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Treatment with paclitaxel plus carboplatin, alone or with irradiation, of advanced or recurrent endometrial carcinoma

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Abstract

Objectives. The goal of this study was to evaluate the efficacy and toxicity of paclitaxel plus carboplatin in the treatment of primarily advanced or recurrent endometrial carcinoma.

Methods. Thirty-seven consecutive patients with advanced or recurrent endometrial carcinoma were enrolled in this study. Paclitaxel at a dose of 175 mg/m^2 was administered intravenously over 3 h followed by carboplatin with area under the curve of 5 to 6 over 1 h at 4-week intervals. Five patients were received 50 Gy pelvic irradiation, and 7 were received 50 Gy pelvic and 50 Gy paraaortic irradiation, after adjuvant chemotherapy with paclitaxel plus carboplatin. Eighteen patients had evaluable lesions. Responses were assessed before the use of any irradiation.

Results. Eleven patients (61%) achieved an objective response, including one complete response (5.6%) and 10 partial responses (56%). The most common toxicity was hematologic: grade 3 or 4 leukopenia and neutropenia occurred in 59% and 86% of patients, respectively. Three patients (8%) required granulocyte colony-stimulating factor support. One patient required a platelet transfusion, and four required blood transfusions. There was a single adverse event of anaphylaxis.

Conclusion. The combination of paclitaxel and carboplatin appears to be an effective regimen for the treatment of patients with advanced or recurrent endometrial carcinoma with tolerable toxicity.
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Keywords: Paclitaxel; Carboplatin; Endometrial cancer

Introduction

Endometrial carcinoma is one of the most common gynecologic malignancies. A large percentage, approximately 85%, of patients diagnosed as endometrial cancer has shown to have limited disease that can be cured with surgery alone with or without adjuvant radiation [1]. However, for cases of advanced or recurrent disease, there is no consensus regarding the optimal therapy and systemic chemotherapy is required.

Single-agent chemotherapy regimens including doxorubicin, cisplatin, carboplatin and paclitaxel have been employed in this setting and have been reported to have response rates of 25%, 20%, 33% and 36% [2–6]. Studies examining combination regimens have shown to have higher response rates. The results from a Gynecologic Oncology Group phase III trial showed that the combination of cisplatin and doxorubicin improved response rate (42% vs. 25%) and progression-free survival compared with doxorubicin alone with a negligible impact on overall survival and increased toxicity [2]. Because of its low response rate, cardiac and renal toxicity, and requirement of hydration, identification of new chemotherapy regimens is necessary.

In recent years, several studies demonstrated efficiency of paclitaxel plus carboplatin in the treatment of endometrial cancer [7–11]. The purpose of this study was to evaluate further the efficacy of paclitaxel plus carboplatin, with or without irradiation, in the treatment of primarily advanced or recurrent endometrial carcinoma. We also investigated the adverse effects of this combination chemotherapy.

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Patients and methods

This study includes 37 patients with histologically confirmed endometrial carcinoma treated between 1999 and 2005 at the Department of Obstetrics and Gynecology of the University of Tokyo. Patients were eligible if they met any of the following criteria: (1) newly diagnosed, primarily advanced, i.e. surgical stages IIIB, IIIC, or IV; clinical stage IV; IIIA with macroscopic ovarian involvement; or (2) recurrent after surgery and/or radiotherapy. Additional eligibility criteria included Gynecologic Oncology Group performance status of 2 or better, an interval of 3 weeks or more since any prior tumor-directed therapy, recovery from recent surgery or radiotherapy or chemotherapy, and adequate white blood cell count ($\geq$3000/μl), platelet count (=100,000μl), and renal function test (serum creatinine level $\leq$ 1.5 mg/dl).

All patients underwent a pretreatment regimen, designed to abrogate hypersensitivity reactions of paclitaxel, which consisted of dexamethasone 20 mg, diphenhydramine 50 mg, and ranitidine 50 mg. Paclitaxel at a dose of 175 mg/m^2 was infused intravenously in 500ml of normal saline over 3 h, followed by carboplatin with an area under the curve (AUC) of 5 (patients who had been previously treated with chemotherapy and/or irradiation) or 6 (patients who had not received any chemotherapy and irradiation) in 250 ml of 5% glucose over 1 h. All treatment was repeated every 28 days. Five to six cycles were administered for patients with recurrent tumor and for primary advanced cases of clinical stage IVb, unless there was documented disease progression, undue toxicity. For primary advanced cases of clinical stage III or IVa, three cycles of chemotherapy were given, followed by whole pelvis 50 Gy irradiation and/or 50 Gy irradiation at the paraaortic nodal region depending on their nodal involvement [12].

Response to the chemotherapy was assessed in those with measurable disease using radiographic and clinical assessment. Responses were assessed before the use of any irradiation. A complete response (CR) required disappearance of all clinically detectable disease for at least 4 weeks. A partial response (PR) required >50% reduction in the sum of the products of the two largest perpendicular dimensions of bidimensionally measurable lesions for at least 4 weeks. Stable disease was defined as a steady state of response, either less than a partial response or progression of less than 25%. All other cases were considered to have progressive disease. Response duration was defined as the time from partial or complete response to the appearance of progressive disease. Time to progression was measured from the time of initiation of treatment to the time of last patient contact or documented progressive disease. Survival was calculated from the time of initiation of therapy to the last patient contact or death [13]. Time to progression and survival curves were constructed using the Kaplan–Meier product limit method [14].

Toxicity was graded according to National Cancer Institute-Common Toxicity Criteria. Toxicity was recorded as the worst ever per patient for this treatment regimen. For the purpose of this report, it is only recorded for the chemotherapy cycles and not for the radiotherapy component.

Table 1
Patient characteristics

Characteristic	No. of patients	
	Primary	Recurrent
Age (years)		
Median	58	62
Range	30–80	42–81
Stage (FIGO)		
IIIa	4	N/A
IIIc	10	N/A
IVb	9	N/A
Histology type		
Endometrioid	17	13
Serous	1	1
Clear cell	2	0
Mixed	1	0
Undifferentiated	2	0
Grade (Endometrioid type)		
G1	2	5
G2	10	6
G3	5	2

N/A: not applicable as recurrent disease.

Table 2
Response rates

	Primary	Recurrent	Total
CR	0	1	1
PR	3	7	10
SD	1	3	4
PD	0	3	3
Response rates	75%	57%	61%

Results

Thirty-seven endometrial cancer patients were treated with paclitaxel and carboplatin during the study period. The median age of the patient population was 59 years (range: 30 to 81 years). The main characteristics of the patients are summarized in Table 1. Twenty-three patients (62%) presented with an advanced FIGO stage (III or IV). Five patients were received 50 Gy pelvic irradiation, and 7 were received 50 Gy pelvic and 50 Gy paraaortic irradiation, after adjuvant chemotherapy with paclitaxel plus carboplatin. One of them received this regimen as a neo-adjuvant chemotherapy. Eighty-one percent of histology of the tumors were endometrioid adenocarcinoma. Ten of 14 recurrent cases had received prior chemotherapy: seven patients had been treated with cyclophosphamide, doxorubicin and cisplatin, one had received cyclophosphamide, doxorubicin and carboplatin, and another two had used paclitaxel and carboplatin. The median number of cycles of paclitaxel plus carboplatin administered was 4 (range: 1 to 9 cycles).

Response

Eighteen patients (49%) had measurable disease. Patients with measurable disease were analyzed for the response assessment. The overall response rate was 61% (95% confidence interval [CI]: 36% to 86%). The great majority of responses were partial (Table 2). Of the 10 patients who had received prior chemotherapy, 5 patients (50%) had a partial response. Because of this small number of patients and the variety of subgroups, it is not possible to perform meaningful subset analysis of response.

Survival

Of 23 primarily advanced patients, 4 individuals have died of progressive cancer and 4 are currently alive with disease. The remaining 15 patients are currently without clinical evidence of disease. The median overall and progression-free survival time for primarily advanced patients has not yet been reached, with a 77% 3-year overall survival rate (Fig. 1). The median progression-free survival time for those with recurrent was 7 months (range: 1 to 33 months). The median overall survival time for those patients has not yet been reached (Fig. 2).

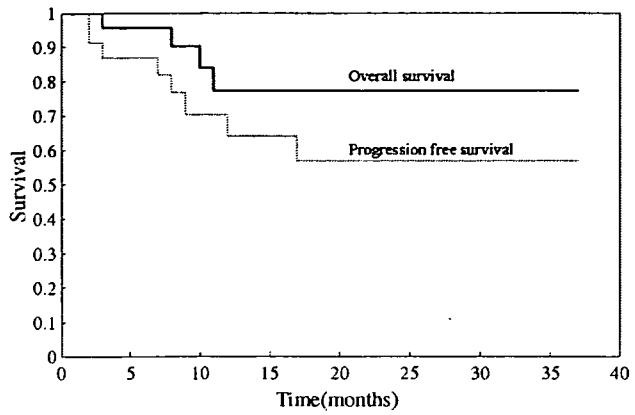

Fig. 1. Overall and progression-free survival time in patients with primarily advanced cancers.

Toxicity

There were no treatment-related deaths. The most common toxicity was hematologic (Table 3), with 59%, 86% and 11% of patients experiencing grade 3 or 4 leukopenia, neutropenia and thrombocytopenia, respectively. Three patients (8%) required granulocyte colony-stimulating factor (G-CSF) support. One patient (3%) had a platelet transfusion, and four (11%) had blood transfusions during the chemotherapeutic portion of the regimen. Non-hematologic toxic reactions consisted of grade 3 nausea and emesis in 2 patients (5%), peripheral neuropathy in 3 patients (8%), diarrhea in 1 patient (3%), general fatigue in 1 patient (3%) and dyspnea in 1 patient (3%). Alopecia was observed in all patients. A single patient encountered severe hypersensitivity reactions: this patient and one patient who suffered grade 3 peripheral neuropathy required to discard continuation of the chemotherapy.

Discussion

Both paclitaxel and carboplatin have been reported to have activity against endometrial carcinoma [4–6]. The purpose of this study was to evaluate the activity and toxicity of the combination of paclitaxel and carboplatin in women with primarily advanced or recurrent endometrial cancers. There are several studies which demonstrated activity of paclitaxel plus carboplatin in the treatment of endometrial cancer (Table 4) [7–11]: for example, Hoskins et al. reported the response rate being 61% in 46 patients with either advanced or recurrent disease treated by this chemotherapy regimen alone or with irradiation.

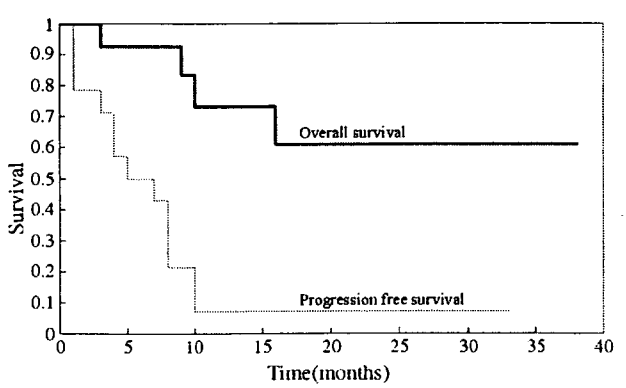

Fig. 2. Overall and progression-free survival time in patients with recurrent cancers.

Table 3
Hematologic toxicity

Toxicity	% of patients affected				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	0	14	27	51	8
Neutropenia	0	3	11	27	59
Anemia	0	19	57	16	8
Thrombocytopenia	48	41	0	11	0

In this study, we observed objective responses in 61% of patients, including complete responses in 6% of them. Our data about overall response rate were in line with other reports. One of the reasons for relatively lower rate of CR in our study was that 14 of 18 patients with measurable lesions were recurrent and that 10 of them had received prior chemotherapy. Considering these backgrounds of the patients, this combination chemotherapy with paclitaxel and carboplatin is highly effective against endometrial cancer. This study also supports the results of two other reports of secondary chemotherapy with paclitaxel and/or platinum in patients with endometrial carcinoma [15,16].

Toxicity with this regimen was tolerable, the most common one being hematologic side-effects. The fact that only limited number of patients required G-CSF support and/or blood transfusion supports the feasibility of this regimen in control of endometrial cancer. Only one patient who required discontinuation of this regimen due to grade 3 peripheral neuropathy was recurrent and had been treated with 9 cycles of cyclophosphamide, doxorubicin and cisplatin (CAP regimen) and 2 cycles of cyclophosphamide and cisplatin (CP regimen), prior to the entry into this study. Accumulation of neurotoxicity due to prior treatment with 11 cycles of chemotherapy using cisplatin might have a role in the occurrence of serious neuropathy. In addition, severe hypersensitivity reactions (HSRs) were observed in one patient though standard anti-allergic pretreatments had been given her. The incidence of severe HSRs to paclitaxel has been reported. Sendo et al. reported that in 105 patients with ovarian cancer during the chemotherapy of paclitaxel and carboplatin, the frequency of

Table 4
Chemotherapy results for primary advanced or recurrent endometrial carcinoma

	No. of patients	Response (%)		
		CR	PR	Total
Price [7]	8	0	63	63
Hoskins [8][a]	46	15	46	61
Nakamura [9]	11	45	27	73
Akram [10]	18	35	28	63
Michener [11]	17	41	41	82

[a] Included irradiation.

HSRs that led to cessation or discontinuation of the chemotherapy was 13.3% [17]. It has been reported that pemirolast is potentially useful for prophylaxis of paclitaxel-induced HSRs [18]. Revision of the current protocol for premedication is requisite.

Our current study included a case that the combination of paclitaxel and carboplatin was administered as neo-adjuvant chemotherapy. Her uterine body and cervix were enlarged and left hydronephrosis due to left ovarian metastasis was observed. In addition, there was a metastasis of the liver so that her clinical stage was IVB. After 4 cycles of paclitaxel and carboplatin, both the primary and metastatic lesions showed a marked decrease in size. She underwent complete surgery of total abdominal hysterectomy, bilateral salpingo-oophorectomy, partial omentectomy and partial resection of liver. Adjuvant chemotherapy was performed and there is no evidence of disease so far. Though there is no consensus regarding to the neo-adjuvant chemotherapy as the treatment of endometrial carcinoma, the existence of such a case and higher responses of this combination chemotherapy suggest that neo-adjuvant chemotherapy with paclitaxel plus carboplatin is effective to advanced endometrial cancer.

The results of our study indicate that the combination of paclitaxel and carboplatin, alone or with irradiation, is effective against primarily advanced and recurrent endometrial cancer. Long-term follow-up and additional prospective randomized studies are necessary to be better able to predict the efficacy of the chemotherapy with this regimen.

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ORIGINAL ARTICLE

Explanation for the failure of neoadjuvant chemotherapy to improve outcomes after radiotherapy for locally advanced uterine cervical cancer from the standpoint of the tumor regression rate

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Abstract
Purpose. Treatment outcomes for patients with locally advanced cervical cancer are no better with neoadjuvant chemotherapy (NAC) combined with radiotherapy (RT) than with RT alone. We investigated the reason for this failure from the standpoint of the tumor regression rate (RR).
Materials and methods. A total of 48 patients with clinical stage IIB-IVA cervical squamous cell carcinoma were treated clinically with cisplatin-based NAC plus RT (n = 15) or RT alone (n = 33). The RR was defined as the slope of a tumor shrinkage curve derived with magnetic resonance images. The local control rate (LCR) and disease-free rate (DFR) were estimated by clinical stage (IIB vs. III-IVA), pretreatment volume (≤ median vs. > median), lymph node status (negative vs. positive), treatment type, overall treatment time (≤8 weeks vs. >8 weeks), and RR (≤ median vs. > median) using univariate and multivariate analyses.
Results. RR during NAC or during NAC and RT (n = 15) was not significantly higher than RR by RT alone (n = 33). Low RR and positive nodal status were significantly powerful prognostic factors for both the LCR and DFR, whereas the others were not.
Conclusion. Although effective in reducing tumor volume prior to RT, NAC showed no overall effect in increasing the RR, which was shown to be the most powerful prognostic factor.

Key words Cervical cancer · Neoadjuvant chemotherapy · Radioresponse · Chemoradiotherapy · Cross-resistance

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Introduction

Radiotherapy (RT) has long played a central role in the treatment of uterine cervical cancer. The U.S. National Cancer Institute recommends concurrent use of RT and cisplatin or cisplatin plus fluorouracil chemotherapy rather than conventional RT alone for locally advanced cervical cancer.[1] Concurrent use of these chemotherapeutic agents at low doses (chemoradiotherapy, CRT) is believed to sensitize tumor cells to RT and thereby increase local control, and the efficacy of this treatment has been confirmed by systematic review and meta-analysis.[2] In contrast, the efficacy of high-dose neoadjuvant chemotherapy (NAC) before RT or surgery has not

been demonstrated by either systematic review or meta-analysis, at least with regard to survival.[3,4]

Our group has treated patients with locally advanced disease using CRT since 2001 and currently limits use of NAC to patients with clinically evident distant metastasis and those who present with radiologically probable metastasis to paraaortic lymph nodes, given this finding's association with distant metastasis. During the period between 1997 and 2000, we specifically used NAC for patients with locally advanced cervical cancer, when considered feasible, in the expectation of tumor shrinkage in advance of RT. The clinical experience gained suggested that NAC offered no advantage over RT-alone treatment. Here, we retrospectively compared treatment outcomes for those receiving NAC plus RT versus those given RT alone. Assessment included the tumor radioresponse, expressed in terms of the tumor regression rate (RR) (i.e., speed of shrinkage), in addition to standard prognostic factors including clinical stage, pretreatment tumor volume, overall treatment time, and pelvic nodal status.[5–7] We then used these findings to attempt to explain why NAC has failed to produce a net benefit.

Materials and methods

Patients

The study comprised 48 patients selected from 141 consecutive patients with cervical squamous cell carcinomas in clinical stages IB1-IVB (International Federation of Gynecology and Obstetrics staging system) treated definitively by RT alone ($n = 96$), NAC ($n = 38$), or CRT ($n = 7$) between 1993 and 2002. Eligibility criteria were clinical stages IIB-IVA, treatment with NAC plus RT (NAC group) or RT alone (RT-alone group), and magnetic resonance (MR) images eligible to assess RR. Excluded patients ($n = 93$) were those with stage IB1-IIA ($n = 21$) or stage IVB ($n = 8$) disease, those referred for RT after failure of surgical care ($n = 4$), those treated by CRT ($n = 7$), or those without complete MR images ($n = 53$). Eligibility with MR images include (1) availability of pre-RT and post-RT MR images for the RT-alone group or pre-NAC, post-NAC but pre-RT, and post-RT MR images for the NAC group; (2) all pretreatment MR images were obtained within 1 month of treatment; and (3) MR images identified a tumor with sufficient clarity for measurement. The 53 patients were excluded because they did not have a complete set of MR images ($n = 29$; most lacked post-RT images), pretreatment MR images were not obtained within the specified time ($n = 22$), or their tumors were difficult to identify ($n = 7$). Patient age ranged from 32 to 95 years (median 60 years), and clinical stages were IIB ($n = 11$), IIIA ($n = 0$), IIIB ($n = 29$), and IVA ($n = 8$).

Treatment

Radiotherapy consisted of external RT and intracavitary RT, which was identically applied irrespective of treatment group. External RT was performed with a 10-MV X-ray in 1.8-Gy fractions at five fractions per week. The initial clinical target volume was the normal whole pelvis including up to the common iliac bifurcation ($n = 45$) or the small pelvis including up to the internal and external iliac bifurcation for elderly patients ($n = 3$). Individual pelvic central doses and boost doses via external RT as well as the timing and number of intracavitary RT insertions were determined in consideration of the pretreatment tumor volume, tumor response, and lymph node status. After total doses of 20.0–61.0 Gy (median 30.6 Gy) were reached, central shielding was placed in the pelvic RT field ($n = 46$) for the start of intracavitary RT. Two patients underwent intracavitary RT after the completion of external RT without central shielding (50.4 Gy and 61.0 Gy). After a total dose of 50.4 or 50.0 Gy was reached, boost RT was administered to parametrial invasion or radiologically detected lymphadenopathy at 3.6–16.0 Gy ($n = 26$; median 10.0 Gy). Thus, the total dose with pelvic external RT ranged from 45.0 to 66.4 Gy ($n = 48$; median 54.0 Gy). Paraaortic RT was performed in 10 patients (6 RT-alone patients and 4 NAC patients) at 1.8-Gy fractions, five fractions per week, to total doses ranging from 45.0 to 57.6 Gy (median 45.0 Gy) definitively ($n = 6$) or prophylactically ($n = 4$), concurrently ($n = 8$) or sequentially ($n = 2$). Intracavitary RT was performed with a high-dose-rate remote afterloading system. The prescribed dosage to reference point A was 6.0 Gy per insertion at two ($n = 3$), three ($n = 14$), four ($n = 59$), or five ($n = 20$) weekly insertions per patient. Typically, a patient with a stage IIIB disease underwent 54.0–57.6 Gy of external RT with central shielding after 30.6 Gy and four insertions of intracavitary RT over 7.5 weeks. Overall treatment time ranged from 38 to 71 days (median 50 days). Prolongation of overall treatment (>56 days), which is known to be associated with poor local control,[6] occurred in 12 patients (median 61 days; 11 RT alone, 1 NAC).

NAC regimens were not consistent owing to historical and multiinstitutional use, but they were all cisplatin-based. NAC was delivered intravenously ($n = 14$) or intraarterially ($n = 1$). The most frequent intravenous regimen ($n = 11$) was cisplatin or platinum analog (80 mg/m^2, day 1), ifosfamide (1000 mg/m^2, days 1–5), and

peplomycin (5 mg, days 1–6) administered every 4 weeks for two cycles. Other intravenous regimens included cisplatin (90 mg/m^2; days 1, 8, and 15) plus irinotecan (90 mg/m^2; days 1, 8, and 15) administered every 4 weeks for two cycles (n = 2) and cisplatin plus other agents administered every 4 weeks for two cycles (n = 1). The intraarterial regimens were platinum analog (100 mg/injection), mitomycin C (8 mg/injection), and peplomycin (30 mg/injection) administered for one cycle (n = 1). The time between the start of NAC and start of RT ranged from 35 to 67 days (median 52 days).

Estimation of tumor volume and response

Tumors identified on T2-weighted MR images as high-intensity lesions were measured three-dimensionally on axial and sagittal images for maximum width, thickness, and length. Tumor volume was calculated on the assumption that the tumor mass was ellipsoid.

Tumor shrinkage curves were derived from the pretreatment volume, the post-NAC but pre-RT volume (NAC group only), and the post-RT volume by plotting on a semilogarithmic scale graph with the start of RT set as day 0. Tumors that disappeared, that were recognized as a remnant only, and that remained as a high-intensity "scar" which was difficult to measure were all regarded as 0.05 cm^3, corresponding to a 0.5 cm diameter tumor; they were regarded as representing a complete response. RR was defined as the exponent (day^{-1}) of an exponential regression equation fitted to a shrinkage curve on the assumption that tumors regressed exponentially.

The RR was estimated for RT-alone tumors during RT and for NAC-group tumors during both NAC and RT and during NAC (RR_{NAC}). The RR of the NAC-group tumors was estimated by omitting the post-NAC but pre-RT volume for tumors that did not respond completely during NAC and was equal to that for the RR_{NAC} for tumors that responded completely during NAC.

Assessment of pelvic nodal status

Pelvic nodal status was radiologically assessable by pretreatment computed tomography (CT) in 47 patients. Lymph nodes ≤10 mm were considered negative for metastasis, and those >10 mm were considered positive.[8]

Statistical analysis

Differences between treatment groups in terms of patient age, clinical stage (stage IIB or stages III–IVA), pretreatment volume, and nodal status (negative or positive) were tested by the Mann-Whitney U-test or Fisher's exact test. The RR of the RT-alone group was compared to the RR of the NAC group and to RR_{NAC}, and the statistical difference was tested by the Mann-Whitney U-test.

Treatment outcomes, including the local control rate (LCR) and disease-free rate (DFR), were estimated by the Kaplan-Meier method; and differences were tested by the log-rank test. The LCR was calculated by counting local recurrence in the irradiated pelvis including the cervix, parametrium, pelvic lymph nodes, and upper vagina as an event and by censoring death from metastasis or other causes with no evidence of local recurrence and survival at the time of the last follow-up examination with no evidence of local recurrence. DFR was calculated by counting any recurrence as an event and censoring death from other causes with no evidence of recurrence. The time to an event was measured from the start of treatment.

The LCR and DFR were estimated in relation to prognostic factors, including clinical stage (stages IIB vs. stages III–IVA), pretreatment volume [small (≤ median) vs. large (> median)], nodal status (negative vs. positive), overall treatment time [normal (≤56 days) vs. prolonged (>56 days)], treatment type (NAC vs. RT alone), and RR [high (> median) vs. low (≤ median)]. The strength of these factors was assessed using Cox's proportional hazards regression model. StatView 5.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Patients were significantly younger in the NAC group (median 49 years) than the RT-alone group (median 70 years) ($P < 0.0001$) (Table 1). Clinical stages were IIB in 11 patients (8 RT-alone, 3 NAC) and IIIB–IVA in 37 patients (25 RT-alone, 12 NAC). There was no difference in clinical stage between the treatment groups ($P > 0.9999$). Pretreatment volume ranged from 5.8 to 301.6 cm^3 (n = 48, median 56.5 cm^3) and tended to be larger in the NAC group (median 70.6 cm^3) than in the RT-alone group (median 37.9 cm^3; $P = 0.0572$). Nodal status was assessed as negative in 31 patients (64.6%) including 22 RT-alone (66.7%) and 9 NAC (60.0%) patients and as positive in 16 patients including 10 RT-alone (32.3%) and 6 NAC (40.0%) patients. It was unknown in one RT-alone patient. There was no significant difference in nodal status between the treatment groups ($P = 0.7422$). Treatment prolongation occurred

Table 1. Patient characteristics by treatment group

Characteristic	Treatment group		
	RT alone (n = 33)	NAC (n = 15)	P
Age (years), range and median [total: 32–95 (60)]	34–95 (70)	32–72 (49)	<0.0001
Clinical stage (no. of patients)			
Stage IIB	8 (24.2%)	3 (20.0%)	>0.9999
Stages III–IVA	25 (75.8%)	12 (80.0%)	
Stage IIIA, IIIB, IVA	0, 19, 6	0, 10, 2	
Pretreatment volume (cm^3), range and median [total: (n = 33, 15); 5.8–301.6 (56.5)]	5.8–301.6 (37.9)	29.1–245.0 (70.6)	0.0572
Overall treatment time (days), range and median			
Total 38–71 (50)	38–71 (50)	43–62 (50)	0.7895
Prolonged > 56 days (no. of patients)	12 (33.3%)	1 (6.7%)	0.0729
RR value, range (median) day^{-1}			
RR (n = 33, 15); 0.004–0.069 (0.021)	0.004–0.069 (0.022)	0.004–0.065 (0.020)	0.4169
RR_{NAC} (n = –, 15)	—	0.003–0.065 (0.013)	0.1784*

RR, tumor regression rate; RR_{NAC}, RR during NAC; NAC, neoadjuvant chemotherapy; RT, radiotherapy. (n = RT-alone, NAC)
* P was calculated for RR (RT alone) vs. RR_{NAC}

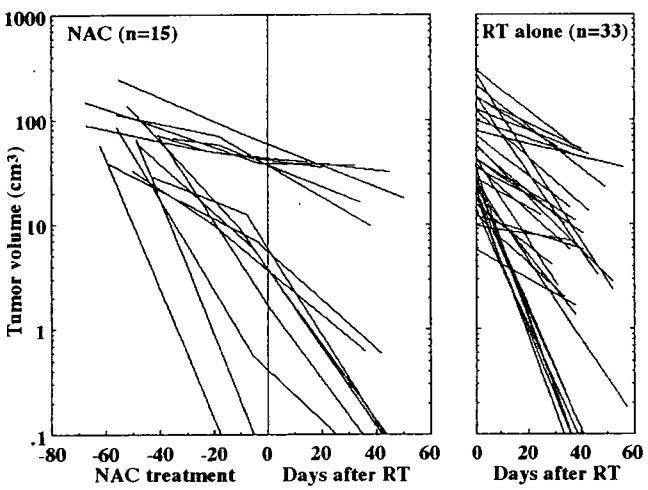

Fig. 1. Tumor regression curves for tumors treated with neoadjuvant chemotherapy plus radiotherapy (*NAC*) or radiotherapy alone (*RT alone*). The start of RT was set as day 0

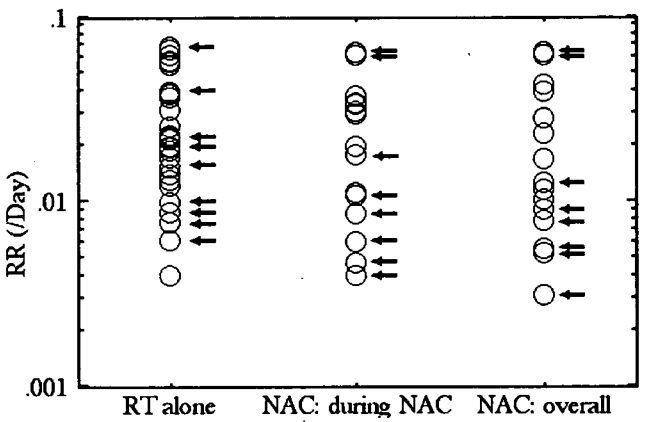

Fig. 2. Relation between the RR for RT alone (n = 33), during NAC (n = 15), and overall in the NAC group (n = 15) and local recurrence (RT alone, n = 9; NAC, n = 8) shown with *arrows*

in 12 RT-alone patients (33.3%) and 1 NAC patient (6.7%) (P = 0.0729).

RR analysis

Tumor regression curves are shown in Fig. 1. Two NAC patients achieved a complete response before RT. Overall, a complete response was observed on posttreatment MR images in 13 patients and tended to be more frequent in the NAC group (n = 7, 46.7%) than in the RT-alone group (n = 6, 18.2%) (P = 0.0766).

The RR ranged from 0.004 to 0.069 day^{-1} (n = 48; median, 0.021 day^{-1}) (Table 1). The RR did not significantly differ between the RT-alone group (range 0.004–0.069 day^{-1}; median 0.022 day^{-1}) and the NAC group (range 0.004–0.065 day^{-1}; median 0.020 day^{-1}) (P = 0.4169) (Table 1). RR_{NAC} ranged from 0.003 to 0.065 day^{-1} (median 0.013 day^{-1}), which did not differ significantly from the RR of the RT-alone group (P = 0.1784).

Treatment outcomes

Follow-up periods ranged from 3.1 to 127.9 months (median 27.8 months), at the end of which 27 patients were alive with (n = 3) or without (n = 24) evidence of cancer; the remaining 21 had died of the cancer (n = 17) or of incidental disease without evidence of cancer (n = 4) (March 2006) (Table 2). Local recurrence was identified in 17 patients (9 RT-alone, 8 NAC). Local recurrence is shown in relation to RR in Fig. 2. Extrapelvic recurrence was identified in 11 (8 RT-alone, 3 NAC), most fre-

Table 2. Local control rate and disease-free rate, by various characteristics

Characteristic	No. of patients	LCR (2 years) %	LCR (2 years) *P*	DFR (2 years) %	DFR (2 years) *P*
Entire	48	64.6		55.3	
Clinical stage					
Stage IIB	11	60.0	0.4669	60.0	0.8558
Stages III–IVA	37	66.6		55.1	
Pretreatment volume					
Small (≤ median)	24	76.5	0.0797	61.4	0.2069
Large (> median)	24	53.8		49.7	
Pelvic nodal status					
Negative	31	79.0	0.0145	71.4	0.0044
Positive	16	42.9		30.0	
Overall treatment time					
Normal (≤56 days)	36	67.0	0.4685	54.3	0.9537
Prolonged (>56 days)	12	58.3		58.3	
Treatment type					
RT alone	33	70.5	0.3391	63.1	0.1635
NAC	15	53.3		40.0	
RR					
High (> median)	24	81.7	0.0093	72.9	0.0127
Low (≤ median)	24	47.4		38.3	

LCR, local control rate; DFR, disease-free rate; RR, tumor regression rate
Pelvic nodal status was assessed in 47 patients

quently at the paraaortic nodes ($n = 7$) followed by the lung ($n = 3$). Recurrence was isolated to the pelvis ($n = 8$) or to extrapelvic sites ($n = 4$), or it was combined ($n = 7$). Of the 27 patients alive, 7 were lost to follow-up within 24 months, with (2 NAC) or without (5 RT alone) evidence of recurrence at the time of the last follow-up. Altogether, 28 patients were assessed as disease-free after treatment, with follow-up periods ranging from 3.1 to 127.9 months (median 46.5 months). LCR and DFR were calculated as 64.6% and 55.3% at 2 years, respectively.

Prognostic factors

In relation to prognostic factors (Table 2), the LCR (2 years) was significantly higher in the high-RR group than in the low-RR group (81.7% vs. 47.4%, $P = 0.0093$) and in the node-negative group than in the node-positive group (79.0% vs. 42.9%, $P = 0.0145$) but marginally not higher in the small-tumor group than the large-tumor group (76.5% vs. 53.8%, $P = 0.0797$). Clinical stage, overall treatment time, and treatment type were not significant prognostic factors for the LCR ($P = 0.4669$, 0.4685, and 0.3391, respectively). The DFR (at 2 years) was significantly higher in the node-negative group than in the node-positive group (71.4% vs. 30.0%, $P = 0.0044$) and in the high-RR group than in the low-RR group (72.9% vs. 38.3%, $P = 0.0127$). However, clinical stage, overall treatment time, pretreatment volume, and treatment type were not significant prognostic factors for the DFR ($P = 0.8558$, 0.9537, 0.2069, and 0.1635, respectively).

Comparison of the strength of prognostic factors

Multivariate analysis of prognostic factors was performed in 47 patients excluding 1 patient whose nodal status was unknown (Table 3). The hazard ratio for the LCR was high for low RR [3.43; 95% confidence interval (CI) 1.03–11.4; $P = 0.0448$]. The hazard ratio in DFR was high for positive nodal status (3.07; 95% CI 1.11–8.49; $P = 0.0304$) followed by low RR (2.71; 95% CI 1.01–7.28; $P = 0.0481$). Stepwise analysis showed that significant prognostic factors were RR (hazard ratio 3.46; $P = 0.0348$) and nodal status (hazard ratio 3.07; $P = 0.0349$) for LCR and nodal status (hazard ratio 3.35; $P = 0.0104$) and RR (hazard ratio 2.85; $P = 0.0349$) for DFR.

Discussion

A subset of patients with locally advanced cervical cancer are considered to have distant micrometastasis at diagnosis. Locally advanced cervical cancer must therefore be treated with consideration to the control of both locoregional disease and micrometastasis. Theoretically, NAC is used to eradicate micrometastasis and to shrink tumors prior to RT so intracavitary RT, which has a steep dose-fall-off profile, is beneficially applied. The

Table 3. Comparison of prognostic factors regarding local disease control rate and disease-free rate assessed by Cox's proportional hazards regression model

Prognostic factor (control vs. object)	Local control rate		Disease-free rate	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Clinical stage (IIB vs. III–IVA)	0.35 (0.08–1.57)	0.1687	0.70 (0.18–2.71)	0.6066
Pretreatment volume (small vs. large)	2.72 (0.70–10.6)	0.1494	1.43 (0.49–4.15)	0.5157
Pelvic nodal status (negative vs. positive)	2.71 (0.84–8.76)	0.0958	3.07 (1.11–8.49)	0.0304
Overall treatment time (normal vs. prolonged)	1.82 (0.41–8.11)	0.4338	1.01 (0.27–3.82)	0.9905
Treatment type (RT alone vs. NAC)	0.58 (0.15–2.32)	0.4403	0.64 (0.19–2.11)	0.4637
RR (high vs. low)	3.43 (1.03–11.4)	0.0448	2.71 (1.01–7.28)	0.0481

CI, confidence interval

Significant prognostic factors after stepwise analysis were pelvic nodal status (hazard ratio 3.07, $P = 0.0349$) and RR (hazard ratio 3.46, $P = 0.0348$) for local control rate and pelvic nodal status (hazard ratio 3.35, $P = 0.0104$), and RR (hazard ratio 2.85, $P = 0.0349$) for disease-free rate

effect of NAC on micrometastasis, however, cannot be measured during treatment; rather, it can be assessed only indirectly via the response of the measurable lesion (i.e., the cervical tumor mass). Assessing the cervical tumor response to NAC is therefore particularly important.

Here, we assessed tumor response by the RR instead of the more commonly used degree of tumor shrinkage,[9] such as complete response and partial response. The rationale for this is that RR is a function of time and consequently analyzable over time, whereas the degree of shrinkage is independent of time and thus represents the response at the time of verification only.[10] Gong et al. observed a wide range in RR (0.015–0.26 day^{-1}), as did we (0.004–0.069 day^{-1}), on MR images obtained weekly during RT.[11] Tumors in their series were shown to shrink exponentially, a characteristic we assumed here. In contrast, patterns of tumor shrinkage during NAC have rarely been studied. If they could be determined, the chemoresponse of a tumor during the early course of NAC could be estimated, which in turn would allow differentiation of nonchemoresponsive tumors from chemoresponsive tumors during NAC.

The use of NAC before RT in our clinical practice did not improve the LCR or DFR; rather, it conversely decreased the LCR. Univariate analysis of prognostic factors of LCR among all cases showed that the pretreatment volume and nodal status were significant, whereas clinical stage and prolongation of overall treatment time were not. Moreover, LCR was contrarily worse for NAC treatment than for RT-alone treatment. Regarding the DFR, nodal status was the only significant prognostic factor. On multivariate analysis, nodal status was shown to be a significant factor of both LCR and DFR, suggesting that a positive nodal status is associated with difficulty controlling pelvic node metastasis and a propensity for distant micrometastasis. These observations imply that a decrease in LCR with NAC treatment was compensated for by suppressive effects on micrometastasis, resulting in the absence of a significant difference in DFR between the treatments.

Several studies have described the use of NAC before surgery to convert unresectable disease to resectable disease, with tumors responding to NAC treated by surgery and those not responding treated by RT.[12–15] Minagawa et al. reported that the 3-year DFR for 26 patients with stage IIIB disease after intraarterial NAC was 72% for 18 patients who underwent surgery and 25% for the remaining 8 patients who underwent RT.[14] Sugiyama et al. reported that the 4-year DFR in 22 patients with stage IIIB disease treated by intra-arterial NAC was 75% for 16 patients who underwent surgery and 44% for 6 patients who underwent RT.[15] Although these reported DFRs are significantly higher in patients treated by surgery than in those treated by RT, they are comparable to those of our patients in both the high- and low-RR groups. We believe that chemoresponsive tumors are treatable by RT without surgery. Preoperative use of NAC thus poses a management problem for patients whose tumors are not converted to being resectable.

The tumors in our series showed a considerable response to NAC treatment, as shown by the finding that the RR_{NAC} did not differ significantly from the RR of the RT-alone group. Nevertheless, the use of NAC did not increase the RR overall. The results indicate that RR is a better determinant of LCR than the better-known prognostic factors. Taken together, our observations imply a number of things: NAC does not further improve the LCR in radioresponsive tumors with a high RR because they are treatable by RT alone; nonradioresponsive tumors may benefit from NAC if they are chemoresponsive; and nonchemoresponsive tumors might be exacerbated by the possible induction of tumor cell cross-resistance by NAC.[16] These findings strongly

suggest that the presence of these conditions offsets the potential benefits of NAC and would explain why the use of NAC before RT is not effective overall.

Conclusion

Because NAC here was used in clinical practice and not according to a protocol study, the regimens used varied widely with regard to the type and dose of chemotherapeutic agents, the number and length of cycles, and drug delivery method. We believe that the fact that tumors did respond to NAC no matter which NAC regimen was used, however, validates our conclusions. Our study of the RR in cervical cancer patients suggested that NAC is effective in reducing tumor volume prior to RT but is not effective overall in enhancing the RR, which was shown to be a strong prognostic factor for the LCR and DFR. These findings may explain at least in part why NAC does not contribute to any significant improvement in survival in patients with locally advanced cervical cancer.

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REVIEW ARTICLE

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Management of lymph nodes in the treatment of vulvar cancer

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Abstract The management of vulvar cancer has changed in the past two decades. Modern treatment of vulvar cancer must be individualized, and less radical surgery is now being performed if possible, in consideration of the quality of life (QOL) of patients. Even though the treatment now tends to be conservative, lymph nodes in all patients should be appropriately treated, with the only exception being microinvasive cancer with less than 1-mm stromal invasion. Here we review the up-to-date management of lymph nodes in patients with vulvar cancer.

Key words Vulvar cancer · Inguinal lymph node · Femoral lymph node · Pelvic lymph node · Surgery · Radiation

Introduction

Vulvar cancer is a relatively rare cancer in gynecologic malignancies, mainly affecting elderly women. The majority of vulvar cancers are squamous cell carcinomas, which account for 90% of all vulvar cancers. Traditionally, "en-bloc" radical vulvectomy with bilateral groin node dissection has been the choice of treatment for vulvar cancer since Taussig[1] in the United States and Way[2] in Great Britain introduced this systemic surgical procedure. The rationale for the surgery was based on the analysis of patients with vulvar cancer showing that the pattern of dissemination of this cancer is predominantly lymphogenic to the inguinofemoral lymph nodes and subsequently to pelvic lymph nodes. The radical vulvectomy with bilateral groin node dissection with butterfly incision was established in the 1940s and it has been a gold standard for a long time in the management of invasive vulvar cancer. However, management of the primary tumor and its lymphatic drainage has changed dramatically in the past two decades, to more individualized and conservative procedures. The changes have been developed based on many excellent clinical and clinicopathological studies performed for vulvar cancer.

The present review focuses on the recent management of the lymph node system of vulvar cancer in relation to the status of the primary lesions.

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Lymphatic spread of vulvar cancer

Lymph node metastasis may occur when the depth of invasion exceeds 1 mm in the primary tumor. The dermal lymphatic network of the vulva courses superiorly to the area of the mons pubis and then turns laterally to drain into the superficial inguinal lymph nodes.[3] The superficial inguinal lymph nodes are located in the femoral triangle formed by the inguinal ligament superiorly, the border of the sartorius muscle laterally, and the border of the adductor longus muscle medially. The nodes lie along the great saphenous vein, superficial epigastric vein, superficial circumflex iliac vein, and lateral accessory saphenous vein, between Camper's fascia and the cribriform fascia overlying the femoral vessels. Lymphatic drainage from the lateral sites of the vulva is to the ipsilateral groin. Crossover drainage to the opposite groin is rare. Drainage from the midline dermis could be to the bilateral groins.[4] Intraoperative lymphatic mapping has demonstrated that lymphatic drainage from vulvar lesions proceeds initially to a "sentinel" node located in the superficial inguinal lymph nodes.[5]

The lymphatic channels from the superficial group then perforate the cribriform fascia (fascia lata covering the femoral vessels) to the deep inguinal nodes located medial to the femoral vein. The node situated most cephalad of the deep inguinal node group beneath the inguinal ligament is called Cloquet's node. The deep inguinal nodes drain cephalad into the medial portion of the external iliac nodes and then through pelvic to aortic chains. The numbers of lymph nodes are around ten in the superficial lymph node group and three to five in the deep inguinal lymph node group.

Recent important observations regarding the nodal metastasis of vulvar cancer note the following: (1) the superficial inguinal nodes are the most common site of lymphatic metastasis; (2) the phenomenon of lymphatic embolization to the groin nodes is the initial lymphatic metastasis, because in-transit metastases within the vulvar skin are extremely rare; (3) metastasis to the contralateral groin or pelvic nodes is unusual in the absence of ipsilateral groin metastases; and (4) nodal involvement generally proceeds in a stepwise fashion from the superficial inguinal to the deep inguinal and then to the pelvic/aortic nodes. However, metastasis to the deep inguinal nodes without involvement of the superficial inguinal nodes has been reported.[6-10]

Management of groin nodes in early vulvar cancer

Tumors confined to the vulva without clinically suspicious lymph nodes may be considered early. Groin nodes of early vulvar cancer should be appropriately managed, based on the clinicopathological findings of the primary tumor, and the management should be individualized. Two facts have become apparent according to an increasing number of clinicopathological reports. First, the only patients without significant risk of groin node metastasis are those whose tumor invades the stroma to a depth of 1 mm or less.[11] On the contrary, there is a significant risk of groin node metastasis in those patients whose tumor invades the stroma to a depth of more than 1 mm. Second, patients with recurrent disease in the undissected groin nodes have a very high mortality rate.[12-15] Based on these important observations, all patients with more than 1-mm stromal invasion require inguinal lymphadenectomy.

Stage IA vulvar cancer

Stage Ia is defined as a single lesion measuring 2 cm or less in diameter with a depth of invasion of 1.0 mm or less. This lesion is safely managed by a wide local excision. Groin dissection is not necessary for stage Ia vulvar cancer, as mentioned above.

Stages T1b and T2 vulvar cancer

A randomized prospective phase III trial by the Gynecologic Oncology Group (GOG) demonstrated that groin dissection with or without postoperative radiation was superior to primary groin irradiation for the patients with nonsuspicious groin nodes, in respect to progression-free survival and overall survival.[16] Therefore, groin dissection is the choice of treatment in early vulvar cancer.

All patients with T2 lesions and T1b lesions should undergo at least an ipsilateral thorough inguinofemoral lymphadenectomy. Even in patients with early vulvar cancer, deep inguinal lymph node dissection should not be omitted. According to the prospective study conducted by the GOG, six groin recurrences among 121 patients with T1N0 or N1 tumors were reported after a superficial groin dissection and negative pathological results.[17] The results indicate that modification of the groin dissection increases groin recurrence and mortality.

Unilateral groin dissection is appropriate for the lateral T1 tumor, because the incidence of positive contralateral nodes in such lesions is less than 0.5%.[11] On the contrary, bilateral groin dissection is required for the patients with midline tumors and for those with tumors involving the anterior labia minora, because of the more frequent contralateral lymph flow from these regions.[4] Large lateral tumors with a positive ipsilateral node should also be candidates for bilateral groin dissection.

Surgical technique for groin dissection

It is recommended, in the Good Practice Guidelines (GPG) issued by the International Federation of Gynaecology and Obstetrics (FIGO) and the International Gynecologic Cancer Society (IGCS) that both the superficial and the deep inguinal nodes be removed, as superficial inguinal node dissection alone is associated with a higher incidence of groin recurrence, as mentioned previously.[17,18] Groin dissection is preferably performed through a triple incision approach, as this method should reduce the wound complications.[19,20] An en-bloc approach may still be useful for clitoral or periclitoral lesions. In a separate incision technique, a linear incision is made along a line between the anterior superior iliac spine and the pubic tubercle. Micheletti et al.[21] propose that the superficial circumflex iliac vessel is a lateral landmark of the incision, because no lymph nodes are present beyond this vessel. The anatomic boundaries of the femoral triangle for superficial lymphadenectomy are 2 cm above the inguinal ligament superiorly, the border of sartorius muscle laterally, and the border of the adductor longus muscle medially. The fatty tissue including lymph nodes between the superficial subcutaneous fascia (Camper's fascia) and the fascia lata is removed over the femoral triangle. The superficial subcutaneous fat is left attached to the skin to provide blood supply and avoid skin necrosis. The great saphenous vein is tied off at the apex of the femoral triangle and at its point of entry into the femoral vein, or it may be preserved to reduce morbidity, such as cellulitis, wound breakdown, and chronic lymphedema.[22,23] Surgical removal of the deep inguinal nodes is performed as an extension of a superficial node dissection. Traditionally, the fascia lata along the sartorius muscle is opened and then mobilized medially as a part of the specimen. After uncovering the entire femoral vessels, the deep inguinal nodes lying medial to the femoral vein are removed in continuity with the superficial nodes. Transposition of the sartorius muscle may be performed to cover the exposed femoral vessels.[24]

Recently, less radical femoral dissection has been recommended, based on the following observations.[11] Micheletti et al.[25] suggest that there is no need to remove the fascia lata lateral to the femoral vessels, because all of the deep

inguinal nodes are consistently located medial to the femoral vein in the opening of the fossa ovalis. Rouzier et al.[26] also showed techniques of groin node dissection that preserved the fascia lata and great saphenous vein; these techniques were associated with a decreased risk of postoperative morbidity, without reducing survival.

Management of patients with positive groin nodes

In the past, pelvic lymphadenectomy had been considered in patients with positive groin nodes. In 1986, Homesley et al.[27] reported GOG data demonstrating superior results for pelvic and groin radiation compared to pelvic node dissection for patients with grossly positive groin nodes, or more than one microscopically positive node. In that trial, patients with positive groin nodes were randomized to either ipsilateral pelvic node dissection or bilateral pelvic-plus-groin irradiation. Patients randomized to radiation received a 4500- to 5000-cGy midplane dose to the pelvis between the superior border of the obturator foramina and the L5-S1 interspace at a rate of 180–200 cGy per day. Radiation therapy encompassed the groin as well as the obturator, and external and internal iliac pelvic node areas. The survival rate for the radiation group was 68% at 2 years, which was significantly better than that for the pelvic lymphadenectomy group, which was 54% at 2 years. Groin recurrence occurred in only 5.1% of patients treated with radiation compared to 23.6% of patients treated with lymphadenectomy alone. The study also highlights the value of postoperative prophylactic groin irradiation in preventing groin recurrence for patients with multiple positive groin nodes.

Subsequent studies have further clarified the prognostic significance of the morphology of positive groin nodes, particularly the size and the extracapsular spread of metastatic lymph nodes.[28–30] According to these studies, GPG by FIGO and IGCS recommended the indication of postoperative irradiation as follows:[18]

(1) Patients with one and possibly two micrometastases (<5 mm) do not require adjuvant radiation therapy
(2) Patients should receive bilateral pelvic and groin irradiation for the following indications:
    a) one macrometastasis (>5 mm diameter)
    b) presence of extracapsular spread of the groin lymph node
    c) two (possibly three) or more micrometastases (< = 5 mm).

Management of groin nodes in advanced vulvar cancer

Patients with T3, T4 tumors and bulky positive groin nodes are considered to have advanced vulvar cancer. In these patients, management of the groin and pelvic nodes should be individualized regarding the status of lymph nodes, after thorough pretreatment evaluation including computed tomography (CT) scan and/or magnetic resonance imaging (MRI). If no suspicious nodes are recognized in the groin and there are no enlarged nodes and no evidence of metastasis on frozen section, bilateral inguinofemoral lymphadenectomy should be performed. Adjuvant radiation for groin and pelvis should be considered for those with positive groin nodes, as for early vulvar cancer mentioned previously.

The management of bulky positive groin and pelvic nodes is controversial. Hacker.[11] and Hyde et al.[31] have recommended the following strategy in the management of patients with bulky positive groin nodes. All enlarged groin nodes with metastasis are surgically removed from a separate incision approach without full lymphadenectomy. Any enlarged pelvic nodes seen on CT scan or MRI should be removed by an extraperitoneal approach. After the removal of large metastatic groin and pelvic nodes, radiation is planned to both sides of the groin and pelvis. Nodal debulking instead of full groin dissection results in similar survival in patients with clinically suspicious groin nodes.

Patients with ulcerated of fixed groin nodes should be treated with primary radiotherapy with or without concurrent chemotherapy. When feasible, the nodes should be resected following radiation. Montana et al.[32] reported the GOG trial that prospectively evaluated the efficacy of preoperative chemoradiation for advanced vulvar cancer with N2/N3 lymph nodes. Forty-two patients completed concurrent chemoradiation with cisplatin/5-fluorouracil (5-FU). High resectability (96%) and local control (97%) were obtained in that study.

Sentinel lymph node in vulvar cancer

The concept of lymphatic mapping during surgery has been employed in the treatment of vulvar cancer, in order to attempt to decrease the morbidity associated with full inguinal lymphadenectomy.[33,34] The sentinel node is identified by the intradermal injection of isosulfan blue dye around the primary vulvar lesion, either alone or in combination with intradermal radioactive technetium 99m. After the injection, the node is isolated in the dissected groin visually or by gamma counting.

Marker et al.[35] reviewed the literature on sentinel node biopsy and reported the successful identification of the sentinel node in 82.5% of patients using the blue dye technique, and 100% using lymphoscintigraphy. Although promising, these results await confirmation by a prospective randomized controlled trial, because false-negative sentinel nodes have also been reported.[36,37] An intergroup randomized study of sentinel node versus full inguinofemoral lymphadenectomy in patients with vulvar cancer is currently being carried out by the European Organization for Research and Treatment of Cancer. We need to wait for the results. At present, we should consider sentinel lymph node identification and biopsy as an experimental procedure in the treatment of vulvar cancer.

Table 1. Evidence to support recent changes in the management of vulvar cancer

Evidence	Level of evidence[a]	Reference no.
1. Individualization of the management for T1, 2 carcinoma of the vulva.		
1) Separate incision approach instead of "en-bloc" radical vulvectomy and groin lymph node dissection	B	19, 20
2) Radical local excision for the T1 lesion of lateral and posterior aspects of the vulva instead of radical vulvectomy	C	11, 13, 38
3) Omission of groin dissection in T1a carcinoma of the vulva	C	11, 38, 39
4) Omission of contralateral groin dissection of lateral T1, 2 tumor	C	11
5) Full inguinofemoral lymphadenectomy for T1b, 2 tumor	A	17
6) Modification of deep inguinal lymphadenectomy	C	25
7) Superior results of groin dissection compared with groin irradiation for nonsuspicious groin node	A	16
8) No additional treatment for one microscopically positive groin node	C	40
2. Survival advantage of pelvic and groin irradiation in comparison with pelvic dissection in patients with clinically evident or more than one positive groin node	A	27
3. Better survival in patients with groin node metastasis less than 5 mm diameter compared with those with metastasis of 5 mm or more	C	28, 9
4. Poor survival in patients with positive groin node with extracapsular spread	C	30
Preoperative radiation therapy for locally advanced vulvar cancer with fixed or ulcerated groin nodes	C	32

[a] A, randomised controlled trial; B, prospective (cohort) study with a comparison group; C, retrospective follow-up study

Summary

Vulvar cancer is an uncommon cancer, and therefore, individual institutions have relatively little experience with this disease. However, changes have been made in the management of this disease in order to achieve less radical with better results in regard to the QOL of the patients. These changes have been made based on high-quality retrospective analyses and prospective multiinstitutional trials. Table 1 summarizes some of the recent evidence that supports the changes in the management of vulvar cancer in the past two decades. Efforts are still ongoing to pursue improvements in the management of vulvar cancer. We should continuously watch for advances in management for the sake of patients with this relatively rare disease.

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Retinoic acid receptor β2 is epigenetically silenced either by DNA methylation or repressive histone modifications at the promoter in cervical cancer cells

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Abstract

To elucidate the silencing mechanism of *retinoic acid receptor* β2 (*RAR*β2) in cervical carcinogenesis, we investigated *RAR*β2 expression and the status of both DNA methylation and histone modifications at the promoter in cervical cancer cell lines. *RAR*β2 was frequently repressed in cancer cell lines and in primary cancers of the cervix. Although the majority of *RAR*β2-negative cancers had methylated promoter, *RAR*β2 was repressed with hypomethylated promoter in a substantial fraction of the cancers. The *RAR*β2-negative cells with hypomethylated promoters showed a repressive histone modification pattern at the promoter. *RAR*β2 was reactivated by a histone deacetylase inhibitor, accompanied by formation of active histone modifications. The repressive modification was also observed in cells repressed with hypermethylated promoter, but *RAR*β2 was reactivated only by DNA demethylating agent and not by histone deacetylase inhibitor. Our results suggest that *RAR*β2 is silenced by either of the two key epigenetic pathways, DNA methylation or repressive histone modifications, depending on the individual cancer cells.
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1. Introduction

During carcinogenesis, tumor suppressor genes (TSGs) are inactivated by genetic changes including gene mutations, deletions, and genomic rearrangements. However, in many human cancers, these genes are also frequently silenced by epigenetic alterations [1,2]. DNA methylation and histone modifications

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are two major mechanisms of epigenetic regulation of gene expression. DNA methylation is normally involved in long-term silencing of certain genes, such as inactivated X chromosome and imprinted genes [3]. In carcinomas, aberrant DNA hypermethylation usually occurs at CpG islands located in the 5′ regulatory regions of genes, which are normally unmethylated, and causes transcriptional silencing of TSGs. Some genes are, however, repressed without apparent hypermethylation at the promoters [4–6]. Histone modifications also play critical roles in regulating gene expression [7–11]. Deacetylation of H3 and H4, demethylation of H3 lysine 4 (H3K4), and methylation of H3 lysine 9 (H3K9) are markers of transcriptionally silent heterochromatin. These repressive histone modifications correlate with promoter DNA methylation and silencing of TSGs in cancer cell lines [12].

Retinoic acid is essential for the maintenance of normal epithelial differentiation, and its effect is exerted mainly via members of the nuclear receptor superfamily, the retinoic acid receptors (RARs) and the retinoid X receptors [13]. RAR consists three subtypes, α, β, and γ, that are encoded by distinct genes in vertebrates. Each subtype has some isoforms with different N-terminal domains, produced from each *RAR* gene by alternating usage of multiple promoters and alternative splicing. The biologically active RARβ2 isoform is expressed from the P2 promoter of the *RAR*β gene (also known as the *RAR*β2 promoter), containing a high-affinity retinoic acid response element [13–15]. As a TSG, *RAR*β2 is expressed in most normal cells and tissues but is repressed in a variety of epithelial carcinomas [16–19]. However, neither gross rearrangements nor deletions of this gene have been reported to date, suggesting that the repression of *RAR*β2 is due to epigenetic regulation at the transcriptional level. Studies in recent years have revealed that a significant proportion of *RAR*β2 repressions are associated with promoter hypermethylation in several epithelial carcinomas [20–23]. One investigation of lung cancer cell lines reported that the loss of *RAR*β2 expression is linked to aberrant histone H3 acetylation [24].

Cervical cancer is the leading cause of cancer-related mortality in women worldwide. In addition to human papillomavirus infection – the well-known critical event in the malignant transformation and immortalization of cervical epithelial cells – other factors, such as inactivation of tumor suppressors, are involved in its carcinogenesis [25]. Recent studies on cervical carcinoma have demonstrated that promoter hypermethylation of TSGs, including *RAR*β2, is a frequent epigenetic event [26,27] and that promoter hypermethylation is correlated with transcriptional repression of *RAR*β2 [28]. However, no extensive study has yet been made of the involvement of another major epigenetic determinant, histone modifications, in the *RAR*β2 repression. In the present study we found that *RAR*β2 was sometimes silenced by formation of repressive histone modifications without DNA methylation in cervical cancers.

2. Materials and methods

2.1. Cell lines and clinical tissues

Four squamous cell carcinoma cell lines (HT-3, C33A, SKG-IIIa, and SiHa) and five adenocarcinoma cell lines (HeLa, HeLa-TG, OMC-4, JSK-1, and TMCC-1) from cervixes were routinely cultured in Dulbecco's modified Eagle's medium (Sigma, USA) plus 10% fetal calf serum. In the drug treatment experiments, cells were cultured for 12–24 h before treatment in each of the following three conditions: (1) 1 μM 5-aza-2′-deoxycytidine (DAC, purchased from Sigma, USA) for 120 h, (2) 0.6 μM trichostatin A (TSA, purchased from Wako, Japan) for 36 h, (3) 1 μM DAC for 120 h plus 0.6 μM TSA during the last 36 h. The media were changed every 24 h, and at least two independent experiments were performed. In the pilot experiments the cells were treated with different concentrations of the drugs, and significant cell-death was observed with 3 μM and 1.2 μM of DAC and TSA, respectively. The expression and the histone modifications were analyzed with different concentrations of the drugs up to these concentrations. Essentially the same results as those shown in Fig. 3A and B were obtained with the maximal concentrations.

Six normal epithelial tissues of cervix and 17 tissues of primary invasive cervical cancers (Ib ~ IIIa FIGO stages) were collected from the Hospital of Saga University in Japan and the First Hospital of Xi'an Jiaotong University in China. Clinical samples were obtained by biopsy or at the time of therapeutic surgery. The samples were immediately frozen in liquid nitrogen and stored at −80 °C. This research with human samples was conducted under the approval of the Ethical Committee for Research on Human Materials of the universities. Informed consent was obtained from all individuals for the use of the samples.

2.2. RT-PCR analysis

Total RNA of cells and tissues was isolated with an ISOGEN Kit (Nippon Gene Co., Japan) and treated with RNase-free DNase I (Boehringer Mannheim, Germany). Reverse transcription was performed to synthesize single-stranded cDNA according to the manufacturer's protocol (RNA PCR Kit AMV, TaKaRa, Japan). The

primers, 5′-ACTGTATGGATGTTCTGTCAG and 5′-GTCGACAGTATTGGCATCGA, are specific for *RAR*β2 mRNA (GenBank Accession No. NM_000965) and were used in RT-PCR to produce 405 bp cDNA fragment. The PCRs were performed in a 10-μl volume containing ~100 ng template DNA, 0.5 μM of each primer, 0.2 mM of each dNTP, and 0.5 U of LA Taq polymerase (TaKaRa) in 1× LA-PCR buffer with 2.0 mM $MgCl_2$. The amplification reaction was in 28 cycles of 96 °C for 30 s, 60.5 °C for 30 s, and 72 °C for 30 s. This PCR mixture and condition were used as the standards in all other PCR experiments in this paper, unless stated otherwise. The primers 5′-ATCGTCACCAACTGGGACGA and 5′-TCCATCACGATGCCAGTGGT (annealed at 58.5 °C, 23 cycles) were used to amplify β-actin cDNA of 239 bp as the internal control.

2.3. Bisulfite sequencing assay and COBRA

Two CpG islands (CGI1 and CGI2) in the human *RAR*β2 promoter region (GenBank Accession No. X56849) [15] were identified at the website http://www.ebi.ac.uk/emboss/cpgplot/. The CpG islands are located at the nt 417–nt 684 region upstream and at the nt 909–nt 1058 region downstream of the transcription initiation site at nt 844, respectively (Fig. 1A).

Genomic DNA from cultured cells and clinical tissues was isolated using a QIAamp Tissue Kit (Qiagen, Germany). Sodium bisulfite treatment was done as described [29], with some modifications [30]. To analyze the methylation status of CGI1, the primers 5′-TAGAGGAATTTAA AGTGTGGG and 5′-CTTCAAATAACCCAACTAATC (annealed at 59 °C, 40 cycles) were used in a PCR with bisulfite-treated DNA (BS-PCR1 in Fig. 1A), and the PCR products were cloned into pT7Blue vectors and sequenced (bisulfite sequencing assay). For CGI2, semi-nested PCR was performed. The primers BSF1 (5′-GAGTTGGTGATGTTAGATTAG) and BSR (5′-A AAATCCCAAATTCTCCTTCC, annealed at 60 °C, 40 cycles) were used in the first PCR. The second PCR (BS-PCR2 in Fig. 1A) was done using 1 μl of 30-fold diluted first PCR products with the primers BSF2 (5′-A GATTAGTTGGGTTATTTGAAG) and BSR (annealed at 58 °C, 35 cycles). The semi-nested PCR product was used for the bisulfite sequencing assay and for the combined bisulfite restriction analysis electrophoresis (COBRA) [31,32] with a restriction enzyme, *Acc*II. CpGenome™, universally methylated human genomic DNA (CHEMICON International, USA), was used as methylation-positive control in the methylation studies.

2.4. ChIP assay

ChIP assays were performed to analyze histone modifications as previously described using four antibodies: anti-acetylated histone H3, anti-acetylated histone H4, and anti-dimethylated histone H3K4 purchased from Upstate Biotechnology, USA and anti-dimethylated histone H3K9 [6,33]. The primer pairs 5′-AGACAGAA AGGCGCACAGAG/5′-GTCTGACATCACCAACTC CC (annealed at 64 °C, 31 cycles) and 5′-ATGCGAGC TGTTTGAGGACTG/5′-AAAGATCCCAAGTTCTC CTTCC (annealed at 60 °C, 32 cycles) were used in ChIP-PCRs at CGI1 and CGI2, respectively (See ChIP-1 and ChIP-2 in 1A). The 5′ CpG island of *G6PD* was amplified as a positive control for H3Ac, H4Ac, and H3me2K4 and as a negative control for H3me2K9. The human chromosome 16 centromere region (*16CEN*) was an inverse control for those of *G6PD* [33,34]. Three independent ChIPs were performed for each analysis, and PCR was done twice for each of the ChIP-DNA. Fold enrichment in each immunoprecipitation was determined as previously described [35,36]. Differences among each cell line (HT-3 versus each of the *RAR*β2 negative cell lines) were calculated by Student's *t*-test. Probability levels of <0.05 were considered statistically significant.

3. Results

3.1. RARβ2 gene is frequently repressed in cervical cancer cells

To find out whether the promoter hypermethylation is the main cause of *RAR*β2 repression in cervical cancer, we analyzed the expression of *RAR*β2 and the methylation of CpG islands in the promoter region in nine cervical cancer cell lines, along with six normal epithelial tissues from cervixes (Fig. 1). The expression of *RAR*β2 was investigated

Fig. 1. The status of the expressions of *RAR*β2 and the methylation of *RAR*β2 CpG Islands. (A) Schema of the *RAR*β2 promoter region. The 5′ part of the first exon and the two CpG Islands (CGI1 and CGI2) are shown with open and solid boxes, respectively. A horizontal arrow indicates the transcription initiation site. BS-PCR1 and BS-PCR2 are the regions for bisulfite-sequencing analyses. ChIP-1 and ChIP-2 are the PCR-regions for ChIP assays. Shown is the position of the *Acc*II site for COBRA. (B) RT-PCR analyses of *RAR*β2 mRNA in cervical cancer cell lines. The mRNA is negative or significantly decreased in eight of the nine cervical cancer cell lines. CVX, mixture of four normal cervical samples; Neg, negative control without a reverse transcriptase reaction. (C) DNA methylation analyses of CGI2 in cancer cells by COBRA. Four of six *RAR*β2 silenced cell lines have methylated promoters, and the others are unmethylated or hypomethylated. Me, a positive control with genomic DNA methylated in vitro; U, unmethylated; and M, methylated. (D) Detailed methylation analyses of the CpG islands by bisulfite sequencing. Each circle graph represents the percentage of methylation of each CpG (number of methylated clones/number (10 ~ 15) of analyzed clones × 100%). (E) RT-PCR analyses of *RAR*β2 in cervical cancer tissues. (F) DNA methylation analyses of CGI2 in cancer tissues by COBRA.

by semi-quantitative RT-PCR. It was expressed at high levels in all the normal tissues but was repressed in eight cancer cell lines. Six cell lines had no expression of *RARβ2*, and two cell lines, OMC-4 and C33A, showed a significant decrease of expression, that is, approximately 10% of the normal level. Only HT-3 expressed a relatively high level of *RARβ2* although it was approximately 70% of those in the normal tissues (Fig. 1B). To know if the