actual management during a fixed one-year period and the prescription of adjuvant therapy for endometrial cancer in each JGOG member institution. In October 2005, we mailed the questionnaire to all 226 JGOG member institutions, and the last date for receipt of responses was set as December 9, 2005. The following parameters were assessed: (1) number of patients who underwent primary surgery in 2004; (2) type of adjuvant therapy, such as chemotherapy (CT), radiotherapy (RT), or hormonal therapy (HT); (3) indication criteria for adjuvant modalities, especially in relation to pathologic findings, such as surgical stage, histologic grade, non-endometrioid subtype, and LVSI; and (4) the first recommended regimen for adjuvant CT. A standardized computer software package (SPSS version 11.0, SPSS Japan Inc., Tokyo, Japan) was used for statistical analysis. We used two-tailed *t*-test, Chi-square test, and Fisher's exact test and considered $p<0.05$ as statistically significant.

Results

Annual caseload of endometrial cancer

Table 1 summarizes the demographic data of the respondents. Of the 226 JGOG member institutions who received the survey questionnaire, 199 (88%) responded. They included 89 academic institutions (44.7%), such as university hospitals or cancer centers, and 110 general hospitals (55.3%). Two institutions were excluded as ineligible and the subsequent analyses were conducted using responses from the remaining 197 institutions. Overall, 4063 endometrial cancer patients underwent primary surgery at the respondent institutions, and 66.8% of these institutions performed more than 10 primary surgeries for endometrial cancer in 2004. Of the total, 2096 patients (51.6%) received postoperative adjuvant therapy, and the number of patients that received CT (1675 patients) was significantly greater than the number that received RT (273 patients, $p<0.01$) or HT (148 patients, $p<0.01$). All adjuvant RT in respondents was performed as whole pelvic irradiation, none of the respondents received chemoradiation therapy or combination therapy consisting of CT and RT, CT and HT, or RT and HT.

Chemotherapeutic regimen for adjuvant therapy

Table 2 shows the first-line CT regimens preferred for patients with endometrial cancer after primary surgery. For first-line CT, the most frequently used regimen was a combination of paclitaxel and carboplatin (TC) (59.5%), followed by combinations of cyclophosphamide, adriamycin, and platinum (7.8%); docetaxel and carboplatin (5.3%); adriamycin and cisplatin (5.0%); and other regimens containing platinum (22.4%). The majority using TC administered it thrice weekly (51.1%), while those using the paclitaxel/cisplatin combination administered it weekly (8.4%). Furthermore, a majority of the regimens containing platinum were combinations of paclitaxel/doxorubicin/cisplatin and paclitaxel/doxorubicin/carboplatin.

Table 1
Caseload of endometrial cancer in JGOG member institutions.

Total number of JGOG member institutions	226
Total number of responded institutions	199
Academic institutions	89
General hospitals	110
Annual[a] treatment cases of surgery	4063
No. of patients by performed adjuvant modalities[b]	
Total	2096
Chemotherapy (%)	1675 (79.9)
Radiotherapy (%)	273 (13.0)
Hormonal (%)	148 (7.1)

JGOG; Japanese Gynecologic Group. Academic Institution; University hospital or Cancer center.
[a] During January 1st to December 31st, 2004.
[b] Multiple answers available.

Table 2
Regimens of adjuvant chemotherapy.

Regimens of chemotherapy	Annual[a] treatment case number (%)
Paclitaxel + Carboplatin	
Tri-weekly	997 (59.5)
Weekly	856 (51.1)
Cyclophosphamide + Adriamycin + Cisplatin	141 (8.4)
Docetaxel + Carboplatin	131 (7.8)
Adriamycin + Cisplatin	88 (5.3)
Cyclophosphamide + Epirubicin + Cisplatin	84 (5.0)
Others	84 (5.0)
	291 (17.4)

[a] During January 1st to December 31st, 2004.

Criteria to administer adjuvant therapy

Tables 3 and 4 show percentages of respondent institutions that administered adjuvant therapy on the basis of surgical stage, histologic grade (G), and results of LVSI in patients with endometrioid type (Table 3) and non-endometrioid type (Table 4) endometrial cancer. The present survey reveals that more than 50% of the institutions administered adjuvant therapy beyond FIGO IB/G3/positive LVSI, and more than 90% administered adjuvant therapy beyond FIGO IC/G3/positive LVSI when the histologic subtype of tumor was endometrioid. Similarly, more than 50% of the institutions administered adjuvant therapy beyond FIGO IB/G3/regardless of LVSI, and more than 90% of institutions administered adjuvant therapy from FIGO IC/G3/regardless of LVSI when the histologic subtype of tumor was non-endometrioid. Therefore, a majority of respondent JGOG member institutions considered that some form of adjuvant therapy should be administered for not only high-risk but also intermediate-risk endometrial cancer. Moreover, the present survey also revealed that the respondent institutions regard LVSI as an important clinicopathologic prognostic factor of endometrial cancer other than the FIGO stage and histopathologic grade.

Discussion

Indications for adjuvant therapy for patients with endometrial cancer have been determined by postoperative clinicopathologic prognostic factors such as histologic subtype, histologic grade, lymph node metastasis, depth of myometrial invasion (MI), and LVSI. Based on these clinical factors, endometrial cancer is classified into three risk groups: low, intermediate, and high [3]. In general, adjuvant therapy has been administered in high-risk endometrial cancer but not in low-risk cases. However, there was no clinical consensus regarding the need for adjuvant therapy in intermediate-risk endometrial cancer.

Practice guidelines in oncology by the National Comprehensive Cancer Network (NCCN) [4] recommend vaginal brachytherapy

Table 3
Percentage of institution adopted indication criteria for adjuvant therapy on endometrioid histology.

LVSI	Histological grade	FIGO surgical stage								
		IA	IB	IC	IIA	IIB	IIIA	IIIB	IIIC	IV
Negative	1	0.0	7.7	73.8	76.7	86.7	97.8	97.8	98.9	100
	2	2.2	20.4	86.7	80.1	87.4	97.8	97.8	98.9	100
	3	18.7	48.4	89.1	88.5	90.2	98.9	97.8	98.9	100
Mild	1	5.0	20.3	76.6	82.2	88.4	97.8	97.8	98.9	100
	2	8.3	30.0	88.8	85.0	88.4	98.9	97.8	98.9	100
	3	21.7	53.3	90.7	88.4	90.1	99.5	97.8	99.5	100
Severe	1	14.4	41.2	88.1	89.0	90.7	99.5	97.8	99.5	100
	2	18.2	50.0	91.9	89.5	90.7	99.5	97.8	99.5	100
	3	29.3	68.5	92.4	90.7	91.3	99.5	97.8	99.5	100

LVSI; lymphatic-vascular space invasion, FIGO; International Federation of Obstetrics and Gynecology.

Table 4
Percentage of institution adopted indication criteria for adjuvant therapy on non-endometrioid histology.

LVSI	Histological grade	FIGO surgical stage								
		IA	IB	IC	IIA	IIB	IIIA	IIIB	IIIC	IV
Negative	1	9.9	29.3	79.8	81.1	89.5	97.8	97.8	98.9	100
	2	12.6	39.2	88.1	84.0	89.6	97.8	97.8	98.9	100
	3	22.5	55.5	90.2	90.2	92.3	98.9	98.9	98.9	100
Mild	1	14.9	39.0	82.1	86.7	90.6	97.8	98.9	98.9	100
	2	18.2	47.2	89.7	87.8	90.6	98.9	98.9	98.9	100
	3	26.7	60.6	91.8	90.1	92.3	98.9	98.9	99.5	100
Severe	1	25.6	53.3	89.2	91.7	92.9	98.9	98.9	99.5	100
	2	29.8	59.9	93.0	91.7	92.9	98.9	98.9	99.5	100
	3	35.4	70.7	93.5	92.3	93.4	98.9	98.9	99.5	100

LVSI; lymphatic-vascular space invasion, FIGO; International Federation of Obstetrics and Gynecology.

and/or pelvic irradiation for intermediate-risk endometrial cancer and vaginal brachytherapy and/or CT for patients having FIGO IC/G3 with adverse risk factors (>60 years of age, positive LVSI, tumor size, or lower uterine involvement) and FIGO IIB/G3. Moreover, the endometrial cancer treatment guidelines of JSGO also recommend pelvic irradiation or CT as an adjuvant therapy for intermediate-risk endometrial cancer. Although publication of an analysis and summary of the present survey was delayed because missing data needed to be confirmed and/or reminders needed to be sent to non-respondents, the present survey indicated that CT was the preferred postoperative adjuvant modality in most of the respondents from JGOG institutions. The reason for the preference of CT over RT as adjuvant therapy for endometrial cancer in Japan unlike in Western countries remains unknown. However, considering that LVSI is considered an important clinicopathologic prognostic factor among members of JGOG, CT would be a suitable adjuvant therapy because as positive LVSI is suggestive of systemic spread. Moreover, JGOG2033 trial [5] has shown that although there was no significant difference between CT and RT in a low- to intermediate-risk group defined as stage IC patients younger than 70 years with grade 1/2 endometrioid adenocarcinoma, compared with whole pelvic irradiation, CT achieved significantly higher progression-free survival (PFS) and higher overall survival (OS) in a high- to intermediate-risk group defined as either (1) stage IC in patients older than 70 years or with grade 3 endometrioid adenocarcinoma or (2) stage II or IIIA. Furthermore, GOG122 trial [6] has also shown that CT significantly improved PFS and OS compared with whole abdominal irradiation in stage III or IV endometrial carcinoma having a maximum residual disease of 2 cm. We suspect that results of these phase III trials, especially those of JGOG2033, would be a reason for the preference of CT over RT among JGOG members. Furthermore, more recent meta-analysis of the MRC ASTEC and NCIC CTG EN.5 trials [7] indicated that adjuvant external beam radiotherapy is not recommended for patients with intermediate-risk or high-risk early-stage endometrial cancer because there was no evidence that overall survival with external beam radiotherapy was better than that observed. Therefore, the tendency to perform CT by JGOG members rather than RT would be higher for high-risk endometrial cancer as an adjuvant therapy in the future.

Regarding indication criteria for adjuvant therapy, over 50% of JGOG members administered adjuvant therapy beyond FIGO IB/G3. A survey conducted among members of the Society of Gynecologic Oncologists (SGO) in 2005 concerning the use of adjuvant RT in endo>metrial cancer also showed similar results. In that survey, the percentages of SGO members who administered adjuvant RT when patients were FIGO IA/G3, FIGO IB/G2, and FIGO IB/G3 were 42.5%, 30.5%, and 62.6%, respectively. Furthermore, a majority of the SGO members administered adjuvant RT for FIGO stage IC [8]. Therefore, our present survey revealed that there is both agreement and disagreement regarding the indication criteria of adjuvant therapy for patients with endometrial cancer among SGO and JGOG members: (1) over 50% of members administered adjuvant therapy when patients was FIGO IB/G3 while over 90% of members administered adjuvant therapy to FIGO IC patients; (2) SGO members prefer RT, while JGOG members prefer CT; and (3) JGOG members considered LVSI as an important clinicopathologic factor to administer adjuvant therapy in endometrial cancer. Interestingly, the present survey revealed that the frequencies of adjuvant therapy were not drastically higher in cases of non-endometrioid histology compared with cases of endometrioid histology. Although the specific reasons for this finding are unknown, it was speculated that JGOG members do not consider histologic subtype as an independent prognostic factor when other clinicopathologic prognostic factors are not included. The present survey also revealed that, although anthracycline/platinum-based CT is recommended for advanced or recurrent endometrial cancer as per the JSGO guidelines—based on meta-analysis of previous clinical trials [9]—59.5% of JGOG members used TC more frequently as an adjuvant CT regimen, despite the lack of evidence from a Phase III trial. A randomized GOG trial reported the comparison of survival benefits from the combination of paclitaxel/adriamycin/cisplatin (TAP) with G-CSF as compared to adriamycin/cisplatin (AP) in women with advanced or recurrent endometrial cancer [10]. Although TAP is being compared with TC in an ongoing GOG randomized trial (GOG 209) [11], TAP has still not been accepted as the standard CT in routine clinical practice in Japan due to concerns of toxicity, including severe neuropathy, congestive heart failure, and severe bone marrow suppression. Cyclophosphamide/adriamycin/cisplatin (CAP) [12] has been considered the most common anthracycline/platinum-based CT regimen for patients with advanced endometrial cancer in Japan based on the results of a JGOG 2033 in which 425 patients with more than 50% MI were randomly administered whole pelvic irradiation or three or more courses of CAP [6]. Although it remains unclear whether TC may have greater efficacy and lesser toxicity than anthracycline/platinum combinations such as AP or CAP, JGOG members prefer TC as the CT regimen for advanced endometrial cancer. Recently, we conducted a Phase III trial (JGOG 2043) [13], in which over 600 patients with surgically treated endometrial cancer were randomly assigned to six courses of AP, TC, or combined docetaxel/cisplatin based on the results of a previous randomized Phase II trial (JGOG 2041) for advanced or recurrent endometrial cancer [14]. The results of JGOG 2043 should resolve the disparity between evidence-based standards and help in revising the JSGO guidelines, thus establishing a standard regimen of CT to improve the prognosis of advanced endometrial cancer.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

References

[1] Watanabe Y, Aoki D, Kitagawa R, Takeuchi S, Sagae S, Sakuragi N, et al. Status of surgical treatment procedures for endometrial cancer in Japan: results of a Japanese Gynecologic Oncology Group Survey. Gynecol Oncol 2007;105:325–8.
[2] Japan Society of Gynecologic Oncology. Endometrial Cancer Treatment Guidelines 2006 (http://www.jsgo.gr.jp/09_guideline/guideline/e_taigan.pdf).
[3] Lurain JR. Uterine cancer. In: Berek JS, editor. Novak's gynecology 13th ed. Philadelphia: Lippincotto Williams & Wilkins; 2002. p. 1143–7.
[4] National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology V2. 2009 (http://www.nccn.org/professionals/physiciangls/PDF/uterine.pdf).
[5] Susumu N, Sagae S, Udagawa Y, Niwa K, Kuramoto H, et al. Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combination chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study. Gynecol Oncol 2008;108:226–33.
[6] Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2006;24:36–44.
[7] ASTEC/EN.5 Study Group. Adjuvant external beam radiotherapy in the treatment of endometrial cancer (MRC ASTEC and NCIC CTG EN.5 randomised trials): pooled trial results, systemic review, and meta-analysis. Lancet 2009;373:97–9.

[8] Naumann RW, Coleman RL. The use of adjuvant radiation therapy in early endometrial cancer by members of the Society of Gynecologic Oncologists in 2005. Gynecol Oncol 2007;105:7–12.
[9] Humber CE, Tierney JF, Symonds RP, Collingwood M, Kirwan J, Williams C, Green JA. Chemotherapy for advanced, recurrent or metastatic endometrial cancer: a systemic review of Cochrane collaboration. Ann Oncol 2007;18:409–20.
[10] Fleming GF, Brunetto VL, Cella D, Look KY, Reid GC, et al. Phase III trial of doxorubicin plus cisplatin with or without paclitaxel plus filgrastim in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2004;22:2159–66.
[11] Fleming GF. Major progress for a less common cancer. J Clin Oncol 2006;24:6–8.
[12] Maggi R, Lissoni A, Spina F, Melpignano M, Zola P, et al. Adjuvant chemotherapy vs. radiotherapy in high-risk endometrial carcinoma: results of a randomized trial. Br J Cancer 2006;95:266–71.
[13] UMIN000000522. JGOG2043: a randomized phase III trial of AP versus DP, TC regimens for high risk group of endometrial carcinoma (http://center.umin.ac.jp:80/cgi-o19n-bin/).
[14] Nomura H, Aoki D, Takahashi F, Katsumata N, Watanabe Y, et al. Randomized phase II study comparing docetaxel plus cisplatin, docetaxel plus carboplatin, and paclitaxel plus carboplatin in patients with advanced or recurrent endometrial carcinoma: Japanese Gynecologic Oncology Group trial (JGOG 2041). J Clin Oncol 2008;26 (May 20 suppl; abstr 16526).

Association of Metastin/a G-protein-coupled Receptor Signaling and Down Syndrome Critical Region 1 in Epithelial Ovarian Cancer

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Abstract. *It has been revealed that metastin/a G-protein-coupled receptor (AXOR12) signaling enhances the expression of Down syndrome critical region 1 (DSCR1), known to be duplicated in Down syndrome, and suppresses tumor metastasis in in vitro study. The aim of this study was to evaluate whether gene expression of metastin/AXOR12 signaling system is correlated with that of DSCR1 and consequently affect prognosis of patients with epithelial ovarian cancer. Patients and Methods: The expression levels of metastin, AXOR12, DSCR1 isoform 1 (DSCR1-1), DSCR1 isoform 4 (DSCR1-4), calcineurin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression were analyzed by real-time quantitative reverse transcription-polymerase chain reaction in 102 epithelial ovarian cancer surgical specimens. Results: Patients were dichotomized into two groups having low and high expressions by using the median value as the cut-off. A good agreement was noticed between metastin and AXOR12 gene expression levels (kappa coefficient; 0.73), however, the gene expression of metastin/AXOR12 signaling system was not significantly correlated with that of DSCR1-4. By univariate Cox regression analysis, the prognosis of the patients with low metastin and low AXOR12 gene expression was significantly worse than that of those with high metastin and high AXOR12 gene expression, respectively (p=0.04 and 0.018). Combination of metastin and AXOR12 gene expression also had significant impact on patient prognosis (p=0.045). The DSCR1-1, DSCR1-4 and calcineurin gene expressions did not significantly affect the prognosis. Conclusion: The precise mechanism of metastin/AXOR12 signaling for suppression of the invasive phenotype in vivo, especially in epithelial ovarian cancer, is still uncertain. Genes such as DSCR1 that are duplicated in Down syndrome might not play an important role in tumorigenesis of epithelial ovarian cancer.*

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Key Words: Metastin, AXOR12, DSCR1, gene expression, real-time quantitative RT- PCR, epithelial ovarian cancer.

The KiSS-1 protein is predicted to consist of 145 amino acids, with a secretory signal sequence located at the *N*-terminus, suggesting that KiSS-1 functions as a secretory protein (1). 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) (2). Furthermore, metastin was identified as a ligand for an orphan G-protein-coupled receptor, designated as AXOR12 (3). Jiang *et al.* (4) reported the differential expression of KiSS-1 and AXOR12 in human ovarian cancer cell lines. SKOV3 cells expressed AXOR12, but lacked expression of KiSS-1. They established a KiSS-1-infected SKOV3 cell line, and found that KiSS-1 expression inhibited migration of SKOV3 cells and reduced colony formation of SKOV3 cells without affecting cell proliferation (4). These results suggest that KiSS-1 serves as a metastasis suppressor for ovarian cancer. Recently, 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. Moreover, high expression of both *metastin* and *AXOR12* genes was significantly associated with improved patient prognosis (5). Therefore, metastin/AXOR12 signaling might suppress the aggressive tumor phenotype in epithelial ovarian cancer. Similar results have been reported in melanoma (6), thyroid cancer (7), esophageal carcinoma (8), urinary bladder cancer (9), and gastric carcinoma (10).

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To better characterize the mechanism of action of metastin/AXOR12 *in vitro*, Stathatos *et al.* (11) created an AXOR12 overexpression model in *AXOR12*-null thyroid cancer cells to identify AXOR12-regulated genes that are potentially responsible for its antimetastasis effect using cDNA microarray. Consequently, they determined that AXOR12 stimulated by recombinant metastin enhanced the expression of myocyte-enriched calcineurin-interacting protein 1 (MCIP-1). Additional studies demonstrated that metastatic thyroid cancer tissue was characterized by loss of MCIP-1 expression, consistent with escape from a metastasis inhibitory effect (11). MCIP-1 is identical to Down syndrome critical region 1 (DSCR1) (12). The *DSCR1* gene was initially isolated independently from the candidate region of human chromosome 21, trisomy of which causes Down syndrome (13, 14). People with Down syndrome are more susceptible to childhood leukemia and germ cell cancer, but a reduced risk of solid tumors in all age groups is reported (15, 16). These findings may open the possibility for future clinical application of DSCR1 protein for prevention of cancer invasion and metastasis, and thus may improve patient prognosis. These results provoked us to evaluate the expression of *metastin/AXOR12* signaling system and *DSCR1* 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 gynecological tumor (17).

In this study, we sought to determine mRNA expression of the *metastin/AXOR12* signaling system and *DSCR1* using a real-time quantitative reverse transcription-polymerase chain reaction (RT- PCR) in cases of epithelial ovarian cancer. The gene expression of *metastin/AXOR12* signaling system was correlated with that of *DSCR1* and their impact on patient survival was evaluated. Moreover, cellular expression of *DSCR1* was examined by immunohistochemistry.

Patients and Methods

Patients. Patients with epithelial ovarian cancer treated between January 1990 and February 2008 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, if they had undergone any kind preoperative therapy, had cancer 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 102 eligible patients was 56 years (range, 29-84 years). Thirty-one of them were premenopausal. Patients were staged according to the 1987 criteria recommended by FIGO (18). There were 48 stage I patients, 4 stage II patients, 43 stage III patients, and 7 stage IV patients. The staging system defined by FIGO, as described elsewhere (19, 20), assumes that an adequate staging operation has been performed. Tumors were classified histologically according to the World Health Organization (WHO) criteria (21) as serous (n=54), mucinous (n=25), endometrioid (n=12), clear cell (n=10) and transitional cell (n=1). The tumors were classified histologically as either being well differentiated (n=65), or moderately differentiated (n=19), or poorly differentiated (n=7) (22). The number of poorly differentiated tumors was smaller than that of well differentiated tumors. This seems to be unusual compared to European series. However, this is a typical population in Japanese ovarian cancer (5, 19, 20, 23).

The surveillance for recurrent disease usually consisted of physical examination, Papanicolaou smear and serology with tumor 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 computed tomography (CT) scan or ultrasonography were obtained every 6 months for 5 years after surgery and every year thereafter, and, if necessary, magnetic resonance imaging (MRI) was performed. Recurrent disease was confirmed either pathologically, radiographically, or serologically. Follow-up information was obtained from medical records, letter, or by telephone contact with patients, and information from the referring physician. Survival data were available for all patients (median follow-up 51 months, range 4-218 months). Of these, 99 patients received platinum and/or paclitaxel-based chemotherapy. Two patients with stage Ia tumors of endometrioid adenocarcinoma and mucinous cystadenocarcinoma, and one with stage IV tumor of serous cystadenocarcinoma had no further treatment after surgery.

Seventy-six of the patients had participated in a previous study (5).

Tissue specimens 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 quatification of mRNA expression.

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 1,000 ng of total RNA using a First-Strand cDNA Synthesis Kit (Pharmacia-LKB, Uppsala, Sweden). Real-time quantitative PCR was performed using the TaqMan system (Applied Biosystems). The gene expression levels of *metastin*, *AXOR12*, *DSCR1 isoform 1* (*DSCR1-1*), *DSCR1 isoform 4* (*DSCR1-4*), *calcineurin*, and internal reference *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) were measured by using TaqMan probes labeled with 6-carboxyfluorescein (FAM) or VIC, respectively. The primers and TaqMan probes of *metastin* and *AXOR12* were designed using Primer Express v 2.0 software (Applied Biosystems). The sequences of primer and TaqMan probe (forward primer, reverse primer, TaqMan probe) were: *metastin*, 5'-GCAGGTCCTTCTCCCGCT-3', 5'-GCCAGATCCCCGCACC-3', 5'-CACCAGCACCGCGCCCTG-3'; *AXOR12*, 5'-TGGCACCCACG CAGCTA-3', 5'-AGTTGCTGTAGGACATGCAGTGA-3', 5'- CCGC CTACGCGCTTAAGACCTGG-3'. We also purchased the Pre-Developed TaqMan Assay Reagents, *DSCR1-1*, *DSCR1-4*, *calcineurin*, and *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 quantity of cDNA for each experimental gene was normalized 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 tumor specimen in triplicate. All samples with a coefficient of variance higher than 10% were retested.

Immunohistochemstry. Immunohistochemistry was performed using the Histofine Simple Stain MAX-PO MULTI (Nichirei Biosciences, Tokyo, Japan) method on 4 μm-thick sections from formalin-fixed, paraffin-embedded blocks. The primary antibody used was anti-DSCR1 mouse monoclonal antibody (generated using the DSCR-1, 94-197 amino acids fragment (24)) at a dilution of 1:500. Mayer's hematoxylin was used as a counterstain. Specificity controls included the omission of primary antibody as negative control and the use of normal placenta as positive control.

Statistical analysis. The kappa coefficient value was used as a measure of agreement between end-points, according to the following criteria: poor agreement (<0.20), fair (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80), and very good (>0.80) (25). 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 (26). Kaplan-Meier curves were compared by the univariate Cox regression analysis. All *p*-values presented are two-sided. A *p*-value of less than 0.05 was considered significant.

Results

Gene expression and clinicopathological features. The median gene expression and (range) was metastin, 0.047 (0.0005-13.566); AXOR12, 4.002 (0.011-135.845); DSCR1-1, 1.019 (0.121-8.920); DSCR1-4, 0.475 (0.026-21.297); and calcineurin, 3.990 (0.343-62.709), respectively. The patients were divided into low or high groups for each gene expression using the median value as the cut-off, respectively. The results of kappa statistic are shown in Tables I-III. A good agreement was noted between *metastin* and *AXOR12* gene expression levels (kappa coefficient: 0.73) (Table I), however, the gene expression of *metastin/AXOR12* signaling system was not significantly correlated with that of *DSCR1-4* (Table II). An agreement between *DSCR1-4* and *calcineurin* gene expression levels was moderate (kappa coefficient: 0.49) (Table III). The values of each gene expression in epithelial ovarian carcinomas were classified according to patient age at diagnosis, stage of disease, presence or absence of residual tumor mass after initial surgery, histological subtype and grade. The patient's age at diagnosis was significantly associated with *metastin* gene expression (p=0.0151) (data not shown). Presence of residual tumor was negatively associated with *metastin* (p=0.0043) and *AXOR12* (p=0.0099) gene expression (data not shown).

Table I. *Agreement between metastin and AXOR12 gene expression.*

	AXOR12 gene expression		Kappa statistic
	High	Low	
metastin gene expression			
High	44	7	0.73 (good)
Low	7	44	

Table II. *Agreement between metastin/AXOR12 signaling system and DSCR1 isoform 4 gene expression.*

	DSCR1 isoform 4 gene expression		Kappa statistic
	High	Low	
metastin and *AOXR12* gene expression			
Both high	23	21	NS
Both low	18	26	

NS, p-Value for kappa statistic was not significant, indicating that agreement was not different from that expected by chance.

Table III. *Agreement between DSCR1 isoform 4 and calcineurin gene expression.*

	Calcineurin gene expression		Kappa statistic
	High	L ow	
DSCR1 isoform 4 gene expression			
High	38	13	0.49 (moderate)
Low	13	38	

Gene expression and prognosis. As shown in Table IV, we found the prognosis of the patients with low *metastin* and low *AXOR12* gene expression to be significantly worse than that of those with high *metastin* and high *AXOR12* gene expression by univariate Cox regression analysis (p=0.04 and 0.018, respectively). Combination of *metastin* and *AXOR12* gene expression also had significant impact on patient prognosis (p=0.045). However, the *DSCR1-1, DSCR1-4, calcineurin* gene expression did not significantly affect the prognosis. FIGO stage (stage III–IV; p<0.0001), residual

Table IV. *The results of univariate Cox regression analysis.*

Variables	Hazard ratio interval	95% confidence	*p*-Value
Age at the time of diagnosis	1.02	0.99-1.05	0.174
FIGO stage			
I-II (n=52)	Referent		
III-IV (n=50)	14.62	4.43-48.21	<0.0001
Residual disease			
Negative (n=59)	Referent		
Positive (n=43)	14.07	4.92-40.24	<0.0001
Histological subtype			
Other (n=48)	Referent		
Serous (n=54)	1.37	0.67-2.79	0.395
Histological grade			
Other (n=95)	Referent		
Poorly differentiated (n=7)	4.92	2.11-11.46	0.0002
metastin gene expression ratio			
High (n=51)	Referent		
Low (n=51)	2.15	1.04-4.48	0.040
AXOR12 gene expression ratio			
High (n=51)	Referent		
Low (n=51)	2.47	1.17-5.21	0.018
Combination of *metastin* and *AXOR12* gene expression levels			
Both high (n=44)	Referent		
Other (n=58)	2.20	1.02-4.76	0.045
DSCR1 isoform 4 gene expression ratio			
High (n=51)	Referent		
Low (n=51)	1.12	0.56-2.24	0.747
Calcineurin gene expression ratio			
Low (n=51)	Referent		
High (n=51)	0.53	0.26-1.07	0.075
DSCR1 isoform 1 gene expression ratio			
High (n=51)	Referent		
Low (n=51)	1.49	0.74-3.00	0.261

Table V. *The results of multivariate Cox regression analysis.*

Variables	Hazard ratio interval	95% confidence	*p*-Value
FIGO stage			
I-II (n=52)	Referent		
III-IV (n=50)	303.63	9.86-9349.49	0.001
Residual disease			
Negative (n=59)	Referent		
Positive (n=43)	0.42	0.37-4.83	0.488
Histological grade			
Other (n=95)	Referent		
Poorly differentiated (n=7)	11.69	2.14-63.97	0.005
Metastin gene expression ratio			
High (n=51)	Referent		
Low (n=51)	11.82	1.16-120.60	0.037
AXOR12 gene expression ratio			
High (n=51)	Referent		
Low (n=51)	0.71	0.80-6.29	0.757
Combination of *metastin* and *AXOR12* gene expression levels			
Both high (n=44)	Referent		
Other (n=58)	0.35	0.02-8.17	0.516

disease (positive; $p<0.0001$), and histological grade (poorly differentiated; $p=0.0002$) were found to be significantly associated with a poor prognosis in univariate Cox regression analysis (Table IV). Older age at the time of diagnosis and serous tumors type are generally thought to be more aggressive (27). However, no significant association for these variables could be found in this study (Table IV). Multivariate Cox regression analysis revealed that low *metastin* gene expression ($p=0.037$), FIGO stage (III–IV; $p=0.001$) and histological grade (poorly differentiated; $p=0.005$) were the independent prognostic factors in this series (Table V).

Cellular DSCR1 expression. DSCR1 was mainly localized in the cytoplasm of the carcinoma cells. An example of DSCR1 expression in a section of endometrioid adenocarcinoma is shown in Figure 1.

Discussion

Although the detrimental effects of Down syndrome such as mental retardation and congenital heart defects are well known to clinicians, less familiar are the apparent benefits seen in many patients with the condition, such as lower incidence of diabetic retinopathies, atheromas and some types of cancer (particularly solid tumors) (28). As regards the occurrence of ovarian cancer, including non-epithelial, higher than expected numbers were seen but were not significant (15). Previous reports have suggested a positive link between ovarian dysgerminoma and Down syndrome (29, 30). Faruqi *et al.* (31) reported a case of a highly aggressive, grade III, poorly differentiated serous adenocarcinoma of the ovary which was determined to exhibit trisomy 21 as the sole chromosomal abnormality. They commented that the presence of trisomy 21 suggested an aggressive phenotype for that tumor and showed the importance of this chromosome in the tumorigenic process of ovarian malignancy (31). *DSCR1* is one of more than 50 genes present in that portion of chromosome 21 that is duplicated in trisomy 21, the chromosomal abnormality

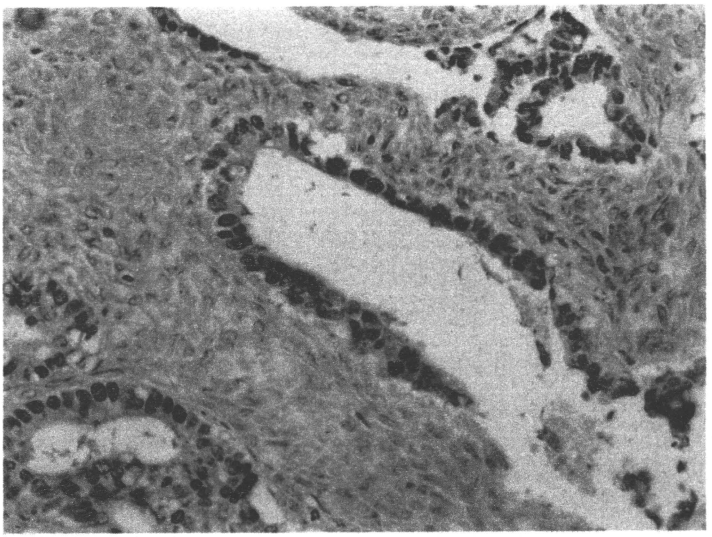

Figure 1. *Immunohistochemical staining for DSCR1 in a section of an endometrioid adenocarcinoma. There is cytoplasmic staining of the tumor cells (original magnification ×200).*

responsible for Down syndrome (13, 14). It has been revealed that metastin/AXOR12 signaling induces consistent increases in the expression of DSCR1 (MCIP-1) and subsequently DCSR1 suppresses metastasis in human cancer *in vitro* (11). High expression of both *metastin* and *AXOR12* identified and confirmed by real-time RT-PCR was significantly associated with improved patient prognosis in epithelial ovarian cancer (5). Therefore, we directed our attention to the relationship between metastin/AXOR12 signaling system and DSCR1 in epithelial ovarian cancer.

The *DSCR1* gene comprises seven exons, the first four of which (exon 1-4) can serve as start sites that then combine with exons 5 to 7 to produce four different mRNA isoforms (14, 32). Since exons 5-7 are likely to be common in each mRNA isoform, for nomenclature simplification Ermak *et al.* (32) proposed to assign them numbers, as isoform 1 (exon composition: 1, 5, 6, 7), isoform (exon composition: 2, 5, 6, 7), isoform 3 (exon composition: 3, 5, 6, 7), and isoform 4 (exon composition: 4, 5, 6, 7). The metastin/AXOR12 signaling increased mRNA levels of the *DSCR1* gene that was found to be DSCR1-4, consequently DSCR1-4 protein product inhibited calcineurin activity (11). It has been also demonstrated that the metastin/AXOR12 pathway chronically inhibits calcineurin activity in human papillary thyroid carcinoma cells and that inhibition of calcineurin inhibits cell motility *in vitro* (11). In this study, we reconfirmed that overexpression of metastin/AXOR12 signaling system suppresses the tumor aggressive phenotype in epithelial ovarian cancer. However, significant association between metastin/AXOR12 signaling and DSCR1-4 as reported in the *in vitro* study was not noted in human epithelial ovarian cancer tissue. Moreover, DSCR1-4 expression did not affect the prognosis. DSCR1 was found to be mainly localized in the cytoplasm of the carcinoma cells, but metastin/AXOR12 signaling in concert with DSCR1 might not be responsible for the inhibition of celluar invasion and metastasis in epithelial ovarian cancer. The precise mechanism of metastin/AXOR12 signaling for suppression of the invasive phenotype *in vivo*, especially in epithelial ovarian cancer is still uncertain.

DSCR1-4 expression blocked cell migration and division in laboratory cells. It also blocked blood vessel formation and tumor progression in mice. Moreover, DSCR1-4 acts by binding to and inhibiting the intracellular molecule calcineurin, which is part of a cell signaling pathway. When vascular endothelial growth factor (VEGF) or thrombin binds

to cell surface receptors, it prompts calcineurin to bind to proteins that move to the nucleus and turn on genes that encourage cell migration, proliferation and angiogenesis (28, 33). But these proteins turn on the *DSCR1-4* gene, and the encoded DSCR1-4 protein then binds to calcineurin and blocks it from participating in the signaling cascade. Therefore, DSCR1-4 provides a negative feedback loop and keeps cell growth in check (34). *DSCR1-1* and *DSCR1-4* represent distinct isoforms of the same gene that are regulated by different promoters and that have opposite effects on tumor growth *in vitro* (35). How these conflicting activities are balanced in tumor growth *in vivo* remains to be determined. Several *DSCR1* isoforms have different expression patterns and likely different functions and regulatory mechanisms (14, 32). Moreover, the expression of different *DSCR1* isoforms by different types of tumors could explain the puzzling finding that, in patients with Down syndrome, the incidence of certain types of malignancy increased, where that of others decreased (15, 16, 29-31). Therefore, we also measured the *DSCR1-1* gene expression in addition to *DSCR1-4* gene expression in this study. However, the prognosis of the patients with epithelial ovarian cancer was not influenced by the gene expression of *DSCR1-1*. These results suggest that genes such as *DSCR1* that are duplicated in Down syndrome may not play an important role in tumorigenesis of epithelial ovarian cancer.

References

1 Harms JF, Welch DR and Miele ME: KISS1 metastasis suppression and emergent pathways. Clin Exp Metast *20*: 11-18, 2003.

2 Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O and Fujino M: Metastasis suppressor gene *KiSS-1* encodes peptide ligand of a G-protein-coupled receptor. Nature *411*: 613-617, 2001.

3 Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, A. Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CGC, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL and Harrison DC: AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J Biol Chem *276*: 28969-28975, 2001.

4 Jiang Y, Berk M, Singh LS, Tan H, Yin L, Powell CT and Xu Y: KiSS1 suppreses metastasis in human ovarian cancer *via* inhibition of protein kinase C alpha. Clin Exp Metast *22*: 369-376, 2005.

5 Hata K, Dhar DK, Watanabe Y, Nakai H and Hoshiai H: Expression of metastin and a G-protein-coupled receptor (AXOR12) in epithelial ovarian cancer. Eur J Cancer *43*: 1452-1459, 2007.

6 Shirasaki F, Takata M, Hatta N and Takehara K: Loss of expression of the metastasis suppressor gene *KiSS1* during melanoma progression and its association with LOH of chromosome 6q16.3-q23. Cancer Res *61*: 7422-7425, 2001.

7 Ringel MD, Hardy E, Bernet VJ, Burch HB, Schuppert F, Burman KD and Saji M: Metastin receptor is overexpressed in papillary thyroid cancer and activates MAP kinase in thyroid cancer cells. J Clin Endocrinol Metab *87*: 2399-2402, 2002.

8 Ikeguchi M, Yamaguchi K and Kaibara N: Clinical significance of the loss of *Kiss-1* and *orphan G-protein-coupled receptor* (*hOT7T175*) gene expression in esophageal squamous cell carcinoma. Clin Cancer Res *10*: 1379-1383, 2004.

9 Sanchez-Carbayo M, Capodieci P and Cordon-Cardo C: Tumor suppressor role of KiSS-1 in bladder cancer: loss of KiSS-1 expression is associated with bladder cancer progression and clinical outcome. Am J Pathol *162*: 609-617, 2003.

10 Dhar DK, Naora H, Kubota H, Maruyama R, Yoshimura H, Tonomoto Y, Tachibana M, Ono T, Otani H and Nagasue N: Down-regulation of Kiss-1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma. Int J Cancer *111*: 868-872, 2004.

11 Stathatos N, Bourdeau I, Espinosa AV, Saji M, Vasko VV, Burman KD, Stratakis CA and Ringel MD: Kiss-1/G protein-coupled receptor 54 metastasis suppressor pathway increases myocyte-enriched calcineurin interacting protein 1 expression and chronically inhibits calcineurin activity. J Clin Endocrinol Metab *90*: 5432-5440, 2005.

12 Rothermel B, Vega RB, Yang J, Wu H, Bassel-Duby R and Williams RS: A protein encoded within the Down syndrome critical region is enriched in striated muscles and inhibits calcineurin signaling. J Biol Chem *275*: 8719-8725, 2000.

13 Fuentes JJ, Pritchard MA, Planas AM, Bosch A, Ferrer I and Estivill X: A new human gene from the Down syndrome critical region encodes a proline-rich protein highly expressed in fetal brain and heart. Hum Mol Genet *4*: 1935-1944, 1995.

14 Fuentes JJ, Pritchard MA and Estivill X: Genomic organization, alternative splicing, and expression patterns of the *DSCR1* (Down syndrome candidate region 1) gene. Genomics *44*: 358-361, 1997.

15 Hasle H, Clemmensen IH and Mikkelsen M: Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet *355*: 165-169, 2000.

16 Boker LK and Merrick J: Cancer incidence in persons with Down syndrome in Israel. Downs Syndr Res Pract *8*: 31-36, 2002.

17 Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ: Cancer statistics, 2008. CA Cancer J Clin *58*: 71-79, 2008.

18 International Federation of Gynecology and Obstetrics (FIGO): Changes in definitions of clinical staging for carcinoma of the cervix and ovary. Am J Obstet Gynecol *156*: 263-264, 1987.

19 Hata K, Kamikawa T, Arao S, Tashiro H, Katabuchi H, Okamura H, Fujiwaki R, Miyazaki K and Fukumoto M: Expression of the *thymidine phosphorylase* gene in epithelial ovarian cancer. Br J Cancer *79*: 1848-1854, 1999.

20 Hata K, Fujiwaki R, Nakayama K and Miyazaki K: Expression of *endostatin* gene in epithelial ovarian cancer. Clin Cancer Res *7*: 2405-2409, 2001.

21 Serov SF, Scully RE and Sobin LH: International Histological Classification of Tumours, No. 9: Histological Typing of Ovarian Tumours. Geneva, World Health Organization, 1973.

22 Arao S, Suwa H, Mandai M, Tashiro H, Miyazaki K, Okamura H, Nomura H, Hiai H and Fukumoto M: Expression of multidrug resistance gene and localization of P-glycoprotein in human primary ovarian cancer. Cancer Res *54*: 1355-1359, 1994.

23 Hata K, Nakayama K, Fujiwaki R, Katabuchi H, Okamura H and Miyazaki K: Expression of the *angiopoietin-1, angiopoietin-2, Tie2,* and *vascular endothelial growth factor* gene in epithelial ovarian cancer. Gynecol Oncol *93*: 215-222, 2004.

24 Minami T, Miura M, Aird WC and Kodama T: Thrombin-induced autoinhibitory factor, Down syndrome critical region-1, attenuates NFAT-dependent vascular cell adhesion molecule-1 expression and inflammation in the endothelium. J Biol Chem *281*: 20503-20520, 2006.

25 Altman DG: Practical Statistics for Medical Research. London, Chapman & Hall, 1991.

26 Preston DL, Lubin JH and Pierce DA: EPICURE: risk regression and data analysis software. Seattle, HiroSoft International Corporation, 1990.

27 Beale PJ and Friedlander ML: Prognostic variables in ovarian cancer. *In*: Epithelial Cancer of the Ovary. Lawton FG, Neijt JP, and Swenerton KD (eds.). London, BMJ Publishing Group, pp. 96-111, 1995.

28 Hampton T: Down syndrome protein deters cancer: scientists reveal molecular mechanism. JAMA *293*: 284-285, 2005.

29 Smucker JD, Roth LM, Sutton GP and Hurteau JA: Trisomy 21 associated with ovarian dysgerminoma. Gynecol Oncol *74*: 512-514, 1999.

30 Satge D, Honore L, Sasco AJ, Vekemans M, Chompret A and Rethore MO: An ovarian dysgerminoma in Down syndrome. Hypothesis about the association. Int J Gynecol Cancer *16 (Suppl 1)*: 375-379, 2006.

31 Faruqi SA, Noumoff MJ, Deger RB, Jalal SM and Antoniades K: Trisomy 21 as the only recurrent chromosomal anomaly in a clinically aggressive ovarian carcinoma. Cancer Gent Cytogenet *138*: 165-168, 2002.

32 Ermak G, Harris CD and Davies KJA: The DSCR1 (Adapt78) isoform 1 protein calcipressin 1 inhibits calcineurin and protects against acute calcium-mediated stress damage, including transient oxidative stress. FASEB J *16*: 814-824, 2002.

33 Minami M, Horiuchi K, Miura M, Abid MR, Takabe W, Noguchi N, Kohro T, Ge X, Aburatani H, Hamakubo T, Kodama T and Aird WC: Vascular endothelial growth factor- and thrombin-induced termination factor, Down syndrome critical region-1, attenuates endothelial cell proliferation and angiogenesis. J Biol Chem *279*: 50537-50554, 2004.

34 Rothermel BA, Vega RB and Williams RS: The role of modulatory calcineurin-interacting proteins in calcineurin signaling. Trend Cardiovasc Med *13*: 15-21, 2003.

35 Qin L, Zhao D, Liu X, Nagy JA, Hoang MV, Brown LF, Dvorak HF and Zeng H: Down syndrome candidate region 1 isoform 1 mediates angiogenesis through the calcineurin-NFAT pathway. Mol Cancer Res *4*: 811-820, 2006.

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Radical hysterectomy for invasive cervical cancer during pregnancy: A retrospective analysis of a single institution experience

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Summary

Purpose: To evaluate long-term prognosis and patient safety for a radical hysterectomy in pregnant women with invasive cervical cancer. *Patients and Methods:* We retrospectively analyzed 12 cases of radical hysterectomy (RH) performed for invasive cervical cancer during pregnancy. Four patients underwent RH with the fetus in situ and another eight patients underwent RH followed by cesarean section. *Results:* The median treatment period was 17 weeks of gestation (range: 9 to 39), the mean blood loss was 550.1 ± 162.5 g (range: 275 to 850). Pelvic lymph node metastases were observed in three patients and parametrial invasion was observed in one patient. Although one patient experienced a recurrence at the vaginal stump, all patients were alive at a median follow-up interval of 105 months (range: 61 to 234). *Conclusion:* RH during pregnancy can be safely performed even with the fetus in situ and a subsequent cesarean section.

Key words: : Cervical cancer; Pregnancy; Radical hysterectomy.

Introduction

A recent trend in Japan for gynecologic malignancies is the occurrence of cervical cancer in the younger population, particularly under 30 years. The annual report of the Gynecologic Cancer Committee of the Japan Society of Obstetrics and Gynecology in 2005 noted that 34.8% of International Federation of Gynecology and Obstetrics (FIGO) Stage I and 13.3% of FIGO Stage II cervical cancer cases occurred in women under 30 in Japan [1]. Although no systematic survey of advanced cervical cancer in pregnant women has been reported, several reports of radical hysterectomies for pregnant women with invasive cervical cancer are present in the English literature [2-4]. We report the long-term prognosis and patient safety for radical hysterectomy (RH) with pelvic nerve preservation [5] in pregnant women with invasive cervical cancer.

Methods

This retrospective study was based on the clinical information found in patient records from 1990 through 2001; these patients underwent radical hysterectomy with pelvic nerve preservation during pregnancy at the Kinki University School of Medicine. Cases treated postpartum were excluded. The two-sample t-test was used for statistical analysis and a p-value less than 0.05 was determined to be statistically significant.

Results

Clinicopathologic characteristics of the patients are presented in Table 1. Seven patients underwent a radical hysterectomy with the fetus in situ (FIS-RH) and another four patients underwent a radical hysterectomy followed by cesarean section (CS-RH). The results of radical hysterectomies for pregnant women with invasive cervical cancer are presented in Table 2. When FIS-RH and CS-RH cases were compared, no significant differences were found in mean blood loss, mean surgical time, and number of blood transfusions. Postoperative complications included a retroperitoneal lymph cyst in one patient (FIS-RH), and a wound seroma in one patient (CS-RH). However, all patients recovered urinary function and also achieved an effective resolution of postoperative urinary retention. Pelvic lymph node metastases were observed in three patients (25.0%) and parametrial spread was observed in one patient (8.3%). The clinicopathologic characteristics of patients with pelvic lymph node metastasis were: 1) Non-keratinizing squamous cell carcinoma (NKSCC) in two patients and adenosquamous cell carcinoma (ADSQ) in one patient; 2) The FIGO stage of all patients was Ib2, the number of positive metastatic lymph nodes was two in two patients (NKSCC and ADSQ) and one patient (NKSCC) had four metastatic lymph nodes; and 3) All cases were FIS-RH. Although one patient (ADSQ) had a vaginal stump recurrence, 11 patients (91.7%) achieved disease-free survival at a median follow-up interval of 105 months.

Discussion

A review of cytologic screening in Japanese women under the age of 30 revealed that FIGO Stage 0 and Ia cervical cancer were most frequently observed in pregnant women. Only a few studies exist in the English literature in regard to radical hysterectomy for pregnant patients with invasive cervical cancer. Monk *et al.* retro-

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Table 1. — *Characteristics of patients.*

Periods analyzed	1990-2001
Total no. of cases	12
Mean age (range)	32.7 ± 3.9 - years (27-39)
Mean no. of prior pregnancies	2.5 ± 1.7 (0-11)
Mean no. of prior labors	1.2 ± 0.9 (0-5)
Median gestational weeks	21.7 week
Histological subtype	
NKSCC	9
KSCC	1
MUC	1
ADSQ	1
FIGO (1994) stage	
IB1	1
Ib2	9
IIa	1
IIb	1
Surgical procedure	
FIS-RH	8
CS-RH	4
Median follow-up period (range)	105 months (61-234)

NKSCC: non-keratinizing squamous cell carcinoma; KSCC: keratinizing squamous cell carcinoma; MUC: mucinous carcinoma; ADSQ: adenosquaomus carcinoma; FIS-RH: radical hysterectomy with the fetus in situ; CS-RH: radical hysterectomy followed by cesarean section.

Table 2. — *Results of surgical treatment.*

Mean blood loss (range)	550.1 ± 162.5 g (275-850)
FIS-RH	500.3 ± 162.3 g (275-850)
CS-RH	637.3 ± 102.3 g (450-829)
Mean time of surgery (range)	150.9 ± 27.0 min (95-185)
FIS-RH	159.3 ± 36.5 min (95-220)
CS-RH	151.3 ± 23.1 min (105-175)
No. of cases needing intra- or postoperative blood transfusion (%)	2/12 (16.7)
FIS-RH (%)	1/8 (12.5)
CS-RH (%)	1/4 (25.0)
Postoperative complication (%)	2/12 (16.7)
Retroperitoneal lymph cyst	1/12 (16.7)
Wound seroma	1/12 (16.7)
Mean periods of urinary retention < 50 ml (range)	12.1 days (9-18)
Prognosis	
Disease-free survival (%)	11 (91.7)
Alive with disease (%)	1 (8.3)
Died due to disease progression (%)	0 (0.0)

FIS-RH: radical hysterectomy with the fetus in situ; CS-RH: radical hysterectomy followed by cesarean section.

spectively studied 13 cases of RH-treated pregnant women with invasive cervical cancer [3], and reported that CS-RH cases suffered a significantly greater blood loss when compared to FIS-RH cases; however, they concluded that RH offers immediate treatment for early-stage cervical cancer during pregnancy, with a low morbidity and acceptable survival. Hopkins *et al.* [4] also retrospectively evaluated the management of 53 pregnant women with cervical cancer, and reported that 21 patients were treated by RH. According to previous studies, both FIS-RH and the CS-RH can be safely performed in pregnant women with invasive cervical cancer. Although biological behavior of cervical cancer is no different than it is in non-pregnant women, several characteristic changes occur during pregnancy, such as non-specific lymph node swelling, decidual changes in the pelvic lymph nodes [6], dilation of pelvic veins, and cervical softness. Therefore, when performing RH on pregnant women, accurate determination of lymph node metastases, attention of venous injury, and accurate recognition of the cervix to ensure surgical margins are important factors. Via a retrospective case-controlled study of 30 women with cervical cancer associated with pregnancy, Sood et al. reported that a planned delay in therapy is safe when patients have Stage 1 disease [7]. Although the number of case reports is small, affirmative results of treatment delay [8-10] or neoadjuvant chemotherapy [11-12] in pregnant patients with invasive cervical cancer has been reported. However, because maternal deaths following treatment delay have been reported, treatment delay for pregnant women with invasive cervical cancer remains experimental and it should only be performed with clear indications and full informed consent. All of our cases were cervical macrocarcinomas and preoperatively diagnosed as greater than FIGO Stage Ib2. Although pelvic lymph node metastases were observed in three of the 12 cases (25.0%), and one case had a vaginal stump recurrence, no deaths occurred; therefore, a RH can be performed in pregnant women with macrocarcinoma staged higher than FIGO Stage Ib2. However, RH during pregnancy, particularly a FIS-RH has the possibility of patient trauma. For example, one patient who underwent a FIS-RH required antidepressant medication after surgery. Although RH can be safely performed on pregnant patients, mental support and counseling are indicated both before and after surgery. Furthermore, to reduce the number of RHs performed during pregnancy, cytological screening for cervical cancer is essential.

References

[1] Gynecologic Cancer Committee in the Japan Society of Obstetrics and Gynecology: "Annual report of gynecologic cancer in 2005". *J. Obstet. Gynaecol. Res.*, 2007, *59*, 901.

[2] Nisker J.A., Schbat M.: "Stage IB cervical carcinoma and pregnancy: report of 49 cases". *Am. J. Obstet. Gynecol.*, 1983, *145*, 203.

[3] Monk B.J., Montz F.J.: "Invasive cervical cancer complicating intrauterine pregnancy: treatment with radical hysterectomy". *Obstet. Gynecol.*, 1992, *80*, 199.

[4] Hopkins M.P., Morley G.W.: "The prognosis and management of cervical cancer associated with pregnancy". *Obstet. Gynecol.*, 1992, *80*, 9.

[5] Sasaki H., Yoshida T., Noda K., Yachiku S., Minami K., Kaneko S.: "Urethral pressure profiles following radical hysterectomy". *Obstet. Gynecol.*, 1982, *59*, 101.

[6] Ashlaf M., Boyd C.B., Beresford W.A.: "Ectopic decidual cell reaction in para-aortic and pelvic lymph nodes in the presence of cervical squamous cell carcinoma during pregnancy". *J. Surg. Oncol.*, 1984, *26*, 6.

[7] Sood A.K., Sorosky J.I., Krogman S., Anderson B., Benda J., Buller R.E.: "Surgical management of cervical cancer complicating pregnancy: a case-control study". *Gynecol. Oncol.*, 1996, *63*, 294.

[8] Duggan B., Muderspach L.I., Roman L.D., Curtin J.P., d'Ablaing G., Morrow C.P.: "Cervical cancer in pregnancy: reporting on planned delay in therapy". *Obstet. Gynecol.*, 1993, *82*, 568.

[9] Sorosky J.I., Squatrito R., Ndubisi B.U., Anderson B., Podczaski E.S, Mayr N. *et al.*: "Stage I squamous cell cervical carcinomain pregnancy: planned delay in therapy awaiting fetal maturity". *Gynecol. Oncol.*, 1995, *59*, 207.

[10] Takushi M., Moromizato H., Sakumoto K., Kanazawa K.: "Management of invasive carcinoma of the uterine cervix associated with pregnancy: outcome of intentional delay in treatment". *Gynecol. Oncol.*, 2002, *87*, 185.

[11] Tewari K., Cappuccini F., Gambino A., Kohler M.F., Pecorelli S., Disaia P.J.: "Neoadjuvant chemotherapy in the treatment of locally advanced cervical carcinoma in pregnancy: a report of two cases and review of issues specific to the management of cervical carcinoma in pregnancy including planned delay of therapy". *Cancer*, 1998, *82*, 1529.

[12] Bader A.A., Petru E., Winter R.: "Long-term follow-up after neoadjuvant chemotherapy for high-risk cervical cancer during pregnancy". *Gynecol. Oncol.*, 2007, *105*, 269.

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CLINICAL INVESTIGATION **Cervix**

PROSPECTIVE STUDY OF ALTERNATING CHEMORADIOTHERAPY CONSISTING OF EXTENDED-FIELD DYNAMIC CONFORMATIONAL RADIOTHERAPY AND SYSTEMIC CHEMOTHERAPY USING 5-FU AND NEDAPLATIN FOR PATIENTS IN HIGH-RISK GROUP WITH CERVICAL CARCINOMA

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Purpose: To assess the efficacy of alternating chemoradiotherapy combined with extended-field conformal radiotherapy for patients with high-risk cervical cancer.

Methods and Materials: Patients with previously untreated cervical cancer, with Stage III/IVA disease, or Stage IB/II with high-risk factor (primary tumor diameter ≥50 mm or positive lymph node) were entered into this study. Three cycles of chemotherapy with 3,500 mg/m^2 of 5-fluorouracil (5-FU) and nedaplatin (NDP) were accompanied with pelvic irradiation of 45.6–51.3 Gy in 24–27 fractions over 6 weeks. Prophylactic (36 Gy/20 fractions) or definitive (45–56 Gy) irradiation for para-aortic region was followed by pelvic irradiation.

Results: Between 1998 and 2004, 40 patients were recruited for this protocol study. Eighteen patients from Phase I setting were registered. Twenty-two patients were treated with NDP of 140 mg/m^2 (the recommended dose) in the Phase II segment. Twenty-five patients had T3 disease, and 25 patients had nodal disease including para-aortic involvement ($n = 5$). Overall/progression-free survival rates at 5 years were 78.8 and 66.5%, respectively. The median follow-up time was 61.8 months (25.5–106.7). Hematologic and gastrointestinal Grade 3 or more toxicities were relatively high rate (27.5–45%); however, they were well manageable. Two for bladder toxicity of Grade 3 were noted. Comparing the data from historical control group evaluated by magnetic resonance imaging, alternating chemoradiotherapy revealed a significant favorable factor for survival and disease recurrence in multivariate analysis ($p < 0.05$).

Conclusion: Acquired results from our unique protocol for cervical cancer with high-risk factor were thought to be promising, considering that the majority of our cohort consisted of high-risk population.

Extended field, Alternating chemoradiotherapy, Nedaplatin, Cervical cancer, Conformational radiotherapy.

INTRODUCTION

Standard treatment for patients with advanced-staged cervical carcinoma is now believed to be concurrent chemoradiotherapy. Chemoradiotherapy improves overall survival (OAS) and progression-free survival (PFS), whether or not platinum was used. Absolute benefit was reported as 10% advantage of OAS and 13% of PFS (1). Chemoradiation showed a significant benefit for local recurrence and a suggestion of a benefit for distant recurrence, although this trend was more markedly noted among patients with Stage I-II disease compared with those of Stage III -IVA (2–5). Contents of chemotherapy regimen was varied much, although weekly administration of cisplatin was now widely used because Gynecologic Oncology Group (GOG) 120 could not showed an apparent advantage of addition of 5-fluorouracil (5-FU) compared with single use of cisplatin (2, 6).

Nedaplatin (NDP) is an active agent for cervical carcinoma (7), shown to have treatment effects equivalent to those of the widely used cisplatin but with less renal and gastrointestinal toxicity (8). Its dose-limiting toxicities (DLT) are thrombocytopenia and myelosuppression, and its recommended dose (RD) in Japan is 100 mg/m^2. However, we have reported the possibility of dose escalation of NDP when used in combination with 5-FU before the administration of NDP. In our previous report, the RD of NDP was 150 mg/m^2 (9). Theoretically, the antitumor effect of concurrent administration is

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identical, but the increasing acute toxicity is an important problem. Thus the intensity of both radiotherapy and chemotherapy would be compromised in this setting. Alternating chemoradiotherapy (ALCRT) is a method for resolving this problem; avoiding the concurrent usage of these two modalities may reduce the acute toxicity, allowing the full dose of chemotherapy to be maintained. We have also reported excellent outcomes of ALCRT in nasopharyngeal cancer (10). As with nasopharyngeal cancer, patients with cervical cancer with advanced stage had hazard of metastatic disease progression, so intensity of chemotherapy is thought to be an important issue for patient management.

To investigate the efficacy and feasibility of ALCRT for high-risk cervical carcinoma, we performed a Phase I/II study at our institution.

METHODS AND MATERIALS

Eligibility criteria

Previously untreated patients with histologically diagnosed as squamous cell carcinoma of uterine cervix were entered into this study. Eligible patient was defined as having a high risk factor (Stage I-II; tumor size ≥50 mm or positive pelvic node OR all Stage III-IV disease); good performance status (PS), adequate organ function; age 20–75; and informed consent. Importance of prognostic indicator of magnetic resonance imaging (MRI) has been reported multi-institutional study (11, 12), and we take account for patient selection for this protocol. Patients with lymph node metastasis limited to para-aortic region who were diagnosed by imaging are also included this study.

Before enrollment, each patient underwent complete physical, laboratory, and stage assessments. The laboratory examinations consisted of complete blood count, serum chemistry, 24-h creatinine clearance, and electrocardiography. The staging workup included chest radiography, computed tomography (CT) of the whole abdomen, and pelvic MRI. Lymph nodes measuring 10 mm or more along the long axis on CT or MRI scan was defined as metastatic nodes. Patients were required to have a white blood cell count ≥3,000/μL, platelet count ≥100,000/μL, hemoglobin level ≥10.0 g/dL, normal hepatic (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level <2.5 times the upper normal limit) and renal function (24-h creatinine clearance level ≥60 mL/min), and normal electrocardiogram. Written informed consent was obtained from all patients. The protocol was approved by the institutional review board.

Response and toxicity evaluations

To evaluate responses and toxicity, all patients underwent complete blood count and serum chemistry analysis one to two times per week. The response evaluation was judged 2 months later from last day of whole treatment. Response evaluation was done with physical examination with smear cytology, pelvic MRI scan, and whole-abdominal CT scan.

Magnetic resonance imaging was repeated every 3–4 months for the first 2 years and twice per year thereafter. A CT scan of the whole abdomen was repeated every 6 months. Toxicity was assessed and graded using the National Cancer Institute Common Toxicity Criteria, version 3.0. The grading of late urinary and gastrointestinal toxicities due to radiotherapy was in accordance with the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer toxicity criteria (13). The DLT were defined as Grade 4 hematologic toxicities or any nonhematologic Grade 3 or higher toxicities, except diarrhea, nausea, and vomiting. The chemotherapy dose and schedule modifications for toxicity are shown in Table 1.

Phase I component

The primary end point of the Phase I part of the study was to determine the maximum tolerated dose (MTD) and the RD of NDP for the Phase II segment, when combined with 120-h infusion of 3,500 mg/m^2 5-FU and definitive radiotherapy on an alternating schedule, for patients with cervical cancer with high-risk factors.

Dose escalation scheme

The starting dose of NDP was 100 mg/m^2, as suggested by a previous study (9). Additional increases of 20 mg/m^2 up to the MTD were permitted. According to our previous report, the dose of NDP did not exceed 150 mg/m^2 (9). At least 3 patients were treated at each dose level. The end point to close the study was a DLT if observed in 2 of 3 patients or in 3 of 6 patients at the same dose levels.

Table 1. Chemotherapy and radiotherapy dose and schedule modifications for toxicity

Toxicity	Modifications
Chemotherapy	
Grade 4 leukopenia, granulocytopenia	25% reduction of both nedaplatin and 5-FU
Grade ≥3 thrombocytopenia	25% reduction of both nedaplatin
Grade 2 renal dysfunction	
Grade ≥3 diarrehea	25% reduction of 5-FU
Grade 2 liver dysfunction	
Grade ≥3 liver or renal reaction	Withheld additional chemotherapy
Nonhematologic Grade >3: toxicity, except for nausea/vomiting	Chemotherapy postponed until recovery
Radiotheraphy	
Grade 4 leukopenia, granulocytopenia	Postponed until recovery to Grade 2
Grade 4 thrombocytopenia	Postponed until recovery to Grade 2
Grade 3 leukopenia, granulocytopenia, and infection or Grade 2 fever	Postponed until recovery of infection and fever
Schedule modification	
Chemotherapy was started with a white blood cell count ≥2, 500 μ^{-1}, platelet count ≥100, 000 μ^{-1}, hemoglobin level ≥8.0 g dl^{-1}, total bilirubin ≤2.0 mg/dL serum creatinine ≤1.2 mg/dL, and esophagitis Grade ≤3. If these data did not fulfill the criteria, radiotherapy was continued until these data recovered . As soon as these data improved, the next cycle of chemotherapy should be started, resting radiotherapy between courses of chemotherapy.	

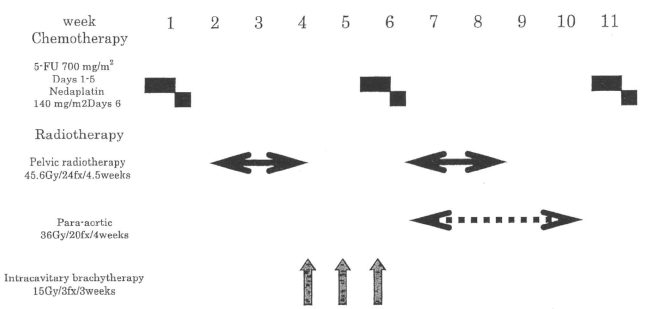

Fig. 1. Treatment scheme of the Phase I/II study of alternating chemoradiotherapy with nedaplatin and 5-FU in patients with advanced cervical carcinoma.

The previous doses before the MTD were considered the RD for the Phase II study.

Phase II component

The primary end point of the Phase II segment of the study was PFS of alternating chemoradiotherapy at the RD. The secondary end points were the OAS and the feasibility of this protocol. The same patient eligibility requirements, treatment schedules, dose and schedule modifications, and response and toxicity criteria as in the Phase I part of the study applied.

Treatment schedule and modifications

Chemotherapy. The treatment scheme is shown in Fig. 1. Prophylactic antiemetics therapy, using a 5-hydroxytryptamine type III receptor blocker and dexamethasone was given to all patients. The details of the administration of chemotherapy have been reported (9, 14). The dose of NDP was elevated to find MTD. MTD was decided to dose limiting toxicities as to Grade 4 of hematologic toxicities and Grade 3 of nonhematologic toxicities excluding diarrhea and nausea/vomiting. After deciding RD, patients were treated with RD of NDP.

Radiotherapy. Radiation therapy using a megavoltage photon beam (6–10 MV) by linear accelerator (CLINAC; Varian Medical Systems) was started 1–2 days after the end of systemic chemotherapy. The gross tumor volume (GTV) was defined as the total volume of the primary tumor evaluated by MRI scan (GTV primary) and the involved lymph nodes (GTV node) assessed by either MRI or abdominopelvic CT scan. A patient with lower vaginal involvement was arranged the adequate inferior margin of radiation field for tumor extent using iodine powder or metallic ring at planning setup. The clinical target volume (CTV) for involved lymph node (CTV node) was defined as the GTV node with 1cm margin in every direction. CTV pelvis was defined as entire uterus and regional pelvic lymph node according to the guidelines of Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer consensus. CTV pan was defined as para-aortic lymph node region located up to upper border of the 12th thoracic spine. In general, CTV pan was included both inferior vena cava and abdominal aorta with 1-cm margin for every direction. The planning treatment volume (PTV) for involved lymph node (PTV node) was defined as the CTV node with a 0.5–1 cm margin. The PTV pelvis and PTV pan was defined as the CTV plus a 0.5–1 cm margin in all directions. Radiotherapy was given with daily 1.9 Gy fractions to 45.6 Gy in 24 fractions for PTV pelvis by biaxial dynamic conformal radiation therapy (11, 12, 15). If patients had a positive pelvic lymph node, they received 51.3 Gy of 27 fractions to PTV pelvis followed by an additional boost dose for PTV node up to a total dose of 57.3 Gy. Patients with positive pelvic lymph node or diagnosed as Stage III or more stage received a prophylactic para-aortic lymph node irradiation of 36 Gy with 20 fractions was planned by dynamic conformal radiotherapy (15). Patients with positive lymph node on para-aortic region receive an additional boost to PTV node up to 54 Gy. Radiotherapy was interrupted during the administration of the second and third cycles of chemotherapy. Intracavitary brachytherapy (ICBT) was accompanied with external beam radiotherapy (EBRT). Both EBRT for PTV primary and ICBT should not be treated in same day. During treatment course, MRI of the pelvis was taken to evaluate response. If primary tumor was thought to shrink to a sufficiently small volume within the high-dose volume of ICBT, brachytherapy was started. All EBRT was planned by radiation treatment planning system FOCUS or XiO (CMS Inc.). Before March 2002, the source of intracavitary brachytherapy was radium, and then was replaced with iridium. High-dose-rate ICBT was delivered using microselectron. The radiation therapy dose and schedule modifications for toxicity are shown in Table 1.

Statistical considerations

The survival time was defined as the period from the start of treatment to death or the last follow-up evaluation, and the PFS was defined as the period from the start of treatment to progression of disease or death, for any reason. The statistical differences between the two groups were assessed with the chi-square test. The OAS and PFS curves were calculated using the Kaplan-Meier method (16). The log–rank test (17) was used to compare survival curves. Cox-proportional hazards model (18) was used for a multivariate analysis.

RESULTS

Characteristics of patients

Between September 1998 and December 2004, 40 patients at the Aichi Cancer Center Hospital, Japan, were enrolled in this Phase I/II study. The patient characteristics of each group are shown in Table 2.

In the Phase I segment, 18 women were enrolled. In the Phase II segment, 22 women were enrolled using RD of NDP.

Phase I study

Dose escalation and toxicity. The principal toxicities observed in the Phase I study are summarized in Table 3. At the first dose level (100 mg/m^2), none of the 3 patients had DLT. At the second dose level (120 mg/m^2), 1 case of Grade 4 thrombocytopenia developed among the 6 patients. This dose level was considered safe, and the dose was increased to the next level. At the third dose level (140 mg/m^2), one case each of Grade 3 liver dysfunction and diarrhea developed among 6 patients. In next dose level (150 mg/m^2), two cases of neutropenia in 3 patients developed, then the MTD was determined to be 150 mg/m^2 and an RD of 140 mg/m^2 was used in the Phase II part.

Completion of therapy. As shown in Table 2, 23 of 40 patients were able to receive three cycles of chemotherapy. Four patients reduced their doses of NDP during the second cycle of chemotherapy. Twenty-three (58%) patients received the third cycle of systemic chemotherapy, but the NDP dose had to be reduced in 4 of these patients. Two patients received only a single cycle of chemotherapy because of toxicities. The 5-FU dose was not reduced in any patients in the Phase II part of the study. Delay or inability to administer the third cycle of chemotherapy was chiefly from hematologic toxicities.

Table 2. Patient characteristics and treatment contents

Factors	Number
Age (y)	54 (34–74)
Performance status	
0	4
1	36
T stage	
1b	
2a	2
2b	10
3a	3
3b	22
N stage	
0	15
I	25
FIGO stage	
I	3
II	11
III	21
IV	5
Maximum tumor size (mm)	61 (35–100)
Radiation theraphy	
EBRT	
Pelvic region (Gy)	53.6 (41.8–64.6)
Paraaortic region	36 (14.4–54)
OTT(days)	51 (34–78)
ICBT	
Source	
Radium	24
Iridium	16
A point dose	23.1 (7.5–27.6)
Fraction	2 (1–4)
Chemotherapy	
Dose of NDP (mg/m^2)	
100–120	9
140	28
150	3
Cycle of chemotherapy	
1	2
2	15
3	23

Table 3. Results of Phase I component

NDP (mg/m^2)	100	120	140	150	Total
Leukopenia	0/3	0/6	0/6	2/3	2/18
Anemia	0/3	0/6	0/6	0/3	0/18
Thrombocytopenia	0/3	1/6	0/6	0/3	1/18
Liver	0/3	0/6	1/6	0/3	1/18
Renal	0/3	0/6	0/6	0/3	0/18
Diarrhea	0/3	0/6	1/6	0/3	1/18
Emesis	0/3	0/6	0/6	0/3	0/18
Vomiting	0/3	0/6	0/6	0/3	0/18
Fever	0/3	0/6	0/6	0/3	0/18
Stomatitis	0/3	0/6	0/6	0/3	0/18
Total	0/3	1/6	2/6	2/3	5/18

A median dose of 53.6 Gy (range, 41.8–64.6 Gy) was administered to pelvic lesion by EBRT. All patients received ICBT using low-dose-rate or high-dose-rate ICBT. The median dose of sum of point A dose of ICBT was 23.1 Gy ranged from 7.5 to 27.6 Gy. All patients could be treated with planned pelvic radiotherapy including ICBT. The median dose of para-aortic region was 36 Gy (range, 14.4–54 Gy). Para-aortic irradiation stopped in 2 patients at 14.4 Gy and 18 Gy because of acute gastrointestinal toxicity. Five patients received an additional radiotherapy to involved para-aortic lymph node with a dose of 46–54 Gy using cone down technique.

Treatment outcomes

Response and survival. The following 22 patients were treated with dose level of RD. Between 1998 and 2004, 65 patients were treated with this protocol, and 40 patients of 65 were evaluated for treatment efficiency. The reasons for exclusion of 25 patients were patient's age, previous treatment before chemoradiotherapy, and refusal of chemotherapy. Thus we evaluated these 40 patients including Phase I study regarding to treatment outcome and feasibility. At the median follow-up of 61.8 months (range, 8.6–106.7 months), 10 patients had died of the disease, 3 were alive with the disease, and 27 were alive without disease.

The OAS and PFS rates at 5 years were 78.8% (95%CI, 65.6–92.1%) and 66.5% (95%CI, 51.4–81.6%), respectively.

Four patients had residual tumor or disease progression at the primary site, and 5 patients had relapses at the pelvic region with or without local failures. Eight patients had distant metastasis during the follow-up period. The OAS and PFS rates were not significantly different between patients received three cycles of chemotherapy and those with one or two cycles ($p > 0.05$).

Table 4. Adverse event of acute adverse event in alternating chemoradiotheraphy with all 40 patients

	1	2	3	4	% of toxicities Grade 3
Leukopenia	4	10	25	1	65
Neutropenia	4	14	14	5	47.5
Anemia	4	21	7	7	35
Thrombocytopenia	12	8	10	8	45
Liver	13	10	3	0	7.5
Renal	10	1	0	0	0
Diarrhea	14	15	9	2	27.5
Emesis	4	19	17	0	42.5
Vomiting	15	25	0	0	0
Fever	0	13	1	0	2.5

Toxicity

The toxicities observed in 40 patients during treatment and follow-up are shown in Table 4. The most common toxicity was leukopenia. Grade 3 or higher leukopenia and granulocytopenia occurred in 26 and 19 patients, respectively. Grade 3 or higher thrombocytopenia and anemia occurred in 18 and 14 patients, respectively. Grade 3 or higher diarrhea occurred in 11 patients. Significant increase of neutropenia and diarrhea was noted in patients with three cycles of chemotherapy compared to those of one or two cycles ($p < 0.05$). There was no treatment-related death. We experienced two cases of Grade 3 of urinary bladder and six Grade 2 of the rectum regarding to late adverse event. No patients developed with Grade 3 or higher of late rectal toxicity. Late toxicity of the rectum and bladder showed no significant difference between patients with three cycles of chemotherapy and those with one to two cycles.

Comparison of historical control group

Between 1986 and 1998, we treated 43 patients with radiotherapy alone who were thought to be eligible for this protocol criteria using staging workup including MRI. During this period, systemic chemotherapy is not generally planned in our institutes; the majority of patients visited during this period were recruited in this cohort. In addition, MRI study was routinely performed to evaluate tumor volumetry in this period. This group (*historical control group*) was compared with the ALCRT group. Patient's characteristics of both groups were summarized in Table 5. Age and radiation dose of the historical control group proved to be significantly higher compared with those of ALCRT ($p < 0.05$). Stage distribution and tumor size did not show a significant difference between the two groups, although tumor size of ALCRT group had a slightly larger than that of the historical control group. ALCRT group showed a tendency for larger ratio of patients with positive lymph node compared with that of the historical control group ($p = 0.07$).

OAS and PFS showed a significant improvement in ALCRT group by univariate analysis. The 5-year OAS rate of ALCRT group is 78.8% (95%CI, 65.6–92.1%) and that of the historical control group is 48.8% (95%CI, 33.9–63.8%; $p = 0.02$, Fig. 2). The 5-year PFS rate of ALCRT group is 66.5% (95%CI, 51.4–81.6%) and that of historical control group is 37.2% (95%CI, 22.8–51.7%; $p = 0.006$, Fig. 3).

Table 5. Patient characteristics of both protocol group and historical control group

Factor	Protocol group	Historical control
Age (median: y)	54*	67
Size (median: mm)	61	55
Pelvic radiation (mean: Gy)	53.6**	59.2
Stage III-IV (%)	65	69.8
Lymph node–positive (%)	62.5***	42.9

* $p < 0.0001$.
** $p = 0.017$.
*** $p = 0.07$.

In multivariate analysis, ALCRT also showed a significant reduction both death and disease progression (Table 6). Hazard ratio of the ALCRT group was 0.639 (95%CI, 0.41–0.96; $p = 0.03$) in OAS and 0.534 (95%CI, 0.35–0.81; $p = 0.002$) in PFS. Late adverse event according to bladder and rectum showed no significant increase in ALCRT group compared with those of historical control group ($p < 0.05$).

DISCUSSION

To the best of our knowledge, this is the first report of successful outcome of chemoradiotherapy using extended-field radiotherapy. The OAS and PFS rates at 5 years were 78.8% (95%CI, 65.6–92.1%) and 66.5% (95%CI, 51.4–81.6%), respectively. Our results of OAS and PFS are thought to be quite comparable to the reported data of concurrent chemoradiotherapy (5, 6, 19) (Table 7). Our protocol has shown acceptable treatment compliance without increasing late toxicities with relatively long follow-up (median, 61.8 months). In addition, our cohort has a higher proportion of both advanced clinical stage and lymph node involvement including para-aortic region compared with reported data (1, 5, 6, 19).

We believe dynamic conformal radiotherapy have a benefit to reduce toxicities especially for chemoradiotherapy setting

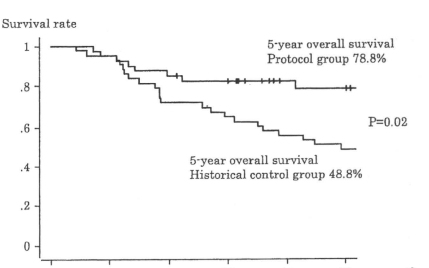

Fig. 2. Overall survival curves of groups of protocol treatment and historical control.

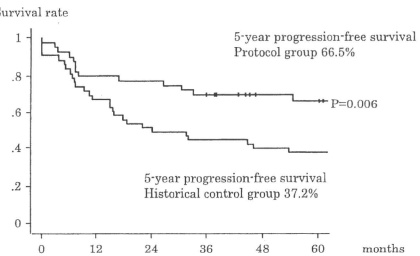

Fig. 3. Progression-free survival curves of groups of protocol treatment and historical control.

with large treatment volume such as extended-field radiotherapy (15, 20, 21). In many reports, researchers used a contiguous field technique for extended field treatment (22–26). This method had an advantage in a short treatment period and an accurate treatment volume. Sequential method such as ours is thought to have a deficit in longer treatment time and would have a potentially loss of disease control. Although patient number was small ($n = 5$), all patients with positive para-aortic disease are well controlled in our protocol. Thus we believe no apparent clinical disadvantage as to sequential radiotherapy for pelvic and para-aortic irradiation. There is another problem of sequential method as to field matching. Both pelvic and para-aortic field should be arranged carefully, because a gap between two fields had a potential risk of underdose or overdose. In this report, we did not experience both regional failure on gap area and late toxicity from excessive dose by overlapping. We also have reported acceptable outcome using sequential EBRT for para-aortic region in definitive and postoperative intent (15, 27). In these reports, para-aortic field was treated with four-field technique (27) or dynamic conformal radiotherapy (15) in sequential setting. In fact, many reports have failed to improve clinical results by simultaneous extended-field chemoradiotherapy (22–24). RTOG 0116 recruited patients with cervical carcinoma and high common iliac or para-aortic metastasis (22). Patients received extended contiguous field radiotherapy up to 54–59.4 Gy with concurrent administration of 40 mg/m^2 of weekly cisplatin. A total of 26 patients were entered, and

Table 6. Mulivariate analysis of several prognostic factor regarding to overall and progression-free survival

	Overall survival		Progression-free survival	
Factor (reference group)	Hazard ratio	*p*-value	Hazard ratio	*p*-value
Age (<62 y)	0.922	0.664	0.927	0.684
Stage (I–II)	1.10	0.600	1.073	0.673
Size (<60 mm)	1.10	0.597	1.409	0.049
Lymph node (no)	1.12	0.597	0.857	0.336
Modality (CRT)	0.639	0.031	0.534	0.0024

Table 7. Comparison clinical results of chemoradiotherapy with or without extended-field radiation

Author	Number	5-year survival	Toxicity (Grade 3 or more)
Varia	95	39 (3 y)	37.7
Grigsby	30	29 (4 y)	80
Maltefano	13	69	0
podczaski	33	31	6
Small	26	60 (18 months)	40
Present	40	78	5
chemoradiotherapy without extended field radiotherapy			
GOG85*	177	NS	4
GOG120			
Weekly CDDP	192	70	2.7
CDDP+5FU*	191	70	0.9
RTOG9001	193	73	13

Abbreviations: GOG = Gynecologic Oncology Group; CDDP = cisplatin; NS = not stated; * = same chemotherapy regimen; RTOG = radiation therapy oncology group.

developed 40% of late Grade 3/4 toxicity, including 8 patients requiring surgical intervention. Estimated OAS at 18 months was 60%. The majority of failure of these studies was based on low compliance from acute or late severe gastrointestinal toxicity. These reports also could not acquire comparable clinical results with standard chemoradiotherapy (22, 24). We reported promising clinical efficacy without increasing toxicity, so we believe sequential para-aortic irradiation should be taken into consideration in practice.

As for method of chemotherapy, cisplatin is now widely accepted as standard care for chemoradiotherapy for cervical cancer (2, 4, 6, 19). The GOG 120 study compared with definitive radiotherapy and hydroxyl-urea and concurrent chemoradiotherapy with cisplatin (6). In the GOG 120 study, two chemoradiotherapy arms were applied—such as weekly cisplatin and combination of 5FU and cisplatin (same arm of GOG 85). In recent report, there was no apparent benefit of addition of 5FU within both two arms, although dose of cisplatin varied much (100 mg/m^2 for the combined arm vs. 240 mg/m^2 for the weekly arm). In the RTOG 9001 study, 5-FU and cisplatin were used with concurrently in chemoradiotherapy arm. The sum of cisplatin of RTOG 9001 study was 225 mg/m^2. RTOG 9001 reported a subset analysis for Stage IB-II versus III-IV, statistical significance only for Stage IB-II subset was noted, leading some to suggest that chemoradiotherapy was not effective in more advanced disease stage (28). The update of RTOG 9001 demonstrated that, because the early stage of disease accrued to the protocol, a strong trend only was noted in the patients with more advanced disease (Stage III-IV) (5). Among three studies (GOG 85, GOG 120, RTOG 9001), the ratio of Stage III-IV disease ranged from 30% to 53.8%, and that of positive lymph node was 12.5–24%. In our cohort, the ratio of both advanced stage disease (III-IV: 65%) and positive lymph node was larger ratio (62.5%) compared with those reported study (4, 6, 19).

One of the reasons of our successful result regardless worse prognostic population of our ALCRT experience was sufficient dose intensity of systemic chemotherapy.

This method had an advantage of intensive drug administration because of minimizing acute toxicities, especially for mucosa and intestine; therefore, patients having potentially distant microscopic disease are thought to be better candidates for ALCRT. In previous report, major failure site of patient with Stage III disease in our institute was distant metastasis (12, 20), then we believe our treatment protocols are promising, especially for advanced disease and extended lymph node involvement with potentially hazards of para-aortic region. Using the ALCRT method, we could achieve high-dose administration (1.4 times higher than domestic standard dose of NDP) of a multidrug agent with successful compliance without increasing toxicity.

Finally, we have used NDP, the derivatives of cisplatin developed in Japan. This antitumor agent had a promising activity for cervical cancer (7, 8) and less toxicities of renal and gastrointestinal (29). We believe one of the reasons of our successful result of ALCRT was lower toxicity of NDP compared with cisplatin. In fact, our cohort showed no significant increase gastrointestinal toxicity and could archive a acceptable compliance of protocol compared with reported data using cisplatin (22). Again we should emphasize our reported effective outcomes of ALCRT with NDP for other malignancies (14, 30).

Our protocol seemed to have a promising advantage for patients with advanced disease or positive lymph node patients. However, this study has a definite limitation because of the retrospective comparison to historical matched control group. The several biases regarding patient selection and treatment content should be considered. In addition, our historical control group received radiotherapy alone, which was not present standard care.

But we believe that an acquired result of ALCRT was quite comparable, slightly better (78% vs. 70–73% in 5-year survival; Table 7) than those of standard chemoradiotherapy without para-aortic irradiation. Compared with their reported data, we should emphasize that our cohort had worse prognostic factors. To evaluate clinical efficacy of ALCRT, especially for more advanced disease or positive lymph node, properly randomized controlled trial comparing ALCRT with NDP with concurrent chemoradiotherapy using cisplatin should be tested in the future.

CONCLUSION

Using both dynamic conformational technique and ALCRT setting, extended-field radiation therapy could be successfully combined with intense multiagent chemotherapy. ALCRT is thought to significantly reduce both recurrence and mortality of patients with advanced cervical carcinoma, chiefly with Stage III or positive lymph nodes. We believed that our promising data of the Phase II study warranted advancing to Phase III study comparing ALCRT with NDP to standard concurrent chemoradiotherapy using cisplatin.

REFERENCES

1. Green JA, Kirwan JM, Tierney JF, *et al.* Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: A systematic review and meta-analysis. *Lancet* 2001;358:781–786.
2. Rose PG, Bundy BN, Watkins EB, *et al.* Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *N Engl J Med* 1999;340:1144–1153.
3. Peters WA 3rd, Liu PY, Barrett RJ 2nd, *et al.* Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. *J Clin Oncol* 2000; 18:1606–1613.
4. Morris M, Eifel PJ, Lu J, *et al.* Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *N Engl J Med* 1999;340: 1137–1143.
5. Eifel PJ, Winter K, Morris M, *et al.* Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: An update of radiation therapy oncology group trial (RTOG) 90-01. *J Clin Oncol* 2004;22: 872–880.
6. Rose PG, Ali S, Watkins E, *et al.* Long-term follow-up of a randomized trial comparing concurrent single agent cisplatin, cisplatin-based combination chemotherapy, or hydroxyurea during pelvic irradiation for locally advanced cervical cancer: A Gynecologic Oncology Group Study. *J Clin Oncol* 2007; 25:2804–2810.
7. Adachi S, Ogasawara T, Wakimoto E, *et al.* Phase I/II study of intravenous nedaplatin and intraarterial cisplatin with transcatheter arterial embolization for patients with locally advanced uterine cervical carcinoma. *Cancer* 2001;91:74–79.
8. Yoshinaga K, Niikura H, Ogawa Y, *et al.* Phase I trial of concurrent chemoradiation with weekly nedaplatin in patients with squamous cell carcinoma of the uterine cervix. *Gynecol Oncol* 2007;104:36–40.
9. Fuwa N, Kodaira T, Kamata M, *et al.* Phase I study of combination chemotherapy with 5-fluorouracil (5-FU) and nedaplatin (NDP): Adverse effects and recommended dose of NDP administered after 5-FU. *Am J Clin Oncol* 2002;25:565–569.
10. Fuwa N, Kano M, Toita T, *et al.* Alternating chemoradiotherapy for nasopharyngeal cancer using cisplatin and 5-fluorouracil: A preliminary report of phase II study. *Radiother Oncol* 2001;61: 257–260.
11. Kodaira T, Fuwa N, Kamata M, *et al.* Clinical assessment by MRI for patients with stage II cervical carcinoma treated by radiation alone in multicenter analysis: Are all patients with stage II disease suitable candidates for chemoradiotherapy? *Int J Radiat Oncol Biol Phys* 2002;52:627–636.
12. Kodaira T, Fuwa N, Toita T, *et al.* Comparison of prognostic value of MRI and FIGO stage among patients with cervical carcinoma treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2003;56:769–777.
13. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). *Int J Radiat Oncol Biol Phys* 1995;31:1341–1346.
14. Kodaira T, Fuwa N, Kamata M, *et al.* Single-institute phase I/II trial of alternating chemoradiotherapy with 5-FU and nedaplatin for esophageal carcinoma. *Anticancer Res* 2006;26:471–478.
15. Kodaira T, Fuwa N, Nakanishi T, *et al.* Long-term clinical outcomes of postoperative pelvic radiotherapy with or without prophylactic paraaortic irradiation for stage I-II cervical