patients (24.0%) were above 60 years of age (Table 1). The follow-up period varied from 1 to 126 months, with a median of 57 months. One hundred and nine patients (90.6%) had stage I disease, two had stage II disease, and nine had stage III and IV disease (Table 1). The dominant histopathological subtypes were mucinous (91 cases; 75.2%) and serous (27 cases; 22.3%) (Table 1). Only three tumours (2.5%) were of endometrioid type. Seventy-five (82.4%) and 16 of the 91 mucinous borderline tumours were intestinal and endocervical types, respectively. Radical treatment was performed in 68 (56.2%) patients, and 53 (43.8%) patients underwent conservative management (Table 1). Complete surgical staging was performed in 43 (35.5%) patients (Table 1). Adjuvant chemotherapy was given to 30 (24.8%) patients (Table 1). Seventeen patients were lost to follow-up, and two patients died of the other diseases (Table 1). Four patients had a mucinous tumour with pseudomyxoma peritonei and were excluded from the present study because presence of pseudomyxoma peritonei changes the scope of management and the category of pseudomyxoma peritonei is recognised as tumour that can simulate primary mucinous borderline ovarian tumour (Ronnett *et al*, 2004).

Among 102 patients who were finally evaluated for clinical outcome and prognostic factors, eight had tumour recurrence but none of them died of the disease (Table 1). The median time to recurrence was 46 ± 33 months (range 14–107 months). The 5- and 10-year disease-free survival rates were 91.7 and 69.2% for stage I diseases, respectively, and the 5- and 7-year disease-free survival rates were 100 and 66.7% for stage II–IV diseases, respectively (Figure 1A). The 10-year disease-free survival rate was 91.5 and 36.0% for mucinous and serous tumours, respectively (Figure 1B). Although no significant differences in disease-free survival rate were seen between different clinical stages, the difference between serous and nonserous (mucinous and endometrioid) types was significant. On the other hand, the 10-year disease-free survival rate was 89.1% for the radical surgery group and 57.4% for the conservative surgery group (Figure 1C). This difference was significant ($P<0.05$). In univariate analysis, serous type and conservative surgery were found to be important variables affecting recurrence of disease (Table 2). Frequency of recurrence was not influenced by clinical stage, staging laparotomy, and postoperative adjuvant chemotherapy (Table 2). Multivariate analysis showed that only conservative surgery had independent prognostic value regarding recurrence of disease (Hazard ratio 2.2, 95% confidence interval, 0.02–0.52) (Table 2). Subsequently, risk factors for recurrence were evaluated among 43 patients who underwent conservative surgery (Table 3). Of these patients, six had tumour recurrence (Table 3). Three of eight patients who had cystectomy and three of 35 patients who had adnexectomy experienced tumour recurrence (Table 3, $P<0.03$). Recurrence occurred more frequently in patients with serous tumour than with nonserous tumour (Table 3). No correlation was found between recurrence and the factors such as clinical stage, staging laparotomy, or postoperative adjuvant chemotherapy among conservative surgery group (Table 3). Multivariate analysis confirmed cystectomy and serous type as an independent risk factor for recurrence of disease among the patients who underwent conservative surgery (Table 3). Table 4 shows estimated relative risk of having recurrence of disease for different combination of procedure of conservative surgery and histopathological subtype. For example, the relative risk for a patient receiving cystectomy for her serous tumour is 4.33 times greater than the risk for a patient receiving adnectomy for her nonserous tumour.

The clinical and pathological features of the eight patients who developed recurrence were demonstrated in Table 5. None of these eight patients died of progression of their disease. Three of the four serous tumours with recurrence were a noninvasive peritoneal implant, one of which was diagnosed as a serous adenocarcinoma at recurrence. The case developed adenocarcinoma in contralateral ovary 107 months after cystectomy. All mucinous tumours with recurrence were of intestinal subtype. All patients with recurrence who were initially treated conservatively are free of disease after secondary surgical treatment.

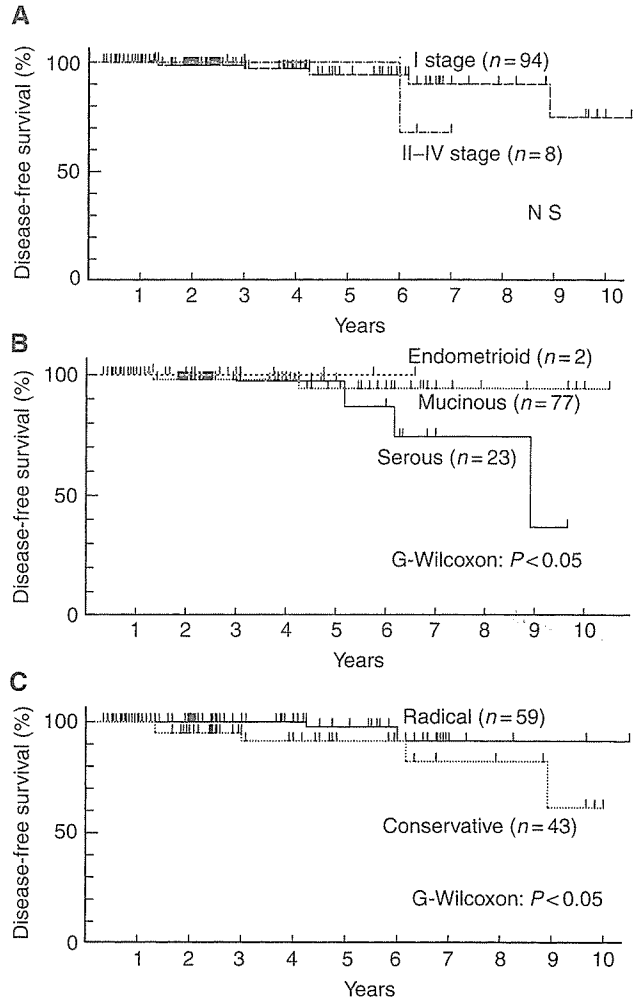

Figure 1 (**A**) Clinical stages and disease-free survival in patients with borderline ovarian tumour. There is no significant difference between two curves. (**B**) Histological type and disease-free survival in patients with borderline ovarian tumour. There is significant difference in disease-free survival between serous and nonserous (mucinous and endometrioid) type ($P<0.05$). (**C**) Surgical procedure and disease-free survival in patients with borderline ovarian tumour. There is significant difference between two curves ($P<0.05$).

DISCUSSION

It has been shown that the 5-year survival rate was 95–97% for stage I and 65–87% for stages II and III (Trope *et al*, 2000; Trimble *et al*, 2002; Sherman *et al*, 2004) suggesting that the prognosis for borderline ovarian tumours depends on extraovarian extension of the tumour. In addition, prognostic factors included clinical stage, histopathological subtype, and residual tumour, but the surgical method was not regarded as a prognostic factor (Trope *et al*, 2000; Gilks, 2003). The results of the present study, however, showed neither the stage nor the histopathological subtype of the disease was related with long-term prognosis, but showed that disease-free survival rates were significantly lower in cases managed by conservative surgery (Figure 1).

Table 2 Risk factors for recurrence in borderline tumours

Factors	Recurrence ($n=8$)	No recurrence ($n=94$)	Univariate P	Multivariate P
Mean age (years)	42.2±13.7	43.5±16.2		
Histology, n (%)				
Serous	4 (50)	19 (20.2)		
Nonserous	4 (50)	75 (79.8)	0.053	0.09
Surgical procedure, n (%)				
Radical	2 (33.3)	57 (60.6)		
Conservative	6 (66.7)	37 (39.4)	0.05	0.031
Staging laparotomy, n (%)				
Staged	3 (37.5)	36 (38.3)		
Unstaged	5 (62.5)	58 (61.7)	0.96	0.58
Stage, n (%)				
I	7 (87.5)	87 (92.6)		
II–IV	1 (12.5)	7 (7.4)	0.61	0.79
Adjuvant chemotherapy, n (%)				
Yes	4 (50)	22 (23.4)		
No	4 (50)	72 (76.6)	0.098	0.33

Table 3 Risk factors for recurrence in the patients who underwent conservative surgery for borderline tumours

Factors	Recurrence ($n=6$)	No recurrence ($n=37$)	Univariate P	Multivariate P
Surgical procedure, n (%)				
Cystectomy	3 (50)	5 (13.5)		
Adnexectomy	3 (50)	32 (86.5)	0.03	0.047
Staging laparotomy, n (%)				
Staged	1 (16.7)	3 (8.1)		
Unstaged	5 (83.3)	34 (91.9)	0.51	0.137
Adjuvant chemotherapy, n (%)				
Yes	3 (50)	7 (18.9)		
No	3 (50)	30 (81.1)	0.095	0.593
Stage, n (%)				
Ia	3 (50)	23 (62.2)		
Ic	3 (50)	14 (37.8)	0.57	0.198
Histology, n (%)				
Serous	3 (50)	6 (16.2)		
Non-serous	3 (50)	31 (83.8)	0.059	0.041

Table 4 Relative risk of recurrence in borderline tumours

Conservative surgery	Histological type: Nonserous	Histological type: Serous
Adnexectomy	1	2.11
Cystectomy	2.05	4.33

In our study, surgical procedure was found to be an independent risk factor for recurrence and the risk could be reduced by radical surgery (Table 2). Because borderline tumours are seen more frequently in younger females than definitive carcinomas (Harris *et al*, 1992), whether conservative surgery is appropriate for

Table 5 Eight patients with borderline tumour who developed recurrence

Age	Histological type	Stage	Initial surgery	Staging procedure	Adjuvant chemotherapy	Time to recurrence	Site of recurrence	Treatment after recurrence	Histology of recurrence site	Status
33	SBT, noninvasive	Ia	Conservative (adnexectomy)	(−)	(+)	74	Intrapelvis	Surgery alone	SBT	NED
35	SBT, noninvasive	Ia	Conservative (adnexectomy)	(−)	(−)	36	Contralateral ovary	Surgery alone	SBT	NED
36	SBT, noninvasive	Ia	Conservative (cystectomy)	(−)	(+)	107	Contralateral ovary	Surgery+chemotherapy	Serous adenocarcinoma	NED
46	SBT, invasive	IIIa	Radical	(+)	(+)	62	Perihepatic	Chemotherapy alone	Unknown	AWD
28	MBT, intestinal	Ic	Conservative (cystectomy)	(−)	(−)	14	Ipsilateral ovary	Surgery alone	MBT, intestinal	NED
35	MBT, intestinal	Ic	Conservative (cystectomy)	(+)	(+)	16	Intrapelvis	Surgery alone	MBT, intestinal	NED
58	MBT, intestinal	Ic	Conservative (adnexectomy)	(−)	(−)	20	Intrapelvis	Surgery alone	MBT, intestinal	NED
67	MBT, intestinal	Ia	Radical	(+)	(−)	35	Lung	None	Unknown	AWD

SBT = serous borderline tumour; MBT = mucinous borderline tumour; NED = no evidence of disease; AWD = alive with disease.

Clinical Studies

borderline ovarian tumours is an important matter to be resolved. Zanetta *et al* (2001) reported that only three of 119 stage I (2.5%) cases that underwent radical surgery recurred, whereas 20 out of 164 stage I (12.1%) cases that underwent conservative surgery recurred, with one case resulted in death from the disease. Morice *et al* (2001) demonstrated that the majority of recurrent cases, including stages II and III, were cured completely by subsequent surgery, and few cases resulted in death. More over, Donnez *et al* (2003) reported that although recurrence was commoner in cases treated by conservative surgery (3 out of 16, 18.7%) than by radical surgery (0 out of 59, 0%), subsequent treatment resulted in no tumour-related deaths, and 63.6% of conservative surgery cases subsequently became pregnant, suggesting that conservative surgery can be an option for management of borderline malignant ovarian tumours in young subjects who need to reserve fertility. However, it is also reported that all of deaths as a result from recurrence were seen in cases treated by conservative surgery (Morris *et al*, 2000; Zanetta *et al*, 2001). Therefore, it is of quite importance to investigate underlying risk factors for recurrence after conservative surgery. As shown in Table 3, we found that cystectomy and serous tumours were independent risk factors for recurrence in patients who received conservative surgery. Previous reports have shown that recurrence after cystectomy did not necessarily occur ipsilaterally (Morris *et al*, 2000, 2001; Zanetta *et al*, 2001). So it seems that the residual tumour during cystectomy is solely responsible for recurrence. Then, it may be rational for young women who wish pregnancy to select cystectomy as an option if the surgical margin is free of tumour. However, results by Morice *et al* (2001) did not support this as they found that the recurrence rate was high after cystectomy compared with adnexectomy. Morris *et al* (2000) also demonstrated that recurrence was higher in cases treated by cystectomy rather than by adnexectomy. The present study confirmed this and further demonstrated for the first time that a difference in pathohistology affects the recurrence rate. As shown in Table 3, it was revealed that serous tumour is a significant risk factor for recurrence in cases managed by conservative surgery. Morris *et al* (2000) also showed similar tendency, but they regrettably missed statistical analysis. As shown in Table 4, the present study clearly demonstrated that the risk of recurrence when serous tumours were treated by cystectomy was approximately four times higher than for adnexectomy of nonserous tumours.

As a prognostic factor for borderline ovarian serous tumours, the concept of peritoneal implant is attracting attention (Bell *et al*, 1988). When estimating the prognosis of borderline ovarian serous tumours, peritoneal lesions should be explored and biopsied at the time of the surgery – in other words, accurate surgical staging is required. Clinical stage is one of the most important prognostic factors in borderline ovarian tumours (Kliman *et al*, 1986), and an accurate surgical staging is indispensable for follow-up after conservative surgery, as well as selecting postoperative therapy. Winter *et al* (2002) compared 48 cases that underwent complete surgical staging, and 45 cases without surgical staging – a higher stage was found in 17% (8 out of 48) of those assessed by surgical staging, but there was no difference in recurrence and survival rates between the groups. Camatte *et al* (2002) found metastasis to lymph nodes in 19% (8 out of 42) of cases. All cases with metastases were seen with borderline ovarian serous tumours associated with peritoneal dissemination, but no cases resulted in death – there was no difference in prognosis when compared with cases without metastases. The presence or absence of a peritoneal lesion is an important predictive factor of recurrence as well as an important prognostic factor, and we do not deny the importance of surgical examination of the abdominal cavity where possible. However, many reports have indicated that the presence or absence of lymph node metastasis is not related to the prognosis for borderline ovarian tumours (Camatte *et al*, 2002; Winter *et al*, 2002), and it is still debatable whether or not to perform a biopsy or dissection of the lymph nodes. As shown in Table 3, the present study could not show a significant relevance to risk of recurrence. The limitation of the present study is that surgical staging was not considered beforehand in all cases so that our data may be biased in this respect. Further studies using a prospective design with emphasis on surgical staging are required to investigate the risk of recurrence in borderline ovarian serous tumours after conservative surgery. Therefore, it is important that conservative surgery should only be performed in cases that truly require conservative surgery, after giving a full explanation of the risk of recurrence.

Barakat *et al* (1995) reported that cisplatin-based chemotherapy induced complete remission in six of 23 (26%) advanced cases with macroscopic diseases, and in 17 of 25 (68%) cases with microscopic disease, and proposed that adjuvant chemotherapy could be considered as a therapeutic option although a life-extension effect of chemotherapy was not clear. In the present study, the regimen or frequency of chemotherapy used was not uniform, and differed among institutions, and no relationship was found between the presence or absence of postoperative adjuvant chemotherapy and recurrence (Table 2). Kaern *et al* (1993) showed that adjuvant chemotherapy did not improve neither recurrence free survival nor overall survival rate in 364 cases without residual tumour. Morice *et al* (2001) demonstrated that postoperative chemotherapy did not improve the survival rate in 80 cases of advanced borderline ovarian serous tumour in stages II and III with extraovarian extension, and that deaths were more closely related to the treatment than to the tumour. Thus, the efficacy of chemotherapy for borderline ovarian tumours is not yet established.

In conclusion, although recurrence was detected in eight out of 102 cases with borderline ovarian tumour that were available for follow-up, no tumour-related deaths were found, and there was a favourable long-term prognosis. Although the relative risk of recurrence is high, conservative surgery appears to be worth trying to preserve fertility, considering the favourable prognosis. When considering conservative surgery, special care should be given when cystectomy is chosen as a surgical procedure or the histological subtype is borderline serous ovarian tumour. Consensus has not been reached on such issues as to the significance of surgical staging, the indication for postoperative adjuvant chemotherapy, or the indications for conservative surgery. To reinforce the present study results, we expect that a large scaled prospective clinical study involving many institutions will be designed to obtain more evidence.

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Clear cell carcinoma of the ovary: a retrospective multicentre experience of 254 patients with complete surgical staging

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A retrospective analysis was performed to evaluate the clinical characteristics and prognostic factors in the patients with clear cell carcinoma (CCC) of the ovary. After central pathological review and scanning of the medical records of nine Japanese institutions between 1992 and 2003, a total of 254 patients with CCC of the ovary were enrolled in the present study. Mean age was 52.4 years (range 23–73 years). Tumours were 13% (33/254) stage Ia, 36% (92/254) stage Ic, 13% (33/254) stage II, 30% (80/254) stage III, and 6% (16/254) stage IV. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively. Retroperitoneal lymph node metastasis was observed in 9% in pT1a tumours, 7% in pT1c tumours, 13% in pT2 tumours, and 58% in pT3 tumours, respectively. There was no survival benefit according to chemotherapeutic differences in the patients who received complete surgical staging procedures and conventional chemotherapy. Peritoneal cytological status was an independent prognostic factor in stage Ic patients ($P = 0.03$) and only residual tumour diameter was an independent prognostic factor in stage III, IV patients ($P = 0.02$). Our results suggest that cytoreductive surgery resulting in no residual tumour only could improve the prognosis of advanced CCC patients.
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Cancer of the ovary has the worst prognosis of all gynaecological malignancies in the United States (Edwards *et al*, 2005) and Europe (Bray *et al*, 2005). Survival rate of patients with ovarian cancer has dramatically improved after introduction of platinum-based chemotherapy, but there still exist a large number of patients showing no response to the treatments. Although response to anticancer drugs is not easy to predict, *in vitro* studies suggested that acquired resistance to cisplatin has been associated with increased levels of glutathione and glutathione-S-transferase activity, increased metallothionein and decreased accumulation of cisplatin (Kikuchi *et al*, 1998). Histological subtypes such as clear cell carcinoma (CCC) and mucinous adenocarcinoma had been suggested as one of the most reliable criteria predicting the ineffectiveness of chemotherapy.

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Clear cell carcinoma (CCC) was initially termed as mesonerhroid in 1939 (Schiller, 1939), and since 1973 it was strictly defined by World Health Organization as lesions characterised by clear cells growing in solid/tubular or glandular patterns as well as hobnail cells (Serov *et al*, 1973). Since then, many literatures have identified the distinctive behaviour of the tumors as compared with other histological subtypes of ovarian neoplasms. The most distinctive difference is that patients with CCC of the ovary have lower response rate to anticancer drugs. To our knowledge, only a few clinical studies have evaluated the response rates for CCC patients with measurable disease. The response rate of chemotherapy for CCC was 11.1% with platinum-based regimens (Sugiyama *et al*, 2000) and 22–56% with paclitaxel plus carboplatin. (Enomoto *et al*, 2003; Ho *et al*, 2004).

Another factor that might contribute to prognosis of ovarian cancer is the degree of cytoreductive surgery including lymphadenectomy. Complete surgical staging including para-aortic lymphadenectomy might influence the prognosis in early-stage CCC cases (Ho *et al*, 2003). Furthermore, the patients with pure-type CCC had worse overall survival than those with mixed-type CCC (Ho *et al*, 2004).

To evaluate the clinical characteristics of the patients with CCC of the ovary and to determine the impact of surgery and

chemotherapy on prognosis of those patients, we conducted a retrospective study over 11-year period of a sample of 254 patients diagnosed with pure-type CCC in the departments of nine Japanese institutions.

MATERIALS AND METHODS

Patients and tumours

Between 1992 and 2002, 254 patients with CCC of the ovary were identified by scanning the medical records of the collaborating institutions and central pathological review. Patients received initial treatment and follow-up at nine institutions belonging to Japan Clear Cell Carcinoma Study Group; National Defence Medical College Hospital, Tohoku University Hospital, Aichi Cancer Center Hospital, Osaka Medical College Hospital, Osaka City General Hospital, Jichi Medical College Hospital, Tottori University Hospital, Kobe National Hospital, Iwate Medical College Hospital.

Initially, 337 patients were accrued from medical records of each institution. All pathological specimens from primary surgery were reviewed under central pathological review by two independent pathologists with no knowledge of patients' clinical data. Tumours were diagnosed as CCC if typical clear or hobnail cells growing in a papillary, solid, or tubulocystic pattern appeared in >90% of all pathological specimens. After pathological review, three cases were excluded; two diagnosed as mixed epithelial ovarian cancers and the other diagnosed as CCC derived from mature cystic teratoma, and 334 cases were identified as the patients with pure-type CCC of ovary. In those patients, 80 patients were excluded owing to insufficient surgery lacking complete surgical staging procedures: 13 cases in pT1a tumours, 51 cases in pT1c tumours, 16 cases in pT2 tumours, respectively. The rest 254 patients were enrolled on the present study. Patients of FIGO stage Ic were classified into three subtypes according to pathological characteristics; Ic (capsule ruptured) for the patients with ruptured capsule at laparotomy, Ic (ovarian surface) for those with tumour on ovarian surface, and Ic (ascites/malignant washing) for those with positive malignant cells in the ascites or positive peritoneal washing.

All 254 patients underwent complete surgical staging procedures including hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing, omentectomy, pelvic lymphadenectomy and paraaortic lymphadenenctomy. Staging was based on the FIGO classification. The resected lymph node counts were not considered for the completion of the lymphadenectomy. A pN1 case was determined as having one or more lymph node metastasis in pelvic or paraaortic lymph nodes.

Chemotherapy

Two hundred and forty-two (95.3%) patients received postoperative chemotherapy after initial surgery. Second look operation or second reductive surgery was done by surgeon's preference. Combination therapy of cyclophosphamide and doxorubicin and cisplatin (CAP) was as follows: one cycle consisted of a drip infusion of 50–75 mg m^{-2} cisplatin for 3 h accompanied by an i.v. injection of 50 mg m^{-2} doxorubicin and 500 mg m^{-2} cyclophosphamide and six cycles were given every 4 weeks. Paclitaxel and platinum regimen consisted of an infusion of 175–180 mg m^{-2} of paclitaxel and 50–75 mg m^{-2} of cisplatin or carboplatin (AUC = 5–6). Other regimens included the combination chemotherapy irinotecan hydrochloride and cisplatin (40 cases) and irinotecan hydrochloride and mitomycin C (20 cases) and irinotecan hydrochloride and etoposide (3 cases). One cycle of irinotecan hydrochloride and platinum regimen consisted of a drip infusion of 50–60 mg m^{-2} of cisplatin on day 1 and 50–60 mg m^{-2} of CPT-11 on day 1, 8, 15 and 1 week off and it was repeated every 4 weeks.

Response was evaluated with CT or MR images for patients with measurable disease. A complete response (CR) was defined as the complete disappearance of all detectable disease for at least 4 weeks. A partial response (PR) was defined as a >50% decrease in tumour size for at least 4 weeks. Stable disease (SD) was defined as the absence of any significant change in measurable lesions for at least 4 weeks. Progressive disease (PD) was defined as the appearance of a new lesion or a >25% increase in tumour size. Serum levels of tumour markers including CA125 were not used for response evaluation of chemotherapy in the present study.

The time to progression was defined as the interval from the date of primary surgery until the date of recurrence or tumour progression (PD). Survival duration was determined as the time from the date of primary surgery until death or the date of last follow-up contact.

Statistical methods

Kaplan–Meier method was used for calculation of patient survival distribution. The significance of the survival distribution in each group was tested by a generalized Wilcoxon test and the log-rank test. The χ^2-test and Student's t-test for unpaired data were used for statistical analysis. A P-value of <0.05 was considered statistically significant. The Stat View software ver.5.0 (SAS Institution Inc., Cary, NC, USA) was used to analyse the data.

RESULTS

Patients and tumours

The characteristics of the study population are summarized in Table 1. Mean age was 52.4 years (range 23–73 years). Tumours were 13% (33/254) stage Ia, 36% (92/254) stage Ic, 13% (33/254) stage II, 31% (80/254) stage III, and 6% (16/254) stage IV, respectively. There is no case with stage Ib tumours. Among 92 cases of stage Ic, there were 45 cases (49%) of Ic (capsule

Table 1 Characteristics of the patients

Characteristics	No. of patients (%)
All cases	254
Age (years)	
<55	147 (57.9)
>55	107 (42.1)
FIGO Stage	
Ia	33 (13.0)
Ic (ovarian surface)	3 (1.2)
Ic (capsule ruptured)	45 (17.7)
Ic (ascites/malignant washing)	44 (17.3)
II	33 (13.0)
IIIa,b	5 (2.0)
IIIc	75 (29.5)
IV	16 (6.3)
Residual tumour diameter	
0 cm	176 (69.3)
<1 cm	18 (7.1)
>1 cm	60 (23.6)
Postoperative chemotherapy	
CAP[a]	76 (29.9)
Paclitaxel+platinum	103 (40.6)
Others	63 (24.8)
None	12 (4.7)

[a]CAP, cyclophosphamide+doxorubicin+cisplatin.

ruptured), 3 cases (3%) of Ic (ovarian surface) and 44 cases (48%) of Ic (ascites/malignant washing), respectively. In 75 stage IIIc tumours, 15 cases (20%) were upstaged to stage IIIc because of retroperitoneal lymph node metastasis and 20 patients (27%) had both retroperitoneal lymph node metastasis and intra-peritoneal disease. Residual tumour diameter after primary debulking surgery was 0 cm in 176 cases (69%), less than 1 cm in 18 cases (7%), and more than 1 cm in 60 cases (24%), respectively.

Postoperative chemotherapy was offered for all patients, and 242 patients (95%) received anticancer drugs. Eight patients in stage Ia and four patients with stage Ic (capsule ruptured) refused postoperative chemotherapy.

Precise lymph node status according to pT distribution was documented in Table 2. Lymph node metastasis was documented in 3 of 36 patients (9%) in pT1a tumours, 7.1% in pT1c tumours, 13% in pT2, and 58% in pT3 tumours, respectively. Retroperitoneal lymph node metastasis in pT3 tumours was observed significantly more frequent than in pT1, 2 tumours (58.0 *vs* 8.7%, $P<0.001$, χ^2-test).

Response of chemotherapy

Response judged with CT or MRI images was assessable in 73 cases (29%) in 242 patients who received postoperative chemotherapy. Only 5 of 30 cases (16%) responded to CAP regimen. Progressive disease was documented in 23 patients (77%) and SD was observed in 2 patients (7%). In 28 patients treated with paclitaxel and platinum, response was observed in nine cases (32%) including one case with CR. In the patients treated with other regimens, response was observed in 3 of 10 patients (30%) treated with irinotecan hydrochloride and cisplatin. There is no responder in seven assessable patients who received combination with irinotecan hydrochloride and mitomycin C.

The median duration of progression-free survival for the patients with measurable disease was 4 months (range, 1–20 months) in CAP regimen, 5 months (range, 1–21 months) in paclitaxel and platinum, and 3 months (range, 2–20 months) in irinotecan hydrochloride and cisplatin, respectively.

Table 2 Rates of lymph node metastasis according to pT status

pT status	pN1	pN0	Rate of Lymph Node metastasis (%)
pT1a ($n=36$)	3	33	9.1
pT1c ($n=99$)	7	92	7.1
pT2 ($n=38$)	5	33	13.1
pT3 ($n=81$)	47	34	58.0
Total ($n=254$)	62	192	24.4

Clinical course

Average follow-up for all CCC patients in the present study is 47.4 months. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively (Figure 1). Although there is no statistically significant difference in progression-free survival between patients with stage Ic (capsule ruptured) and those with stage Ia ($P=0.11$), progression-free survival of the patients with stage Ic (ascites/malignant washing) and Ic (ovarian surface) was significantly worse than that of stage Ic (capsule ruptured) ($P=0.04$)(Figure 2). Multiple regression survival analysis for stage Ic patients with CCC revealed that positive peritoneal cytology was the only independent prognostic factor ($P=0.03$; Relative risk, 3.40; 95% CI, 1.14–10.18). Cumulative progression-free survival of pT1M0 patients with positive node was significantly lower than those with negative node ($P<0.01$). Five-year progression-free survival was 84% in pT1N0 patients and 56% in pT1N1 patients, respectively.

Progression-free survival curves of stage III, IV patients according to the residual tumour diameter were shown in Figure 3. Median progression-free survival duration was 39 months in the

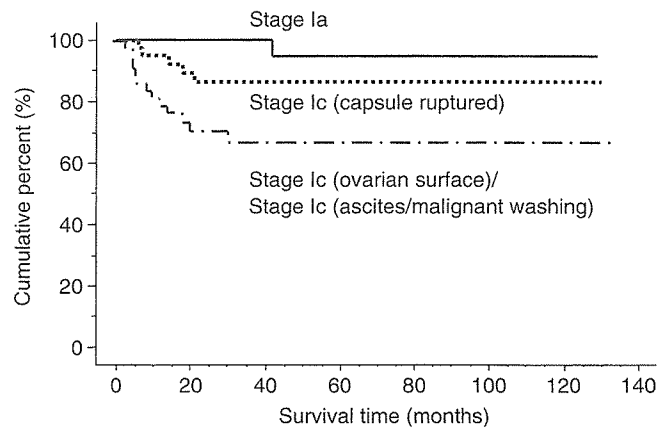

Figure 2 Progression-free survival of patients with FIGO stage I patients. There is no significant difference between patients with stage Ic (capsule ruptured) and those with stage Ia ($P=0.11$). Survival of the patients with stage Ic (ascites/malignant washing) and Ic (ovarian surface) was significantly worse than that of stage Ic (capsule ruptured) ($P=0.04$).

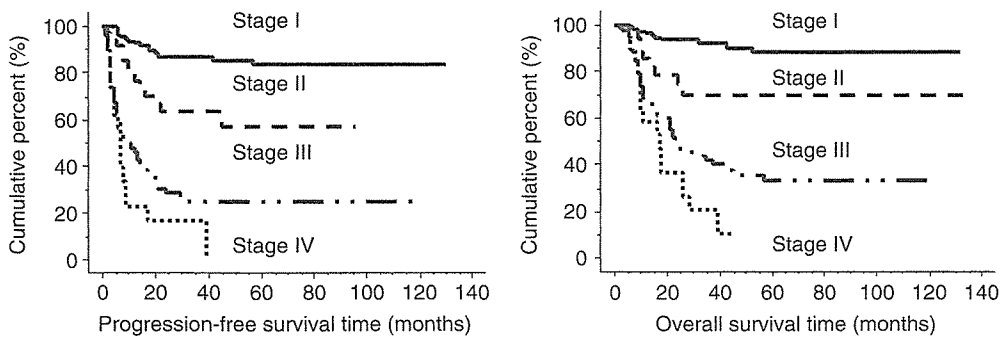

Figure 1 Progression-free survival and overall survival of patients depending on their FIGO stage. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively. *P*-values in progression-free survival were as follows: Stage I *vs* stage II, $P<0.01$; stage II *vs* stage III, $P<0.01$; stage III *vs* stage IV, $P=0.35$. *P*-values in overall survival were as follows: Stage I *vs* stage II, $P<0.01$; stage II *vs* stage III, $P<0.01$; stage III *vs* stage IV, $P=0.17$.

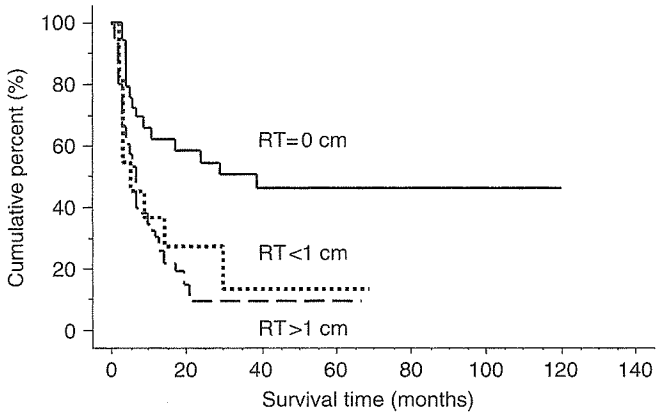

Figure 3 Progression-free survival of stage III, IV patients according to the residual tumour (RT) diameter. There is no significant prognostic difference between the patients with the tumour diameter less than 1 cm and those with the tumour diameter more than 1 cm ($P=0.40$). The patients with no residual tumour had significantly better progression-free survival than those with the tumour less than 1 cm ($P=0.04$) or those with tumour diameter more than 1 cm ($P<0.01$), respectively. Median progression-free survival duration was 39 months in the patients with no residual tumour, 7 months in those with the tumour diameter less than 1 cm, and 5 months in those with residual tumour diameter more than 1 cm, respectively.

Table 3 Multiple regression survival analysis for stage III, IV patients with CCC

Variables	Hazard ratio	95% confidence interval	P
Age (years)			0.96
<54	1		
>55	0.99	0.60; 1.61	
PS			0.67
0	1		
1,2	1.06	0.79; 1.43	
FIGO stage			0.22
III	1		
IV	1.47	0.80; 2.70	
Residual tumour			0.02
None	1		
<1 cm	2.23	0.89; 5.54	
>1 cm	3.17	1.68; 6.00	
Chemotherapy			0.24
CAP[a]	1		
Paclitaxel+platinum	0.56	0.48; 1.88	
Others	0.95	0.32; 1.22	

[a]CAP, cyclophosphamide+doxorubicin+cisplatin.

patients with no residual tumour, 7 months in those with the tumor diameter less than 1 cm, and 5 months in those with residual tumour diameter more than 1 cm, respectively. There is no significant prognostic difference between the patients with the tumour diameter less than 1 cm and those with the tumour diameter more than 1 cm ($P=0.40$). The patients with no residual tumour had significantly better progression-free survival than those with the tumour less than 1 cm ($P=0.04$) or those with tumour diameter more than 1 cm ($P<0.01$), respectively.

Multiple regression analysis in stage III and IV patients revealed that chemotherapeutic regimen was not an independent prognostic factor ($P=0.24$) and only residual tumour diameter was an independent prognostic factor in stage III and IV patients ($P=0.02$) (Table 3).

DISCUSSION

The present study and previous studies support that CCC of the ovary tended to present at earlier stages. Proportion of stage I/II tumours ranged from 59 to 71% (Yoonessi *et al*, 1984; Crozier *et al*, 1989; Jenison *et al*, 1989; Kennedy *et al*, 1989; O'Brien *et al*, 1993; Behbakht *et al*, 1998; Sugiyama *et al*, 2000). One of the reasons for the early detection was explained by the slow growing tumour behaviour (Itamochi *et al*, 2002a) and frequent presentation of the tumours as relatively large pelvic masses (Kennedy *et al*, 1989; Behbakht *et al*, 1998). In the present study, the status of peritoneal cytology was identified as an independent prognostic factor in FIGO stage Ic patients. Although tumour progression was observed in 5 (11%) of 45 stage Ic (capsule ruptured) tumours and one (3%) of 33 stage Ia tumours, there is no significant survival difference between two groups. Recent report analysing prognosis of early-staged ovarian cancer including only 25 CCC cases (26.6%) in 94 carcinomas showed no statistical significant difference between stages Ic preoperative *vs* intraoperative rupture (Leitao *et al*, 2004). Another report including higher ratio of CCC patients identified that stage Ic (capsule ruptured) patients showed significantly poorer survival than stage Ia patients (Mizuno *et al*, 2003). The present study implied the importance to remove the tumour mass without intraoperative rupture, especially in CCC patients.

Even in stage I ovarian cancer including all histological subtypes, the incidence of positive lymph nodes was not low, ranging from 5.1 to 20% (Sakuragi *et al*, 2000; Cass *et al*, 2001; Morice *et al*, 2003). It was reported that serous tumour had a higher incidence of lymph node involvement than non-serous tumors (Takeshima *et al*, 2005). Although the true incidence of lymph node metastasis in CCC tumour had not been clear, the present study revealed the frequency of metastasis in a large number of the CCC patients. Lymph node metastasis was observed in 3 of 36 patients (9.1%) in pT1a tumours, 7.1% in pT1c tumours, 10.8% in pT2 tumours, respectively. Fifteen (8.7%) of 173 patients who had pT1 or pT2 tumors were upstaged as stage IIIc tumours based on lymph node status. In general, prognostic significance of retroperitoneal lymph node metastasis in early-staged ovarian cancer patients was controversial. Survival rates with node-positive disease were significantly lower in clinical stage I and II disease (Kanazawa *et al*, 1999; Sakuragi *et al*, 2000; Negishi *et al*, 2004). In contrast, another report showed that the prognoses for clinical stage I/II patients with or without lymph node metastasis were similar (Onda *et al*, 1998). In pT1 CCC patients of the present study, lymph node status was identified as a strong prognostic factor and it is essential to accurately evaluate the lymph node status through complete surgical staging procedures. The study, called Adjuvant ChemoTherapy in Ovarian Neolasm (ACTION), revealed that no benefit of adjuvant chemotherapy was observed in early-stage ovarian cancer with optimal surgical procedures (Trimbos *et al*, 2003). In the present study, 12 patients with stage Ia or stage Ic (capsule ruptured) refused to receive chemotherapy, but there was no evidence of recurrence in median follow-up period of 44 months (range: 6–63 months), which might support the results of ACTION study.

Previous Japanese report have shown that the chemotherapeutic effect was assessable in only 27 patients (26.7%) in 101 CCC cases, in contrast it was assessable in 47% of serous adenocarcinoma (Sugiyama *et al*, 2000). In our series of CCC patients, patients with residual tumour diameter more than 1 cm were documented in only 60 (18%) of 254 cases, and the chemotherapeutic effect was assessable in only in 73 cases (29%) in 242 patients who received adjuvant chemotherapy. As the residual tumour after debulking surgery often lacked measurable tumour diameter to evaluate the

effects of adjuvant chemotherapy in CCC patients, it has been quite difficult to select superior regimen.

There have been only a few reports to document the response of anticancer agents for CCC patients, but each of them included relatively small number of cases. The present study confirmed that CAP regimen showed a low response rate and quite a high incidence of PD in CCC patients as described previously (Sugiyama *et al*, 2000). The combination chemotherapy consisting of paclitaxel and platinum has been established as standard therapy for ovarian cancer. One report of paclitaxel and platinum regimen for CCC patients revealed that the response was observed in two of nine cases (22%) (Enomoto *et al*, 2003), and the other report of paclitaxel plus platinum chemotherapy showed the response was observed in 9 of 15 cases (56%) (Ho *et al*, 2004). These two studies including the present study suggested that paclitaxel plus platinum regimen had higher response rate compared to platinum-based chemotherapy. One report showed survival benefit of conventional chemotherapy with paclitaxel and platinum after complete surgery in CCC patients (Ho *et al*, 2003). However, the results from our series of CCC patients showed that there was no survival benefit with chemotherapy with paclitaxel and platinum compared with CAP regimen in both early and advanced cases. Irinotecan hydrochloride was preliminary introduced for CCC patients in clinical settings (Shimizu *et al*, 1998; Adachi *et al*, 1999; Kita *et al*, 2000), but there is no large clinical trial for the treatment of CCC patients of the ovary. Further studies are needed to establish the candidate regimen for CCC of the ovary.

Recent studies have suggested that CCC tumour showed a distinctive molecular behaviour from other histological subtypes. *In vitro* study suggested that paclitaxel and irinotecan hydrochloride were the candidates for anti-neoplastic agents for CCC (Itamochi *et al*, 2002b), but the present study has failed to prove the survival benefit of these two drugs in CCC patients. Another strategy for CCC tumours might be the additive use of molecular targeting agents. It was reported that hepatocyte nuclear factor-1 beta (HNF-1 β) was a CCC-specific marker and had anti-apoptotic effects in CCC cell lines (Tsuchiya *et al*, 2003). Another candidate marker could be ABCF2, which belongs to the ATP-binding cassette gene superfamily and is highly expressed in CCC and non-responders for chemotherapy (Tsuda *et al*, 2005). Suppression of CCC-specific molecular markers such as HNF-1 β or ABF2 may be another strategy for the treatment of CCC of the ovary. The present study clarified the significant prognostic importance of positive peritoneal cytology in early-stage CCC disease, and no macroscopic residual tumour in advanced CCC tumours, respectively. However, there was a little impact of chemotherapeutic effects on both early and advanced diseases. Although further studies are needed to identify effective agents in both anti-neoplastic agents and molecular targeting agents, our study provides the fundamental characteristics of CCC of the ovary.

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Paclitaxel–platinum combination chemotherapy for advanced or recurrent ovarian clear cell adenocarcinoma: a multicenter trial

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Abstract. Utsunomiya H, Akahira J, Tanno S, Moriya T, Toyoshima M, Niikura H, Ito K, Morimura Y, Watanabe Y, Yaegashi N. Paclitaxel–platinum combination chemotherapy for advanced or recurrent ovarian clear cell adenocarcinoma: a multicenter trial. *Int J Gynecol Cancer* 2006;16:52–56.

The therapeutic effect of a combination of paclitaxel (PTX) and platinum (PLT) in ovarian clear cell adenocarcinoma (CC) patients with measurable disease has yet to be elucidated. In this study, we used retrospective review to evaluate the results of treatment with a combination of PTX and PLT in CC patients with measurable disease. A total of 28 patients with measurable residual CC (15 cases with primary disease, 13 cases with recurrent disease) treated with combination PTX-PLT chemotherapy was identified through medical records from ten institutions. Clinical response to chemotherapy was evaluated using Response Evaluation Criteria in Solid Tumors criteria. Of the 28 cases, 8 of 15 patients with primary disease (53.3%) and 3 of 13 patients with recurrent disease (23.1%) responded to PTX-PLT chemotherapy. The response rate for cases with late recurrent disease (>12 months) was 20% (1/5), whereas the rate was 25% (2/8) for cases with early recurrent (<12 months) or refractory disease. Our results indicate that the combination of PTX and PLT may have greater efficacy against CC than conventional PLT-based chemotherapy that does not include PTX.

KEYWORDS: chemotherapy, clear cell adenocarcinoma, ovary, paclitaxel, platinum.

Epithelial ovarian cancer is the leading cause of death among gynecological malignancies in the great majority of developed countries[1]. Treatment for this disease has improved over the past 30 years with advances in surgery and in platinum (PLT)-based chemotherapy. However, most women with ovarian cancer still develop recurrent disease and die within 5 years. This high mortality is considered to be, in large part, due to the high frequency of advanced-stage disease at time of diagnosis. However, many clinical studies have reported that there are other prognostic factors such as histologic type, degree of primary surgical cytoreduction, and response to chemotherapy[1–3].

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Standard chemotherapy for ovarian cancer has been a combination of cyclophosphamide and a PLT agent, with or without doxorubicin[4]. Recently, the therapeutic effect of a combination of cisplatin (CDDP) and paclitaxel (PTX) was shown to be superior to that of a combination of cyclophosphamide and CDDP, with a clinical response rate (RR) for the PTX-CDDP combination of roughly 70%[5]. An increasing amount of evidence shows that this general combination of PTX-PLT chemotherapy seems to improve overall and disease-free survival not only for primary ovarian cancer patients but also for patients with relapsed disease[6–8].

Ovarian clear cell adenocarcinoma (CC) has been recognized as a distinct histologic entity in the World Health Organization classification of ovarian tumors since 1973[9]. The incidence of CC among epithelial ovarian carcinomas is cited as 3.7–12.1%, with approximately 60% of CC patients presenting with

early-stage disease[3,10–14]. Recently, CC has increased in prevalence. It now accounts for 18.5–20% of all epithelial ovarian cancers in Japan[15,16]. It has become obvious that chemosensitivity of ovarian cancers is closely related with histologic type. Several reports indicate that CC has a poor response to conventional therapies such as chemotherapy and irradiation and has a significantly worse prognosis than other histologic types of ovarian carcinoma[17–21]. However, those previous studies were conducted mainly for general ovarian cancer patients and with combination chemotherapy that did not include PTX. Moreover, prognosis for CC patients is considered to be influenced not only by chemotherapeutic factors but also by operative procedure[22]. In this study, we conducted a multicenter, retrospective analysis to evaluate true chemosensitivity for PTX-PLT (with either CDDP or carboplatin [CBDCA]) combination chemotherapy for CC patients who had measurable target lesions.

Materials and methods

In April 2003, ten Japanese institutions received questionnaires regarding CC cases treated with chemotherapy between 1998 and 2003: Tohoku University Hospital, Kinki University Hospital, Tsuboi Hospital, Miyagi Municipal Cancer Center, National Sendai Hospital, Takeda Hospital, Yamagata Municipal Central Hospital, Yuri-kumiai Hospital, Ichinoseki Hospital, and Hachinohe Municipal Hospital. All the selected institutions from which patients were enrolled in this study are considered highly specialized in gynecologic oncology. Patient data were collected from patient chart review by a responsible person at each institution.

Eligible patients included those with primary advanced disease (eg, stage II/III/IV) and those with recurrent or persistent CC disease. Both patient groups had to have measurable disease before chemotherapy. Tumors were diagnosed as CC if the following appeared in >50% of all histologic specimens: small to large sheets of polyhedral clear cells with delicate fibrovascular septa; tubules and papillae; clear, hobnail, or eosinophilic cells of organoid appearance; or clear cells with coalescent vacuoles containing "targetoid" eosinophilic, periodic acid–Schiff stain–positive globules. Histologic evaluation was performed under central pathologic review by one of the authors (T.M. or J.A.). All patients underwent complete surgical staging including intraperitoneal cytology, bilateral salpingo-oophorectomy, hysterectomy, omentectomy, pelvic and/or para-aortic lymphadenectomy, and aggressive cytoreductive surgery as initial treatment. Each chemotherapy cycle consisted of PTX 175 mg/m^2 and CBDCA with an area under the curve equal to five or PTX 175 mg/m^2 and CDDP 50 mg/m^2 after initial suboptimal surgery (residual tumor >1 cm) in cases of primary CC disease. The same therapy was administered for patients with persistent or recurrent measurable disease. For patients with recurrent or persistent CC disease, the number and regimens of previous chemotherapy were not used as exclusion criteria. Patients received more than three cycles of PTX-PLT combination chemotherapy every 3 weeks. In addition, to evaluate the specific response to PTX-PLT, patients who received only this regimen were enrolled in the study.

Clinical response to chemotherapy was evaluated by two of the authors (K.I. and H.N.) according to Response Evaluation Criteria in Solid Tumors[23] with use of computed tomography after three cycles of planned chemotherapy and/or when all planned chemotherapy was finished. Toxicity of chemotherapy was not evaluated in this study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan. Each survival curve was obtained by the Kaplan–Meier method.

Results

A total of 28 patients were identified who met all the study eligibility criteria described above. Patient characteristics are summarized in Table 1. Of the 28 patients included in the study, 15 women with primary disease (53.6%) received PTX-PLT as first-line chemotherapy and 13 women with recurrent or refractory disease (46.4%) received PTX-PLT as second- or third-line chemotherapy. The details of previous

Table 1. Patient characteristics

	Clear cell	
	Primary (n = 15)	Recurrence (n = 13)
Age (years)		
Median (range)	52 (43–74)	54 (48–68)
Performance status		
0	12	11
1	3	1
2	0	1
Stage at primary treatment		
1	0	7
2	1	4
3	12	1
4	2	1

chemotherapy and other elements of recurrence status for the 13 recurrent or refractory cases are summarized in Table 2.

Response to PTX-PLT chemotherapy among all patients is shown in Table 3. Of the 28 patients, 11 women (39.3%) responded to the combination. The RR for primary disease cases was 53.3% (8/15) in contrast to 23.1% (3/13) for recurrent disease cases. Progressive disease was documented in six cases of primary disease (40%) and nine cases of recurrent disease (69.2%). The RR for cases of late recurrent disease (>12 months) was 20% (1/5), whereas RR for cases of early recurrent (<12 months) or refractory disease was 25% (2/8).

Median survival time in patients with primary CC and recurrent CC was 20 and 28 months, respectively.

Table 3. Clinical Response to chemotherapy

Status	Total	CR	PR	SD	PD	RR (95% CI)
Primary	15	5	3	1	6	53.3% (26.6–78.7)
Recurrence	13	0	3	1	9	23.1% (5–53.8)
Early (<12 months)	8	0	2	0	6	25% (3.2–65.1)
Late (>12 months)	5	0	1	1	3	20% (0.5–71.6)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Discussion

A number of previous studies support the concept that CC demonstrates clinical behavior that is distinct from that of other epithelial ovarian carcinomas, particularly in terms of its chemoresistance and poor prognosis with advanced disease[11,17,19,20]. However, this fact has not been regarded as a serious issue clinically because CC is relatively rare among epithelial ovarian cancer and approximately 60% of CC cases are diagnosed as stage I disease[17,21], which means that only a small percentage of patients with this specific neoplasm require chemotherapy for residual disease. These characteristic features of CC make it difficult to evaluate real response to chemotherapy, and there have been no large prospective studies targeted exclusively at CC patients. In the present study, we retrospectively analyzed response to PTX-PLT chemotherapy among CC patients who had measurable residual disease. Also, to exclude interobserver differences between institutions, histologic types of all surgical specimens were confirmed by central pathologic review and residual disease was reevaluated by Response Evaluation Criteria in Solid Tumors criteria.

To our knowledge, few clinical studies have evaluated response to PLT-based chemotherapy in patients with measurable residual CC. Goff *et al.*[19] reported a higher rate of progressive disease with PLT-based chemotherapy in CC patients with measurable or nonmeasurable disease (16/23; 70%) compared with patients with serous adenocarcinoma (10/34; 29%). Sugiyama *et al.*[17] compared CC patients with stage III disease with serous adenocarcinoma patients with stage III disease using cyclophosphamide–PLT combination chemotherapy with or without doxorubicin. The RR to PLT-based chemotherapy in patients with CC was significantly lower than that in patients with serous adenocarcinoma (RR in CC patients, 11.1%). Although the two reports analyzed CC patients with measurable disease, the combination chemotherapy used for their patients did not include PTX. Recently, Enomoto *et al.* presented the results of a study on PTX-CBDCA combination chemotherapy for epithelial

Table 2. Summary of data for cases of recurrent or refractory disease

Case	Stage	Recurrence site	Previous regimen	Time to recurrence	Therapy effect
1	IC	Lung	CDDP, CPM, ADR	>12 months	SD
2	IC	Para-aortic LN	CDDP, CPM, ADR	<12 months	PR
3	IC	PC	CDDP, CPT-11	<12 months	PD
4	IIIC	Pelvic cavity	PTX, CBDCA	<12 months	PR
5	IC	Lung	MMC, VP-16, CDDP	<12 months	PD
6	IA	Para-aortic LN	CDDP, CPM, ADR	<12 months	PD
7	IIC	Abdominal cavity	PTX, CBDCA	<12 months	PD
8	IIC	Abdominal cavity	CDDP, CPM	>12 months	PR
9	IV	Lung	CDDP, CPM, ADR	>12 months	PD
10	IC	Abdominal cavity	CDDP	<12 months	PD
11	IIC	PC	PTX, CBDCA	<12 months	PD
12	IC	Para-aortic LN	PTX, CBDCA	>12 months	PD
13	IIC	Abdominal cavity	PTX, CBDCA	>12 months	PD

LN, lymph nodes; PC, peritonitis carcinomatosa; CPM, cyclophosphamide; ADR, adriamycin; MMC, mitomycin C; VP-16, etoposide; SD, stable disease; PR, partial response; PD, progressive disease.

ovarian cancer at the 2003 ASCO meeting[24]. This group concluded that CC (RR, 22%) was not as sensitive to the chemotherapy combination as serous adenocarcinoma (RR, 81%). This appeared to be especially true for women with measurable disease, although the authors analyzed only nine such CC cases. On the other hand, Behbakht *et al.* reported on six stage III/IV CC patients with measurable residual tumor, 67% (4/6) of whom partially responded to PTX-PLT[20]. In contrast to previous studies on conventional PLT-based chemotherapy without PTX, the RR for primary residual CC in our study was relatively high (53.3%), although this rate was lower than the 73% RR found in suboptimal patients with advanced ovarian cancer including serous adenocarcinoma[5]. RR for PLT-refractory disease or early (<12 months) recurrent disease and late (>12 months) recurrent disease was 25% and 20%, respectively, in our study. Guastalla *et al.* reported RRs for PLT-refractory patients and those with early (≥3 and <12 months) and late (>12 months) relapsing disease as 24%, 33%, and 70%, respectively. The RR in our study on limited CC patients was lower than those given in previous reports on patients with previously treated advanced ovarian cancer, especially in cases of late recurrent disease[25].

In the present study, median survival time in patients with primary CC disease was 20 months. Sugiyama *et al.* reported that median survival time in primary stage III CC patients treated with PLT-based chemotherapy was 12.7 months. In previous reports for total primary advanced epithelial ovarian cancers including serous adenocarcinoma treated with PTX-PLT, median survival time was 38 months[5]. Our study suggests that RR for primary residual CC was relatively high and median survival time was slightly longer than that reported for stage III CC patients treated with PLT-based chemotherapy, but not as long as survival times given in reports including other histologic types such as serous adenocarcinoma. We conclude that chemosensitivity of CC may not contribute too much to survival. However, it is noteworthy that a significant proportion of CC patients with primary disease responded to PTX-PLT combination chemotherapy. Guastalla *et al.* reported that in recurrent ovarian cancer treated with PTX-PLT, median survival time was 14 months[26]. In the present study, median survival time in patients with recurrent disease was longer (28 months) than in patients with primary disease in spite of a lower RR (23.1% for recurrent disease vs 53.3% for primary disease). We speculate that most patients with recurrent disease in our study were stage I or II and may have had slow-growing carcinoma. This characteristic may be related to the chemoresistance (RR 20%) seen in late recurrent serous adenocarcinoma, a tumor that is generally chemosensitive.

In *in vitro* studies, Ohta *et al.*[26] reported that cyclophosphamide was more effective against serous adenocarcinoma cells than CC cells, whereas PTX was effective against CC cells and ineffective against serous adenocarcinoma cells. Cloven *et al.*[27] reported clear cell tumors had the lower rates of the Extreme Drug Resistance assay to PTX and cyclophosphamide than papillary serous tumors. In addition, Itamochi *et al.*[28] demonstrated that PTX and camptothecin (CPT-11) were effective against three of five CC cell lines while only one CC cell line was sensitive to CDDP. Thus, they concluded that PTX and CPT-11 may be effective agents against CC. In a clinical trial, Shimizu *et al.*[16] reported that chemotherapy including CPT-11 demonstrated significant activity (RR, 52%) in PLT-refractory CC patients, and they advocated that CPT-11 should be given as front-line chemotherapy for CC patients. Our present clinical data also support the *in vitro* studies; the relatively high RR observed in our study seems to be accounted for by the addition of PTX to the chemotherapy regimen. Together with our clinical data, PTX may have the potential to play a key role in the treatment of CC even though chemosensitivity may be lower than that of serous adenocarcinoma. The main limitation of the current study was that the number of cases was too small and the follow-up period too short to form definite conclusions about the contribution of PTX-PLT combination chemotherapy to survival. We speculate that therapy with only PTX may be not sufficient to get better response and prognosis, so new combinations such as PTX and CPT-11 with or without PLT may be considered for future clinical trials.

However, our results with CC patients do indicate that the combination of PTX and PLT has some efficacy compared with conventional PLT-based chemotherapy for this population, and there seem to be some candidates for alternative regimens to improve treatment. A large-scale, prospective trial may be necessary in order to confirm our observation.

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Expression of estrogen-responsive finger protein (Efp) is associated with advanced disease in human epithelial ovarian cancer

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Abstract

Objective. The estrogen-responsive ring finger protein (Efp) gene, one of estrogen receptor (ER) target genes, is considered to be essential for estrogen-dependent cell proliferation. To understand the estrogenic action on ovarian cancer, we studied the relationships between Efp and ERs expressions and the correlations of Efp expression with clinicopathological parameters in epithelial ovarian cancer.

Methods. The protein expressions for Efp, ERα and ERβ were examined by immunoblotting in 12 ovarian cancer cell lines. Efp mRNA expressions were evaluated by quantitative RT-PCR in 12 ovarian cancer cell lines. A total of 100 surgical specimens diagnosed as epithelial ovarian cancer were examined immunohistochemically using antibodies for Efp, ERα and ERβ.

Results. Efp protein was detected in 8 out of 12 cell lines. In Efp protein-positive cell lines, Efp mRNA was expressed higher than that in negative ($P = 0.021$). All of the Efp protein-positive cell lines simultaneously expressed either ERα or ERβ protein. By immunohistochemical staining, Efp immunoreactivity was detected in 63 out of 100 ovarian cancer specimens and positive signals were in the cytoplasm of carcinoma cells. There were significant correlations between Efp and ERα, ERβ immunoreactivity (Efp and ERα, $P = 0.022$; Efp and ERβ, $P = 0.032$). Efp expression was significantly higher in a subgroup with serous adenocarcinoma ($P = 0.010$) and with advanced disease ($P = 0.026$). No significant relationship was detected between Efp immunoreactivity and overall survival.

Conclusion. The expression of Efp was detected in human epithelial ovarian cancer and high expression of Efp was correlated with advanced disease and serous adenocarcinoma, and ERs status.

Keywords: Efp; Ovarian cancer; Immunohistochemistry; Estrogen receptor

Introduction

Estrogen and progesterone are sex steroid hormones that are secreted from the ovary and cause the development of the female sex organs. They are also recognized as a significant modifier of the growth, development, invasion and metastasis of gynecological cancers. The actions of estrogen are mediated through specific ligand receptors. There are two receptor subtypes for estrogen, estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) [1,2]. It is assumed that these receptors, members of the steroid/thyroid hormone receptor superfamily mediate these actions by binding ligand dependently to the estrogen-responsive element (ERE) that is located in the promoter region of target genes, thus directly regulating their transcription [3,4]. A variety of estrogenic functions are characterized by the expression of the estrogen-responsive genes following the binding of receptor protein to EREs [5,6].

Estrogen-responsive finger protein (Efp) is a member of the Ring-finger B-box Coiled-Coil family that is thought to be involved in the regulation of various celluer functions, including cell-cycle regulation and gene transcription [5,7].

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Efp has been isolated from human genomic DNA binding-site cloning using a recombinant ER protein [5]. Efp gene has an estrogen-responsive element (ERE) in an exon corresponding with the 3′-untranslated region of mRNA [5,8]. Efp is widely expressed in various organs and structures such as the genital tracts, thyroid gland, aorta, spleen, kidney and brain [9]. Estrogen-induced Efp expression is found in the uterus, brain and mammary gland cells [5,8], and its expression is co-localized with ER [9]. A study of knock-out mice has revealed that Efp is essential for cell growth mediated by estrogen in the uterus [6], suggesting that Efp is essential for estrogen mediated cell growth.

Efp expression in the context of cancer has been studied predominantly in breast cancer. Efp mRNA was detected in the MCF-7 human breast carcinoma cell line, where it was induced by estrogen treatment within 0.5 h [10], suggesting that Efp can mediate estrogen actions such as cell growth as a primary responsive gene in breast cancer [10,11]. Recently, it has been suggested that negative cell-cycle regulators, such as 14-3-3 sigma are reduced in Efp-positive breast cancer cells because Efp targets 14-3-3 sigma for proteolysis as an ubiquitin ligase [11]. Thus, Efp may play roles not only as an estrogen target gene but also as a cell-cycle regulator.

Epithelial ovarian cancer is the leading cause of death due to a gynecological malignancy in the great majority of developed countries [12,13]. Sex steroid hormones have been implicated in the etiology and/or progression of some epithelial ovarian cancers, but the possible biological significance of steroid hormone actions in these cancers remains controversial [14–17]. The expression of Efp has not been examined in human epithelial ovarian cancer tissues, and thus the biological significance of Efp expression and correlation between the expression of Efp and ERs expression in this cancer have not yet been studied. We need to understand the new molecular targets or biological makers related to estrogenic actions for ovarian cancer as well as for those associated with breast cancer. In the current study, we examined the expression of Efp in human ovarian cancer tissues and cell lines.

Materials and methods

Cell lines

We used 12 ovarian carcinoma cell lines, two normal ovarian surface epithelial cell lines and one breast cancer cell line as follows. The seven cell lines OVCAR3, Caov3, SKOV3, TOV112D, TOV21G, OV90 and ES2 (adenocarcinoma OVCAR3, SKOV3; serous adenocarcinoma Caov3, OV90; clear cell adenocarcinoma TOV21G, ES2; endometrioid adenocarcinoma TOV112D) were purchased from American Type Culture Collection. The five cell lines JHOS2, JHOS3, HTOA, OMC3 and JHOC5 (serous adenocarcinoma JHOS2, JHOS3, HTOA; mucinous adenocarcinoma OMC3; clear cell adenocarcinoma JHOC5) were purchased from Riken cell bank (Tsukuba, Japan). Two cell lines OSE2 and OSE4 established from normal ovarian epithelial cells were kindly provided by the Department of Obstetrics and Gynecology, Kumamoto University School of Medicine, Kumamoto, Japan [18]. MCF-7 (the human breast cancer cell line) was provided by the Institute of Department, Aging and Cancer, Tohoku University, Sendai, Japan. Cell lines were maintained in DMEM/F12 medium (Invitrogen, CA, USA), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen) and incubated in a 5% CO_2 atmosphere at 37°C.

Surgical specimens and clinical data

We examined surgical specimens from a total of 100 cases of common epithelial ovarian carcinoma obtained from patients treated between 1988 and 2000 at Tohoku University Hospital, Sendai, Japan. Information regarding age, performance status on admission, histology, stage, grade, residual tumor after primary surgery and overall survival was retrieved from the review of patient charts. Median follow-up time for patients was 59 months (range, 4–120 months). Of the 100 patients, 77 (77%) received optimal cytoreduction at time of surgery, 84 (84%) patients received platinum-based chemotherapy postoperatively. Patients with stage Ia disease, low grade-disease (G1, G2) or poor performance status did not receive platinum-based chemotherapy. Performance status was defined according to WHO criteria (World Health Organization, 1979). Histology, stage and grade were according to FIGO criteria (International Federation of Gynecology and Obstetrics; [19]). Residual tumor was determined by the amount of unresectable tumor left following primary cytoreductive surgery. Optimal cytoreduction was defined as no gross residual tumor or less than 2 cm in diameter, whereas suboptimal cytoreduction was defined as any gross residual tumor remaining 2 cm or residual tumor greater than 2 cm in diameter. Overall survival was calculated from the time of initial surgery to death or the date of the last contact. Survival times of patients still alive or lost to follow-up were censored as of December 2002. All of these archival specimens were retrieved from the surgical pathology files at Tohoku University Hospital, Sendai, Japan. The informed consent was obtained from each patient. These specimens were all fixed in 10% formalin and embedded in paraffin. The research protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan.

Quantitative reverse transcription-PCR

Total RNA was isolated from cells by phenol-chloroform extraction using Isogen (Nippon gene, Tokyo, Japan). RNA was treated with RNase-free DNase (Roche Diagnostics; 1 μg/μl) for 2 h at 37°C, followed by heat inactivation at 65°C for 10 min. A reverse transcription (RT)-PCR kit (SUPER-

SCRIPT II First-strand synthesis system, Invitrogen) was used and cDNA synthesis was carried out according to the manufacturer's instructions. cDNAs were synthesized from 5 μg of total RNA using random hexamer and RT was carried out for 50 min at 42°C with SUPERSCRIPT II reverse transcriptase. Quantitative PCR was performed using an iCycler system (Bio-Rad, Tokyo, Japan). For the determination of Efp cDNA content, a 25 μl-reaction mixture consisting of 23 μl iQ™SYBR Green MasterMix, 1 μl each primer and 1 μl of template cDNA was prepared. PCR conditions were as follows: 2-min denaturation at 90°C, 30-s annealing at 60°C (for Efp), 62°C (for β-actin) and 1.5-min extension at 72°C. Primers for PCR reactions were as follows: Efp-F, 5′-CGTGGAGTGGTTCAACAC-3′ and Efp-R, 5′-GAGCAGATGGAGATGGTG-3′ (1689–1923, 234 base pairs, bp); β-actin-F, 5′-CCAACCGCGAGAAGATGAC-3′ and β-actin-R, 5′-GGAAGGAAGGCTGGAAGAGT-3′ (382–841, 459 bp). In initial experiments, following amplification, PCR products were purified and subjected to direct sequencing to verify amplification of correct sequences (ABI prism 310 Genetic Analyzer, Applied Biosystems, CA, USA). β-Actin primers were utilized as a positive control and Efp expression level was calculated as value of Efp RT-PCR divided by value of β-actin RT-PCR. Negative controls without RNA and without reverse transcriptase were also performed.

Immunoblotting

Cells were grown to 70% confluence in 10-cm plates and after removal of culture medium with Phosphate-buffered saline (PBS). Whole-cell protein concentration was measured by Model 680 microplate reader (Biorad, USA) using BradFord reagent (Biorad). A rabbit polyclonal antibody against Efp protein was made by one of the authors (SI). Mouse monoclonal antibody for ERα was purchased from NOVOCASTRA (Newcastle, UK). Mouse monoclonal antibody for ERβ was purchased from GeneTex, Inc. (TX, USA). In all, 20 μg of protein of each sample was mixed with an equal volume of 2× concentrated sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, boiled and then electrophoresed on 7% ready-made gels containing SDS (Mini Protein II Western blotting system, Biorad). Proteins were then transferred to nitrocellulose membrane (Hybond PDVF, Biorad). The membranes were incubated in blocking solution (PBS containing 5% nonfat milk and 0.05% Tween-20), then incubated in 1:4000 dilution of Efp antibody (1:100 for ERα, 1:1500 for ERβ and 1:1000 for β-actin) in blocking solution overnight at 4°C. After incubation with horseradish peroxidase (HRP)-labeled anti-rabbit IgG (anti-mouse IgM for ERα and ERβ) (Vector Laboratories, USA), the antigen–antibody complex was visualized with ECL system (Amersham, Germany). The MCF-7 breast cancer cell line was used as positive control. Actin (Ab-1, Oncogene) was used as an internal positive control.

Immunohistochemistry and scoring of immunostaining

Immunohistochemical analysis was performed using a streptavidin–biotin amplification method using the Histofine Kit (Nichirei, Tokyo, Japan). For antigen retrieval, slides were heated in an autoclave at 120°C for 5 min in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate gyhydrate, pH 6.0). The dilutions of primary antibodies for Efp, ERα and ERβ were 1:2000, 1:50 and 1:1500, respectively. The antigen–antibody complex was visualized with 3,3′-diaminobenzidine (DAB) solution (1mM DAB, 50mM Tris–HCl buffer (pH 7.6) and 0.006% H_2O_2), and counterstained with hematoxylin. The ER positive normal breast tissue was used as a positive control. For statistical analysis of Efp immunoreactivity, we classified carcinomas into two groups: +, positive carcinoma cells; and −, no immunoreactivity. For evaluation of ERα and ERβ immunoreactivity, we used the H score system to count carcinoma cells as described previously [20,21]. Scores were generated as follows: (3 × [percentage of strongly staining cells]) + (2 × [percentage of moderately staining cells]) + (1 × [percentage of weakly staining cells]). This scoring system yielded results ranging from 0 to 300. Evaluation was carried out independently by two of the authors (MS and JA) for at least 500 cells.

Statistical analysis

Statistical analysis was performed using Stat View 5.0 (SAS Institute Inc., NC, USA) software. The correlation between expression of Efp mRNA and protein was also assessed using the Mann–Whitney *U* test. The statistical significance between Efp immunoreactivity and clinicopathological parameters was evaluated using Friedman's χ_{2r}-test. The correlation between Efp and ERα, ERβ immunoreactivity was assessed using the Mann–Whitney *U* test. The univariate analysis of prognostic significance was performed using the log-rank test after each survival curve was obtained by the Kaplan–Meier method. All patients who could be assessed were included in the intention-to-treat analysis. A result was considered significant when the *P* value was less than 0.05.

Results

First, we examined Efp expression in ovarian cancer cells. By immunoblotting with anti-Efp antibody, immunoreactive bands corresponding to Efp, sized at approximately 70 kDa, were detected in 8 out of 12 ovarian cancer cell lines (Fig. 1). Efp expression in cell lines was supported with data obtained by quantitative RT-PCR study. Of the 12 ovarian cancer cell lines, 8 were positive for Efp protein expression by immunoblotting showing relatively higher levels of Efp-mRNA than seen in the 4 cell lines negative for Efp protein expression (Fig. 2) ($P = 0.021$). From these

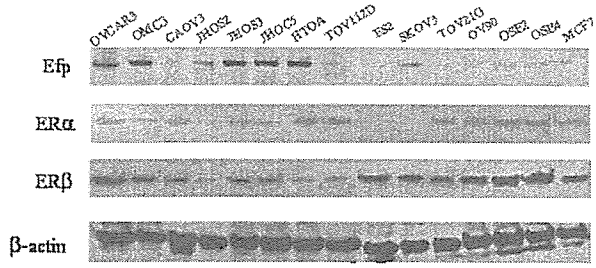

Fig. 1. Immunoblotting with variable cell lines, the top panel with anti-Efp antibody, the second panel with anti anti-ERα antibody, the third panel with anti-ERβ antibody and the bottom panel with anti β-actin antibody. OVCAR3, OMC3, CAOV3, JHOS2, JHOS3, JHOC5, HTOA, TOV112D, ES2, SKOV3, TOV21G and OV90 were derived from ovarian cancer. OSE2 and OSE4 were derived from normal ovarian surface epithelium. MCF-7 was a cell line derived from breast cancer and used as a positive control of Efp and ERs expressions. β-Actin was used as an internal positive control of antibody.

results, we were convinced that cell lines established from not only normal ovarian epithelium but also ovarian cancers expressed Efp genes at various levels similar to results seen with breast cancer cell lines.

Because the Efp gene was expressed by estrones through estrogen receptors, we next examined ERs protein expression in these cell lines (Fig. 2). By immunoblotting with anti-ERα antibody, 10 out of 12 ovarian cancer lines and the 2 cell lines from normal ovarian epithelium showed positive bands. All cell lines positive for Efp protein expression, except SKOV3, were simultaneously positive to ERα. Similarly, all cell lines showed positive bands for ERβ by immunoblotting with anti-ERβ antibody. From these results, we knew that all of the Efp-immunoreactive cell lines simultaneously expressed either ERα or ERβ protein.

Then, we performed immunohistochemical staining with anti-human Efp antibody for the 100 surgical specimens diagnosed as ovarian cancer to confirm Efp expression in ovarian cancer tissues. Efp protein expression was detected in 63 out of 100 specimens (63%). Positive staining was observed in the cytoplasm of ovarian cancer cells (Figs. 3A, D).

We then compared Efp expression and various clinicopathological parameters; results are summarized in Table 1. Differences by histological types were detected in Efp expression, i.e., the subgroup of serous adenocarcinomas showed significantly higher incidence of Efp positivity than other subgroups ($P = 0.010$). Similarly, the subgroup of advanced-stage disease showed a significantly higher incidence of Efp positivity than the subgroup consisting of early-stage disease ($P = 0.026$). There were no significant relationships between Efp immunoreactivity and patient age, performance status, histological grade or residual tumor (Table 1).

We decided to examine simultaneous ER and Efp expression in cancer tissues because Efp is mainly transactivated by ERs. Immunohistochemical studies showed that all ovarian cancer tissues were positive for both ERα and ERβ to a greater or lesser extent, and immunopositive signals were confined exclusively to the nuclei of tumor cells (Figs. 3B, C, E, F). The median H scores for ERα in Efp-immunopositive and Efp-immunonegative tumors were 80.1 ± 70.3 and 39.5 ± 59.4 (mean ± SD), respectively, indicating that Efp-positive cancers expressed significantly higher levels of ERα than Efp-negative cancers ($P = 0.022$). In the same way, the median H score for ERβ in Efp-immunopositive and Efp-immunonegative tumors was 67.7 ± 56.2 and 43.0 ± 43.7 (mean ± SD), respectively, indicating that Efp-positive cancers expressed significantly higher levels of ERβ than Efp-negative cancers ($P = 0.032$). Interestingly, the subgroup of serous adenocarcinomas showed significantly higher H scores (112.1 ± 60.7) than those of the other subgroups (29.7 ± 51.8) ($P < 0.0001$). In contrast, this tendency was not observed in immunoreactivity of ERβ among each histologic subgroup (data not shown).

Finally, we examined the possibility of Efp as a clinical prognostic factor by univariate analysis. As shown in Table 2, clinical variables including histologic type, grade, stage and residual tumor size were all significantly related with overall survival. These results seem to be consistent with data described previously [13,22,23]. With regard to analysis of Efp expression, we did not find any significant correlation between Efp immunoreactivity and overall survival ($P = 0.78$).

Discussion

In this study, we found strong correlations between Efp and ERα and between Efp and ERβ in ovarian cancer tissues; we also found that both ERα and ERβ proteins were expressed in most cancer cell lines with positive for Efp protein. Efp mRNA and protein are up-regulated by

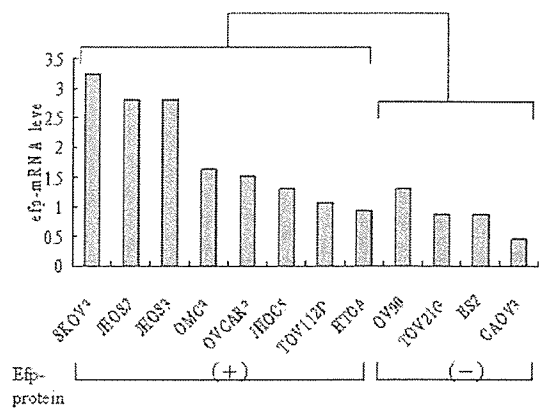

Fig. 2. Quantitative RT-PCR for expression of Efp mRNA in ovarian cancer cell lines. RT-PCR reactions were performed for each samples, and the ratio of Efp; β-actin was calculated and normalized. The left 8 cell lines were positive for Efp protein by immunoblotting, as determined from results of Fig. 1. Efp mRNA expression among cell lines positive for Efp protein was significantly higher than those among cell lines that are negative.

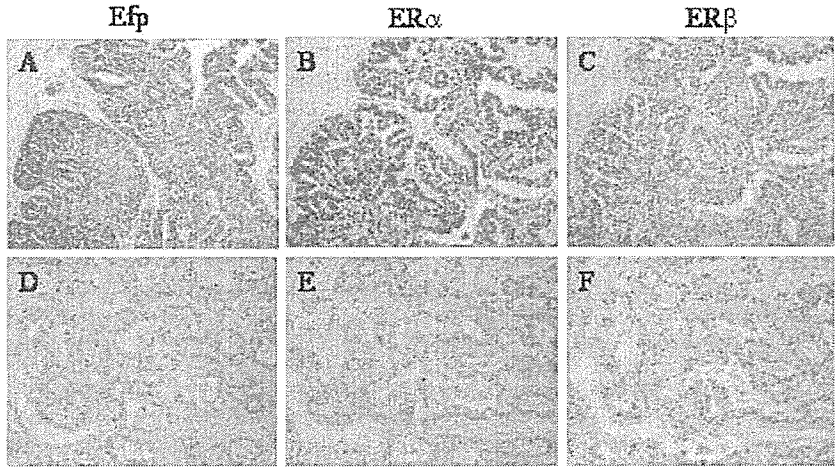

Fig. 3. Immunohistochemistry for Efp, EPα and ERβ in ovarian cancer tissues. Serial sections of each surgical specimens were stained with anti-Efp (A and D), anti-ERα (B and E) and anti-ERβ (C and F) antibodies, respectively. Representative data positive for each antibody (A–C) and negative (D–F) were shown. Positive signals for Efp were detected on the cytoplasm of cancer cells and positive signals for both ERα and ERβ were confined to the nuclei of cancer cells.

estrogen treatment in vivo [5,8]. Estrogen-responsive proliferation of uterine cells which express abundant ERα was impaired in Efp knock-out mice, suggesting that Efp is a mediator of cell proliferation as one of the direct targets of ERα [6]. In breast cancer, which is considered one of the sex steroid hormone-related malignant neoplasms, the Efp promoter could be enhanced by both ERα and ERβ in the setting of estrogen treatment. It has been suggested that Efp responds to estrogen as a common downstream gene of ERα and ERβ and that Efp mediates estrogen action in both ERα- and ERβ-positive breast cancer [10]. Results from our present study are consistent with these previous reports focusing on breast cancer [6,10], and suggest that Efp may mediate estrogen action through ERα and ERβ in some human epithelial ovarian cancer tissues and cell lines.

However, responsiveness and prognosis impact of steroid hormone in ovarian cancer may be different from those in breast cancer. Responsiveness to hormonal manipulation clearly results in favorable feature of breast cancer. It is known that most of the ER-positive breast cancers are primarily responsive to endocrine therapy, but breast cancer lacking any ER expression often reveals more aggressive phenotypes and is resistant to endocrine therapy [24]. Human epithelial ovarian cancer is believed to be a sex steroid hormone-related neoplasm, although the biological significance and the prognostic impact of hormone receptors are still controversial [14–17]. Previous studies have suggested a relation between progesterone receptor expression and favorable prognosis of epithelial ovarian cancer; especially, tumors of ER-negative/PR-positive showed a significantly superior prognosis when compared with the other combinations [14,25,26]. These evidences suggest that

Table 1
Association between Efp immunoreactivity and clinicopathological parameters in human epithelial ovarian cancer

	Total	Efp immunoreactivity		*P* value
		+	−	
		63	37	
Age				
≦50	50	33	17	
>50	50	30	20	NS
Performance status				
0, 1	70	44	26	
2, 3, 4	30	19	11	NS
Histological type				
Serous	43	34	9	
Mucinous	14	10	4	
Endometrioid	15	7	8	
Clear cell	27	11	16	
Squamous cell	1	1	0	0.01
Histological grade				
Grade 1	41	23	18	
Grade 2	35	22	13	
Grade 3	24	18	6	NS
Stage				
I, II	55	23	22	
III, IV	45	40	15	0.026
Residual tumor				
≦2cm	59	34	25	
>2cm	41	29	12	NS
ERα H score		80.1 ± 70.3	39.6 ± 59.4	0.022
ERβ H score		67.7 ± 56.2	43.0 ± 43.7	0.032

Histological type: serous, serous adenocarcinoma; mucinous, mucinous adenocarcinoma; endometrioid, endometrioid adenocarcinoma; clear cell, clear cell adenocarcinoma; squamous, squamous cell carcinoma.

Table 2
Univariate analysis of overall survival

Variable	*P* value
Efp immunoreactivity (+ vs. −)	0.78
Histological type	0.018
Histological grade	0.0085
Stage	<0.0001
Residual tumor	<0.0001