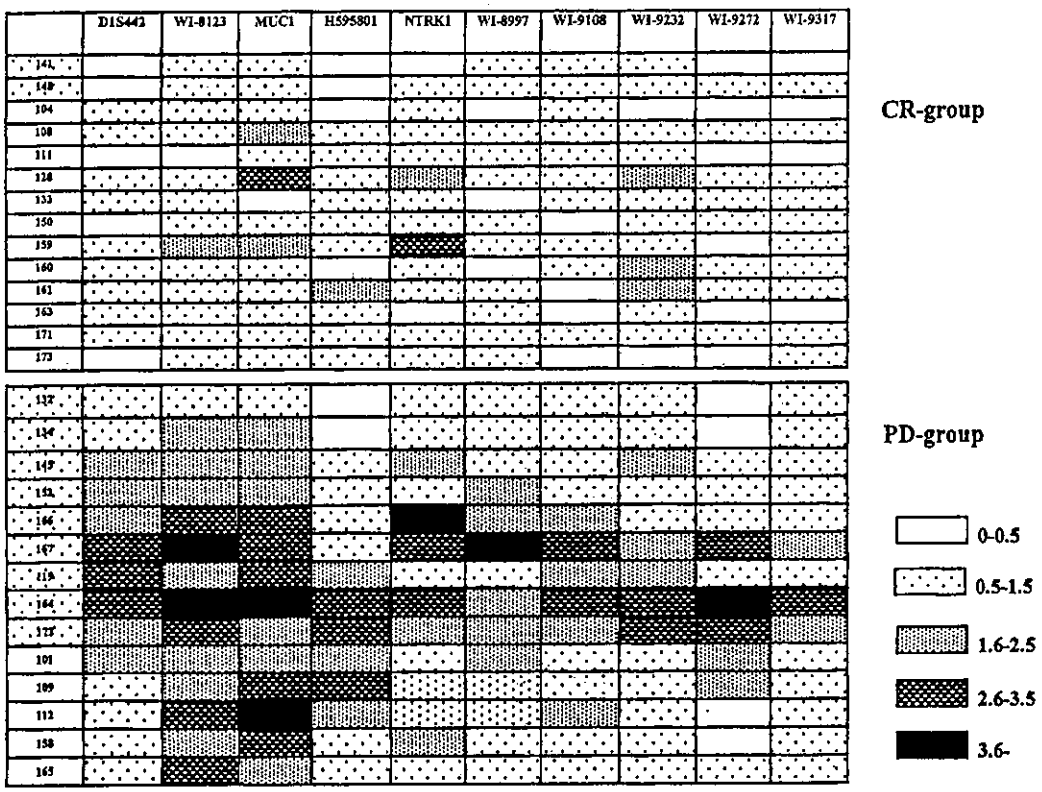

Figure 1. Levels of amplfication or deletion at each primer-set located on chromosomal region including 1q21-q22. Each column represents expression ratio compared with internal standard (beta2-microglobulin); amplified values are as shown in the figure. Patient numbers with 1q21-q22 gain by CGH analysis (case 141 and 144 in CR-group and case 132, 134, 145, 152, 166, 119, 164, and 172 in PD-group) are shaded. Amplifications were rarely observed in chemosensitive (CR-group) patients (upper columns) with the real-time PCR analysis. Chemoresistance (PD-group) patients (lower columns) showed moderate to high amplification in the real-time PCR analysis with the primers located on chromosomal 1q including 1q21-q22. PD-group showed high expression of the genes located in the region between WI-8123 and MUC1.

used between 5 and 10 ng/μl per well. Each standard and sample was done in triplicate.

Primers and probes. Primers and probes for the genes used in real-time PCR were chosen with the assistance of Primer Express (Perkin-Elmer Applied Biosystems, CA, USA). Sequences of the primers are shown in Table II. D1S442 is located near centromere of chromosome 1q, and WI-9317 is on telomeric side of 1q. We conducted BLASTN searches dbEST and GenBank to confirm the total gene specificity and the absence of DNA polymorphisms. All primer oligonucleotides were purchased from Espec-oligo Service Co. (Tsukuba, Ibaraki, Japan).

Immunohistochemical staining. Tumor specimens of ovarian cancer patients were immunohistochemically stained by the antibody of MUC1 protein. Tumor samples were obtained from 28 patients as used in real-time PCR analyses. There were patients whose chemosensitive tumor disappeared after platinum-containing chemotherapy (CR-group, n=14) and those whose chemoresistant tumor progressed after chemotherapy (PD-group, n=14). Representative paraffin-embedded blocks containing tumor from each case were sectioned at 4 micrometers, affixed to slides and dried. Sections were dewaxed in xylene and incubated in Dako ChemMate Antigen Retrieval buffer (Dako, Kyoto, Japan) and heated at 121°C in autoclave for 15 min and stored at room temperature for 20 min. The sections were rehydrated through descending graded alcohols to Tris-buffered saline, pH 7.4 (TBS). After washing in TBS, sections were treated for 10 min with 3% (v/v) H_2O_2, 18% (v/v) methanol in TBS for inhibition of endogenous peroxidase activity. The sections were incubated with 4% (w/v) non-fat skim milk powder in TBS for 15 min to reduce non-specific antibody binding. The sections were incubated with anti-human CA15-3 murine monoclonal antibody (Dako, Kyoto, Japan) diluted with Antibody Diluent buffer (Dako). The degree of MUC1 expression was judged by the overall proportion of positively stained tumor cells for MUC1 antibody. Staining of cell membrane and cytoplasm was judged respectively. Rates of MUC1 positive cancer cells were counted in >50 high-powered fields. Two pathologists who were blinded to clinical characteristics and pathologic grade of response performed evaluation of sections. The results for antibody reactivity with regard to the localization and proportion of staining were compared with the chemosensitivity to cisplatin-containing regimen.

Statistical analysis. The Chi-square test or Fisher's exact method was used analyze the statistical differences between several variables and the genetic alterations. Mann-Whitney test was used for comparison of MUC1 immunoreactivities. The overall survival curves were estimated by Kaplan-Meier method, and statistical significance was analyzed by log-rank

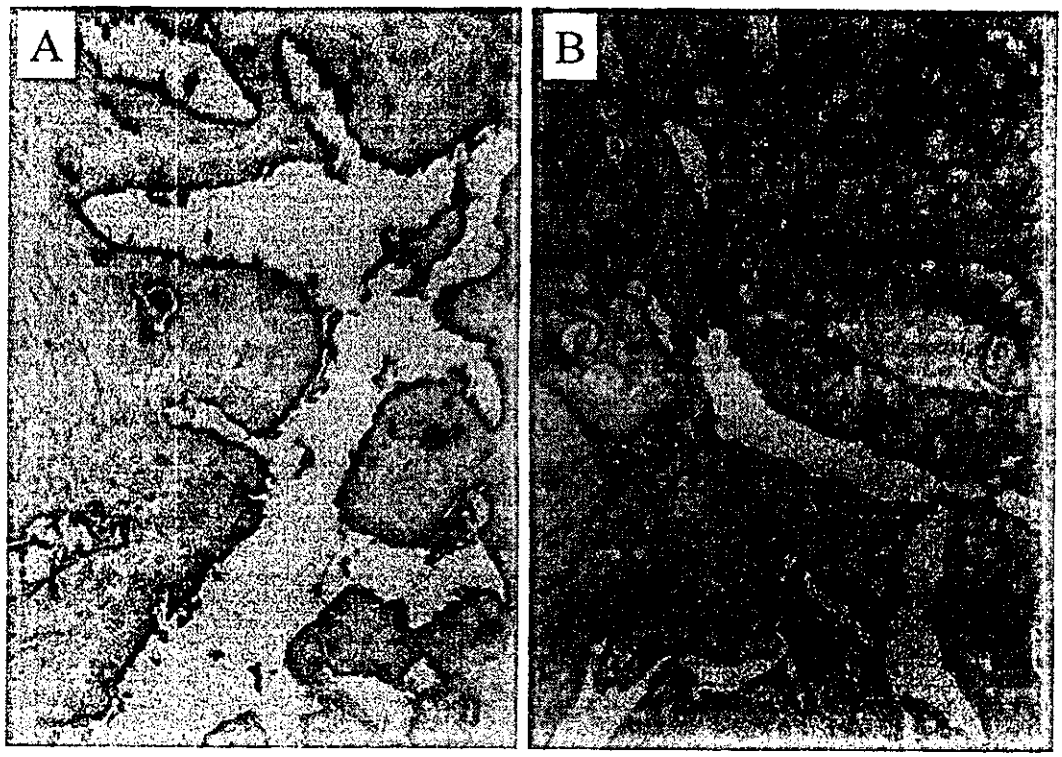

Figure 2. A) Representative immunohistochemical MUC1 staining of a case of CR-group ovarian cancers. Most of chemosensitive (CR-group) ovarian tumors showed MUC1 reactivity in only the apical membrane. Cytoplasms of cancer cells had no reactivity for MUC1 protein. B) Representative immunohistochemical MUC1 staining of a case of immunohistochemical staining of MUC1 protein in PD-group ovarian cancer. Chemoresistant (PD-group) ovarian cancer showed MUC1 reactivity in the apical membrane and the cytoplasm concurrently.

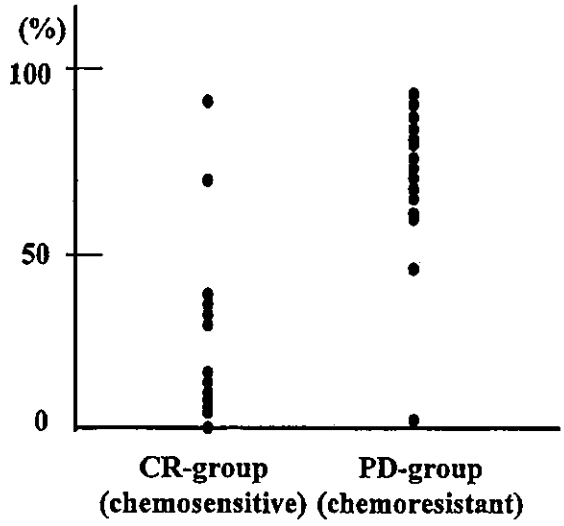

Figure 3. Rates of MUC1 cytoplasm-positive cancer cells in CR-group and PD-group. Rates of cancer cells with high expression of cytoplasmic MUC1 were significantly higher in the chemoresistant (CR-group) patients compared to those in the chemosensitive (PD-group) patients. ($p<0.01$, Mann-Whitney test).

statistics. Cox's proportion hazard model was used in multivariate regression analysis of survival data. Differences were considered statistically significant when the probability value was <0.05.

Table III. Clinicopathological findings and expression rate of MUC1 protein as prognostic factors in ovarian cancers.

Variables	Multivariate analysis p-value
Stage (II / III, IV)	0.172
Histology (S,E /C,M)[a]	0.065
MUC 1 expression (<50% / >50%)	0.046
Residual tumor (<2 cm / >2 cm)	0.579

[a]S, serous cystadenocarcinoma; E, endometrioid adenocarcinoma; C, clear cell adenocarcinoma; M, mucinous cystadenocarcinoma.

Results

Real-time PCR. We analyzed 14 cases of the CR-group and 14 cases of the PD-group. Of 11 cases with 1q21-q22 gains, cases 141 and 144 were chemosensitive (CR-group) and cases 132, 134, 145, 152, 166, 119, 164, and 172 were chemoresistant (PD-group). Levels of amplification and deletion at each primer-set are summarized in Fig. 1. Although two cases of CR-group had chromosomal gains of 1q21-q22 by CGH analysis, no amplification was observed

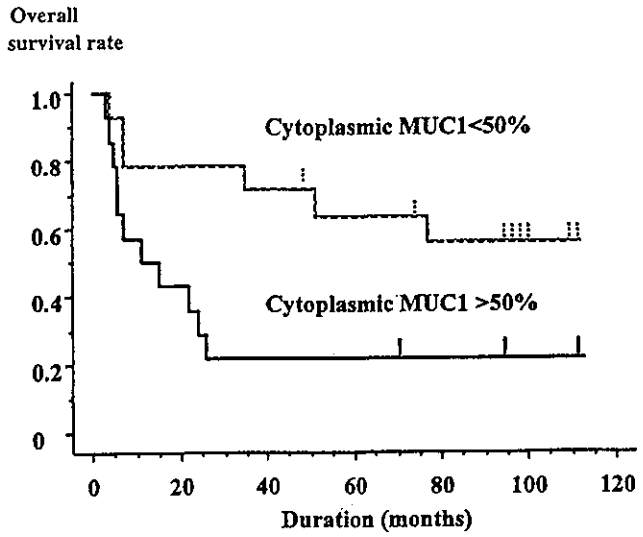

Figure 4. Kaplan-Meier survival curves by MUC1 expression. Overall survival rate of patients with cytoplasmic MUC1 immunoreactivity >50% (solid line) was significantly lower than that of patients with MUC1 immunoreactivity <50% (dotted line) (p=0.019, log-rank test).

with the real-time PCR analysis by the primers we selected. PD-group represented moderate to high amplification in the real-time PCR analysis with these primers. DNAs of seven from nine PD-group tumors showed high expression of the genes located in the region between WI-8123 and *MUC1*. Relative copy number of MUC1 over 1.5 was observed in 13 (92%) of 14 PD-group tumors and 3 (21%) of 14 CR-group tumors ($p<0.05$). High expression was rarely observed in the chromosomal region between H595801 and WI-9317 in both CR-group and PD-group.

Immunohistochemical staining. We have analyzed the expression of MUC1 protein by immunohistochemical staining. In all cases of ovarian cancer, cytoplasmic membrane of cancer cells showed strongly positive staining. Representative immunohistochemical staining results of a case of CR-group are shown in Fig. 2A, indicating positive staining in only the cell membrane. In most of PD-group, MUC1 reactivity was observed in the apical membrane and also in the cytoplasm concurrently as shown in Fig. 2B. MUC-1 staining rates were not correlated with histological type, stage, and histological grade (data not shown). The average rates for MUC1 cytoplasm-positive cancer cells were 29% in CR-group and 67% in PD-group, respectively. Rates of MUC1 cytoplasm-positive cancer cells in the PD-group were significantly higher ($p<0.01$) than those in the CR-group (Fig. 3).

Survival analysis. The effects of different variables on patient prognosis were studied by multivariate analysis. Cytoplasmic immunoreactivity in >50% cancer cells was the only independent prognostic factor (p=0.046) (Table III). Overall survival rate of patients with cytoplasmic MUC1 immunoreactivity >50% was significantly lower than that of patients with MUC1 immunoreactivity <50% (p=0.019 log-rank test) (Fig. 4).

Discussion

The genetic characteristics of chemo-resistance in human carcinomas, especially in clinical samples, are poorly understood. CGH, initially described by Kallioniemi *et al* (9) is a powerful tool with which we can detect genome-wide alterations in one experiment. Comparing different types of DNAs, potentially important genes not only for the expression of tumor phenotype but also for tumor progression are found. Such gene analyses in drug-sensitive and -resistant tumor may allow identification of genes related with resistance to anti-cancer agents. Previous studies revealed that the increased copy number at 8q24, 3q26, and 20q13 were frequently observed in several cancer tissues (6-8). Moreover, the increased copy number at 12q12, 1q31-32 and 5p14 and the decreased copy number at 16q23, 17p and 17q21-22 were commonly observed in ovarian cancer so that these genetic alterations seemed to be ovarian-cancer specific changes. Wasenius *et al* (10) reported that in six pairs of acquired-resistant and parent human ovarian cancer cell lines, cisplatin-resistant cells showed more frequently increased copy numbers at 2q14-33, 4p15-13, 4q22-25, 6q13-16 and 8q12-21 and decreased copy numbers at 2pter-p22, Xp22-21, 7p21-14, 11cen-p14 and 13q21, compared with the parental counterparts.

In the present study, we conducted real-time PCR analysis to determine the highly amplified regions on 1q21-q22 by using the primers we selected. Although Southern and blot analysis techniques have traditionally been used to quantify the copy number of specific DNA sequence, we have adopted real-time PCR which is rapid and requires only small amount of DNA. The region between WI-8123 and *MUC1* was highly expressed in the chemoresistant group (Fig. 1). Eight of nine PD-group tumor specimens with 1q21-q22 gains had genetic gains at WI-8123 and *MUC1* regions. Frequent genetic gains were not observed on the chromosomal region between H595801 and WI-9317. We selected MUC1 as a candidate for chemoresistance-related gene, and conducted an immunohistochemical study using anti-human CA15-3 murine monoclonal antibody which had high affinity to extra-cellular domain of MUC1 protein. In all ovarian cancer patients, cytoplasmic membranes of cancer cells were markedly stained for MUC1 antibody, but the degree of MUC1 stainings for cytoplasm was varied from case to case with ovarian cancer. Rates of MUC1 cytoplasm-positive cancer cells in chemoresistant group were significantly ($p<0.01$) higher than those in the chemosensitive group (Fig. 3), suggesting that chemoresistance to platinum-containing regimen was closely associated with high amplification of MUC1 protein in cytoplasm of cancer cells. Although the study was limited to a small sample group, cytoplasmic MUC1 expression >50% cancer cells was a stronger indicator for overall survival than histologic type or residual tumor diameter in multivariate analysis.

The epithelial mucin coded by MUC1 gene is a transmembrane molecule expressed by most glandular epithelial cells (11). With immunohistochemical staining, MUC1 protein is widely expressed by not only an apical surface of normal glandular epithelial cells but also of breast and ovarian cancer cells and some other cancers (12-14). MUC1 has been reported

to be inhibitory to E-cadherin-mediated cell interactions and to enhance adhesion by interacting with beta-catenin, consequently promoting metastasis (15,16). Several studies have shown the correlation of MUC1 and survival in breast, colon, and lung cancer (17-19). MUC1 might contribute to poor prognosis due to the ability to inhibit human T-cell proliferation (20) or to enhance tumor angiogenesis (21). In ovarian cancer, the correlation between prognosis of the patients and expression of MUC1 remained controversial. Dong *et al* reported that low cytoplasmic expression of MUC1 could be a predictor for good prognosis probably due to loss of metastatic ability of cancer cells (22). On the other hand, northern analyses of ovarian tumor tissue revealed that expression of MUC1 had no correlation with tumor histology, stage, and prognosis of cancer patients (23) and RT-PCR analyses indicated MUC1 expression had no relation to chemotherapeutic effects in ovarian cancer (24). But the authors included patients of recurrent ovarian cancer who hardly responded to chemotherapy (24) and might thus draw the controversial conclusion. In the present study, all the patients were primary epithelial tumors without previous chemotherapy and the determination of the MUC1 expression level was based on the immunohistochemical intensity of cytoplasmic MUC1 protein of cancer cells similar to the former study (22), and it is indicated that overexpression of MUC1 can be a predictor of chemoresistance, resulting in poor prognosis of the patients. One possible mechanisms of MUC1 protein for contributing to chemoresistance might be explained by activation of anti-apoptotic PI3K/Akt and Bcl-xL pathways (25). Further studies are needed to reconfirm the role of cytoplasmic MUC1 on cellular function such as signaling pathways and accumulation of drugs. In conclusion, we identified MUC1 as a new candidate for a biological marker of chemoresistance to platinum-based chemotherapy in ovarian cancer.

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The effect of single weekly paclitaxel in heavily pretreated patients with recurrent or persistent advanced ovarian cancer

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Abstract

Objectives. We have reported that single weekly paclitaxel has moderate activity in heavily pretreated ovarian cancer patients and is associated with a favorable toxicity profile. The purpose of this study was to reconfirm the effect of weekly paclitaxel in more number of cases.

Methods. Although 39 patients were enrolled, 37 patients with recurrent or persistent ovarian cancer previously treated with between one and three chemotherapeutic regimens containing platinum were eligible. Patients had measurable or assessable disease defined by clinical exam, radiographic studies, or serum CA 125. One cycle of treatment consisted of paclitaxel 80 mg/m^2/week in 1-h infusion, 3 weeks on, 1 week off, and repeated at least twice. Two patients were withdrawn because of refusal of further treatment for neuropathy after the first cycle. Clinical responses were defined by established criteria.

Results. Thirty-seven patients were included in this intent-to-treat study. The overall clinical response rate was 45.9% (5 complete responses, 12 partial responses). The clinical response rate in patients with measurable tumor was 25.0% (2 complete responses, 1 partial response), while that in patients without measurable tumor and with assessable CA 125 levels was 56.0% (3 complete responses, 11 partial responses). Clinical response rate in patients with chemotherapy-free interval more than 6 months had about twice higher than that in patients with chemotherapy-free interval less than 6 months. The clinical response rate by number of prior regimens revealed that as number of prior regimens increases, the response rate decreases.

Conclusion. Weekly paclitaxel has significant antitumor activity in heavily pretreated patients with recurrent or persistent ovarian carcinoma and warrants as second or third line chemotherapy in such setting.

Keywords: Weekly paclitaxel; Ovarian cancer; Second line chemotherapy; CA 125

Introduction

Ovarian cancer is the fourth leading cause of cancer death in the female population and the most fatal gynecologic malignancy. The disease is surgically curable when localized (stage I to II). However, the majority of patients present, initially and at relapse, with bulky intra-abdominal disease that is not surgically resectable. Systemic cisplatin-based chemotherapy in combination with debulking surgery has become the standard for initial therapy, with reported response rates that range from 50% to 80% [1]. Unfortunately, the majority of patients eventually die of disease persistence or recurrence, with the abdominal cavity being the most common site of recurrence. The management of tumor recurrence remains a clinical challenge, since the

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chance of response to a secondary treatment is currently less than 20% [2], especially if the disease is platinum-resistant [3]. To improve this outcome, several clinical trials are now exploring the possibility of incorporating new drugs into the first-line chemotherapy regimen [4]. Furthermore, new biological agents and molecularly targeted therapies aimed to overcome drug resistance with less toxic effects are under investigation [5].

Paclitaxel, a unique antimicrotubule agent, has been one of the most promising drugs to enter into clinical trials in the setting of cisplatin-refractory ovarian cancer. Responses have been reported in both heavily and minimally pretreated ovarian cancer patients (20% to 37%) [6,7]. However, myelotoxicity was found to be a major concern even with granulocyte colony-stimulating factor (G-CSF) support. In order to minimize toxicity, paclitaxel can be given weekly instead of triweekly [8,9]; this results in a higher dose intensity of the drug [10]. Two non-randomized trials [11,12] have suggested that the activity of paclitaxel in epithelial ovarian cancer is dose-dependent, and a randomized trial [10] has shown reduced toxicity with weekly scheduling without detriment to efficacy. We have reported that single weekly paclitaxel has moderate activity in heavily pretreated ovarian cancer patients, and 80 mg/m^2 of paclitaxel was recommended as the phase II dose for outpatients [13]. When 80 mg/m^2 of paclitaxel was given, the dose intensity may not be greater than every triweekly. However, continuous low-dose paclitaxel so-called metronomic chemotherapy has been reported to result in antiangiogenic effects and tumor dormancy [14,15]. Thus, we attempted to determine effects of single weekly paclitaxel in heavily pretreated patients with recurrent or persistent ovarian cancer.

Patients and methods

Eligibility criteria

Eligible patients had recurrent or persistent ovarian cancer that was histologically proven at primary diagnosis. All patients had either measurable or assessable disease. Disease was classified as measurable if the patient had bidimensionally measurable disease by computed tomography (CT). Assessable disease was only used in patients with no measurable disease and was defined as a CA 125 ≥ 75 U/ml. Eligibility criteria required the patients to have a baseline leukocyte count >2500, absolute neutrophil count >1500, platelet count >75000, serum creatinine <1.5 mg/dl, serum bilirubin <2.5 mg/dl, and liver function tests <3 times the laboratory standard value. Patients were required to have a life expectancy of at least 2 months and any Gynecologic Oncology Group (GOG) performance score was acceptable for enrollment in this study. Thirty-seven of 39 patients enrolled were eligible for this study. Twenty-three and 14 patients had chemotherapy-free interval ≥6 months and <6 months, respectively. Patients must have had one or more previous chemotherapy regimens (Table 1).

Exclusion criteria included borderline histology, pregnancy, fertility, diagnosis of another malignancy within the past 5 years, prior treatment with weekly paclitaxel, active infection, hepatitis, gastrointestinal bleeding, congestive heart failure, unstable angina, or myocardial infarction in the past 6 months.

Study design

This study was a nonparametric multicenter study of weekly paclitaxel. The investigative sites involved were National Defense Medical College in Saitama and Jichi Medical School in Tochigi, Japan. All investigative sites obtained institutional review board approved and all patients provided signed informed consent.

Treatment plan

Eligible patients who signed informed consent underwent a complete history and physical exam. Pretreatment laboratory tests included a complete blood count (CBC), chemistry panel to include glucose, electrolytes, BUN, creatinine, SGOT, SGPT, bilirubin, alkaline phosphatase, CA 125 level,

Table 1
Patient characteristics

Characteristic	No. of patients	%
Patients		
Enrolled	39	
Eligible	37	
Median age (range)	59 (42–74)	
Original FIGO stage		
Ia	1	2.7
Ic	2	5.4
IIIa	2	5.4
IIIc	23	62.2
IV	9	24.3
Histological type		
Serous	26	70.3
Clear	3	8.1
Mucinous	2	5.4
Endometrioid	2	5.4
Others	4	10.8
Chemotherapy-free interval[a]		
≥6 months	23	62.2
<6 months	14	37.8
Prior regimens		
1	19	51.4
2	14	37.8
3	4	10.8

[a] Interval from prior chemotherapy to start of weekly paclitaxel.

chest X-ray, EKG, and CT scan or magnetic resonance imaging (MRI).

On days 1, 8, and 15 of each 28-day cycle (1 cycle), patients received intravenous infusions of paclitaxel at 80 mg/m^2. Paclitaxel was given as a 1-h intravenous infusion via non-PVC tubing and connectors. Premedications consisted of diphenhydramine (50 mg), cimetidine (300 mg), and dexamethasone (20 mg) intravenously given 30 min before paclitaxel infusion. A minimum of six doses (two cycles) were administered at weekly intervals. Chemotherapy was withheld for white cell counts below 2500/mm^3 or absolute neutrophil counts below 1500/mm^3 and for platelet counts below 75 000 mm^3. Toxicity was assessed by using the GOG scoring system [16]. In patients with progression of disease, chemotherapy was either stopped or changed to another agent. In patients with stable disease or a clinical response, weekly paclitaxel was continued until disease progression or adverse effects necessitated removal from the study. Withdrawal from the study at patient request was allowed at any time.

Response assessment

Although most of the patients had elevated CA 125 levels, many did not have measurable disease on CT, MRI, or clinical exam. Hence, the criteria for response was based on declining CA 125 levels as described by Rustin et al. [17]. Partial response was defined by reduction of CA 125 by more than 50% after two samples or greater than 75% serial reduction over three consecutive samples, with the final sample taken at least 28 days after the previous sample. This has been correlated to standard response criteria as defined by the Gynecologic Oncology Group (GOG) in patients with measurable disease [18]. Partial response by CT scan was defined as a 50% reduction in the sum of the two perpendicular diameters of all measurable tumors for at least 1 month. Complete response was defined as total disappearance of all clinically or radiologically measurable tumors with normalization of CA 125 levels (<35) for at least 1 month. Progression of disease was defined as appearance of new lesions or an increase of more than 50% in the sum of two perpendicular diameters of any existing lesion or increase in CA 125 levels on two consecutive measurements. The term stable disease was used for any response that fell in between progression and a partial response. For statistical comparison, the Mann–Whitney two-sample test and Fisher's Exact Test have been used. Time to progression (TTP) was measured as interval from prior chemotherapy to start of the weekly paclitaxel for progression. Survival was measured from start of the weekly paclitaxel to the date of death or last contact if the date of death is unknown.

Table 2
Clinical response (N = 37 evaluable patients)

Response	No. of patients	%
Complete response	5	13.5
Partial response	12	32.4
Stable disease	16	43.2
Progression	4	10.8

Table 3
Response with tumor regression (N = 12 evaluable patients)

Response	No. of patients	%
Complete response	2	16.7
Partial response	1	8.3
Stable disease	6	50.0
Progression	3	25.0

All patients had morphologically measurable tumor.

Results

From April 1999 to September 2002, 39 patients were enrolled in this prospective trial and received weekly paclitaxel therapy. Two patients were withdrawn because of refusal of further treatment for neuropathy after the first cycle. Demographics for the 37 evaluable patients are listed in Table 1. Twenty-three patients (62.2%) had chemotherapy-free interval ≥6 months. All of 14 (37.8%) patients with chemotherapy-free interval <6 months had platinum-based chemotherapy. The number of patients with one prior chemotherapy regimen was 19, that with two prior regimens was 14, and that with three prior regimens was 4. Primary chemotherapy consisted of 30 patients with combination chemotherapy by cisplatin, Adriamycin, and cyclophosphamide (CAP), 4 patients with combination chemotherapy by paclitaxel and carboplatin (TJ), and 3 patients with combination chemotherapy by carboplatin and cisplatin (JP). Performance status (GOG) of all patients enrolled was 0 or 1.

All 37 patients were evaluable for response. Five patients (13.5%) showed a complete response, 12 (32.4%) showed a partial response. Total response rate was 45.9% (Table 2).

Two (16.7%) out of 12 patients with measurable tumor had complete response and 1 (8.3%) had partial response. The response rate was 25.0% (Table 3). Regarding response based on CA 125 levels, 3 (12.0%) of 25 patients had complete response and 11 (44.0%) had partial response. The

Table 4
Response based on CA 125 levels (N = 25)

Response	No. of patients	%
Complete response	3	12.0
Partial response	11	44.0
Stable disease	10	40.0
Progression	1	4.0

No patient had morphologically measurable tumor.

Table 5
Clinical response according to chemotherapy-free interval ($N = 37$)

	Chemotherapy-free interval			
	<6 months[a]		≥6 months	
Total	14		23	
Response				
Complete	1	7.1%	3	13.0%
Partial	3	21.5%	10	43.5%
Stable	9	64.3%	7	30.5%
Progression	1	7.1%	3	13.0%

[a] All patients received platinum-based chemotherapy.

response rate was 56.0%, showing more than two times of response rate of patients with measurable tumor (Table 4). One (7.1%) of 14 patients with chemotherapy-free interval <6 months had complete response, while 3 (13.0%) of 23 patients with chemotherapy-free interval ≥6 months had complete response. Three patients (21.5%) with chemotherapy-free interval <6 months had partial response, while 10 patients (43.5%) with chemotherapy-free interval ≥6 months had partial response. The response rate (56.5%) of patients with chemotherapy-free interval ≥6 months was about two times higher than that (28.6%) with chemotherapy-free interval <6 months (Table 5). Clinical response rate according to number of prior regimens showed that as number of prior regimens increases, the response rate decreases (Table 6). Median TTP and overall survival were 12 months and 21 months, respectively.

A total of 468 doses (range, 6–39) of weekly paclitaxel were administered to the 37 patients. Toxicity data was available for all the 37 patients. Hematological toxicity more than grade 2 was observed in about 25%, while non-hematological toxicity was observed in 1 (2.7%) of 37 patients (Table 7). Nine patients (24.3%) had a grade 3 or 4 neutropenia. Four patients had treatment delays and two patients required granulocyte colony-stimulating factors intermittently for severe neutropenia, but there were no hospital administrations for neutropenic fever. Four patients had a grade 3 anemia, and two of them required blood transfusion. During treatment with weekly paclitaxel, one patient had a grade 3 neuropathy and the chemotherapy had to be stopped. There was no evidence for cumulative hematological and non-hematological toxicity.

Table 6
Clinical response according to number of prior regimens

	Number of prior regimens					
	1		2		3	
Total	19		14		4[a]	
Response						
Complete	4	21.2%	1	7.1%	0	
Partial	7	36.8%	4	28.6%	1	25.0%
Stable	7	36.8%	7	50.0%	2	50.0%
Progression	1	5.3%	2	14.3%	1	25.0%

[a] All patients with three prior regimens had measurable tumor.

Table 7
Toxicity profiles

Hematological toxicity	No. of patients
Neutropenia	
Grade 3	7
Grade 4	2
Leukopenia	
Grade 3	9
Grade 4	1
Thrombocytopenia	
Grade 3	0
Grade 4	0
Anemia	
Grade 3	4
Grade 4	0
Non-hematological toxicity	**No. of patients**
Peripheral neuropathy	
Grade 2	5
Grade 3	1
Alopecia	
Grade 2	11
Grade 3	0

Discussion

The treatment of recurrent and refractory cancer is a challenging problem because recurrent or refractory disease is almost never curable. The majority of patients who initially respond will develop chemotherapy-resistant disease and ultimately die. Thus, the primary treatment objectives in the salvage setting are prolonging remission and maintaining quality of life. These goals may be attainable through the evaluation of different dosing and timing regimens of standard chemotherapeutic agents.

Introduction of paclitaxel into the armamentarium of drugs to treat platinum-resistant ovarian cancer has been one of the more significant advances in the treatment of ovarian cancer in the last decade. Paclitaxel has a unique mechanism of action, is cell-cycle-specific, and acts by promoting the stability of the microtubule assembly during mitosis. In vitro data suggest that the duration of exposure plays a crucial role in the cytotoxic efficacy of paclitaxel [19,20]. Resistance to paclitaxel-mediated P-glycoprotein (Pgp) [21] has been shown to be significantly reduced by increasing the duration of exposure to paclitaxel from 3 to 96 h in Pgp-expressing paclitaxel-resistant breast cancer cell lines [22].

Weekly administration of paclitaxel has the potential to have an effect similar to that of continuous infusion while

taking advantage of the minimal hematological toxicity associated with shorter infusions. Neutropenia was the most frequent hematological adverse event observed in patients receiving once-weekly intravenous paclitaxel monotherapy. Severe neutropenia was dose-related, occurring in 3% and 15% of patients receiving 80 mg/m^2 monotherapy [23,24]. An absolute neutropenia count of 1000 has been shown to be sufficient for dosing weekly paclitaxel on any given scheduled day of treatment. In the present study, severe neutropenia and leukopenia of grade 4 were observed in 2 (5.4%) and 1 (2.7%) of 37 patients. Other hematological adverse events (grade 4 anemia or grade thrombocytopenia) were not observed. Neuropathy is experienced by most patients receiving once-weekly intravenous paclitaxel monotherapy and is usually mild or moderate [23,24]. The incidence of severe neuropathy with paclitaxel 80 mg/m^2 once weekly was approximately 10% [23,24]. Most patients experienced mild myalgia and/or arthralgia; few patients reported severe symptoms [25]. In the present study, 3/39 (7.7%) containing two patients withdrawn from this trial experienced severe neuropathy. Although alopecia of grade 2 was observed in 11/37 (29.7%), alopecia beyond grade 2 was not observed (Table 7). No patient required dose reduction was observed in this trial. Prolonged exposure to relatively low concentrations of paclitaxel has been shown to induce apoptosis [26]. In addition, prolonged low-dose paclitaxel exposure has been reported to have anti-angiogenic properties [27]. The paclitaxel dose delivered in this regimen is 24 mg/m^2 over 3 weeks as compared to 175 mg/m^2 every 3 weeks with conventional dosing. These features associated with weekly low-dose paclitaxel may explain the response seen in patients with carcinoma refractory to conventionally dosed paclitaxel.

Fennelly et al. [8] did a phase I trial with 18 patients with platinum- and paclitaxel-resistant ovarian cancer and determined that 80 mg/m^2 was the maximally tolerated dose. We also reported in the phase I study that the same dose of 80 mg/m^2 was the maximum recommended dose [13]. Thus, we performed phase II study by single weekly 80 mg/m^2 paclitaxel. Treatment with single weekly 80 mg/m^2. paclitaxel brought about an overall response rate of 45.9%, similar to that of a recent report [28]. It is noteworthy that five complete responses among 37 patients with one or more therapeutic regimens were achieved (Table 2). In addition, 3 (25.0%) of 12 patients with measurable tumor containing two complete responses had response to weekly paclitaxel (Table 3). When based on CA 125 levels, the response rate of 56.0% including a complete response of 12.0% was obtained, showing two times higher response rate compared to that in patients with measurable tumor (Table 4). These results suggest that patients with recurrence detectable only by CA 125 levels (but not morphologically measurable) are more sensible to weekly paclitaxel than those with measurable tumor. It is possible that angiogenesis of detectable tumor only by CA 125 is vulnerable to weekly paclitaxel than that of morphologically measurable tumor. Response rate (56%) of patients with chemotherapy-free interval ≥6 months showed about two times that (28.6%) of those with chemotherapy-free interval <6 months (Table 5). Similarly, a recent report demonstrated that all the responders with paclitaxel-resistant tumors were seen in patients with a paclitaxel-free interval of more than 12 months [28]. Since most of prior regimens used in patients enrolled in the present study were cisplatin-based chemotherapy, weekly paclitaxel seemed to be more effective in patients with longer platinum-free interval. In addition, we examined clinical response according to number of prior regimens. When prior regimen was 1 or 2, the clinical response rate was 58.0% or 35.7%, respectively, whereas in patients with three prior regimens, the responder was only one (25.0%) (Table 6). These results suggest that as number of prior regimens increases, the response rate decreases and therefore patients with less prior regimens may have better be treated with weekly paclitaxel. It is noteworthy that 9 of 14 patients with two prior regimens received chemotherapy containing paclitaxel while all patients with three prior regimens received chemotherapy containing paclitaxel. However, efficacy of weekly paclitaxel was not influenced by kinds of prior chemotherapy regimen.

The choice of second line drug in this present setting is dependent on toxicity and quality of life considerations, in addition to efficacy. Weekly administration of paclitaxel by 1-h infusion has been reported to have less toxicity than other schedules and primary effect in patients with pretreated gynecologic cancers [8,10,29,30]. In addition, a randomized trial comparing the weekly schedules to triweekly paclitaxel for advanced breast cancer is nearing completing in the GALGB. 'Metronomic' dosing or antiangiogenic scheduling of cancer chemotherapeutics has been increasingly recognized to be a potential application of paclitaxel in cancer therapy [31–33].

In conclusion, weekly low-dose paclitaxel used in the present study is considered safe and effective in pretreated patients with recurrent or persistent ovarian cancer. Encouraging response rates in both platinum-sensitive and platinum-resistant patients warrant further studies.

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Clinicopathologic study of 56 patients with endometrial cancer during or after adjuvant tamoxifen use for their breast cancers

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Abstract

Objectives. The aim of this study was to describe the clinicopathologic features and prognosis of endometrial cancer patients diagnosed during or after tamoxifen treatment for breast cancer.

Methods. Fifty-six tamoxifen-related endometrial cancers were identified from 10 hospitals in Japan. Past users were defined as endometrial cancer patients diagnosed more than 12 months after the cessation of tamoxifen treatment for breast cancer. All other users were classified as recent users.

Results. Age at diagnosis of the endometrial cancer ranged from 29 to 81 years. Sixteen (29%) and 19 (34%) patients were nulliparous and overweight, respectively. When the patients were divided into two groups: 30 recent and 26 past users, the distribution of various clinical characteristics, except for age at the time of diagnosis for endometrial cancer and the interval between the diagnoses of two cancers, was similar for two groups. The daily dose, duration and cumulative dose also showed no significant difference between the two groups. Past users had histopathologically more invasive tumors showing prognostically more unfavorable subtypes than recent users. The background lesions including endometrial polyps and diffuse cystic changes were similar for the two groups. The cumulative 3-year survival was significantly worse for past users than for recent users (74.8% and 96.4%, respectively, $P < 0.04$). In multivariate analysis including recentness of tamoxifen use and age at diagnosis of endometrial cancer, the significance of past user disappeared.

Conclusions. Past users had a worse prognosis of endometrial cancer with more invasive histologic features than recent users, probably because they included more elderly patients.

Keywords: Tamoxifen; Clinicopathology; Prognosis; Endometrial cancer; Breast cancer

Introduction

Tamoxifen has been widely used as an adjuvant hormonal treatment for breast cancer over the past two decades and its use has been convincingly shown to improve the disease-free survival as well as overall survival [1]. Tamoxifen has long been considered a safe medication with few serious side effects. Several case-control studies, however, have shown an increased incidence of endometrial cancer in tamoxifen-treated breast cancer patients with or without any positive effects based on the duration of

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tamoxifen use and the cumulative dose on the risk of endometrial cancer [2–6]. Furthermore, the Stockholm Trial showed a continued divergence of the cumulative incidence curves of endometrial cancer for the tamoxifen-treated and control groups even several years after cessation of tamoxifen treatment [5]. Two studies reported that the endometrial cancer risk was similar both during and after tamoxifen treatment [6,7]. Bergman et al. [8] confirmed that the risk did not decrease after a cessation of tamoxifen treatment and it was not modified by other risk factors for endometrial cancer.

The above data seem to recommend that studies concerning the evolution of risk after the cessation of tamoxifen use should be carried out. However, so far, there have been no reports on the different clinicopathologic features and the prognosis of endometrial cancers, which develop in breast cancer patients during or after tamoxifen treatment. Based on the clinicopathologic findings of 56 tamoxifen-related endometrial cancers, we tried to show the different clinicopathologic features between endometrial cancers diagnosed during and after adjuvant tamoxifen use for their breast cancers.

Materials and methods

Case selection

Between October 1991 and April 2003, about 2000 women with endometrial cancer were treated in nine university hospitals and a cancer center hospital in Japan. Fifty-eight endometrial cancers in tamoxifen-treated breast cancer patients were retrospectively found in their medical files. The clinical information and sections of the surgical specimens were sent to the first author for a review. The patients consisted of women diagnosed with endometrial cancer at least 6 months after an initial breast cancer diagnosis. According to the previous studies [7,8], past users were defined as patients in whom endometrial cancer was diagnosed after more than 12 months from the cessation of tamoxifen treatment. All other users were classified as recent users. Overweight patients were defined as those with a body mass index (BMI) of 24.5 or more. Two tumors were not eligible for this study because one was diagnosed as atypical endometrial hyperplasia in the results of a pathological review and another endometrial carcinoma developed in a breast cancer patient with 6 months toremifene treatment in a review of the medical records. All 56 patients underwent a total abdominal hysterectomy. A bilateral salpingo-oophorectomy was performed in 55 patients. A pelvic lymphadenectomy and para-aortic lymph node biopsy or adenectomy was performed in 49 patients. The surgeons from two hospitals did not perform lymph node sampling on five patients when surgeons decided that no myometrial invasion was likely based on the cut surface of the resected uterus during the operation. Two patients with medical complications also did not undergo the lymph node sampling. A cytologic test of the peritoneal fluid was performed in 52 patients. Fifteen patients including six recent and nine past users were treated with adjuvant therapy. Two patients with surgical stage Ic tumor were treated with adjuvant external beam radiation therapy consisting of 50 grays (Gy) to the whole pelvis. Ten patients with surgical stage III tumor and two patients with surgical stage IVb tumor were treated with cisplatin or taxane based combined chemotherapies in three to six courses. One patient with surgical stage IIIc tumor was treated with adjuvant external beam radiation therapy consisting of 40 grays to the whole pelvis.

Histologic evaluation

All hematoxylin and eosin (H&E)-stained sections with a mean of six sections per uterus (range, 1–33 sections) were reviewed by the first author (a member of the International Society of Gynecological Pathologists). Surgical staging was determined using the surgical staging system for corpus cancer established by the International Federation of Obstetrics and Gynecology (FIGO) [9]. The histologic grading method used in the present study has been previously described [10]. The tumors were classified based on the criteria of the World Health Organization [11].

Statistical analyses

Statistical analyses were performed using SPSS for Windows, version 11.0.0 (SPSS Ltd., Chicago, IL). The chi-square test was used to assess the association between categoric variables. The mean ages of the patients, the interval between diagnoses of two cancers, the cumulative dose, and duration of the tamoxifen use were added to assessment by Student's t test. The survival time was calculated from the date of initial surgery for endometrial cancer. The cumulative survivals were determined using the Kaplan–Meier product-limit method. The log rank test was used to test differences in survival within variables. Cox's proportional hazards model was used to identify and simultaneously evaluate any independent prognostic factors associated with relative survival. Statistical significance was considered to exist at a value of $P < 0.05$.

Results

Clinical findings

The clinical characteristics of 56 endometrial cancer patients treated with tamoxifen for their breast cancer are summarized in Table 1. Based on Student's t test, the mean ages of the recent and past users at diagnosis of breast cancer were 53.3 years (range, 25–80 years) and 56.9 years (range, 38–73 years), respectively ($P = 0.17$). The mean

Table 1
Clinical characteristics of tamoxifen-treated breast cancer patients with endometrial cancer

	Case no.	Recent user (%)	Past user (%)	P value[a]
Age at diagnosis of breast cancer (years)				0.33
<49	16	11 (37)	5 (19)	
50–59	22	11 (37)	11 (42)	
≥60	18	8 (26)	10 (39)	
Age at diagnosis of endometrial cancer (years)				
<49	9	7 (24)	2 (8)	0.03
50–59	14	10 (33)	4 (16)	
≥60	33	13 (43)	20 (76)	
Gravidity				0.59
0	12	8 (27)	4 (15)	
1–3	30	15 (50)	15 (58)	
≥4	14	7 (23)	7 (27)	
Parity				0.58
0	16	10 (33)	6 (23)	
1–3	37	19 (64)	18 (69)	
≥4	3	1 (3)	2 (8)	
BMI[b]				0.54
<24.5	37	18 (60)	19 (73)	
24.5–30.4	12	8 (27)	4 (15)	
≥30.5	7	4 (13)	3 (12)	
HRT				0.92
No	54	29 (97)	25 (96)	
Yes	2	1 (3)	1 (4)	
Interval between diagnoses of 2 cancers (months)				0.005
<12	3	2 (7)	1 (4)	
12–59	18	15 (50)	3 (12)	
≥60	35	13 (43)	22 (84)	

[a] P value was determined by chi-square test.
[b] BMI; body mass index (kg/m^2).

ages of the recent and past users at diagnosis of endometrial cancer were 58.3 years (range, 29–81 years) and 65.6 years (range, 48–79 years), respectively ($P = 0.008$). The mean intervals of the recent and past users between diagnoses of two cancers were 62.8 months (range, 7–185 months) and 101.4 months (range, 36–204 months), respectively ($P = 0.02$). The age at diagnosis of endometrial cancer, the interval between the diagnoses of two cancers also showed significant differences between the recent and past users based on chi-square test. The mean months from cessation of tamoxifen treatment to diagnosis of the endometrial cancer were 55.4 months (range, 13–120 months). Using chi-square test, the distributions of gravidity, parity, BMI, and HRT were similar for the recent and past users.

The distributions of daily dose, duration of tamoxifen use, and cumulative dose showed no significant differences between the recent and past users based on the chi-square test (Table 2). The mean durations of tamoxifen use of recent and past users were 59 months (range, 5–204 months) and 48 months (range, 2–144 months), respectively. The mean cumulative doses of the recent and past users were 42 g (range, 3.0–129.6 g) and 35 g (range, 1.2–101.0 g), respectively. These variables also showed no significant differences between the recent and past users based on Student's t test.

Histological findings

The endometrial cancers of recent users included 16 stage Ia, 6 stage Ib, 2 stage Ic, 1 stage IIa, 2 stage IIIa, 2 stage IIIc, and 1 stage IVb. Those of past users included 5 stage Ia, 8 stage Ib, 3 stage Ic, 1 stage IIb, 2 stage IIIa, 6 stage IIIc, and 1 stage IVb. The histopathologic findings of the tamoxifen-related endometrial cancers are summarized in Table 3. The presence of rhabdomyosarcomatous change was confirmed in two carcinosarcomas by immunohistochemical staining of myoglobin. Squamous differentiation was found in 13 endometrioid adenocarcinomas and three carcinosarcomas. Focal mucinous differentiation was found in six endometrioid adenocarcinomas. Prominent psammoma bodies were found in two endometrioid adenocarcinomas and 1 clear cell adenocarcinoma. Advanced stage (Ia vs. more than Ib, $P < 0.01$), Myometrial invasion ($P \leq 0.03$), lymphovascular space invasion ($P < 0.001$) lymph node metastasis ($P = 0.03$) and the prognostically unfavorable histologic subtypes ($P = 0.03$) were more frequently present in past users than in recent users. All serous carcinomas, clear cell adenocarcinomas and carcinosarcomas were found in patients more than 60 years of age. Based on chi-square test, advanced stage (Ia vs. more than Ib, $P < 0.01$) and lymphovascular space invasion ($P < 0.01$) were more frequently present in patients more than 60 years of age than those <60 years of age, but myometrial invasion ($P = 0.30$) and lymph node metastasis ($P = 0.26$) were not.

The histopathologic findings of the background lesions are summarized in Table 4. The largest diameters

Table 2
Tamoxifen treatment

	Case no.	Recent user (%)	Past user (%)	P value[a]
Daily dose (mg)				0.58
20	45	25 (83)	20 (76)	
30	4	2 (7)	2 (8)	
40	6	2 (7)	4 (16)	
80	1	1 (3)	0 (0)	
Duration (months)				0.35
<24	8	6 (20)	2 (8)	
24–59	24	11 (37)	13 (50)	
≥60	24	13 (43)	11 (42)	
Cumulative dose (g)				0.63
<10	7	4 (13)	3 (12)	
10–29	20	9 (30)	11 (42)	
≥30	29	17 (57)	12 (46)	

[a] P value was determined by chi-square test.

Table 3
Histopathologic characteristics of the tamoxifen-related endometrial cancer

	Case no.	Recent user (%)	Past user (%)	P value[a]
Stage				<0.01
Ia	21	16 (53)	5 (19)	
More than I	35	14 (47)	21 (81)	
Grade and histologic subtypes				0.03[b]
Endometrioid grade 1	21	14 (46)	7 (27)	
grade 2	17	9 (30)	8 (31)	
grade 3	2	0 (0)	2 (8)	
Mucinous, grade 1	2	2 (7)	0 (0)	
Serous	4	1 (3)	3 (11)	
Clear	4	2 (7)	2 (8)	
Carcinosarcoma	6	2 (7)	4 (15)	
Myometrial invasion				
None	27	19 (63)	8 (31)	0.03
Less than 1/2	18	8 (27)	10 (38)	
>1/2	11	3 (10)	8 (31)	
Cervical invasion				0.35
None	49	28 (94)	21 (81)	
Superficial	3	1 (3)	2 (8)	
Deep	4	1 (3)	3 (11)	
Lymphovascular space invasion				<0.001
None	37	27 (90)	10 (38)	
Mild	11	2 (7)	9 (35)	
Sever	8	1 (3)	7 (27)	
Lymph node metastasis				0.03
Presence	9	2 (7)	7 (27)	
Absence	40	25 (93)	15 (73)	
Ovarian metastasis				0.84
Presence	1	1 (3)	0 (0)	
Absence	54	29 (97)	25 (100)	
Ascites cytology				0.53
Positive	11	5 (17)	6 (25)	
Negative	41	23 (83)	18 (75)	

[a] P value was determined by chi-square test.
[b] Endometrioid grades 1 and 2 and mucinous vs. endometrioid grade 3, serous, clear, and carcinosarcoma.

of the endometrial polyps ranged from 1 to 7 cm. Diffuse cystic changes composed of cystically dilated atrophic and proliferative glands separated by fibrotic stroma were found in 26 (46%) background endometriums (Fig. 1). The distributions of various characteristics of background lesions were similar for both the recent and past users.

Prognosis

The patients were followed from 2 to 100 months (37.2 ± 24.4 months: mean ± standard deviation) after the surgery for endometrial cancer. Seven patients with 3 stage IIIc endometrioid adenocarcinomas, 2 stages Ib and

Table 4
The background lesions

	Case no.	Recent user (%)	Past user (%)	P value[a]
Endometrial polyp				0.43
Presence	18	11 (37)	7 (30)	
Absence	38	19 (63)	19 (70)	
Diffuse cystic change				0.62
Presence	26	13 (43)	13 (50)	
Absence	30	17 (57)	13 (50)	
Submucosal cystic change				0.25
Presence	10	7 (23)	3 (12)	
Absence	46	23 (67)	23 (88)	
Mucinous metaplasia				0.28
Presence	4	1 (3)	3 (12)	
Absence	52	29 (97)	23 (88)	
Adenomyosis				0.11
Presence	19	13 (43)	6 (24)	
Absence	37	17 (57)	20 (76)	
Myoma				0.71
Presence	18	9 (30)	9 (36)	
Absence	38	21 (70)	17 (64)	

[a] P value was determined by Chi-square test.

Ic carcinosarcomas, 1 stage Ib clear cell carcinoma, and 1 stage IIIc serous carcinoma died of endometrial cancer. Three patients died of breast cancer. Four patients are alive with breast cancer. One patient was died of pulmonary embolism 2 months after the surgery for endometrial cancer. The cumulative 3-year endometrial carcinoma-specific survival was 87.5%. The 3-year cumulative endometrial carcinoma-specific survival (Fig. 2) was significantly worse for past user than for recent user (74.9% and 96.3%, respectively, $P < 0.04$). The 3-year cumulative endometrial carcinoma-specific survivals

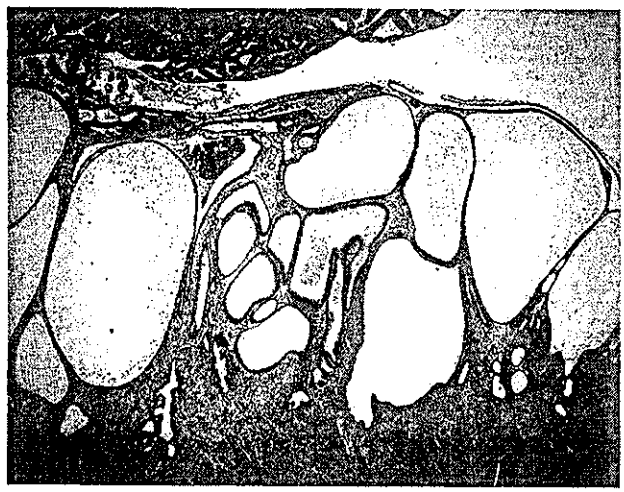

Fig. 1. Diffuse cystic change. Note the cystically dilated atrophic and proliferative glands separated by fibrotic stroma in the background endometrium. The upper portion showed a grade 1 endometrioid tumor (H&E, ×12).

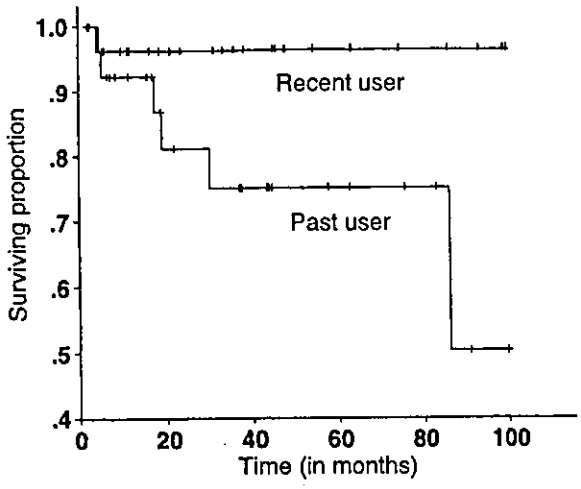

Fig. 2. The cumulative endometrial cancer-specific survival for recent and past users.

for patients <60 years and more than 60 years of ages at diagnosis of endometrial cancer were 95.7% and 81.8%, respectively, (P = 0.08). The 3-year cumulative endometrial carcinoma-specific survivals for the durations of tamoxifen treatment with <60 months and more than 60 months were 87.5% and 84.4%, respectively. The 3-year cumulative endometrial carcinoma-specific survivals for the cumulative doses of <30 g and more than 30 g were 84.7% and 87.2%, respectively. The 3-year cumulative endometrial carcinoma-specific survivals for patients treated with and without adjuvant therapy were 73.5% and 90.1%, respectively (P = 0.16). In a multivariate analysis including recentness of tamoxifen use and age at diagnosis of endometrial cancer (Wald chi-square test: 2.47, P = 0.12 and 1.66, P = 0.20, respectively), the significance of past user disappeared. Furthermore, in a multivariate analysis including the above variables and histopathologic variables (grade, myometrial invasion, cervical invasion, lymphovascular space invasion and histologic type), the presence of lymphovascular space invasion only showed its significance (Wald chi-square test: 3.91, $P < 0.05$). Adjuvant therapy showed no significance in a multivariate analysis.

Discussion

Bergman et al. [8] reported that long-term tamoxifen users had a worse prognosis of endometrial cancers, which seemed to be due to a less favorable histology and a higher stage. No significant relationship between prognosis of the patients and duration of tamoxifen use or cumulative dose was found in the present study, but the cumulative endometrial cancer-specific survival was significantly worse for past users than for recent users. In the prospective randomized trial of Postoperative Radiation Therapy in Endometrial Carcinoma (PORTEC) for stage I disease, Creutzberg et al. [12] reported that a patient age of more than 60 years was an independent predictor of death from endometrial cancer. Several investigators also support older age to be an independent predictor of poor outcome [13,14], but the others do not [15,16]. In this study, the patients more than 60 years of age at diagnosis of endometrial cancer were more frequently found in past user than in recent user. A multivariate analysis including recentness of tamoxifen use and age at diagnosis of endometrial cancer showed the significance of past user to disappear.

In comparing the two groups of patients, one treated and the other not treated with tamoxifen, Magriples et al. [17] reported a high frequency of high grade endometrial carcinomas in patients undergoing tamoxifen treatment. Similar findings have been reported by Silva et al. [18] and Deligdisch et al. [19]. However, Fisher et al. [3] and Barakat et al. [20] showed that tamoxifen-related endometrial cancers did not appear to be of a different type or have a worse prognosis than such tumors in non-tamoxifen-treated patients. In this study, prognostically favorable histologic subtypes and less invasive histologic features were found in most tumors of recent users, whereas unfavorable histologic subtypes and invasive histologic features were more often found in tumors of past users. Advanced stage and lymphovascular space invasion were more frequently present in patients more than 60 years of age than those <60 years of age. Several investigators have reported that older women with endometrial cancer have frequently deep myometrial invasion and/or poorly differentiated histology [12,15].

Silva et al. [18] showed 10 (77%) of 13 postmenopausal patients who developed tamoxifen-related endometrial cancer to have endometrial polyps, whereas 16 (34%) of 47 patients in the comparable group who did not receive tamoxifen had endometrial polyps. Deligdisch et al. [19] reported that 15 (45%) of 33 tamoxifen-related endometrial cancers arose visibly in endometrial polyps. The diffuse cystic change [21] and submucosal cystic change [22] have been reported to be a characteristic findings of the tamoxifen-treated uteri and are considered to be a response to estrogenic effect of tamoxifen [19,23]. In the present study, 18 (32%) of 56 tamoxifen-related endometrial cancers were associated with endometrial polyps. Twenty-six (46%) of them were associated with diffuse cystic change. The incidences of these background lesions were not significantly different between recent and past users.

Carcinosarcomas have been recently reported to develop in tamoxifen-treated breast cancer patients [24]. Several studies have reported a significant association between long-term tamoxifen use and carcinosarcoma [8,24]. Six carcinosarcomas (11%) were found in this study. The durations of tamoxifen use were 144 and 84 months in recent users and 24, 60, 60, and 60 months in past users.

In general, two clinicopathologic types of endometrial cancer have been believed [25]. Type I tumors are low-grade and estrogen-related endometrioid-type adenocarcinoma that usually develop in pre- or perimenopausal women

and coexist with endometrial hyperplasia. In contrast, Type II tumors are nonendometrioid carcinoma, the main prototype of which is serous and clear cell carcinomas, largely occurring in older women. They are aggressive tumors, unrelated to estrogen stimulation and arising in atrophic endometrium. According to this model, endometrial cancer detected in recent user may be considered to be Type I tumor because they are stimulated by a continuous estrogenic effect of tamoxifen. It is interesting whether endometrial cancers detected in patients after cessation of estrogenic effect of tamoxifen are classified as Type I tumors or Type II tumors. Although background lesions including endometrial polyp and diffuse cystic changes are similar for both recent users and past users, past users had a worse prognosis of endometrial cancer with more invasive histologic features than recent user. In a multivariate analysis including recentness of tamoxifen use and age at diagnosis of endometrial cancer, the significance of past user disappeared. The endometrial cancers in past users may partly have a same feature as the Type II tumors. A large case study of endometrial cancers detected in patients after cessation of tamoxifen treatment may give one of the clues to disclose histogenesis of endometrial cancer.

Despite the gynecologic side effects, the benefits of tamoxifen in breast cancer patients in controlling breast cancer or prevention of its relapse have been well established. We are expecting that this study will contribute to prevention and early detection of tamoxifen-related endometrial cancer.

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Chronic Administration of Single Weekly Paclitaxel in Heavily Pretreated Ovarian Cancer Patients

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Abstract: Ovarian cancer patients with paclitaxel-resistance have been reported to respond to a weekly schedule of the same drug. In this report, two cases with long progression free interval by weekly paclitaxel (T) are presented. Case 1. A 41-year-old Japanese woman, gravida 2, para 0, was referred to our hospital in September 16, 1998, because of abdominal mass accompanying large amount of ascites with elevated CA125 (8400 U/ml) and CA19-9 (770 U/ml). Exploratory laparotomy (tumor biopsy plus partial omentectomy) was performed September 21, 1998. After the surgery, the tumor was diagnosed as serous cystadenocarcinoma of the ovary (stage IV) and 6 cycles of treatment consisting of cyclophosphamide, adriamycin and cisplatin (CAP) were performed. The CA 125 level (8400 U/ml) rapidly declined to 150 U/ml by this CAP therapy. After second cytoreductive surgery (SRS) (total hysterectomy and bilateral salpingo-oophorectomy), residual tumor was less than 2 cm. Although 7 cycles of CAP was added, ascites and elevation of CA 125 (5100 U/ml) were observed. Therefore, treatment with single weekly T was performed and CA 125 levels remained between 70-90 U/ml during 13 cycles of this therapy (progression free interval; more than 1 year). Thereafter, she is alive with disease and followed-up. Case 2. A 48-year-old Japanese woman, gravida 3, para 2, was referred to our hospital in July 22, 1998, because of abdominal swelling and pain. Computing tomography (CT) and magnetic resonance imaging (MRI) revealed large amount of ascite and pelvic mass (9 x 7 x 7 cm), and low density area (3 x 3 cm) suggesting metastasis in right lobe of liver. Serum CA 125 level elevated to 5100 U/ml. Bilateral salpingo-oophorectomy and infracolic omentectomy were performed on August 5, 1998. The tumor was diagnosed as endometrioid adenocarcinoma of the ovary, stage IV and chemotherapy with CAP was initiated on September 5, 1998. After 6 cycles of CAP, SRS was performed. After SRS, 3 cycles of CAP were added and changed to weekly T because of damage of renal function. The CA 125 level returned within normal range during weekly T. Total 13 cycles of weekly T were performed and progression free interval was about 18 months. Thereafter, she received treatments with gamma knife and CAP for brain metastasis. She is alive without disease and followed-up. Side effects by weekly T were mild and tolerable despite of long term treatment. In addition, weekly T can be safely used in outpatient setting and even in patients with poor performance status (PS), and warrant long time to progression.

Keywords: Pretreated ovarian cancer, low dose paclitaxel, single weekly treatment.

INTRODUCTION

More than a decade ago paclitaxel was demonstrated to be an active drug in platinum-resistant ovarian cancer [1]. Conventional doses of paclitaxel range from 135 mg/m^2 to 250 mg/m^2 administered during 3 to 24 hours every 3 weeks, and myelosuppression is the main dose-limiting toxicity; however, regimens with shorter infusion schedule have reduced hematologic toxicity [2]. It has been proposed that regimens with phase-specific drugs, such as paclitaxel, may be more active and less toxic when administered continuously or in frequent intervals [3, 4]. An alternative method of increasing the exposure of solid tumor cells to paclitaxel is the delivery of the drug on a more frequent dosing schedule. This strategy (often called a "dose-dense approach") [5] could theoretically permit a larger percentage of the cancer cells to enter the vulnerable phase of their cell cycle when cytotoxic concentrations of paclitaxel are present within the systemic circulation and malignant tissue.

In an effort to minimize bone marrow suppression and other toxicities associated with paclitaxel administration when the agent is delivered on a weekly schedule, both the dose and infusion time have been reduced, compared with the every 3-week treatment schedule. However, despite this reduction in individual dosing, the total dose administered and total duration of exposure of the malignancy to paclitaxel over a 3-week period are increased. As paclitaxel is known to be a cell-cycle-specific agent [6, 7], it has been suggested that increasing the duration of tumor exposure to the drug might enhance cytotoxicity. For example, a 24-hour paclitaxel infusion schedule has been shown to increase bone marrow toxicity, compared with a 3-hour delivery regimen [8].

Thus, it is possible that the administration of paclitaxel in ovarian cancer on a weekly schedule, rather than the

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