Table 7
Clinicopathological factors related to PAN metastasis in ≥2 positive PLN sites with PLN+PAN adenectomy group

	PAN negative (n=7)	PAN positive (n=14)	p value
Age (year)	57.3±11.6	59.2±8.2	0.663
Prognostic factor			
Histological subtype			
Endometrioid	6	12	0.753
Serious/Clear	1	2	
Architectural grade			
Grade 1/2	4	10	0.873
Grade 3	3	4	
Lymph-vascular space invasion			
Nil/minimal	0	2	0.433
Moderate/prominent	7	12	
Myometrial invasion			
≤1/2	2	3	0.557
>1/2	5	11	
Cervical invasion			
Negative/cervical gland	6	10	0.443
Stromal invasion	1	4	
Peritoneal cytology			
Negative	5	12	0.407
Positive	2	2	
Ovarian metastasis			
Negative	7	11	0.274
Positive	0	3	

PLN: pelvic lymph node, PAN: para-aortic lymph node.

difference in the positive PLN sites between these groups (Table 1, p=0.140), the incidence of initial recurrence of PAN was significantly higher in the PLN adenectomy group (24.0%, 6/25) than in the PLN+PAN adenectomy group (2.6%, 1/38; p=0.013, Odds Ratio=11.68) (Table 6).

Clinicopathological factors related to PAN metastasis in ≥2 positive PLN sites with PLN+PAN adenectomy group were also compared (Table 7). The mean age of the PAN negative group (n=7) was 57.3±11.6 years, while that of the PAN positive group (n=14) was 59.2±8.2 years (p=0.663). There were no statistically significant differences between the two groups in the histopathological prognostic factors: histological subtype (p=0.753), architectural grade (p=0.873), lymph-vascular space invasion (p=0.433), myometrial invasion (p=0.557), cervical invasion (p=0.443), peritoneal cytology (p=0.407), and ovarian metastasis (p=0.274).

Discussion

The Federation of Gynecology and Obstetrics (FIGO) announced a surgical staging system that classifies endometrial carcinoma with metastasis to the pelvic and/or para-aortic lymph nodes as stage IIIc in 1988. Lymph node metastasis is one of the most critical prognostic factors of endometrial carcinoma. Many authors have studied the therapeutic role of lymph node adenectomy, focusing mainly on PLN adenectomy in patients with endometrial cancer [1–6]. Although the diagnostic importance of PAN adenectomy has been established, the therapeutic relevance has not yet been clearly evaluated. Therefore, there is no consensus on whether to extend the lymphadenectomy to the para-aortic area. Although many gynecologists would agree that patients with endometrial carcinoma who have a grade I tumor without myometrial invasion do not need lymphadenectomy, there has been no standard method for selecting patients who do not need PAN adenectomy.

In our series of patients with endometrial cancer treated in the same manner, the prognosis of patients with stage I (PLN adenectomy group; n=218, PLN+PAN adenectomy group; n=185), stage II (n=30, n=33), and stage IIIa (n=32, n=41) was excellent, with 5-year DRS of 97.7%, 94.3%, and 86.7%, respectively. For each stage, PAN adenectomy did not improve DRS. Our results on PAN adenectomy were similar to those of Cragun et al. [5]; PAN adenectomy did not improve survival in patients with early-stage endometrial cancer. Therefore, we focused on stage IIIc patients. Mariani et al. [7], based on their study of 51 patients with stage IIIc endometrial cancer, reported that PAN adenectomy had a potential therapeutic role. While in our study PAN adenectomy had a tendency to improve DRS in stage IIIc endometrial cancer patients as a group, this difference was not statistically significant (Fig. 1). This might be due to the number of stage IIIc patients, i.e., according to the statistical analysis, at least 189 stage IIIc patients (negative PAN patients 75, positive PAN patients 114) are necessary to approve the statistical significance of PAN adenectomy in stage IIIc endometrial cancer patients. Since this study consists of 602 endometrial cancer patients (stage I: 403, II: 63, IIIa: 73, IIIc: 63), a total of 1800 endometrial cancer patients are estimated to prove the significance of PAN adenectomy. However, in patients with ≥2 positive PLN sites, PAN adenectomy resulted in a statistically significant improvement in DRS in patients with stage IIIc endometrial cancer (Fig. 3). We also analyzed whether PAN metastasis affects the prognosis in PLN+PAN adenectomy group (Fig. 2). Five-year DRS was 82.4% in the PAN negative group and 43.5% in the PAN positive group (p=0.039). This result was similar to Watari et al. [10] who applied chemotherapy as adjuvant therapy for stage IIIc endometrial cancer patients as same as our study.

Extensive PAN adenectomy added surgical complications in mean operative time and mean estimated blood loss compared with PLN adenectomy alone (Table 2). However, no patients had complications caused by the prolongation of anesthesia. Also, autologous blood reservation before surgery reduced homologous blood transfusion to 12.9% (4/31) in PLN+PAN adenectomy group (7 patients with no available data).

Our patients were treated with adjuvant chemotherapy after surgery, including a combination of adriamycin and cisplatin, with or without cyclophosphamide (CAP/AP), or a combination of paclitaxel and carboplatin (TC). There was no difference in DRS between these two groups (p=0.781).

On multivariate analysis in the present study, the number of positive PLN sites was an independent prognostic factor (Table 3), and PAN metastasis was a confounding factor of the number of positive PLN sites (Table 4). Our result on nodal distribution in stage IIIc patients is similar to the results of recent reports [10–12]; an increasing number of positive PLN sites was

associated with PAN metastasis. Based on this result, it appears clear that incomplete PAN adenectomy in patients with ≥2 positive PLN sites is not likely to confer a therapeutic benefit. Recently, Chan et al. [13] reported that there was a relationship between the number of lymph nodes resected and the survival of patients with intermediate/high risk endometrioid uterine cancer. They stratified the total number of lymph nodes resected into three groups (≤10 nodes, 11–20 nodes, >20 nodes); they concluded that the extent of lymph node resection improved the survival of patients with intermediate/high risk endometrioid uterine cancer. Also, the rate of lymph node metastasis increases in proportion to the number of resected lymph nodes [14]. Conversely, cases false-negative for lymph node metastasis will increase if there are only a few lymph nodes resected. In our study, the median number of pelvic lymph nodes resected was 51 (20–73), and the median number of para-aortic lymph nodes resected was 21 (10–58). This suggests that a sufficient lymphadenectomy was done to permit statistical analysis.

Since our current retrospective analysis demonstrated the therapeutic benefit of PAN adenectomy in patients with ≥2 positive PLN sites, we examined the clinicopathological factors associated with ≥2 positive PLN sites (Table 5). However, we could not identify any. Recently, new molecular markers have attracted attention. Ohkouchi et al. [15] reported that p53 overexpression on immunohistochemical staining was found to be an independent prognostic factor in patients with stage III/IV endometrial cancer; DRS was significantly better in patients without p53 overexpression than in those with p53 overexpression, indicating that p53 missense mutation, which is closely related to immunohistochemical p53 overexpression, has a significant prognostic impact on the survival of patients with advanced endometrial carcinoma. Kanamori et al. [16] reported that PTEN-positive staining was a significant prognostic indicator of favorable survival for patients with advanced endometrial carcinoma who underwent postoperative chemotherapy. Yokoyama et al. [17] reported that high levels of immunoreactivity for vascular endothelial growth factor (VEGF)-D in stromal cells, and its receptor, VEGFR-3, in carcinoma cells, were independent prognostic factors in endometrial carcinoma. They also suggested that the presence of VEGF-D and VEGFR-3 may predict lymph node metastasis in patients with endometrial carcinoma. Although we could not identify risk factors for ≥2 positive PLN sites, further investigation involving molecular biological techniques using pre-operative biopsy specimens may be useful to determine the necessity of PAN adenectomy before radical surgery. Also in the past report, Todo et al. [18] reported that pre-operative volume index of the tumor, CA125 level, and tumor grade/histology were independent risk factors for lymph node metastasis in 211 patients with endometrial cancer. But they could not detect independent risk factors for para-aortic lymph node metastasis. As we focused on stage IIIc endometrial cancer in this study, these clinicopathological prognostic factors were not independent risk factors (data not shown). In our study, we found that para-aortic lymph node metastasis is a confounding factor of pelvic lymph node metastasis, i.e., the incidence of PAN metastasis is frequent in ≥2 positive PLN sites compared with ≤1 positive PLN site. From these analyses, a large prospective multicenter clinical trial needs to be conducted to establish the risk factors for ≥2 positive PLN sites before radical surgery whether to perform PAN adenectomy or not.

We also compared the incidence of initial recurrence of PAN in the PLN adenectomy group and the PLN+PAN adenectomy group (Table 6). The incidence of initial recurrence of PAN was statistically higher in the PLN adenectomy group than in the PLN+PAN adenectomy group, which suggests that PAN adenectomy improves PAN recurrence. Although a different adjuvant therapy was performed between our study and Mariani et al. [7,19], i.e., chemotherapy and extended-field radiotherapy, this result of minimal para-aortic recurrence in patients who had extensive PAN adenectomy was similar to Mariani et al.. These results might suggest that extensive PAN adenectomy with adjuvant chemotherapy has almost the same curative effectiveness as PAN adenectomy with adjuvant radiotherapy. In our study, the 5-year PFS (progression free survival) and DRS in all stage IIIc patients ($n=63$) treated with adjuvant chemotherapy after surgery was 58.7% and 62.6%, respectively, whose results were similar to retrospective study reported by Mariani et al. [7,19]. Recently, Randall et al. [20] reported that adjuvant chemotherapy (doxorubicin and cisplatin) significantly improved PFS and DRS compared with adjuvant radiotherapy for stage III/IV endometrial cancer patients. They also reported that, so far as stage IIIc endometrial cancer patients are concerned, adjuvant chemotherapy improved PFS ($p=0.040$) and DRS ($p=0.044$) compared with adjuvant radiotherapy.

We also examined the clinicopathological factors related to PAN metastasis in ≥2 positive PLN sites with PLN+PAN adenectomy group (Table 7). However, we could not identify any. A large prospective clinical trial needs to be conducted to evaluate the clinicopathological factors.

In conclusion, this is the first report to identify that the number of positive PLN sites is an independent prognostic factor in patients with endometrial cancer, and that PAN adenectomy may be indispensable for improving DRS in patients with ≥2 positive PLN sites. Furthermore, PAN adenectomy improves PAN recurrence, which may be the source of other organ metastases.

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A Change in Promoter Methylation of *hMLH1* is a Cause of Acquired Resistance to Platinum-based Chemotherapy in Epithelial Ovarian Cancer

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Abstract. *Background: Acquired resistance to platinum-based chemotherapy (Pt-chemo) is a major problem for improving the prognosis for patients with advanced epithelial ovarian cancer (EOC). However, the molecular mechanism of acquired resistance to Pt-chemo is not well understood. Materials and Methods: hMLH1 promoter methylation (hMLH1 MET) and hMLH1 protein expression was examined in 36 paired samples of primary and secondary resected tumors by methylation-specific polymerase chain reaction (PCR). Results: No primary tumors exhibited hMLH1 MET, while 56.3% of secondary tumors showed hMLH1 MET. Moreover, no significant correlation was observed between hMLH1 MET and histological subtype, while hMLH1 MET was significantly greater ($p<0.001$) in partially responsive secondary tumors compared with no change or progressive disease, and hMLH1 MET also occurred more frequently ($p=0.059$) in tumors treated with four or more courses of Pt-chemo. Conclusion: A change in hMLH1 MET is a major molecular cause of acquired resistance to Pt-chemo in EOC.*

Platinum-based chemotherapy (Pt-chemo) for advanced primary epithelial ovarian cancer (EOC) has produced survival effects as shown by the results of phase III trials (1). However, the complete remission rate with Pt-chemo has remained at 30% (1)-50% (2). These results mean that more than half of the patients with advanced EOC are left with disseminated tumors even after treatment with Pt-chemo. Therefore, a major problem in improving the long-term prognosis for patients with advanced EOC is resistance to Pt-chemo, especially acquired resistance during postoperative induction chemotherapy. However, although many molecular biological factors, such as *mdr* (3) and *p53* (4), have been investigated, these factors have been found to correlate mainly with intrinsic resistance, and the mechanism of acquired resistance to Pt-chemo is not well understood. We previously reported that tumor microsatellite instability (MSI) changed after Pt-chemo, and that the loss of *hMLH1* protein expression affects this transformation to microsatellite instability (5). However, the reason why *hMLH1* protein changes during Pt-chemo treatment is still unknown. Therefore, in order to investigate the mechanism of change in *hMLH1* expression during Pt-chemo, a comparative study of promoter methylation of *hMLH1* (*hMLH1* MET) in paired specimens of resected primary tumor and secondary tumor resected after treatment with Pt-chemo was conducted.

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Key Words: hMLH1, methylation, acquired resistance, ovarian cancer, platinum-based chemotherapy.

Materials and Methods

Seventy-two specimens were collected from 36 patients with EOC who were treated in our department from 1999 to 2005. Selection criteria were as follows: patients having received at least one course of postoperative chemotherapy and secondary cytoreductive surgery, aged between 19 and 71 years, and with <2 cm of residual tumor to assess the direct effects of postoperative chemotherapy. Tumor samples were collected soon after resection at primary and secondary surgery and stored at -80°C until analysis by polymerase chain reaction (PCR). The assay for microsatellite instability (MSI) of the tumors and criteria of MSI types were according to our previous report (7). For the assay of *hMLH1* promoter methylation (*hMLH1* MET), CpGenome™ Fast DNA Modification Kits (Chemicon International, Temecula, CA, USA) and CpG WIZ hMLH1 Amplification Kits (Chemicon International) were used. After PCR amplification, if a 108 bp PCR product was seen only in reactions performed using an M-primer while no PCR product was seen either as a 124 bp product using a U-primer or a 130 bp product using a W-primer, it was scored as *hMLH1* MET. Immunohistochemical staining of the *hMLH1* protein was also performed using an anti-hMLH1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with

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a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). The Chi-square test was used for statistical analysis and a *p*-value of less than 0.05 was regarded as statistically significant. Full informed consent was obtained from all patients prior to examination of the specimens.

Results

Clinicopathological features of the patients are shown in Table I. Twenty-two patients received paclitaxel 180 mg/m^2 + carboplatin AUC (area under the concentration curve) = 6 every three weeks, eight patients received docetaxel 70 mg/m^2 + cisplatin (CDDP) 60 mg/m^2 every three weeks, and six patients received cyclophosphamide 750 mg/m^2 + CDDP 75 mg/m^2 every three weeks as postoperative chemotherapy. Secondary cytoreductive surgery was performed with a final outcome scored as a partial response (PR), no change (NC), or progressive disease (PD) based on the World Health Organization criteria (1979) by assessment using magnetic resonance imaging every four weeks.

A total of 64 samples from 32 (88.9%) cases produced usable results for *hMLH1* MET. In these cases, no primary tumor exhibited *hMLH1* MET while 18 (56.3%) of the secondary tumors, resected after treatment with Pt-chemo, showed a change from unmethylated *hMLH1* promoter to *hMLH1* MET. Table II shows the microsatellite status and *hMLH1* protein expression in the *hMLH1* MET secondary tumors. No *hMLH1* MET tumor showed expression of *hMLH1* protein, and 11 out of 16 cases where microsatellite status was determined (68.8%) were defined as MSI-H (high frequency of microsatellite instability) tumors. These results suggest that a change in *hMLH1* MET after Pt-chemo in EOC correlates with both loss of *hMLH1* protein expression and microsatellite instability.

Table III shows the correlation between clinicopathological factors and *hMLH1* MET status. The frequency of *hMLH1* MET was significantly greater in cases with a PR to platinum-based chemotherapy than in cases with NC or PD, while no significant differences were found by histological subtype (serous or non-serous), type of platinum agent used (CDDP: cisplatin, or CBDCA: carboplatin), or whether taxanes (paclitaxel and docetaxel) were combined with the Pt-chemo or not. In addition, no *hMLH1* MET was found in secondary tumors after fewer than 4 courses of treatment, while 64.3% of tumors treated with 4 or more courses of Pt-chemo exhibited *hMLH1* MET (p=0.059). These results suggest that *hMLH1* MET occurred after about three courses of Pt-chemo, and that the responsiveness of tumors during Pt-chemo was associated with the development of *hMLH1* MET. Therefore, *hMLH1* MET would not be a cause of intrinsic resistance but a major molecular cause of acquired resistance to Pt-chemo in EOC.

Table I. *Clinicopathological features of the patients.*

Total number of cases	36
Mean age (range)	54.2±8.3 years (21-68)
Histological subtype	
Serous	28
Mucinous	4
Endometrioid	5
Clear-cell	2
Regimens of chemotherapy	
Paclitaxel + CBDCA	22
Docetaxel + CDDP	8
Cyclophosphamide + CDDP	6
Mean treatment courses (range)	5.7±1.6 (1-13)
Direct effects of chemotherapy	
Partial response	19
No change	10
Progressive disease	7
Mean treatment dose of platinum agents (range)	
CDDP (mg)	417.9±90.9 (320-630)
CBDCA (mg)	3543.5±956.4 (1820-6231)

CDDP, cisplatin; CBDCA, carboplatin; therapeutic effect was graded by WHO criteria.

Discussion

Since DNA mismatch repair (MMR)-deficient cell lines exhibit resistance to alkylating agents (6-10), many studies have been conducted to determine correlations between the function of the MMR system and resistance to anticancer agents, especially CDDP resistance. Brown *et al.* (8) reported that the proportion of negative expression of *hMLH1* increased in samples taken after chemotherapy compared to untreated tumors while no significant difference was observed for *hMSH2*, *hMSH6*, or *hPMS2*. Furthermore, Fink *et al.* (9) reported that lack of *hMSH2* activity was also a cause of CDDP resistance. Aebi *et al.* (10) compared characteristics of resistance to CDDP among *hMLH1*, *hMSH2* deleted or mutated MMR-deficient cell lines and MMR-proficient clones by complementation of each gene by chromosome transfer, and reported that loss of either *hMLH1* or *hMSH2* contributed significantly to resistance to CDDP. Therefore, it is generally agreed that loss of DNA mismatch repair genes is a cause of CDDP resistance. However, although these studies have revealed correlations between MMR deficiency and intrinsic resistance to platinum agents, it is still unknown whether MMR deficiency is also correlated with acquired resistance to platinum agents.

Previously, we have reported that MSI in ovarian cancer tissues changed during Pt-chemo, and that the loss of *hMLH1* protein expression affected this transformation to MSI (5). However, it was still unknown when and why MSI changes during Pt-chemo. Since studies on colorectal cancer (11, 12)

Table II. *Microsatellite status and hMLH1 protein expression of cases with hMLH1 promoter methylation.*

Case no.	Histologic subtypes	Regimen of chemotherapy	No. of treatment courses	Direct effects	Microsatellite status	Expression of *hMLH1* protein
1	Serous	CP	4	NC	MSI-H	Negative
2	Serous	DP	4	NC	MSI-L	Negative
3	Serous	TC	4	PR	MSI-H	Negative
4	Serous	TC	4	PR	MSS	Negative
5	Clear	DP	5	PR	MSI-L	Negative
6	Endometrioid	TC	5	PR	MSI-H	Negative
7	Serous	TC	5	PR	MSI-H	Negative
8	Serous	TC	5	PR	MSI-L	Negative
9	Endometrioid	CP	6	NC	MSI-H	Negative
10	Serous	DP	6	PR	MSI-H	Negative
11	Serous	DP	6	PR	NA	Negative
12	Serous	TC	6	PR	MSI-H	Negative
13	Serous	TC	6	PR	MSI-L	Negative
14	Serous	TC	6	PR	MSI-H	Negative
15	Serous	TC	6	PR	MSI-H	Negative
16	Serous	CP	7	PR	NA	Negative
17	Serous	TC	7	PR	MSI-H	Negative
18	Serous	TC	13	PR	MSI-H	Negative

CP, Cyclophosphamide + Cisplatin; DP, Docetaxel + Cisplatin; TC, Paclitaxel + Carboplatin; PR, partial response; NC, no change; MSS, microsatellite stable tumor; MSI-L, low frequency of microsatellite instability; MSI-H, high frequency of microsatellite instability; NA, not amplified.

revealed that hypermethylation occurred frequently in the *hMLH1* promoter, the clinical role of *hMLH1* MET has been studied in several types of cancer. However, the reported influence on survival of loss of *hMLH1* has also been found to be variable: a poor prognostic factor in esophageal cancer (13), but a good prognostic factor in colorectal (14) and gastric cancer (15). Although the correlation between *hMLH1* MET of the primary tumor and patient survival is still unknown, several studies (16, 17) have clearly demonstrated that *hMLH1* expression is reduced by treatment with anticancer agents. Gifford *et al.* (18) studied *hMLH1* MET in plasma DNA after carboplatin/taxoid chemotherapy of patients with EOC who were enrolled in the SCOTROC clinical trial, and reported that acquisition of *hMLH1* MET plasma DNA at relapse predicted poor overall survival. Furthermore, Nadin *et al.* (19) studied DNA damage and *hMLH1* and *hMSH2* protein expression in peripheral blood lymphocytes after chemotherapy and reported that examination of expression of *hMLH1* in peripheral blood lymphocytes is useful in predicting the response to chemotherapy. We have demonstrated that the status of tumor microsatellite regions was changed from stable to unstable by Pt-chemo (5), and in the present study have clarified that *hMLH1* MET is also changed by Pt-chemo and that *hMLH1* MET is the main cause of change of MSI in secondary tumors after Pt-chemo. Furthermore, the present results also show that *hMLH1* MET occurred more frequently in tumors after four or more courses of Pt-chemo than after fewer courses, and *hMLH1* MET was more frequently observed in partially responsive tumors than in tumors with

Table III. *Correlation between clinicopathological factors and hMLH1 methylation in informative cases.*

Factor (n)	Methylated (%)	Unmethylated (%)	*p*-value
Histological subtype			
Serous (23)	15 (65.2)	8 (34.8)	
Non-Serous (9)	3 (33.3)	6 (66.7)	$p=0.102$
Regimen of chemotherapy			
CDDP-based (12)	7 (58.3)	5 (41.7)	
CBDCA-based (20)	11 (55.0)	9 (45.0)	$p=0.853$
Taxanes non-combined (5)	3 (60.0)	2 (40.0)	
Taxanes combined (27)	15 (55.6)	12 (44.4)	$p=0.759$
Direct effects of chemotherapy			
PR (16)	15 (93.8)	1 (6.2)	
NC or PD (16)	3 (18.8)	13 (81.2)	$p<0.001$
Treatment courses			
3 courses ≥ (4)	0 (0.0)	4 (100.0)	
4 courses ≤ (28)	18 (64.3)	10 (35.7)	$p=0.059$

CDDP, cisplatin; CBDCA, carboplatin; Taxanes, paclitaxel or docetaxel; PR, partial response; NC, no change; PD, progressive disease. Direct effects were determined according to the WHO criteria.

NC or PD outcomes, indicating that the presence of *hMLH1* MET was correlated with acquired resistance to Pt-chemo while another mechanism, such as mutation of *p53*, was responsible for intrinsic resistance to Pt-chemo. Therefore, if several of the partially responsive tumors had a change in the *hMLH1* promotor from unmethylated to methylated during Pt-chemo, this would explain the lack of complete response and

the reason for the patient remaining in the PR category. Although why the *hMLH1* promoter is methylated by Pt-chemo is still unknown, we suspect that *hMLH1* MET during Pt-chemo is a temporary change which protects cancer cells from undergoing apoptosis due to exposure to DNA-toxic agents because sensitivity to platinum agents returns after a 6- to 12-month treatment interval from platinum agents (20).

According to the present results, treatment with demethylating agents brings the possibility of improving the effects of Pt-chemo and the prognosis of advanced EOC. Moreover, our *in vitro* study demonstrated that the DNA polymerase reaction inhibitors Ara-C and gemcitabine showed greater efficacy in MMR-deficient cell lines than in MMR-proficient cell lines (21). Large-scale clinical trials using demethylating agents or DNA polymerase reaction inhibitors with evaluation of the *hMLH1* MET status of tumors will be needed to improve the long-term prognosis of advanced EOC.

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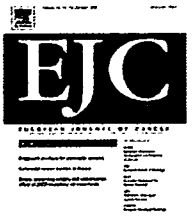

Expression of metastin and a G-protein-coupled receptor (AXOR12) in epithelial ovarian cancer

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ABSTRACT

Background: Metastin, a product of the KiSS-1 gene, is a ligand for a G-protein-coupled receptor (AXOR12) and is a strong suppressant of metastasis. The aim of this study was to evaluate whether *metastin* and AXOR12 gene expressions affect prognosis of patients with epithelial ovarian cancer.

Methods: The expression levels of *metastin*, AXOR12 and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene expression were analysed by the real-time quantitative reverse transcription-polymerase chain reaction in 76 epithelial ovarian cancer surgical specimens. Their expression (metastin/GAPDH and AXOR12/GAPDH ratios) was correlated with the clinical findings. Furthermore, cellular distribution of metastin and AXOR12 mRNA was examined by *in situ* hybridisation on tissue sections.

Results: The median and range of mRNA expression for metastin and AXOR12 were 0.047 and 0.01–13.57, and 4.00 and 0.011–135.13, respectively. Patients were dichotomised into two groups having low and high expressions by using the median value as the cutoff. A good agreement was noticed between *metastin* and AXOR12 gene expression levels (kappa coefficient; 0.74). The presence of residual tumour following resection was negatively associated with *metastin* ($P = 0.0084$) and AXOR12 ($P = 0.0148$) gene expressions indicating an association of low expression of these genes in more aggressive, and advanced tumours. By univariate Cox regression analysis, the prognosis of the patients with low AXOR12 gene expression was significantly worse than those with high AXOR12 gene expression ($P = 0.030$). The combination of *metastin* and AXOR12 gene expression level was also significantly associated with the prognosis ($P = 0.049$). Transcripts for both metastin and AXOR12 were detected in the epithelial ovarian carcinoma cells.

Conclusions: These results present a new insight into the understanding of the biological behaviour of epithelial ovarian cancer. Metastin/AXOR12 signalling may suppress the invasive phenotype of epithelial ovarian cancer.

1. Introduction

KiSS-1 is a human metastasis suppressor gene,[1] which suppresses metastasis of human melanoma[2] and breast carcinoma[3] without affecting tumourigenicity. Ohtaki and colleagues[4] showed that Kiss-1 encodes a carboxy-terminally amidated peptide with 54 amino-acid residues, which have been isolated from human placenta as the endogenous ligand

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of a G-protein-coupled receptor (named AXOR12 or hOT7T175) and is named as metastin. The binding of metastin to its receptor inhibits chemotaxis *in vitro*, enhances the expression and activity of focal adhesion kinase and inhibits the ability of meatstin receptor overexpressing melanoma cells to metastasise *in vivo*.[4] In another model, metastin inhibited chemotaxis, invasion, motility and growth of Chinese hamster ovary (CHO) cells designed to overexpress the metastin receptor, and attenuated pulmonary metastasis of hOT7T175-transfected B16–BL6 melanomas.[5]

Recently, a significant reduction in KiSS-1 or metastin expression has been reported in tumours with high metastatic potential.[6–10] Moreover, reduced KiSS-1 expression became a strong prognostic marker in patients with urinary bladder cancer[9] and gastric carcinoma.[10] These findings may open the possibility of future clinical application of these proteins, KiSS-1, metastin, and AXOR12, for prevention of cancer invasion and metastasis, and thus may improve patient prognosis. These promising results provoked us to evaluate the expression of these genes and their prognostic impact on epithelial ovarian cancer, which is the fourth most common cause of cancer death in women and the most common cause of death in women dying from a gynaecologic tumour.[11]

In this study, we sought to determine mRNA expression of metastin and AXOR12 using the real-time quantitative reverse transcription-polymerase chain reaction (RT- PCR) in cases of epithelial ovarian cancer. The gene expression of metastin and AXOR12 was correlated with clinicopathological parameters and their impact on patient survival was evaluated. Moreover, cellular distribution of metastin and AXOR12 mRNA was examined by *in situ* hybridisation.

2. Patients and methods

2.1. Patients

Patients with epithelial ovarian cancer treated between January 1990 and December 2005 at the Kinki University Hospital, Osaka-Sayama, Japan, were included in this study. Eligible patients had a histological diagnosis of primary epithelial ovarian cancer, and were suitable for adequate surgical staging. Patients were excluded from this study when surgically resected specimens were not available, had undergone any kind of preoperative therapy, had cancers other than ovarian cancer or had severe complications. All research was conducted with patients' informed consent to have their tissue banked for future unspecified studies. The present study conformed with the ethical standards of the Helsinki declaration of World Medical Association. The median age of the 76 eligible patients was 56 years (range, 31–84 years). Twenty-one of them were premenopausal. Patients were staged according to the 1987 criteria recommended by FIGO.[12] There were 34 stage I patients, 3 stage II patients, 35 stage III patients and 4 stage IV patients. The staging system defined by FIGO, as described elsewhere[13,14], assumes that an adequate staging operation has been performed. Tumours were classified histologically according to the World Health Organization (WHO) criteria[15] as serous ($n = 39$), mucinous ($n = 18$), endometrioid ($n = 10$), clear cell ($n = 8$) and transitional cell ($n = 1$). The tumours were classified histologically as either having well differentiated ($n = 46$) or being moderately differentiated ($n = 14$), or poorly differentiated ($n = 7$).[16] The number of poorly differentiated tumours is smaller than that of well differentiated tumours. This seems to be unusual compared to European series. However, this is a typical population in Japanese ovarian cancers.[13,14,17]

The surveillance for recurrent disease usually consisted of physical examination, Papanicolaou smear and serology with tumour marker examination (e.g. CA 125, CA 19-9, carcinoembryonic antigen, sialyl Tn) every month for the first year, every 2 months for the second and third years, and every 3 months for the fourth and fifth years. After 5 years, the patients were examined semiannually. A chest radiograph and CT scan or sonogram were obtained every 6 months for 5 years after surgery and every year thereafter, and if necessary MRI was performed. Recurrent disease was confirmed either pathologically or radiographically or serologically. Follow-up information was obtained from medical record, letter or telephone contact with patients, and information from referring physician. Survival data were available for all patients (median follow-up 36.5 months, range 4–196 months). Of these, 73 patients received platinum and/or paclitaxel-based chemotherapy. Two patients with stage Ia tumours of endometrioid adenocarcinoma and mucinous cystadenocarcinoma, and one with stage IV tumour of serous cystadenocarcinoma had no further treatment after surgery.

2.2. Tissue specimen and RNA preparation

Fresh surgical specimens from all patients were obtained. A dissecting microscope was used to avoid any contamination of cancerous tissue with non-cancerous tissue material. The tissue samples were stored at −80 °C for subsequent quantification of mRNA expression.

2.3. RNA preparation and real-time quantitative RT-PCR procedure

Total RNA was isolated from frozen tissues using a commercially available extraction method (Isogen; Nippon Gene Inc., Tokyo, Japan).

Complementary DNA (cDNA) was prepared by random priming from 1000 ng of total RNA using a First-Strand cDNA Synthesis kit (Pharmacia-LKB, Uppsala, Sweden). We performed real-time quantitative PCR using the TaqMan system (Applied Biosystems). The expression levels of each gene (*metastin* and AXOR12) and internal reference glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were measured by multiplex PCR using TaqMan probes labelled with 6-carboxyfluorescein (FAM) or VIC, respectively. The primers and TaqMan probes were designed using Primer Express v 2.0 software (Applied Biosystems). The sequences of each primer and TaqMan probe (forward primer, reverse primer, TaqMan probe) were *metastin*, 5′-GCAGGTCCTTCTCCCGCT-3′, 5′-GCCAGATCCCCGCACC-3′, 5′-CACCAGCACCGCGCCCTG-3′; AXOR12, 5′-TGGCACCCACGCAGCTA-3′, 5′- AGTTGCTGTAGGACATGCAGTGA-3′, 5′-CCGCCTACGCGCTTAAGACCTGG-3′. We purchased the Pre-Developed TaqMan Assay Reagents, GAPDH primer/probe set from Applied Biosystems. Real-time PCR

amplification and product detection was performed using an ABI PRISM 7300 Sequence Detection System (Applied Biosystems) as recommended by the manufacturer. The simultaneous measurement of each gene-FAM and GAPDH-VIC permitted normalisation of the amount of cDNA added per sample. The quantity of cDNA for each experimental gene was normalised to the quantity of GAPDH cDNA in each sample. Relative expression was determined by using the ΔΔCt (threshold cycle) method according to the manufacturer's protocol (User Bulletin #2). Each assay included a standard curve sample in duplicate, a no-template control and a cDNA sample from the tumour specimen in triplicate. All samples with a coefficient of variance higher than 10% were retested. Furthermore, the sequences of PCR products were analysed and they were identical to the sequence of each gene.

2.4. *In situ hybridisation*

To localise metastin and AXOR12 mRNA, *in situ* hybridisation technique was employed. Parraffin-embedded sections were used for *in situ* hybridisation. Serial sections were used from each patient for sense and antisense probe. A digoxigenin-labelled sense and antisense RNA probe was transcribed by T3 and T7 RNA polymerase, respectively, with a DIG RNA labelling kit according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany). Hybridisation and the immunohistochemical steps were done as we described previously.[10,18] Placental tissue served as a positive control.

2.5. *Statistical analysis*

Kappa statistic was used as a measure of agreement between *metastin* and *AXOR12* gene expression. The kappa coefficient values of up to 0.40 were considered to indicate poor agreement; values between 0.41 and 0.75, moderate to good agreement; and values greater than 0.75, excellent agreement.[19] Mann–Whitney U test and Kruskal–Wallis one-way analysis of variance by ranks were used as appropriate for the evaluation of differences between end-points. The Cox proportional hazards model was used in survival analysis. Maximum likelihood parameter estimates and likelihood ratio statistics (LRS) in the Cox proportional hazards models were obtained with the use of a statistical package, EPICURE.[20] Kaplan–Meier curves were compared by the univariate Cox regression analysis. All *P* values presented were two-sided. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. *Each gene expression and clinicopathological features*

The median and range of mRNA expression were 0.047 and 0.01–13.57, and 4.00 and 0.011–135.13 for metastin and AXOR12, respectively. The patients were divided into low or

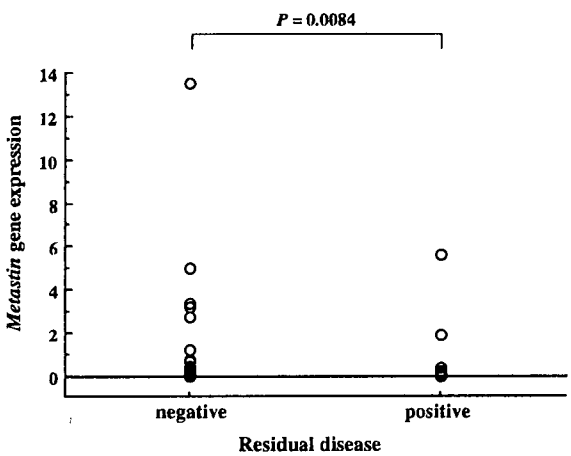

Fig. 1A – *Metastin* gene expression in patients with negative and positive residual disease.

Table 1 – Clinicopathological features and gene expression

Clinicopathological features	Metastin gene expression median (range)	P-value	AXOR12 gene expression median (range)	P-value
Age at the time of diagnosis		0.0058		0.1069
<56 (n = 36)	0.119 (0.001–5.689)		5.683 (0.054–32.309)	
≥56 (n = 40)	0.021 (0.001–13.566)		3.205 (0.011–36.116)	
FIGO stage		0.0539		0.1279
I–II (n = 37)	0.071 (0.001–13.566)		4.529 (0.133–33.588)	
III–IV (n = 39)	0.021 (0.001–5.689)		3.056 (0.011–36.116)	
Residual disease		0.0084		0.0148
Negative (n = 43)	0.097 (0.001–13.566)		4.853 (0.133–36.116)	
Positive (n = 33)	0.010 (0.001–5.689)		2.560 (0.011–25.007)	
Histological subtype		0.0832		0.071
Serous (n = 39)	0.014 (0.001–3.424)		1.651 (0.033–36.116)	
Mucinous (n = 18)	0.160 (0.001–5.689)		5.585 (0.011–23.375)	
Endometrioid (n = 10)	0.073 (0.001–3.256)		10.341 (0.054–33.588)	
Clear cell (n = 8)	0.139 (0.003–13.566)		11.413 (0.133–23.869)	
Histological grade		0.2289		0.1575
Well differentiated (n = 46)	0.054 (0.001–5.689)		4.258 (0.011–33.588)	
Moderately differentiated (n = 14)	0.009 (0.001–1.963)		3.414 (0.033–36.116)	
Poorly differentiated (n = 7)	0.003 (0.001–0.049)		1.856 (0.054–4.441)	
Unclassified (n = 9)	0.133 (0.003–13.566)		6.838 (0.133–23.869)	

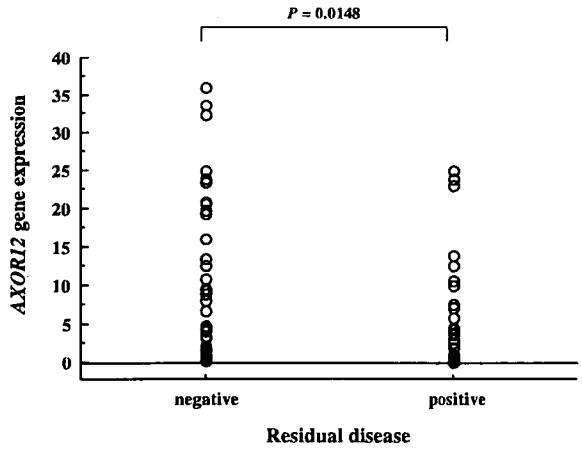

Fig. 1B – A G-protein-coupled receptor (AXOR12) gene expression in patients with negative and positive residual disease.

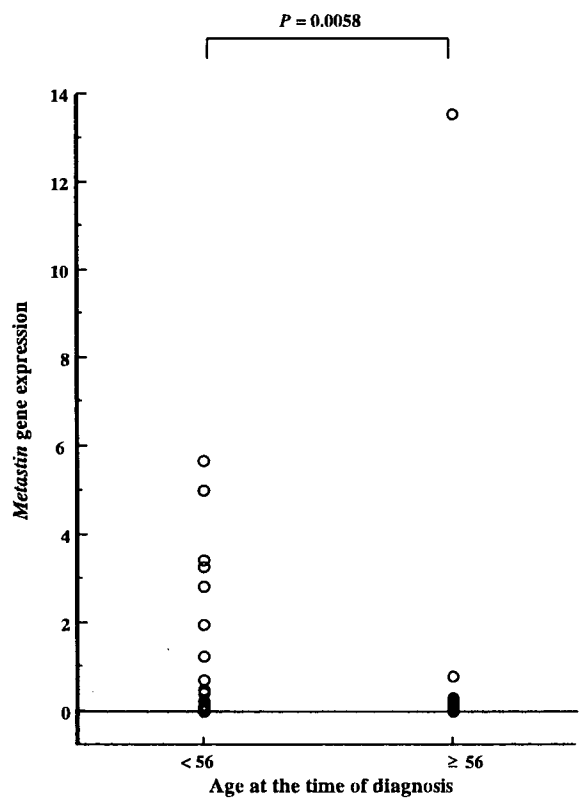

Fig. 1C – Metastin gene expression according to the age at diagnosis (<56 versus ⩾56).

high groups for *metastin* and AXOR12 gene expressions using the median value as the cutoff, respectively. A good agreement was noted between *metastin* and AXOR12 gene expression levels (kappa coefficient; 0.74). The values of *metastatin* and AXOR12 gene expressions in ovarian cancers are classified according to patients' age at diagnosis, stage of disease, presence or absence of residual tumour mass after initial surgery, histological subtype and grade (Table 1). The presence of residual tumour was negatively associated with *metastin* (P = 0.0084) (Fig. 1A) and AXOR12 (P = 0.0148) (Fig. 1B) gene expressions, respectively. The patients' age at diagnosis was significantly associated with *metastin* gene expression (P = 0.0058) (Fig. 1C).

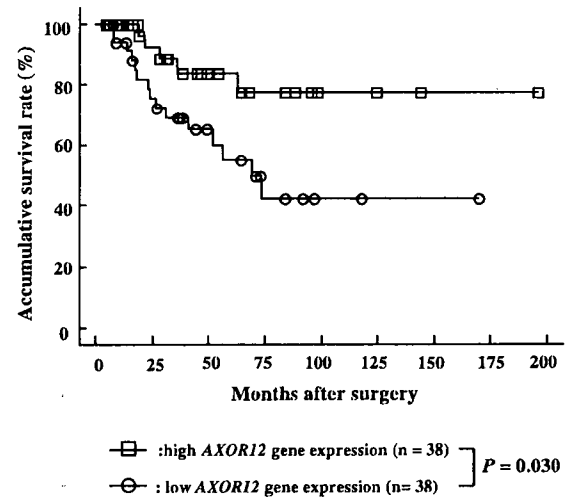

Fig. 2A – Comparison of survivals between groups with a high G-protein-coupled receptor (AXOR12) gene expression and low AXOR12 gene expression according to univariate Cox regression analysis.

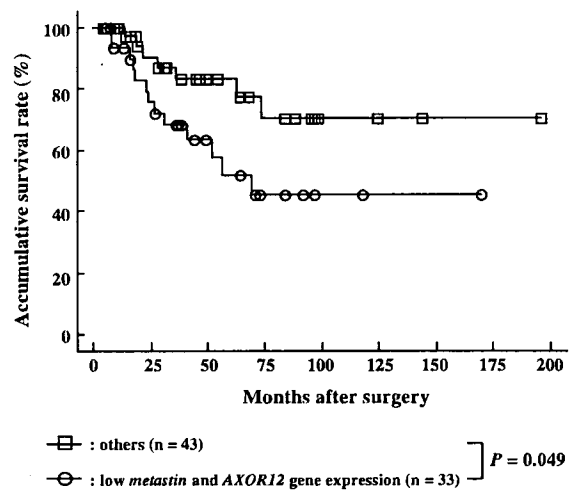

Fig. 2B – Comparison of survivals between groups with both low *metastin* and a G-protein-coupled receptor (AXOR12) gene expression, and others according to univariate Cox regression analysis.

3.2. *Each gene expression and prognosis*

As shown in Fig. 2A, we found the prognosis of the patients with low AXOR12 gene expression to be significantly worse than that of those with high AXOR12 gene expression by univariate Cox regression analysis (P = 0.030). *Metastin* gene

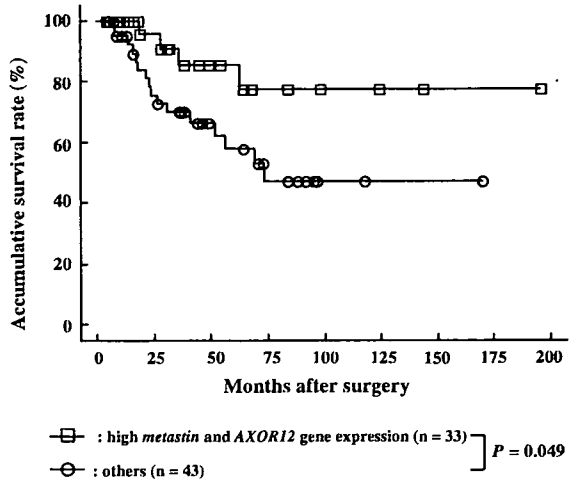

Fig. 2C – Comparison of survivals between groups with both high *metastin* and *a G-protein-coupled receptor (AXOR12)* gene expression, and others according to univariate Cox regression analysis.

Table 3 – The results of multivariate Cox regression analysis

Variables	Hazard ratio	95% confidence interval	P-value
FIGO stage			
I–II (*n* = 37)	Referent		
III–IV (*n* = 39)	12.08	2.39–61.16	0.003
Histological grade			
Others (*n* = 69)	Referent		
Poorly (*n* = 7)	3.08	1.13–8.41	0.028
AXOR12 gene expression ratio			
High (*n* = 38)	Referent		
Low (*n* = 38)	0.35	0.03–4.92	0.439
Combination of metastin and AXOR12 gene expression ratio			
Others (*n* = 43)	Referent		
Both low (*n* = 33)	1.44	0.27–7.64	0.668
Both high (*n* = 33)	Referent		
Others (*n* = 43)	5.26	0.50–55.21	0.167

expression itself had no impact on patient survival, however, combination of *metastin* and AXOR12 gene expression had significant impact on patient prognosis (Figs. 2B and 2C). Moreover, FIGO stage (stages III–IV; $P = 0.001$), residual disease (positive; $P = 0.0004$) and histological grade (poorly; $P = 0.0005$) were found to be significantly associated with a poor prognosis in univariate Cox regression analysis (Table 2). Older age at the time of diagnosis and serous tumours type are generally thought to be more aggressive.[21] However, no significant association for these variables could be found in this study (Table 2). Multivariate Cox regression analysis revealed that FIGO stage (III–IV; $P = 0.003$) and histological grade (poorly; $P = 0.028$) are the independent prognostic factors in this series (Table 3).

3.3. *In situ* hybridisation

Transcripts for *metastin* and *AXOR12* were detected in the syncytiotrophoblasts of placental tissue as positive controls, respectively (Fig. 3). Transcripts for *metastin* and AXOR12 were

Table 2 – The results of univariate Cox regression analysis

Variables	Hazard ratio	95% Confidence interval	P-value
Age at the time of diagnosis value	1.02	0.98–1.06	0.244
FIGO stage			
I–II (*n* = 37)	Referent		
III–IV (*n* = 39)	11.50	2.64–50.01	0.001
Residual disease			
Negative (*n* = 43)	Referent		
Positive (*n* = 33)	14.34	3.31–62.14	0.0004
Histological subtype			
Others (*n* = 37)	Referent		
Serous (*n* = 39)	1.10	0.45–2.69	0.838
Histological grade			
Others (*n* = 69)	Referent		
Poorly (*n* = 7)	5.29	2.07–13.49	0.0005
Metastin gene expression ratio			
High (*n* = 38)	Referent		
Low (*n* = 38)	2.32	0.89–6.05	0.084
AXOR12 gene expression ratio			
High (*n* = 38)	Referent		
Low (*n* = 38)	3.06	1.11–8.43	0.030
Combination of metastin and AXOR12 gene expression level			
Others (*n* = 43)	Referent		
Both low (*n* = 33)	2.52	1.004–6.33	0.049
Both high (*n* = 33)	Referent		
Others (*n* = 43)	3.00	1.004–8.99	0.049

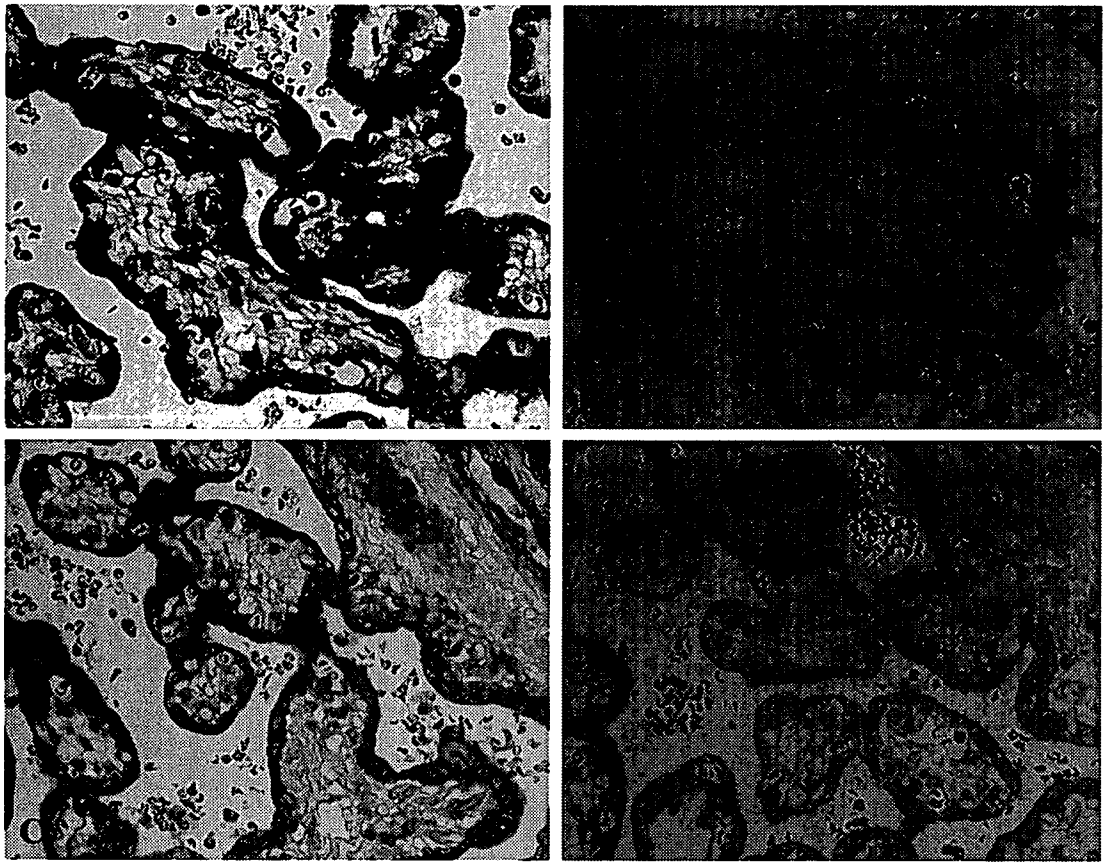

Fig. 3 – *In situ* hybridisation analysis of metastin and a G-protein-coupled receptor (AXOR12) expression in a section of the placenta. Metastin (A, original ×400) and AXOR12 (C, original ×400) mRNA-expressing cells are found in the syncytiotrophoblasts, respectively. In metastin (B, original ×400) and AXOR12 (D, original ×400) sense-control hybridisation, only background colour with no distinction is observed, respectively.

observed in the epithelial ovarian carcinoma cells, respectively (Fig. 4).

4. Discussion

The predicted KiSS-1 proteins consist of 145 amino acids, with a secretory signal sequence located at the N-terminus, suggesting that KiSS-1 functions as a secretory protein.[22] However, the full-length KiSS-1 protein has not been detected in a secreted form. Instead, three truncated fragments of KiSS-1 occur naturally in human placenta and are termed as metastin (54 amino acids), kisspeptin-14 (14 amino acids) and kisspeptin-13 (13 amino acids).[4] Furthermore, metastin was identified as a ligand for an orphan G-protein-coupled receptor, designated as AXOR12.[23] Jiang and colleagues[24] reported the differential expression of KiSS-1 and AXOR12 in human ovarian cancer cell lines. SKOV3 cells expressed AXOR12, but lacked the expression of KiSS-1. They established KiSS-1 infected SKOV3 cell line, and found that KiSS-1 expression inhibited the migration of SKOV3 cells and reduced colony formation of SKOV3 cells without affecting cell proliferation.[24] These results suggest that KiSS-1 serves as a metastasis suppressor for ovarian cancer. In this study, we evaluated the expression level of *metastin* and *AXOR12* genes in epithelial ovarian cancer, and a good agreement was noted between *metastin* and *AXOR12* gene expression levels (kappa coefficient; 0.74). Moreover, high AXOR12 gene expression and high expression of both *metastin* and AXOR12 genes significantly were associated with the improved patient prognosis in this study. Metastin/AXOR12 signalling might suppress the tumour aggressive phenotype in epithelial ovarian cancer. Similar results have been reported in melanoma,[6] thyroid cancer,[7] oesophageal carcinoma,[8] urinary bladder cancer[9] and gastric carcinoma.[10]

More recently, Martin and colleagues[25] noted that KiSS-1 expression is increased in human breast cancer, particularly in patients with aggressive tumours and with mortality. Also, it has been reported that Kiss-1 promotes metastasis in a human breast cancer cell line in an *in vitro* study. These results are in direct contrast to a number of previous studies[6–10] and show that KiSS-1 plays a role beyond the initial metastasis repressor in breast cancer. Ikeguchi and colleagues[26] examined the clinical importance of KiSS-1 and its receptor gene expression in hepatocellular carcinoma. They evaluated 60 surgically resected carcinomas using real-time quantitative RT-PCR and found that there was no loss of KiSS-1 in carcinomas compared to non-cancerous cirrhotic livers. Conversely, they found a high expression of the receptor in

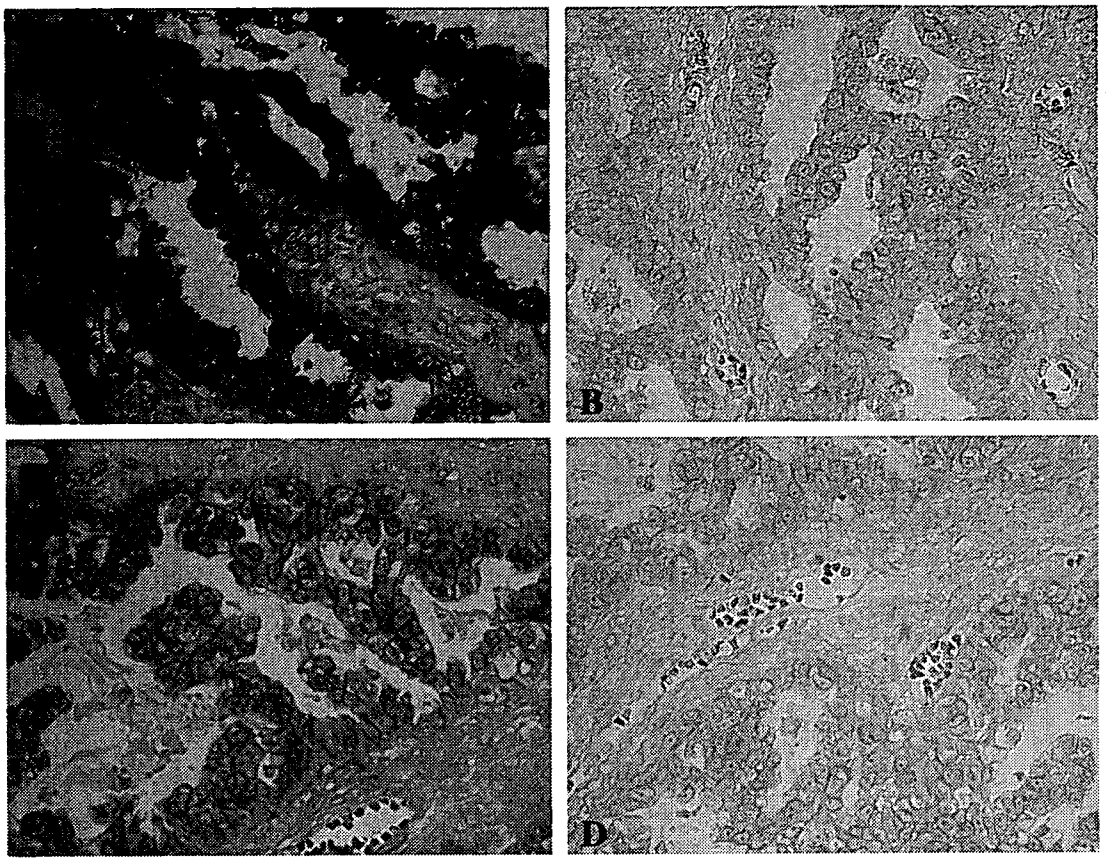

Fig. 4 – Localisation of analysis of *metastin* and a G-protein-coupled receptor (AXOR12) in a section of clear cell carcinoma by *in situ* hybridisation. Metastin (A, original ×400) and AXOR12 (C, original ×400) mRNA-expressing cells are found in the epithelial ovarian carcinoma cells, respectively. In metastin (B, original ×400) and AXOR12 (D, original ×400) sense-control hybridisation, only background colour with no distinction is observed, respectively.

the carcinomas. There was over-expression of KiSS-1 and its receptor in six tumours of advanced stage and those patients had poor survival. These authors concluded that over-expression of KiSS-1 and its receptor was frequently observed and correlated with disease progression.[26] It appears that although KiSS-1 may be a possible metastasis suppressor in melanoma,[6] thyroid cancer,[7] oesophageal carcinoma,[8] urinary bladder cancer,[9] gastric carcinoma,[10] and epithelial ovarian cancer, however, this is not always true as it was found in breast and hepatocellular cancers. Further research is necessary before the true role and effect of Metastin/AXOR12 signalling in each tumour can be elucidated.

KiSS-1 peptides, such as metastin and its receptors were highly expressed in placenta,[4,23] and metastin was isolated from human placental extracts.[4] Metastins were detected in plasma, and their concentrations dramatically increased under certain physiological conditions, such as pregnancy. Histochemical studies detected metastin mRNA in human placenta and immunoreactivity in the syncytiotrophoblasts.[27] These data may indicate that metastin is a novel placenta-derived hormone in humans. There are striking similarities between the behaviour of invasive placental cells and the invasive cancer cells.[28,29] Like tumour cells, cytotrophoblastic cells migrate through and invade the uterine wall at the time of implantation. Unlike tumour invasion, this unique interaction between genetically dissimilar trophoblasts and uterine cells is closely regulated and is limited both temporally and spatially by mechanisms that are largely unknown. Considering the localisation of mRNA for metastin and AXOR12 in syncytiotrophoblasts confirmed in the present study and a dramatic elevation of plasma metastin concentration in the first trimester of pregnancy,[27] it is possible that metastin/AXOR12 signalling may be involved in the negative regulation of trophoblast invasion because unlike the tumour cells trophoblasts never metastasise to distant location. In this study, transcripts for metastin and AXOR12 were detected in the epithelial ovarian carcinoma cells, respectively, by *in situ* hybridisation analysis. Similarly, it might be possible that high expression of both *metastin* and *AXOR12* genes in epithelial ovarian cancer cells, as detected in this study, is responsible for the inhibition of cellular invasion and metastasis; however, this speculation is still putative.

The real-time quantitative RT-PCR method we used for the determination of *metastin* and *AXOR12* gene expressions is convenient because it does not require radioisotopes or relatively large amounts of tumour tissues, and is reliable and accurate. Even biopsy samples could be used for an accurate evaluation of *metastin* and *AXOR12* gene expressions. The

real-time quantitative RT-PCR detection method of these genes might serve as a tool to diagnose the high-risk group of patients with epithelial ovarian cancer who might have worse prognosis. Also the expression pattern of these genes may provide a new insight to understand the biology of epithelial ovarian cancer. Further investigation is necessary in a large number of epithelial ovarian cancer patients before the findings of the present study would be considered for clinical application.

Conflict of interest statement

None declared.

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Hypoxia inducible factor 1-α expression as a factor predictive of efficacy of taxane/platinum chemotherapy in advanced primary epithelial ovarian cancer

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Abstract

To investigate the impact on survival of HIF 1-α expression on primary advanced epithelial ovarian cancer (EOC), we examined the correlations between prognosis and HIF 1-α expression by Western blot analysis in 52 cases of stage III/IV EOC. HIF 1-α expression was confirmed in 36 cases (69.2%) of EOC, and HIF 1-α-expressing tumors had a significantly higher rate of response ($p < 0.01$) to postoperative paclitaxel/carboplatin combination chemotherapy (TC) than tumors without HIF1-α expression. Moreover, patients with HIF 1-α-expressing tumors with suboptimal resection of stage III/IV tumors indicated for postoperative TC exhibited significantly better survival ($p < 0.01$).

Keywords: HIF 1-α; Epithelial ovarian cancer; Chemotherapy; Prognostic factor

1. Introduction

Hypoxia inducible factor 1-α (HIF 1-α) has been reported to be an important predictor of tumor progression for several types of solid cancers [1–5]. However, although several *in vitro* studies have reported correlations between HIF 1-α expression and cell biological features in ovarian cancer, study of the clinical significance of HIF 1-α still has been limited [6]. To determine the clinical usefulness of HIF 1-α expression in treatment of primary epithelial ovarian cancer (EOC), we examined whether HIF 1-α expression can predict effects of postoperative induction chemotherapy and long-term prognosis in patients with stage III/IV advanced EOC.

2. Materials and methods

The study included 52 cases of stage III/IV EOC. Fourteen patients underwent optimal resection (residual tumor <1 cm), while 38 patients underwent suboptimal resection at primary surgery. Furthermore, all patients with suboptimal resection had measurable disease usable for determining direct effects of TC. The clinicopathological characteristics of patients did not differ significantly between optimal resection and suboptimal resection as summarized in Table 1. All of the patients were indicated for postoperative TC (175–180 mg/m^2 paclitaxel and a dose of carboplatin an area under the concentration curve

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Table 1
Correlations between HIF-1 α expression and clinicopathologic factors

Factors	HIF-1 α[a] positive	HIF-1 α negative
Total number of cases	36	16
Mean ages (range)	57.9 ± 8.2 years (34–84)	57.2 ± 7.3 years (39–73)
FIGO stage (%)[b]		
Stage III	31 (68.9)	14 (31.1)
Stage IV	5 (71.4)	2 (28.6)
Histologic subtype (%)		
Serous	19 (65.5)	10 (34.5)
Endometrioid	7 (77.8)	2 (22.2)
Mucinous	3 (60.0)	2 (40.0)
Clear-cell	7 (77.8)	2 (22.2)
Histologic grade (%)[c]		
Grade 1	13 (68.4)	6 (31.6)
Grade 2	10 (71.4)	4 (28.6)
Grade 3	6 (60.0)	4 (40.0)
Surgical status (%)		
Optimal surgery	9 (64.3)	5 (35.7)
Sub optimal surgery	27 (77.1)	11 (28.9)
Overall response rate of postoperative chemotherapy (%)	18 (66.7)	5 (45.5)**
Complete response rate of postoperative chemotherapy (%)	13 (48.1)	2 (18.2)**

[a] HIF, hypoxia inducible factor.
[b] FIGO, Federation of International Gynecology and Obstetrics.
[c] Not including clear-cell carcinomas.
** $p < 0.01$.

by Calvert's formula of 5–6). Direct effects of chemotherapy were assessed using the World Health Organization criteria. HIF 1-α expression was determined by Western blot analysis using anti-HIF 1-α (Novus Biologicals, Littleton, CO) for stocked fresh-frozen tissues, and if an independent positive band in the region of 120 kDa was confirmed on quantification using NIH image analysis, it was taken to indicate HIF 1-α expression (Fig. 1). We obtained fully informed written consent from all patients prior to obtaining the specimens. We used the chi-square test and log-rank test for statistical analysis, with p-values less than 0.05 considered significant.

3. Results

HIF 1-α expression was confirmed in 36 (69.2%) of the patients with FIGO stage III/IV tumors, and no significant correlation was observed between frequency of HIF 1-α expression and patient age, histologic subtype, histologic grade, FIGO stage (III or IV), or surgical status (optimal or suboptimal resection). However, HIF 1-α-expressing tumors exhibited significantly higher overall response rate ($p < 0.01$) and complete response rate ($p < 0.01$) to TC than tumors without HIF 1-α expression (Table 1). Moreover, HIF 1-α predicted prognosis for neither the group of all stage III/IV patients nor that with optimal resection. Although no significant differences were noted in clinicopathologic characteristics between patients with optimal and those with suboptimal resection (Table 2), but among patients in stage III/IV who underwent suboptimal resection at primary surgery and were indicated for postoperative TC, those with HIF 1-α-expressing tumors had a significantly better prognosis than those with tumors without HIF 1-α expression (Fig. 2).

4. Discussion

HIF 1-α expression in malignant tumors has been reported as a predictive factor for tumor progression and a prognostic factor correlated with angiogenesis. However, HIF 1-α expression in solid cancers exhibits marked variation among primary organs in the English literature [1–5]. Generally, HIF 1-α predicts tumor progression, and HIF 1-α-

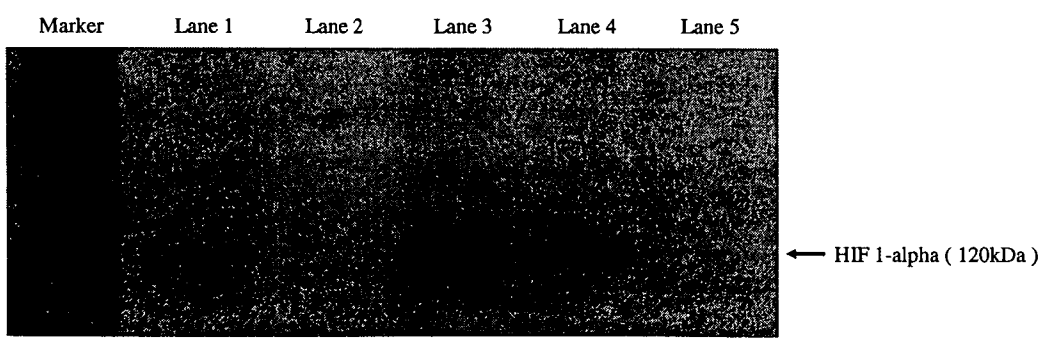

Fig. 1. The expression of HIF 1-α proteins detected by Western blotting. Lane 1: positive control; HCT-116 cell were grown in a chamber containing 1% oxygen, 5% carbon dioxide, and 94% nitrogen at 37 degree for 3 days. Lane 2: negative control; without primary antibody. Lane 3 and 4: HIF 1-α positive cases. Lane 5: HIF 1-α negative case.

Table 2
Clinicopathologic characteristics of all patients

Factors	Optimal	Suboptimal
Total number of cases	14	38
Mean ages (range)	59.6 + 8.3 years (46–84)	57.1 ± 7.6 years (34–74)
FIGO stage (%)[a]		
Stage III	13 (92.8)	32 (84.2)
Stage IV	1 (7.2)	6 (15.8)
Histologic subtype (%)		
Serous	9 (64.3)	20 (52.6)
Endometrioid	2 (14.3)	7 (18.4)
Mucinous	1 (7.1)	4 (10.6)
Clear-cell	2 (14.3)	7 (18.4)
Histologic grade (%)[b]		
Grade 1	6 (50.0)	13 (41.9)
Grade 2	4 (33.3)	10 (32.3)
Grade 3	2 (16.7)	8 (25.8)
Mean treatment courses (range)	5.9 ± 0.3 course (4–6)	5.8 ± 0.9 courses (3–6)
Mean follow up period (range)	58.4 ± 31.4 months (13–135)	48.3 ± 26.3 months (8–110)

[a] FIGO, Federation of International Gynecology and Obstetrics.
[b] Not including clear-cell carcinomas.

expressing cancers tend to have a poor prognosis. However, Nakayama et al. [6] reported finding no relationship between HIF 1-α expression and intratumoral microvessel density, and that vascular endothelial cell growth factor (VEGF) up-regulated HIF 1-α gene, though levels of expression of neither gene affected the survival of patients with EOC. Furthermore, Birner et al. [7] examined HIF 1-α expression in 102 cases of FIGO stage I–IV EOC by immunohistochemical staining, reported that 68.6% of cases of EOC expressed HIF 1-α, and concluded that HIF 1-α protein overexpression also has no impact on prognosis and that response to TC is independent of HIF 1-α expression. However, Escuin et al. [8] recently found that microtubule-targeting drugs, such as taxanes, could be effective in down-regulating HIF 1-α protein via effects on microtubule cytoskeleton that are correlated with HIF 1-α translation activity. For patients with suboptimally resected advanced EOC, survival impact is closely related to effects of postoperative chemotherapy. Therefore, because paclitaxel may exhibit anti-angiogenetic effects through down-regulation of HIF 1-α protein expression, the survival impact of HIF 1-α expression on EOC may be noted only in patients who are stage III/IV, have undergone

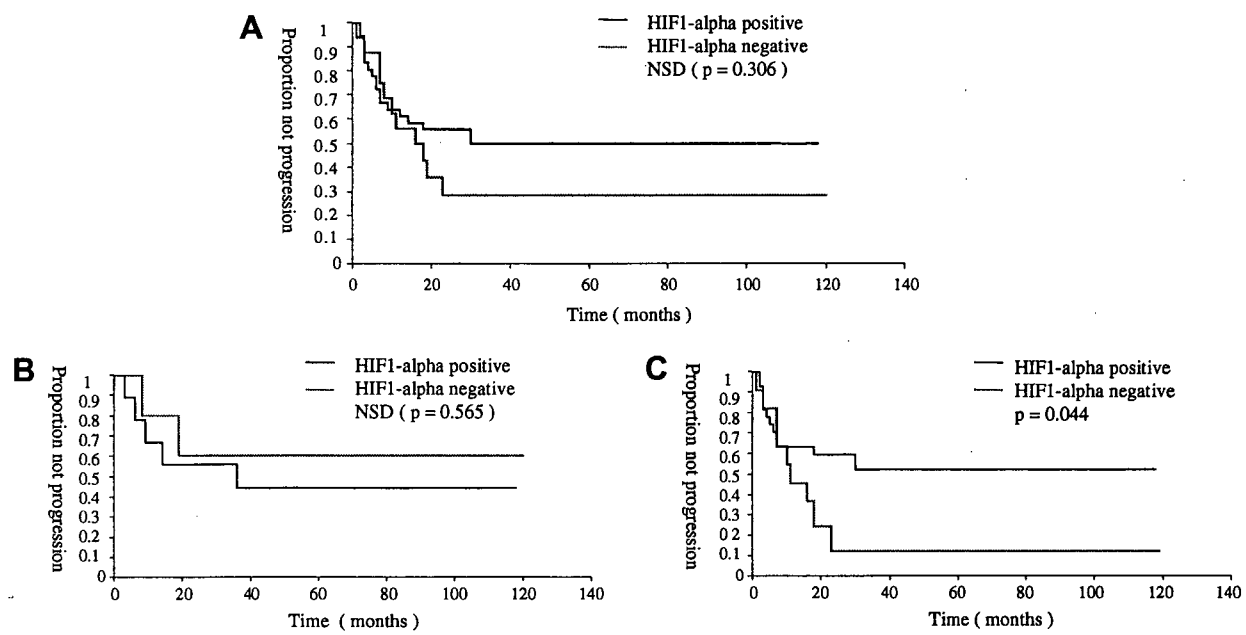

Fig. 2. Correlation between survival and HIF 1-α expression in stage III/IV epithelial ovarian cancer. (A) Progression-free survival in the group of all stage III/IV patients. (B) Progression-free survival of stage III/IV patients who underwent optimal resection at primary surgery and were indicated for postoperative paclitaxel/carboplatin chemotherapy. (C) Progression-free survival of stage III/IV patients who underwent suboptimal resection at primary surgery and were indicated for postoperative paclitaxel/carboplatin chemotherapy. *p*-values were calculated with the log-rank test.

suboptimal resection at primary surgery, and are indicated for postoperative TC. Although TC has been widely used as an effective standard regimen of chemotherapy for primary or recurrent EOC, and TC has achieved a 65–75% overall response rate in several phase 3 clinical trials [9,10], no factors predictive of TC have been found. The present findings suggest that although expression of HIF 1-α is not a factor predictive of survival of patients with early-stage or optimally resected advanced EOC, it does predict the efficacy of chemotherapy using TC. Furthermore, determination of HIF 1-α expression should be useful for devising individualized treatment regimens for advanced EOC. Clinical trials targeting HIF 1-α treatment using taxanes are needed to improve the long-term prognosis of patients with suboptimally resected advanced EOC.

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Ⅲ. 子宮体癌における化学療法

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〔*Jpn J Cancer Chemother* 35(2): 218-223, *February*, 2008〕

はじめに

2006年11月28, 29日英国のマンチェスターにて子宮体癌に関する国際会議が開催され，分子メカニズム，治療法，今後の臨床試験のあり方について，早期がん，進行がん，稀な組織型（明細胞，漿液性腺癌など）の治療，translational research などを対象に討議された[1]。これは英国の NCRI，米国の NCI-US，さらに国際的臨床試験グループである GCIG の共同開催であり，その内容から現在世界の専門家はどのような理解のもとに今後の臨床研究を考えているのかを整理し，特に化学療法に焦点を当てて解説してみたい。

1．原則は手術療法

子宮体癌の治療は，あくまで手術療法の役割が中心である。そこで術後の再発危険因子を理解することが最も重要であり，子宮体癌の術後管理をいかに正確に行うかに直結する課題である。再発危険因子は子宮内因子と子宮外因子に分けられ[2]，表1のごとく多くの因子が存在し，それぞれが FIGO の進行期分類で反映されている[3]。

表1 子宮体癌の予後因子

Uterine Factors	Extrauterine Factors
Histology	Adnexal Metastases
Grade	Intraperitoneal Spread
Myometrial Invasion	Peritoneal Cytology
Cervical-Isthmus Extension	Pelvic Node Metastases
Lymph-Vascular Invasion	Paraortic Node Metastases

昨今はこれらの危険因子を危険度の程度別に，low, intermediate, high risk などとグループ分けされ詳細に検討されている（図1）。そしてこれらが種々の治療法の選択に欠かせない指針となっている。従って，正確な術後進行期の決定がその症例の予後を語るもっとも正確な手段であることは議論の余地がない。

こと手術に関しては，単純子宮全摘術とは異なり，広汎子宮全摘術を子宮体癌で行うことが骨盤内や腟断端への再発を減らすとされ，リンパ節への再発転移をも低くするものとされてきたが，早期であるⅠ期症例への広汎子宮全摘術を支持する証拠は何もない。この手術は明らかな頸管浸潤を伴ったⅡb期症例に限られるべきである[4]。リンパ節郭清の効用は疾患の進行期を決め，そうすることで予後を推測し術後療法の必要性を決めることである。しかしリンパ節を摘出すること自体が治療的意義があるか否かは今日もっとも議論のあるところである[5]。2007年米国での ASCO 総会にて ASTEC 試験の報告[6]があり，二段階の無作為化試験により TAH & BSO 後にリンパ節郭清を行うかどうかと，病理学的に再発高危険群であるが肉眼的に完全に摘出された症例には，放射線の外照射を行うか否かにより，生存期間が比較された（図2）。全生存期間は治療法で差はなかったが，無再発期間はリンパ節郭清のない群で，行った群より優っていた。彼らは多数の症例での成績であり骨盤リンパ節郭清は特に術後療法の存在下では生存期間を延長するものではないと結論した。リンパ節郭清群には無再

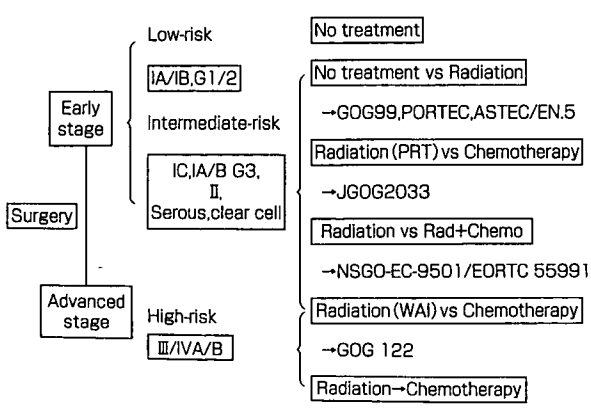

図1 リスク別術後療法のシェーマ

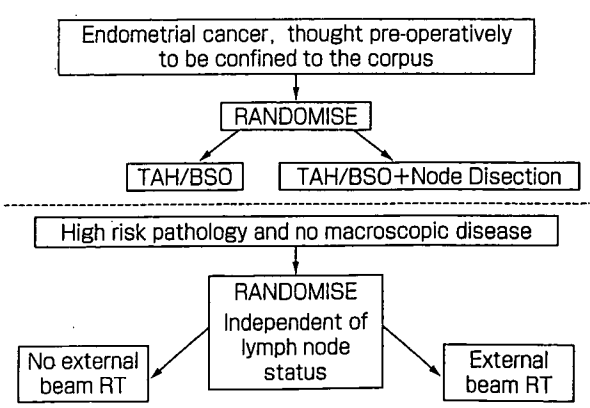

図2 ASTEC 臨床試験 ASCO2007