Table IV. Univariate and multivariate analyses of prognostic factors for overall and disease-free survivals.

	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Tumor grade	3.32 (1.58-7.00)	0.002	1.59 (0.66-3.80)	0.302	3.29 (1.58-6.83)	0.002	1.49 (0.61-3.61)	0.383
Stage	2.64 (1.66-4.21)	<0.001	2.19 (1.21-3.97)	0.010	2.67 (1.66-4.28)	<0.001	2.15 (1.17-3.98)	0.014
MIB-1 labeling index	2.56 (0.34-19.5)	0.364			2.85 (0.38-21.7)	0.311		
CDCP1 expression	0.44 (0.26-0.73)	0.002	0.57 (0.33-0.98)	0.040	0.43 (0.26-0.71)	0.001	0.54 (0.32-0.94)	0.028

HR, hazard ratio; CI, confidence interval.

current study on endometrioid adenocarcinoma, the opposite results were obtained; the high level of CDCP1 expression in the tumor cells was a favorable sign for prognosis. This might suggest a different role of CDCP1 molecules for cancers of endometrium from those of other organs, such as lung, breast, and kidney.

In a physiologic or non-neoplastic condition, lung, breast, and kidney tissues hardly express CDCP1. Whereas, CDCP1 was overexpressed in the cancers of lung, breast, and kidney, suggesting that CDCP1 might render the resistance of cancer cells to anoikis as observed in the cell lines from lung adenocarcinoma (7). In the endometrium, CDCP1 expression was detected in the endometrial glands of both proliferative and secretory phases. The present RT-PCR study showed that level of mRNA expression was well-correlated with that of protein expression as revealed by immunohistochemistry in both the normal and diseases endometrial glands, indicating that the immunohistochemistry is a reliable method for detection of CDCP1. Non-cancerous endometrial tissues, such as endometrial polyp, also expressed CDCP1 at a high level. These findings suggest that CDCP1 plays some physiological roles in the endometrial tissues. In endometrioid adenocarcinoma, the CDCP1 expression level was variable; cancer cells in some cases showed comparable level of expression to that in the normal endometrial glands, whereas others showed a lower level. These findings suggested that a disruption of CDCP1 expression might render a malignant potential to endometrioid adenocarcinoma.

Level of CDCP1 expression correlated with proliferative potential of the lung and breast adenocarcinomas (8,10) but not with that in endometrioid ones. In contrast to cancer cells, proliferative potential of normal endometrial glands correlated with CDCP1 expression level. CDCP1 seems to behave as a cancer suppressive factor in the endometrium, while it promotes cancer development in the lung, breast, and kidney. Because CDCP1 expression in various organs of normal condition has not been studied in detail, further studies are necessary to elucidate whether the disruption of CDCP1 expression is related to malignant progression in tissues that physiologically express CDCP1. First of all, more precise information for action mechanism of CDCP1 in cell survival and death and its regulatory mechanism will shed light on this point.

ER and PgR are abundantly expressed in normal endometrium. Expression levels of ER and PgR are reported to be correlated with a favorable prognosis of endometrioid adenocarcinoma (13,17), although the level of CDCP1 expression was not correlated with those of ER and PgR expressions. ER and PgR might not regulate CDCP1 expression. These findings are consistent with our previous report that CDCP1 expression was transcriptionally regulated by Zfp67, a member of zinc-finger proteins, but not by ER and PgR (9).

In conclusion, low CDCP1 expression is a negative prognostic factor in endometrioid adenocarcinoma.

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ORIGINAL ARTICLE

Endometrial carcinoma: better prognosis for asymptomatic recurrences than for symptomatic cases found by routine follow-up

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Abstract
Background The aim of this study was to determine if there is a prognostic value for the presence of symptoms at the time of recurrence detection in surgically resected endometrial carcinoma patients.
Methods During the study period of 2000–2006, complete surgical removal of endometrial carcinoma was achieved in 271 stage I–IV endometrial cancer cases at the Department of Obstetrics and Gynecology of Osaka University Hospital, Osaka, Japan. A subsequent recurrence was detected in 29 (11%) of these cases. Patient characteristics and clinicopathological features were retrospectively reviewed utilizing their clinical records.
Results Among the 29 cases with a recurrence, 13 (45%) had symptoms, whereas in the other 16 cases (55%) the recurrent disease was found only during routine follow-up procedures. Although the time to detection of recurrence was similar for both asymptomatic and symptomatic cases, progression-free survival after detection in the 16 asymptomatic patients was significantly longer than for the 13 symptomatic patients ($P = 0.017$); this was found to be especially true in those who underwent chemotherapy as their adjuvant therapy ($P = 0.023$).
Conclusions A better prognosis after recurrence was demonstrated in cases that were asymptomatic at the time of recurrence detection than in those in which the tumor was symptomatic. This finding implies that, after the initial surgical resection, intensive follow-up intervention looking for asymptomatic recurrences may significantly improve the prognosis of endometrial carcinoma patients. A further in-depth prospective study is required to establish a standard strategy of follow-up care for endometrial cancer patients.

Keywords Endometrial cancer · Recurrence · Symptomatic · Asymptomatic · Progression-free survival

Abbreviations

OS	Overall survival
PFS	Progression-free survival
TEC or TC	Paclitaxel and carboplatin with or without epirubicin
AP	Doxorubicin plus cisplatin
TV-USG	Transvaginal ultrasonography

Introduction

The incidence of uterine endometrial carcinoma, already the most common malignancy of the female pelvis, and the fourth most common cancer of women in the United States, has increased steadily during the past three decades [1]. The treatment for this cancer generally includes a hysterectomy and bilateral salpingo-oophorectomy, with retroperitoneal lymph node dissection. Cytoreductive surgery is performed for extrauterine disease. The prognostic factors for the disease include tumor histological type and

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differentiation, disease stage, peritoneal cytology, myometrial invasion, and extrauterine metastasis [1]. In the past, patients with poor prognostic factors usually underwent postoperative irradiation therapy.

Fung-Kee-Fung et al. [14] systematically reviewed reports from a number of groups [2–13] regarding the benefits of various follow-up strategies for after the primary surgical treatment of endometrial cancer. During their primary treatment, the patients in their reviewed studies generally received a course of radiation therapy following the surgery, although many at low risk did not undergo an adjuvant therapy. They found that the overall risk of a recurrence for all endometrial cancer patients combined was 13%, and for those considered to be at lowest risk, it was only 3% or less. In these reports, approximately 70% of the recurrences were symptomatic before their diagnostic confirmation. Reviewers in the field have suggested that intensive follow-up examinations are of limited benefit for patients matching certain criteria for having a low risk of recurrence, which was presumed because most recurrences tend to occur in known high-risk patients and it was thought that these tumors present with classic symptoms when they do recur. Thus, following the initial treatments of surgery and radiation, the routine use of various intensive follow-up investigations for asymptomatic patients has not been advocated [15].

Recently, a randomized study by the Gynecologic Oncology Group (GOG #122) has found that a combined chemotherapy of doxorubicin and cisplatin (AP) was significantly superior to whole-abdominal irradiation as the adjuvant therapy for endometrial cancer [16]. In our hospital, we offer a modified regimen known as TEC (paclitaxel 150 mg/m^2, epirubicin 50 mg/m^2, and carboplatin AUC 4) as the adjuvant therapy to endometrial carcinoma patients having significant risk factors for a recurrence. We base this treatment regimen on the results of phase I/II prospective studies we have conducted (the results of which will be described elsewhere).

Considering the recent widespread trend of now using chemotherapy as the preferred adjuvant therapy, instead of radiotherapy, for resected endometrial cancer, we thought that a follow-up strategy should now be investigated specifically when adjuvant chemotherapy is given during the primary treatment. In our current study, all our recent postsurgery recurrent cases (with or without adjuvant radiation or chemotherapy) over a given time period were retrospectively investigated. The clinical argument for intensive routine follow-up intervention was evaluated by a comparison between the prognoses of asymptomatic recurrences, found only because of prescheduled follow-up examinations, with the prognosis of cases that were already suggestively symptomatic at the time of confirmatory diagnosis of a recurrence.

Materials and methods

Materials

Patient characteristics

During the study period of 2000–2006 (a 7-year period), 297 endometrial carcinomas were diagnosed in the Department of Obstetrics and Gynecology of the Osaka University Hospital, Osaka, Japan. The patients were all Japanese. The diagnosis was histologically confirmed by pathologists within the Department of Pathology of the Osaka University Hospital. After a written informed consent was obtained, we conducted an exploratory laparotomy for 291 of the 297 patients, with the intent of performing a total abdominal hysterectomy, a bilateral salpingo-oophorectomy, staging, and a maximum cytoreduction, including a retroperitoneal lymph node dissection. Complete surgical removal of the disease was believed to have been achieved in 271 of the 291 laparotomy cases. Following surgery, adjuvant radiotherapy or chemotherapy was indicated in 144 cases we determined to have certain risk factors that included serous and clear cell adenocarcinomas, grade 3 endometrioid carcinoma with myometrial invasion, outer wall myometrial invasion, remarkable lymphovascular involvement, or development of tumors outside the uterus. Twenty-six patients did not agree to receiving adjuvant therapy, 70 patients underwent adjuvant chemotherapy, and 48 patients received adjuvant radiation therapy. Adjuvant therapy was not indicated for the 127 patients with no, or low, risk factors.

After the initial tumor treatment, all patients received intensive follow-up. The number of follow-up visits per year in the first year, the second and third years, the fourth and fifth years, and the sixth year was 12, 4–6, 2, and 1 visit, respectively. Routine physical examinations, including a pelvic-rectal examination, vaginal vault cytology, and transvaginal ultrasonography (TV-USG), were performed at every visit. A computed tomography (CT) scan and chest X-ray was performed semiannually in the first year and annually thereafter. We tested for tumor markers, including CA125, one to four times annually in a subset of the cases. Roughly 90% of the patients in this retrospective study were treated by these follow-up strategies.

Methods

The patients' characteristics and the clinicopathological features of all the recurrent cases of endometrial carcinoma were retrospectively reviewed utilizing their clinical records, including physical examination notes, radiologic reports, operation records, and histology reports. Recurrence of the disease was diagnosed by radiologic or

pathological tests. We measured progression-free survival (PFS) after recurrence from the date of diagnosis of the recurrence to the date of radiologic or pathological relapse of the disease, or, in disease-free patients, to the date of their last follow-up visit. We defined overall survival (OS) after recurrence as the period from the diagnosis of the recurrence to the patient's disease-specific death, or to the date of their last follow-up. We defined a relapse found in either the pelvic cavity or vaginal vault as a local recurrence, and we defined tumors found outside the pelvis as distant recurrences, according to a previous report [14].

Statistical analysis

We conducted a comparison of the clinicopathological characteristics between asymptomatic and symptomatic recurrences by Fisher's exact test and the Mann–Whitney *U* test. Survival curves of the patients of asymptomatic and symptomatic recurrences were constructed using the Kaplan–Meier method, and we evaluated these results for statistical significance by the log-rank test. We considered the results significant when the *P* value was less than 0.05.

Results

Detection of recurrence of endometrial cancer

During the study period, the surgeons achieved complete removal of detectable endometrial carcinoma in 271 of 291 cases. During the median follow-up period of 43 months (range, 2–108 months), a recurrence was detected in 29 (11%) of the 271 resected cases. Twelve cases (41%), 27 cases (93%), and 29 cases (100%) recurred within 1, 2, and 3 years, respectively, of the initial surgery.

A solely local recurrence was found in 6 (23%) of the 26 patients with risk factors who refused adjuvant therapy. In 2 others (8%), both local and distant recurrences were detected. A local recurrence alone was found in 2 (4%) of the 48 patients with risk factors who received adjuvant radiation therapy, and in 6 (13%) of this group of patients, distant recurrences, with or without a local recurrence, were detected. A local recurrence alone was found in 6 (9%) of the 70 patients with risk factors who underwent adjuvant chemotherapy, and in 5 (7%), distant recurrences, with or without local ones, were detected.

Among the total combined cases with a recurrence, 13 of 29 (45%) were accompanied with presenting symptoms. Six patients (46%) visited our outpatient clinic with a complaint of abdominal pain. Intraabdominal disease was subsequently detected in 4 of these cases, and pelvic lymph node involvement was observed in the other 2. Two patients (15%) visited our clinic complaining of lumbar pain, which was found to be caused by metastases to their pelvic and paraaortic lymph nodes. Two other patients suffered from headaches found to be caused by brain metastases. The remaining 3 patients complained, respectively, of leg pain found to be caused by pelvic lymph node involvement, genital bleeding caused by vaginal vault recurrence, and a cough caused by a lung metastasis.

On the other hand, the 16 of 29 (55%) asymptomatic recurrences were found only during our routine intensive follow-up tests (Table 1). In 4 cases (25%), we first detected the recurrence by a routine abdominal CT scan. The initial detection in 2 other cases (13%) was by the combination of a CT scan and tumor marker test performed during the same visit. A physical examination, that included a pelvic-rectal examination, revealed five asymptomatic recurrences (31%). Four recurrences (25%) were detected by cytology from the vaginal vault. None of the asymptomatic recurrences were discovered by chest X-ray.

Clinical characteristics of the recurrent cases

The clinical characteristics of the 29 recurrent cases are described in Table 2. The number of asymptomatic and symptomatic recurrences was 16 and 13, respectively. The distribution of patient age and the time to recurrence following the initial treatment was not significantly different between the asymptomatic and symptomatic groups. The initial tumor stage, adjuvant therapy, and site of recurrence were also not significantly different between groups. The regimen of chemotherapy administered to all 7 asymptomatic patients who received adjuvant chemotherapy, and for 3 of the 4 symptomatic patients who underwent adjuvant chemotherapy, was TEC. The remaining symptomatic

Table 1 Detection of asymptomatic recurrence

Routine follow-up tests	Recurrent cases (%)
Abdominal CT	6 (38)
Physical examination[a]	5 (31)
Vaginal vault cytology	4 (25)
Tumor marker	3 (19)
TV-USG	2 (13)
Chest X-ray	0 (0)

The number of cases in which recurrence was detected by routine intensive follow-up examination is shown. Because in four cases recurrence was detected by two tests conducted during the same routine visit, the sum of the number of the cases in which a recurrence was detected by each routine follow-up examination exceeded the number of the total cases. The most frequent successful examination procedure for detection of an asymptomatic tumor was an abdominal computed tomography (CT) scan

TV-USG transvaginal ultrasonography

[a] The physical examination included a pelvic-rectal examination

Table 2 Characteristics of the asymptomatic and symptomatic cases

Characteristic	Asymptomatic	Symptomatic	*P* value
Number (cases)	16	13	
Median age (year)	60 (53–83)	58 (33–75)	0.50
Histology			
Endometrioid	13	6	0.064
Non-endometrioid	3	7	
Initial stage			
I/II	4	6	0.27
III/IV	12	7	
Adjuvant therapy			
Not performed	4	6	0.27
Performed	12	7	
Median time to recurrence (months)	14 (4–32)	13 (2–30)	0.74
Site of recurrence			
Local alone	10	6	0.47
Distant	6	7	

None of the characteristics exhibited a statistically significant difference between the asymptomatic and symptomatic recurrent cases; however, asymptomatic recurrences tended to be of the endometrioid-type of histology and symptomatic cases tended to be of the non-endometrioid-type

Local, recurrence in the pelvic cavity and vaginal vault; distant, recurrence outside of the pelvis, with or without local recurrence

case received TC therapy. Cases with an endometrioid type of tumor histology tended to relapse as an asymptomatic recurrence; those with a non-endometrioid-type tumor tended to recur with symptoms. However, no statistical significance was detected.

PFS and OS after diagnosis of recurrence

Following diagnosis of recurrence, the differences in prognosis associated with the presence or absence of symptoms was investigated. The median PFS after recurrence of 16 asymptomatic patients was 9 months (0–23 months), which was statistically longer than the 3 months (0–39 months) for the 13 symptomatic patients ($P = 0.017$ by the log-rank test) (Fig. 1). The median OS after recurrence of 16 asymptomatic patients was 37 months (7–51 months), which was longer than the 20 months (2–115 months) of the 13 symptomatic patients; however, again, a statistical significance was not detected ($P = 0.54$ by the log-rank test) (Fig. 2). The median OS from initial treatment of 16 asymptomatic patients tended to be longer than that of the 13 symptomatic patients; however, a statistical significance was not detected ($P = 0.83$ by the log-rank test).

We evaluated the PFS after recurrence for the asymptomatic and symptomatic cases in each group, for those who received adjuvant chemotherapy, those who underwent adjuvant radiation therapy, and those who refused adjuvant therapy. The time to diagnosis of recurrence was not significantly different among these three groups. The median PFS after recurrence for each group is shown in Table 3. For patients for whom adjuvant chemotherapy was performed, the probability of PFS after recurrence of the asymptomatic cases was demonstrated to be significantly higher than that of the symptomatic cases ($P = 0.023$ by the log-rank test). For those who received adjuvant radiation therapy, a similar significant difference in the probability for PFS after recurrence could not be demonstrated ($P = 0.12$ by the log-rank test), nor between

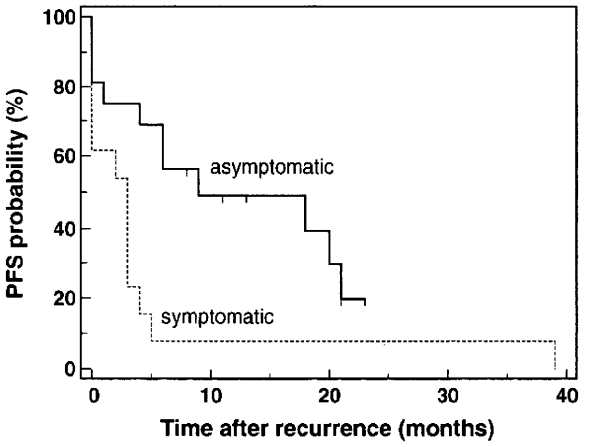

Fig. 1 Progression-free survival (PFS) after recurrence. Probability of PFS after recurrence is shown. Median PFS after recurrence of 16 asymptomatic patients was 9 months (0–23 months), statistically longer than the 3 months (0–39 months) of the 13 symptomatic patients ($P = 0.017$ by the log-rank test). *Solid line* asymptomatic recurrence, *broken line* symptomatic recurrence

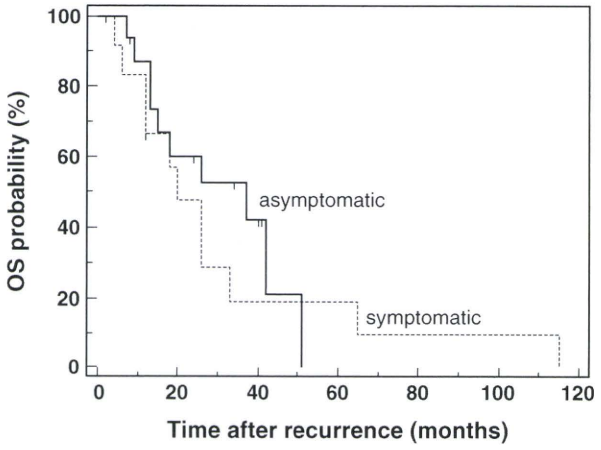

Fig. 2 Overall survival (OS) after recurrence. Probability of OS after recurrence is shown. Median OS after recurrence of the 16 asymptomatic patients was 37 months (7–51 months), longer than the 20 months (2–115 months) of the 13 symptomatic patients; however, a statistical significance was not detected ($P = 0.54$ by the log-rank test). *Solid line* asymptomatic recurrence, *broken line* symptomatic recurrence

Table 3 Progression-free survival (PFS) after recurrence in asymptomatic and symptomatic cases, categorized by adjuvant therapy

	Asymptomatic	Symptomatic	*P* value
Chemotherapy (11 cases)	9 (0–21)	3 (0–5)	0.023
Radiation therapy (8 cases)	6 (0–20)	0 (0–3)	0.12
No adjuvant therapy (10 cases)	9 (0–23)	2 (0–4)	0.28
High risk (8 cases)	9 (0–23)	2 (0–4)	0.28
Low risk (2 cases)	–	21 (3–39)	–

Median PFS (months) after recurrence is shown; in those cases with risk factors for recurrence who received adjuvant chemotherapy, PFS after recurrence was significantly longer in asymptomatic cases than that in symptomatic ones ($P = 0.023$ by the Mann–Whitney U test)

High risk, recurrent cases with risk factors in which no adjuvant therapy was performed; low risk, recurrent cases without risk factors in which no adjuvant therapy was performed; –, PFS and *P* value were not evaluated because there were no low-risk cases that had an asymptomatic recurrence

them and those who received no adjuvant therapy at all ($P = 0.28$ by the log-rank test).

Conclusions

In our study, we detected a recurrence in 29 cases (11%) of 271 patients, and this percentage of recurrence is consistent with a previously reported overall recurrence rate of 13% [14]. Among the 144 high-risk patients, recurrences were detected in 27 (19%) of the patients. Significantly, recurrence was detected in 8 (31%) of 26 patients with risk factors for whom no adjuvant therapy was performed, and 75% of them (6 cases) were local. On the other hand, among the 48 patients with risk factors for whom adjuvant radiation therapy was performed, a recurrence was detected in 8 cases (17%), only 25% (2 cases) of which were at local sites alone; the remaining 75% had distant metastases. These results, although not statistically significant consequent to the small sample size, were compatible with a previous report showing that localized adjuvant radiation was effective in the prevention of local recurrences [14, 17–20]. Among the 70 patients with risk factors for whom adjuvant chemotherapy was performed, a recurrence was detected in 11 cases (16%), a rate comparable to those receiving radiotherapy (17%). Of the recurrences in the chemotherapy cases, 56% (6 cases) were local, compared to the 25% local for those receiving radiotherapy. The 56% to 25% difference in local recurrences implies that the recurrence pattern after adjuvant chemotherapy may depend upon the type of adjuvant therapy received; however, a statistical significance could not be stated because of the small number of cases.

In the current study, recurrence with symptoms was found in 13 (45%) among 29 cases. In previous reports [2–14], asymptomatic disease represented 77% of all the recurrences. The lower overall rate of symptomatic recurrence found in our study, 45% versus 77%, is likely to be in large part the result of our higher rate of early detection of the asymptomatic lesions permitted by our intensive follow-up examinations. The number of routine follow-up visits in our department ranged from 24 to 28 during the 5 years after initial treatment, especially the 20–24 visits in the first to third years when most recurrences occur. In the previous studies, the average was around 9 visits during the 3 years, and 13 visits overall during 5 years of follow-up [14]. Moreover, not only were physical examinations, vaginal vault cytology, and chest X-rays performed, which were also done in the previous studies, but we also conducted additional tests, such as an abdominal CT scan, as part of a routine examination. Our department performs CT scans semiannually in the first year, and annually in the second to fifth years. In addition, we perform tumor marker testing in selected patients once to four times per year. In our retrospective study, we found that our clinicians followed roughly 90% of the patients by these intensive strategies.

In our study, the procedure that most frequently detected asymptomatic recurrences during routine follow-up visits was the CT scan, which found 38% (6/16) of the asymptomatic recurrences. However, we did not evaluate the cost for these examination procedures. Because almost all patients in Japan have some form of private or public health insurance that pays for most of the costs for routine examinations for cancer patients, such intensive

examinations can be performed for all our patients. In other countries, the cost–benefit ratio of the follow-up examination regimen may be quite important. However, in our study, only the medical aspects are addressed.

The question arises as to whether, by our intensive intervention, the early detection of asymptomatic tumor recurrences really improves the prognosis of the patients. There is the probability that with time, even if the patient is not followed up by routine examinations, an asymptomatic recurrence will eventually cause recognizable symptoms. Thus, the symptomatic recurrences reported in our study may be those with a more advanced status, and thus might be expected to have a poorer PFS and OS than the less advanced asymptomatic tumors.

In the current study, to evaluate the beneficial significance of detecting asymptomatic recurrences by intensive routine follow-up procedures, we have analyzed the time to detection of the asymptomatic and symptomatic recurrences from the time of the initial treatment, and we compared the prognosis after detection of those recurrences. The median time to recurrence from the primary therapy of asymptomatic and symptomatic cases was demonstrated to be 14 and 13 months, respectively, exhibiting no significant difference between the two groups ($P = 0.74$ by the Mann–Whitney U test). The median PFS after recurrence of 16 asymptomatic patients was 9 months (0–23 months), statistically longer than the 3 months (0–39 months) for the 13 symptomatic patients ($P = 0.017$ by the log-rank test); however, the treatment strategies for recurrent diseases did not differ in the two groups (data not shown). The median OS after recurrence of asymptomatic patients tended to be longer than that of symptomatic ones; however, a statistical significance was not detected ($P = 0.54$ by the log-rank test). Next, comparison of PFS after recurrence between asymptomatic and asymptomatic patients was conducted in the subgroups of those who underwent adjuvant chemotherapy at the initial treatment, those who received adjuvant radiation, and those who did not undergo any adjuvant therapy. The probability of PFS after recurrence in asymptomatic cases was demonstrated to be significantly higher than that of symptomatic ones in those for whom adjuvant chemotherapy was performed ($P = 0.023$ by the log-rank test). PFS probability after recurrence in asymptomatic cases tended to be significantly higher in those who received adjuvant radiation ($P = 0.12$ by the log-rank test), and in those who did not undergo any adjuvant therapy ($P = 0.28$ by the log-rank test), than that of symptomatic cases; however, a statistical significance was not detected because of the small sample size. Difference in the median OS from initial treatment between asymptomatic and symptomatic patients did not demonstrate a statistical significance ($P = 0.83$ by the log-rank test). Usefulness of routine intensive follow-up examinations was limited in the patients with endometrial carcinomas; however, the results of our current study imply a beneficial significance of intensive examinations to find asymptomatic recurrence, especially for those who underwent adjuvant chemotherapy, as opposed to radiation.

In the present study, we show that PFS after recurrence of those cases with asymptomatic recurrence was significantly longer than that of those with symptomatic recurrence, irrespective of similar duration from initial treatment to recurrence, and we suggest a possible beneficial significance for routine vigorous follow-up examinations to find asymptomatic recurrences. Not only is there a physical benefit achieved by intensive routine follow-up, but the more frequently some patients see their doctors, the more psychological relief they may feel. Further prospective investigations are required to establish a better standard follow-up strategy after initial treatment for endometrial cancer.

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Conflict of interest statement Our Institutional Review Board and Ethics Committee approved this study. There were no known conflicts of interest.

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ORIGINAL ARTICLE

Second-line chemotherapy for advanced or recurrent endometrial carcinoma previously treated with paclitaxel and carboplatin, with or without epirubicin

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Abstract
Purpose A combined chemotherapy of taxane and platinum, with or without anthracycline, has been used as a standard first-line regimen. The purpose of this study was to investigate the effectiveness of second-line chemotherapy for treatment of advanced or recurrent endometrial carcinoma previously treated with a combined chemotherapy of taxane and platinum, with or without anthracycline.
Methods During the 2000–2008 study period, 723 patients were diagnosed with endometrial cancer at the Departments of Obstetrics and Gynecology of the Osaka University and the Osaka Rosai Hospitals, Osaka, Japan. The subset of these cases that eventually required treatment by second-line chemotherapy was retrospectively analyzed.
Results Response rate to second-line chemotherapy was 25%. Treatment-free interval (TFI) of $\geq$ or $<$6 months was demonstrated to be significantly associated with the response to second-line chemotherapy ($P = 0.0026$), progression-free survival ($P = 0.0003$) and overall survival ($P = 0.025$). The second-line chemotherapy similar to the first-line regimen was ineffective in all the 7 cases (100%) whose TFI was shorter than 6 months. Multivariate analysis showed that TFI was the most significantly important factor predicting the effectiveness of second-line chemotherapy (the adjusted hazard ratio of TFI on PFS and OS: 3.482, 95% CI, 1.641–7.388, $P = 0.0012$, and 2.341, 95% CI, 1.034–5.301, $P = 0.042$, respectively).
Conclusions Our present study provides, for the first time, evidence that the majority of refractory or recurrent diseases, if they occur within 6 months of a first-line chemotherapy using taxane and platinum with or without anthracycline, are non-responsive to the current regimens of second-line chemotherapy.

Keywords Endometrial cancer · Second-line chemotherapy · Response · Treatment-free interval · Progression-free survival · Overall survival

Abbreviations

CR	Complete response
HR	Hazard ratio
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
RR	Response rate
SD	Stable disease
TFI	Treatment-free interval
CAP	Cisplatin, Adriamycin and cycloPhosphamide
MPA	MedroxyProgesterone Acetate

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TAP or AP	Anthracycline (doxorubicin) plus Platinum (cisplatin) (with, or without, the Taxane, paclitaxel)
TEC or TC	Taxane paclitaxel and Carboplatin (with, or without, the anthracycline, Epirubicin)
TEP	Taxane (paclitaxel), Epirubicin and the Platinum, cisplatin
TCw, TCm, TECm	TC or TEC administered on a weekly or monthly regimen

Introduction

The incidence of endometrial carcinoma, already the most common malignancy of the female pelvis and the fourth most common cancer among women in the United States, has been increasing steadily during the last three decades [1]. In approximately 75% of patients, the tumor is still confined to the uterus at diagnosis (FIGO stage I), and thus, there is a good prognosis [2]. However, the prognosis for advanced endometrial carcinomas, with extra-pelvic metastatic dissemination, is extremely poor, and the 5-year survival rate is a mere 5–32% [1]. Irradiation was usually performed as post-operative adjuvant therapy for early cases considered being at risk of recurrence and for most advanced cases [3]. Recently, systemic adjuvant chemotherapy, compared to adjuvant irradiation, was reported to significantly improve the prognosis of these cases [4, 5].

About one-fourth of those patients, treated for what was thought to be an early-stage endometrial cancer, go onto develop a recurrence. Unfortunately, 3–19 years after treatment for the recurrence, only 7.7% of the patients were alive and without evidence of disease [3]. Recurrences, restricted to the vaginal vault, are relatively better treated with radiotherapy. However, in most cases of relapse, the disease had spread to other sites, including pelvic and para-aortic lymph nodes, the peritoneum of the pelvis and abdominal cavity, and the lungs. For these cases, systemic chemotherapy is usually required.

Cisplatin and doxorubicin were shown to be the most effective drugs for both advanced and recurrent endometrial carcinomas; paclitaxel was also reported to be useful [6, 7]. A Gynecologic Oncology Group's (GOG) study showed that a tripartite regimen of the Taxane paclitaxel, plus the Anthracycline doxorubicin and the Platinum drug cisplatin (TAP), provided a better response, as measured by progression-free and overall survival rates, than without the taxane (AP). However, there were significant adverse side effects in the TAP group [8]. A modified TAP regimen, with the Taxane paclitaxel and Epirubicin (a semi-synthetic stereoisomer of doxorubicin) plus the Platinum drug cisplatin (TEP), was a more effective combination chemotherapy, with a 73% response rate for advanced endometrial carcinomas, suggesting a possible future role of TEP therapy as the first-line treatment for advanced endometrial carcinomas [9]. A combination chemotherapy of the Taxane paclitaxel and Carboplatin (TC), which is a standard regimen for ovarian carcinoma, was also shown to be a well-tolerated, active adjuvant therapy regimen for advanced endometrial carcinomas [10]. More recently, a combination chemotherapy of the Taxane paclitaxel, Epirubicin and Carboplatin (TEC) was demonstrated to be active and tolerable in patients with advanced metastatic and recurrent endometrial carcinomas [11].

Based on these findings, a combined chemotherapy of taxane and platinum, with or without anthracycline, has been used as a standard first-line regimen for both unresected and recurrent endometrial carcinomas and also as an adjuvant therapy for resected cases with a high risk of recurrence. However, a second-line regimen for the treatment of advanced or recurrent endometrial carcinoma previously treated with a combined chemotherapy of taxane and platinum, with or without anthracycline, has yet to be established. We hoped our findings would change that. In our current study, the effectiveness of second-line chemotherapy and the various clinical factors that associate with the prognosis of advanced or recurrent endometrial carcinoma previously treated with a combination chemotherapy of taxane and platinum, with or without anthracycline, were retrospectively investigated.

Materials and methods

Materials

Patients' characteristics

During the 9-year study period of 2000–2008, we diagnosed 723 endometrial carcinomas in Japanese women at the Departments of Obstetrics and Gynecology of the Osaka University and the Osaka Rosai Hospitals, Osaka, Japan. Traditionally, our department has used either a monthly delivered combination of Taxane (paclitaxel) and Carboplatin (with, or without, the anthracycline Epirubicin, TEC or TC, respectively) or a weekly administered TC regimen as the first-line adjuvant therapy for resected endometrial carcinomas and also as the first-line salvage therapy for unresected and recurrent diseases. Patients were enrolled in the present study, after obtaining their written informed consent, if they were treated by a second-line chemotherapy for their recurrent disease, after first having an initial first-line adjuvant or salvage TEC or TC therapy.

In the initial first-line monthly TEC (TECm) treatment, paclitaxel (150 mg/m^2), carboplatin (AUC = 4) and epirubicin (50 mg/m^2) were administered intravenously every 3–4 weeks. The dose of chemotherapy drugs appropriate for our Japanese population was determined in phase I/II studies we had previously conducted (to be described in detail elsewhere). In the monthly TC (TCm) therapy, paclitaxel (175 mg/m^2) and carboplatin (AUC = 5) were also administered intravenously every 3–4 weeks, based on previous reports [12, 13]. In the weekly TC regimen (TCw), paclitaxel (80 mg/m^2) and carboplatin (AUC = 2) were administered intravenously on days 1, 8 and 15 on a 4-week cycle [14, 15].

The clinicopathological features of these cases, including the age of the patient, the histology and initial stage of the disease and the regimen of the first and second-line chemotherapies, were retrospectively reviewed utilizing their clinical records, including physical examination notes, radiological reports, operative records and histopathology reports. The histological diagnoses were made by authorized pathologists from the Department of Pathology of the Osaka University and the Osaka Rosai Hospitals.

Methods

In order to evaluate the therapeutic effect of second-line chemotherapy, previously described standard criteria from the World Health Organization [16] and others (Pectasides et al. [17–19] were used. The tumors were assessed with a CT scan and/or MRI at baseline and every three treatment courses thereafter. A complete response (CR) was defined as the disappearance of all known disease, determined by two observations not less than 4 weeks apart. Partial response (PR) was defined as a 50% or more reduction in the summed products of the two largest perpendicular dimensions of bi-dimensionally measurable lesions, for at least 4 weeks. Stable disease (SD) was defined as a less than 50% decrease, or a less than 25% increase, of tumor size, with no new detectable lesions. Progressive disease (PD) was defined as a greater than 25% increase in tumor size or as the appearance of new lesions.

Progression-free survival (PFS) was measured from the date of the last administration of chemotherapy to the date of the radiological or pathological relapse or to the date of the last follow-up. Overall survival (OS) was defined as the period from the start of chemotherapy to the patient's disease-specific death or to the date of the last follow-up, as previously described [10]. Treatment-free interval (TFI) was defined as the period between the last administration of first-line chemotherapy and the initiation of the second-line chemotherapy, as previously described [20].

Second-line chemotherapy

Some patients received TECm or TCw (as described above) as second-line chemotherapy. Others were given docetaxel (30 mg/m^2) and CPT-11 (60 mg/m^2) (docetaxel + CPT) on days 1 and 8, on a 3–4-week cycles, or daily oral medroxyprogesterone acetate (MPA) (400–600 mg/day). The dose and schedule of administration of docetaxel + CPT appropriate for our Japanese population was also determined in our previous phase I/II study (to be described in detail elsewhere).

Statistical analysis of effect of second-line chemotherapy

The association between sensitivity to second-line chemotherapy and sensitivity to a TFI was analyzed by Fisher's exact test. PFS curves determined by a TFI were constructed using the Kaplan–Meier method and were evaluated for statistical significance by the log-rank test. The multivariate Cox proportional hazards model was used to calculate the significant factors contributing to PFS after second-line chemotherapy. Results were considered to be significant when the P value was <0.05.

Results

Clinical characteristics of the study cases

During the 9-year study period, 40 patients required a second-line chemotherapy treatment against a recalcitrant or recurrent disease, after having first received an adjuvant or salvage first-line chemotherapy using TECm, TCm or TCw. The clinicopathological characteristics of these 40 patients are shown in Table 1. Eighty percent (32 out of 40 cases) of the patients received first-line TECm therapy; the other eight cases underwent either monthly or weekly TC (TCm or TCw) therapy, prior to the second-line chemotherapy. No cases were canceled due to second-line chemotherapy toxicity.

Outcome of the patients after second-line chemotherapy

Among the 40 patients, 24 patients received second-line TECm, TCm or TCw, 3 received docetaxel + CPT, 7 received oral MPA therapy, and 6 received oral Etoposide therapy (Table 2). The overall response rate for second-line chemotherapy was 25% (0–38%). The PFS was 3.5 months (0–20 months), and the OS was 10 months (2–44 months). The response rate, PFS, and OS were not significantly different among the TECm/TCm/TCw, docetaxel + CPT, MPA and Etoposide groups.

Table 1 Clinical characteristics of the study cases

Characteristics	Patients ($n = 40$)	
	Number	%
Age (years)		
<60	18	45
≥60	22	55
Histology		
Endometrioid	32	80
Serous	4	10
Clear cell	1	3
Others	3	8
Initial stage		
I	10	25
II	6	15
III	19	48
IV	5	13
First-line chemotherapy		
TEC	32	80
TC	7	18
Weekly TC	1	3

All patients received first-line chemotherapy using taxane and carboplatin (with or without epirubicin, TC/TEC)

TECm monthly administration of taxane (paclitaxel), epirubicin and carboplatin, *TCm* monthly administration of paclitaxel and carboplatin, *TCw* weekly administration of paclitaxel and carboplatin

Table 2 Outcome of the patients after second-line chemotherapy

	Cases	Response rate (%)	PFS (months)	OS (months)
TEC/weekly TC	24	38	5.5 (2–20)	13 (3–44)
Docetaxel + CPT-11	3	33	4 (0–5)	6 (4–10)
MPA (oral)	7	0	1 (0–3)	5 (2–22)
Etoposide (oral)	6	0	2 (1–8)	9 (4–11)
Total	40	25	3.5 (0–15)	10 (2–44)

No significant difference was demonstrated among the four groups (TECm/TCm/TCw, docetaxel + CPT, MPA and Etoposide)

TECm monthly administration of paclitaxel, epirubicin and carboplatin, *TCw* weekly administration of paclitaxel and carboplatin, *TCm* monthly administration of paclitaxel and carboplatin, *MPA* oral daily medroxyprogesterone acetate, *Etoposide* oral daily Etoposide, *PFS* progression-free survival, *OS* overall survival

Association between TFI and sensitivity to second-line chemotherapy

The effect of a TFI, after first-line chemotherapy using TC/TEC, on the tumor's sensitivity to second-line chemotherapy was evaluated. Among the 24 patients whose TFI was equal to or longer than 6 months, CR or PR was achieved in 10 patients (42%). However, all 16 cases (100%) whose TFI was shorter than 6 months exhibited either SD or PD against the second-line chemotherapy. This association between TFI and sensitivity to a second-line chemotherapy was statistically significant ($P = 0.0026$ by Fisher's exact test). These results are tabulated in Table 3.

Association between TFI and sensitivity to second-line chemotherapy using taxane and platinum, with or without anthracycline, was, next, analyzed. Among the 17 patients whose TFI was equal to or longer than 6 months, CR or PR was achieved in 9 patients (53%). However, all 7 cases (100%) whose TFI was shorter than 6 months exhibited either SD or PD against the second-line chemotherapy. This association between TFI and sensitivity to a second-line chemotherapy was statistically significant ($P = 0.015$ by Fisher's exact test). These results are tabulated in Table 4.

PFS and OS after second-line chemotherapy by TFI

The differences by TFI in the effectiveness of second-line chemotherapy regimens were investigated. The median PFS was 7 months (1–20 months) for the 26 patients whose TFI was equal to or longer than 6 months, which

Table 3 Association between TFI and effectiveness of second-line chemotherapy

	CR/PR	SD/PD
TFI ≥ 6 months	10 (42%)[a]	14 (58%)
TFI < 6 months	0 (0%)	16 (100%)[a]

Forty-two percent (10 out of 24 cases) of the patients whose TFI was equal to or longer than 6 months exhibited sensitivity to second-line chemotherapy; however, all cases whose TFI was shorter than 6 months were resistant to second-line chemotherapy. This association was statistically significant ($P = 0.0026$ by Fisher's exact test)

TFI treatment-free interval, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a] $P = 0.0026$

Table 4 Association between TFI and sensitivity to second-line chemotherapy using taxane and platinum, with or without anthracycline

	CR/PR	SD/PD
TFI ≥ 6 months	9 (53%)[a]	8 (47%)
TFI < 6 months	0 (0%)	7 (100%)[a]

Fifty-three percent (9 out of 17 cases) of the patients whose TFI was equal to or longer than 6 months exhibited sensitivity to second-line chemotherapy; however, all cases whose TFI was shorter than 6 months were resistant to second-line chemotherapy. This association was statistically significant ($P = 0.015$ by Fisher's exact test)

TFI treatment-free interval, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a] $P = 0.015$

was significantly longer than the 2 months (0–9 months) for the 14 whose TFI was shorter than 6 months ($P = 0.0003$ by log-rank test) (Fig. 1). The median OS was 13 months (3–22 months) for the 26 patients whose TFI was equal to or longer than 6 months, which was significantly longer than the 5.5 months (2–44 months) for the 14 whose TFI was shorter than 6 months ($P = 0.025$ by log-rank test) (Fig. 1).

Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on PFS and OS

In order to further support our finding that a TFI was significantly associated with the effectiveness of second-line chemotherapy, the multivariate Cox proportional hazards model was utilized. The results are listed in Table 4. The adjusted hazard ratio (HR) of TFI (≥6 months versus <6 months) on PFS was 3.482 (95% CI, 1.641–7.388) and that of TFI on OS was 2.341 (95% CI, 1.034–5.301). TFI was demonstrated to be a significant factor in predicting for PFS and OS ($P = 0.0012$ and $P = 0.042$, respectively, based on the multivariate Cox proportional hazards model) (Tables 5 and 6).

Table 5 Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on PFS

Variable	Adjusted HR	95% CI	*P* value
Age (years)			0.14
<60	1		
≥60	0.573	0.274–1.197	
Histology			0.53
Endometrioid	1		
Non-endometrioid	1.331	0.546–3.247	
Initial stage			0.82
I/II	1		
III/IV	1.092	0.517–2.308	
TFI			0.0012
≥6 months	1		
<6 months	3.482	1.641–7.388	

The adjusted HR of TFI < 6 months was 3.482 (95% CI, 1.641–7.388), compared to TFI ≥ 6 months, showing statistical significance ($P = 0.0012$)

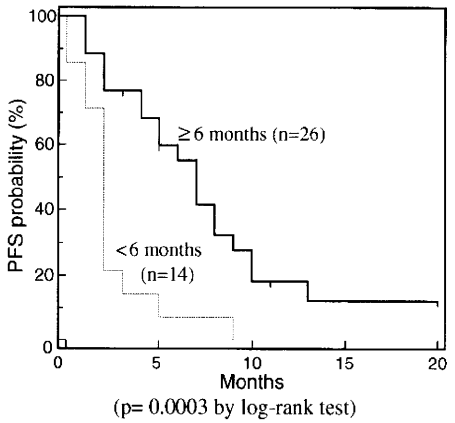
HR hazard ratio, *TFI* treatment-free interval

Conclusions

Endometrial adenocarcinoma is increasingly the most common malignancy of the female pelvis in the United States [1]. Although early-stage endometrial carcinomas have a good prognosis [2], the prognoses for advanced or recurrent cases are extremely poor (except for recurrences limited to the vaginal vault, which can generally be treated successfully with surgery and radiation therapy) [3]. Although a truly successful treatment for advanced or recurrent endometrial carcinomas has yet to be established, taxane and platinum (with or without anthracycline, TC/TEC) have demonstrated at least a partial efficacy [6–11].

Recently, based on these findings, the combined chemotherapy of TC/TEC has been used as a standard first-line regimen for unresected and recurrent endometrial carcinomas as well as an adjuvant therapy for resected cases with a high risk of recurrence. However, an effective second-line regimen for advanced or recurrent endometrial carcinomas previously treated with TC/TEC has not yet been established.

In our present study, we analyzed the outcomes of patients who, after having received a first-line TC or TEC



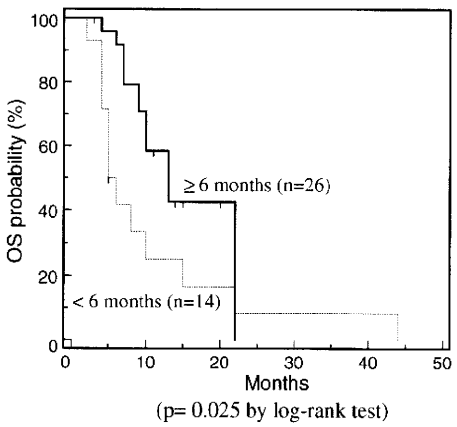


Fig. 1 PFS and OS after second-line chemotherapy by TFI. Progression-free probability and overall probability after second-line chemotherapy of the patients whose TFI was equal to or longer than 6 months were significantly longer than those whose TFI was shorter than 6 months ($P = 0.0003$ and $P = 0.025$, respectively, by the log-rank test)

Table 6 Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on OS

Variable	Adjusted HR	95% CI	*P* value
Age (years)			0.081
<60	1		
≥60	0.497	0.228–1.085	
Histology			0.40
Endometrioid	1		
Non-endometrioid	1.497	0.590–3.800	
Initial stage			0.30
I/II	1		
III/IV	1.613	0.658–3.953	
TFI			0.042
≥6 months	1		
<6 months	2.341	1.034–5.301	

The adjusted HR of TFI < 6 months was 2.341 (95% CI, 1.034–5.301), compared to TFI ≥ 6 months, showing statistical significance ($P = 0.042$)

HR hazard ratio, *TFI* treatment-free interval

chemotherapy, received a variety of second-line chemotherapy against relapsed or recurrent tumors. The overall response rate to second-line chemotherapy was 25% (0–38%). The PFS was 3.5 months (0–20 months), and the OS was 10 months (2–44 months). The response rates, PFS and OS did not differ significantly by the regimen of second-line chemotherapy. Therefore, other factors associated with the response to second-line chemotherapy were investigated.

In the related field of ovarian carcinomas, a TFI has consistently been shown to be the most important factor in the prediction of the response to the second-line chemotherapy [21–24]. Patients with ovarian tumor who relapse within 6 months have a disease that is significantly more likely to be both resistant to the original drugs and to have a lower response rate to new chemotherapy; however, those who relapse after 6 months from a first-line platinum-based chemotherapy have a higher chance of responding well, either to a re-challenge with a platinum-based treatment or to other agents [21].

However, comparable predictive factors for the response to second-line chemotherapy have not been established for endometrial carcinomas. It was yet to be determined whether TFI predicts a response to second-line chemotherapy, and if so, whether 6 months was a reasonable critical threshold that would suggest the clinical outcome after second-line chemotherapy. It was also unclear which current regimen is most effective as a second-line chemotherapy for endometrial carcinoma.

In our study, a TFI greater or less than 6 months was demonstrated to be significantly associated with the tumor's likely responsiveness to a second-line chemotherapy ($P = 0.0026$ by Fisher's exact test). The current study also showed that the association between TFI and sensitivity to a second-line TECm, TCm or TCw was statistically significant ($P = 0.015$ by Fisher's exact test), indicating that second-line chemotherapy using taxane and platinum, with or without anthracycline, was not effective for those whose TFI was shorter than 6 months after initial treatment using taxane and platinum, with or without anthracycline.

PFS and OS were also shown to relate to a TFI ≥ or <6 months ($P = 0.0003$ and $P = 0.025$, respectively, by the log-rank test). As in ovarian tumors, multivariate analysis showed that the TFI for endometrial tumors was also the most significant factor for predicting the effectiveness of a second-line chemotherapy (the adjusted HR of TFI (≥6 months versus <6 months) on PFS: 3.482 (95% CI, 1.641–7.388), and that on OS: 2.341 (95% CI, 1.034–5.301)). TFI was demonstrated to be a significant factor in predicting for PFS and OS ($P = 0.0012$ and $P = 0.042$, respectively, based on the multivariate Cox proportional hazards model).

These results imply, for the first time, that the standard strategy for ovarian carcinoma second-line chemotherapy can now be applied to endometrial carcinoma as well. Endometrial carcinomas that relapse at least 6 months after first-line chemotherapy using TC/TEC have a significantly higher chance of responding to second-line chemotherapy. However, the tumors that relapse before 6 months are likely to be resistant. In fact, none of the currently popular regimens of chemotherapy (including the same as, or other than, the first-line regimen) were effective, and the PFS and OS were only 2 months (0–9 months) and 5.5 months (2–44 months), respectively (data not shown). Palliative care in these cases, rather than second-line chemotherapy, may for now be more appropriate.

Our present study provides, for the first time, good evidence that relapsed or recurrent diseases found within 6 months from a first-line chemotherapy regimen using taxane and platinum (with or without anthracycline) fail to respond to second-line chemotherapy. Further investigation is still required to establish an efficacious strategy for second-line chemotherapy for advanced or recurrent endometrial cancer.

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ORIGINAL PAPER

CRABP1-reduced expression is associated with poorer prognosis in serous and clear cell ovarian adenocarcinoma

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Abstract
Purpose CRABP1 is a modulator of retinoic acid function. The aim of the present study was to investigate CRABP1 expression and its clinical significance in ovarian carcinoma.
Methods Expression of CRABP1 protein was investigated by immunohistochemical analysis in 100 ovarian carcinomas of various histological sub-types, including serous and clear cell adenocarcinomas. Relationship of CRABP1 expression to clinical features, including prognosis, was analyzed.
Results Reduced expression of CRABP1 protein was detected especially frequently in the serous and clear cell adenocarcinomas sub-types, 50% (20 of 40) and 38% (10 of 26) of cases, respectively. We found that in both serous and clear cell adenocarcinomas overall survival was significantly poorer in the cases with reduced CRABP1 expression compared to similar cases where expression was maintained, irrespective of the disease stage ($P = 0.0073$ and 0.049, respectively). Disease-free survival of the serous and clear cell adenocarcinoma cases with reduced CRABP1 expression was significantly poorer, compared to the cases whose CRABP1 expression was maintained ($P = 0.024$). Multivariate analysis showed that reduced expression of CRABP1 was a significantly important prognostic factor (adjusted hazard ratio: 8.189 (95% CI, 2.186–30.672, $P = 0.0019$)).
Conclusions The present study is the first to demonstrate that the reduced expression of CRABP1 has a potential as a prognostic marker for serous adenocarcinoma which is the most frequent histological ovarian tumor type and also for clear cell carcinoma that often exhibits chemo-resistance. Further study is necessary to clarify how CRABP1 protein expression was altered and how CRABP1 affects ovarian carcinoma cells.

Keywords CRABP1 · Ovarian carcinoma · Serous · Clear cell · Prognosis

Abbreviations

CRABP1	Cellular retinoic acid binding protein 1
RA	Retinoic acid

Introduction

Ovarian cancer is the ninth most common cancer in US women (not counting skin cancers, the fifth leading cause of cancer deaths after lung and bronchus, breast, colorectal, and pancreatic cancers) and causes more deaths than any other cancer of the female reproductive system. In 2005, the last year in which accurate statistics were compiled in

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the US, 19,842 women were diagnosed with ovarian cancer and 14,787 died from the disease.

The prognosis of early stage ovarian carcinoma, compared to later stages, is relatively good, and it can be cured by appropriate therapy. The preferred primary management of early ovarian carcinoma is surgical debulking followed by multi-agent adjuvant chemotherapy (DiSaia and Creasman 2002). Unfortunately, in many cases of ovarian carcinoma the tumor has only subtle symptoms or is asymptomatic, and a useful screening test has yet to be established. Thus, three-fourths of all ovarian carcinomas are thus diagnosed at an advanced stage, and the prognosis for these women is generally very poor, with a 5-year survival rate of 23–41% for stage III and only 11% for stage IV (DiSaia and Creasman 2002).

The major histological sub-types of ovarian carcinoma are serous, endometrioid, mucinous, and clear cell adenocarcinomas. In the US, serous adenocarcinomas represent 40–75% of all the ovarian epithelial carcinomas and clear cell adenocarcinomas 5–10% (DiSaia and Creasman 2002; Kurman 1994; Berek 2002). However, we have recently demonstrated that in Japan, clear cell adenocarcinoma accounted for a larger proportion of ovarian carcinoma cases (23% vs 5–10% in US) (our unpublished data). This is an important difference, since clear cell adenocarcinoma has been shown to exhibit a higher resistance to platinum-based chemotherapy, leading to a poor prognosis (Sugiyama et al. 2000). On the other hand, we previously showed that serous adenocarcinoma responded to combined chemotherapy of paclitaxel and carboplatin significantly better than the other tumor sub-types (Ueno et al. 2006).

In 80–85% of serous adenocarcinoma cases, the tumor cells are already disseminated to other pelvic tissues and the peritoneum, or metastasized to regional lymph nodes, at the time of the initial diagnosis; however, up to 60% of clear cell adenocarcinomas are in stage I at diagnosis (Kurman 1994). Thus, it would contribute immensely to the care of patients with serous ovarian adenocarcinoma (which is the most frequent histological type, most wide spread at diagnosis, and yet most responsive to combined chemotherapy of paclitaxel and carboplatin) and those with clear cell adenocarcinoma (which demonstrate the poorest prognosis) to clarify additional diagnostic and prognostic factors for these diseases.

It was recently shown that metabolism of vitamin A, and its active cellular catabolite retinoic acid (RA), was impaired in human ovarian cancer (Williams et al. 2009). RA has the potential to alter the growth and differentiation of a wide range of cell types and was shown to induce the differentiation of many murine teratocarcinoma cell lines (Means et al. 2000). Aldehyde dehydrogenase 1 (ALDH1) that participates in retinoic acid metabolism was shown to be related to prognosis of ovarian carcinoma cases (Chang et al. 2009).

Cellular retinoic acid-binding protein 1 (CRABP1) is a small, well-conserved member of a family of cytosolic lipid-binding proteins; it has a high affinity for RA and is an important modulator of RA signaling (Poulain et al. 2009). Homozygous deletion of the *crabp1* gene was demonstrated to result in decreased intracellular RA concentrations (Boylan and Gudas 1992; Liu et al. 2005). Silencing of *crabp1* by methylation of its promoter CpG island has long been associated with colorectal tumors, and it is one of a small battery of genes often screened in colorectal tumors for indications of the 'CpG island methylator phenotype' (CIMP). *Crabp1* hypermethylation is associated with poor patient survival in thyroid and hepatocellular tumors (Huang et al. 2003; Lee et al. 2009).

A recent study showed that promoter hypermethylation of the *crabp1* gene was detected in 2 of 3 ovarian clear cell adenocarcinomas, but none of 19 serous, 4 mucinous and 16 endometrioid adenocarcinomas (Wu et al. 2007), suggesting that *crabp1* hypermethylation might be an additional potential marker for ovarian clear cell adenocarcinomas. Whether the promoter region hypermethylation was reflected in loss of CRABP1 protein expression, and how this might be linked to patient outcome has yet to be established. Toward that end, in our current study expression of *crabp1* was analyzed in 80 clinical samples of ovarian carcinoma to investigate, first, whether altered expression of *crabp1* was specific to clear cell adenocarcinoma, as suggested, and second, to determine the relationship of loss of *crabp1* expression to clinical features of ovarian carcinomas, including prognosis, which has not yet been analyzed.

Materials and methods

Materials

One hundred cases of ovarian carcinoma were randomly picked from cases diagnosed during 1997 to 2008 at the Department of Obstetrics and Gynecology of the Osaka University Hospital in Osaka, Japan. The 120 cases included 50 serous, 29 clear cell, 26 endometrioid, 12 mucinous, and 3 undifferentiated cases. After asking for informed consent, the tissues from only 100 of these 120 patients were available for our study. They included 40 cases of serous adenocarcinoma, 26 cases of clear cell adenocarcinoma, 24 cases of endometrioid adenocarcinoma, and 10 cases of mucinous adenocarcinoma. The patient age at surgery ranged from 25 to 90 years (median: 54 years). The tumor stages diagnosed following surgery were stage I

in 41 cases, stage II in 19 cases, stage III in 38 cases, and stage IV in 2 cases.

In our institution, for primary ovarian carcinomas, we typically perform a surgical removal of the ovaries, fallopian tubes, uterus, omentum, and the retroperitoneal lymph nodes, followed by giving combination chemotherapy using taxane and platinum. These cases were carefully followed post-operatively with regular exams that included pelvic examinations and tumor marker and radiological tests. The median follow-up period was 42 months (range 1–133 months). Salvage chemotherapy, with or without surgical removal, was performed for recurrent diseases.

Immunohistochemical staining

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue blocks from a total of 100 cases of ovarian cancer, using a LSAB+/HRP kit (Dako, Cambridge, UK) following the manufacturer's instructions. Briefly, after removing the paraffin, the antigens were retrieved by microwave pretreatment in target retrieval solution at 95°C for 5 min. After blocking in peroxidase reagent, the tissues were incubated with an anti-human-CRABP1 primary antibody (Sigma–Aldrich, Saint Louis, MO) at room temperature for 1 h. After washing, the tissues were incubated with secondary antibody, followed by incubation with peroxidase. Visualization was performed with diaminobenzidine with Mayer's hematoxylin. Squamous epithelium of normal uterine cervix was used as a positive control, and tissue sections incubated with only antigen-dilution-reagent were used as negative controls. These controls were used for each staining.

Evaluation of immunohistochemical staining

The slides were observed by light microscopy, with review of the entire histological section from each case, to evaluate for possible tumor microheterogeneity in antigen distribution. Immunohistochemical staining was scored on a 3-tiered scale for both intensity of cytoplasmic staining (grade 1: absent/weak, grade 2: moderate, and grade 3: strong) and extent (grade 1: percentage of positive cells is <10%, grade 2: 10–50%, and grade 3: >50%). The intensity and the extent were then multiplied to give a composite score of 1–9 for each tumor, as described in a previous study (Greenspan et al. 1997). The composite scores of 1–4 were defined as a reduction of CRABP1 protein expression, compared to those of 6–9 (composite scores resulting in the prime numbers 5 and 7 can't mathematically occur). The evaluation of immunohistochemical staining was carried out by two independent pathologists who were unaware of the patient outcomes.

Analysis of patient prognosis

Patient clinical records were reviewed, including histology and surgical records. Overall survival was defined as the time from initial surgery until death or, if still alive, to the date of the last follow-up. Disease-free survival was defined as the time from complete remission of the disease by surgery with/without chemotherapy until documentation of recurrence or, if still free of recurrence, to the date of the last follow-up.

Statistical analysis

The χ^2 test was used for comparison of the distribution of stages between the cases in which CRABP1 expression was reduced and those in which it was maintained. Overall and disease-free survivals were calculated using the Kaplan–Meier method. Univariate and multivariate Cox proportional hazards models (step-wise method) for the factors including age, histology, initial stage, and CRABP1 expression were calculated to evaluate whether reduced expression of CRABP1 was a significantly important factor on OS. A P value <0.05 was considered to be statistically significant.

Approval of the study

This study was approved by our Institutional Review Board and Ethics Committee.

Results

Reduced expression of CRABP1 in various types of ovarian cancer

Staining specific for CRABP1 protein was found in the cytoplasm of all the histological sub-types of ovarian tumor cells. Examples of the immunohistochemical study of CRABP1 expression are shown in Fig. 1. Reduced expression of CRABP1 was observed in 33 (33%) of 100 ovarian cancer cases, and especially frequently in serous and clear cell adenocarcinomas, 20 (50%) of 40 and 10 (38%) of 26 cases, respectively; however, in endometrioid and mucinous adenocarcinomas, only 2 (8%) of 24 and 1 (10%) of 10 cases showed reduced expression. In serous adenocarcinomas, CRABP1 expression was reduced in 4 (44%) of 9 stage I cases, in 4 (57%) of 7 stage II cases, in 11 (48%) of 23 stage III cases, and 1 (100%) of 1 stage IV case. In clear cell adenocarcinomas, CRABP1 expression was reduced in 7 (41%) of 17 stage I cases, in 1 (25%) of 4 stage II case, 1 (25%) of 4 stage III case, and 1 (100%) of 1 stage IV case. The distribution of the stages between the cases in which

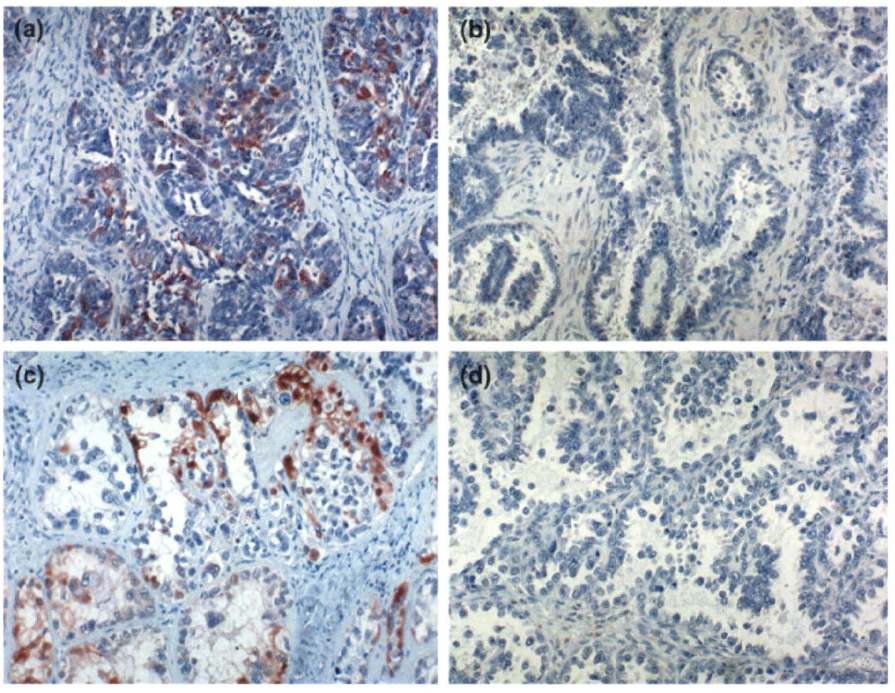

Fig. 1 Examples of immunohistochemical staining of CRABP1 (×400). **a** Maintained CRABP1 expression in a case of serous adenocarcinoma: score 6 (extent: grade 2, intensity: 3). **b** Reduced CRABP1 expression in a case of serous adenocarcinoma: score 1 (extent: grade 1, intensity: 1). **c** Maintained CRABP1 expression in a case of clear cell adenocarcinoma: score 6 (extent: grade 2, intensity: 3). **d** Reduced CRABP1 expression in a case of clear cell adenocarcinoma: score 1 (extent: grade 1, intensity: 1)

CRABP1 expression was reduced and those in which CRABP1 expression was maintained did not exhibit statistically significant differences in either the serous or clear cell adenocarcinoma cases ($P = 0.73$ and $P = 0.52$, respectively, by the χ^2 test). High grade tumors (grade 3) mostly exhibited reduced CRABP1 expression, and CRABP1 expression was frequently maintained in low grade tumors (grade 1 and grade 2) ($P = 0.047$ by Fisher's exact test). Lymph-node metastasis was not associated with CRABP1 expression.

Association of the reduction of CRABP1 expression and overall survival in serous and clear cell adenocarcinoma patients

Overall survival was analyzed in all 40 serous and 26 clear cell carcinoma cases, in which 20 (50%) and 10 (38%) cases, respectively, demonstrated reduction of CRABP1 expression in immunohistochemical analysis. During the median follow-up period of 45.5 months (range 1–133 months), 20 serous adenocarcinoma cases whose CRABP1 expression was reduced exhibited a statistically significant worse prognosis, compared to the other 20 cases whose CRABP1 expression was maintained ($P = 0.0073$ by the Kaplan–Meier method) (Fig. 2). Disease-specific death was documented in only one case (5%) among the 20 cases, which maintained CRABP1 expression. However, disease-linked death occurred in 9 cases (45%) among 20 cases with reduced CRABP1 expression.

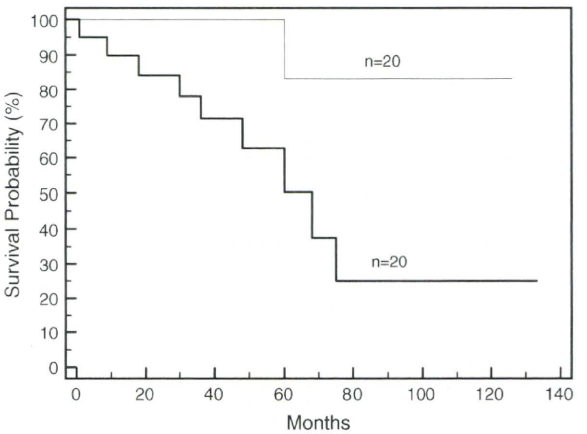

Fig. 2 Overall survival of serous adenocarcinoma cases. The median follow-up period was 45.5 months (range 1–133 months). Overall survival of 20 serous adenocarcinoma cases with reduced CRABP1 expression was significantly worse than that of 20 cases with maintained CRABP1 expression ($P = 0.0073$ by the Kaplan–Meier method). *Broken line* survival probability of maintained-CRABP1-expression cases. *Solid line* survival probability of reduced-CRABP1-expression cases

Similarly, during the median follow-up period of 43 months (3–133 months), 10 clear cell adenocarcinoma cases with reduced expression of CRABP1 exhibited worse prognoses, compared to the other 16 cases whose CRABP1 expression was maintained, with statistical significance ($P = 0.049$ by the Kaplan–Meier method) (Fig. 3). Disease-specific death was documented in only two cases (13%) among the 16 cases with maintained CRABP1 expression.