hysterectomy ranged between 55% and 77%. The most important prognostic factor is the number of positive nodes.

The complications

There were few studies reporting the complications of surgical treatment in patients with stage IIB cervical cancer. The complications increased when the patients received adjuvant radiation after radical hysterectomy compared with surgery alone[2,22]. The complications also increased with radiation dosage. Bowel obstruction, fistula, and rectal hemorrhage occurred in 8.5% and 13.6% of patients receiving radiation of 5000 cGy and 6000 cGy, respectively[2].

Conclusion

Patients with stage IIB cervical cancer may be treated with radical hysterectomy and pelvic lymphadenectomy. Approximately 50–80% of patients are overstaged due to difficulty in differentiation between the parametrial involvement and the inflammatory change of the paracervical tissue. Parametrial invasion significantly correlated with pelvic node metastases. Adjuvant radiation is recommended in patients who have high-risk pathologic factors, ie, positive nodes, parametrial involvement, and involved surgical margins. The strongest prognostic factor is the number of positive nodes. The complications appear to be higher in patients who receive both surgery and adjuvant radiation.

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ORIGINAL ARTICLE

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Preliminary Estimation of Treatment Effect on Uterine Cervical Squamous Cell Carcinoma in Terms of Tumor Regression Rate: Comparison between Chemoradiotherapy and Radiotherapy Alone

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Purpose: We preliminarily estimated the treatment effect on cervical cancer in terms of the tumor regression rate (TRR) achieved with chemoradiotherapy and radiotherapy alone.
Materials and Methods: The study included cervical squamous cell carcinomas treated by radiotherapy alone (n=45) or chemoradiotherapy (concurrent once-a-week cisplatin 30 mg/m^2, n=13). Tumors were measured three-dimensionally on pre- and mid-treatment magnetic resonance images. TRR was defined as the slope of the exponential regression curve of tumor volume (day^{-1}) on the assumption that tumors regressed exponentially with time.
Results: TRR ranged widely (0.004-0.090 day^{-1}) and did not significantly differ between treatment with chemoradiotherapy (median, 0.032 day^{-1}) and with radiotherapy alone (median, 0.024 day^{-1}) (p=0.361). TRR > 0.05 day^{-1} was seen in four chemoradiotherapy tumors (30.8%) and in six radiotherapy-alone tumors (15.0%) (p=0.207), whereas TRR < 0.01 day^{-1} was seen in no chemoradiotherapy tumors (0.0%) and in five radiotherapy-alone tumors (11.1%) (p=0.180). TRR for tumors > 5.0 cm in diameter was greater with chemoradiotherapy (n=5) than with radiotherapy alone (n=12) (p=0.065).
Conclusion: Although the difference did not reach a statistically significant level, our TRR data suggest that concurrent chemotherapy heightens the radioresponse of large-size cervical cancer.

Key words: radiosensitivity, minimum target dose, response rate

Introduction

Radiotherapy (RT) has long played a major role in the treatment of locally advanced uterine cervical cancer as a single treatment modality. The effect of RT on tumors has been estimated subjectively by pelvic examination, and rapid and extensive tumor shrinkage after RT is considered predictive of local disease control. To improve local disease control and survival, the National Cancer Institute of the United States recommends concurrent use of RT and chemotherapy (cisplatin or cisplatin plus fluorouracil), i.e., chemoradiotherapy (CRT), on the basis of the results of five randomized clinical trials.[1] We have treated locally advanced cervical cancer by CRT since September 2001, and we have the impression that tumors regress more rapidly with CRT than with RT alone. Theoretically, concurrent use of cisplatin sensitizes tumor cells to the effect of RT, resulting in an increased tumor regression rate (TRR).

Objective estimation of tumor size is possible with magnetic resonance (MR) imaging, which provides direct tumor visualization.[2] Measuring tumor size with MR imaging weekly, Gong *et al.* observed that cervical tumors regressed exponentially with time during RT.[3] This observation implies that the rate is determined by estimating tumor size before and anytime during RT.

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Part of this study was presented at the 15th International Congress on Anti-cancer Treatment (February 9-12, 2004, Paris) and at the 63rd Annual Meeting of the Japan Radiological Society (April 8-10, 2004, Yokohama).

The present study was a retrospective investigation of whether CRT increases the effect of RT on cervical cancer in terms of tumor regression rate (TRR) estimated from MR images on the assumption that tumors regressed exponentially.

Materials and Methods

Patients

The study included 58 patients: 13 patients treated by CRT and 45 patients treated by RT alone. These patients were selected from among 126 patients with cervical squamous cell carcinoma treated by definitive RT with or without chemotherapy at the Tsukuba University Hospital between January 1996 and May 2003. Before September 2001, pre-RT neoadjuvant chemotherapy was often used, and thereafter CRT was used whenever considered feasible. Elderly patients were treated by RT alone through the period. The 58 patients met the eligibility criteria as follows: treatment with either CRT or RT alone, disease measurable on MR images, and availability of MR images obtained before and during RT. Patients treated by neoadjuvant chemotherapy (n=36), those with tumors that were difficult to measure on MR images (n=5), and those without complete MR images (n=21) were excluded from the study. Patients with pre-RT MR images obtained more than five weeks before RT (n=6) were also excluded. Patient age ranged from 34 to 95 years (median, 68 years). Clinical stages according to the International Federation of Gynecology and Obstetrics (FIGO) were IB1 (n=2), IB2 (n=3), IIB (n=10), IIIA (n=5), IIIB (n=32), and IVA (n=6).

Treatment

Definitive RT consisted of external and intracavitary RT. External RT was performed with 10-MV X-rays delivered through anteroposteriorly opposed or four-field portals in 1.8- or 2.0-Gy fractions at five fractions per week. The treatment field used was the normal field covering the whole pelvis up to the common iliac lymph nodes (n=37), the extended field covering the whole pelvis and the para-aortic nodes (n=15), or the small field covering the pelvis up to the internal and external iliac nodes (n=6). The extended field was used for patients with positive pelvic or para-aortic nodes identified on computed tomograms, and the small field was used for patients in poor condition or more than 80 years old. In 34 patients, boost RT was delivered after 50.4 or 50.0 Gy to the parametrial induration or lymphadenopathy. Thus, total pelvic doses ranged from 45.0 to 66.6 Gy (median, 59.4 Gy) including boost doses. Para-aortic doses ranged from 41.4 to 57.6 Gy (median, 45.0 Gy).

Intracavitary RT was performed with a high-dose-rate remote afterloading system with an iridium-192 source. The prescribed dose to reference point A was 6.0 Gy per insertion, and insertions were performed weekly: two insertions (n=3), three insertions (n=16), four insertions (n=35), and five insertions (n=4). Intracavitary RT was started after a central block was placed on the external RT field. Timing of intracavitary RT depended on the extent of the tumor before treatment and on the tumor response to external RT. A central block was placed after the external RT doses ranging from 19.8 to 61.0 Gy (median, 36.0 Gy). Typically, for medium-sized, normally responsive stage IIIB disease, the pelvic central dose delivered from external RT was 30.6 Gy, and point A dose was 24.0 Gy by four insertions. The overall treatment time for pelvic RT ranged from 38 to 71 days (median, 50 days).

CRT with cisplatin was performed only when patients were considered able to tolerate the drug (acceptable function of the renal, hematologic, and gastrointestinal systems). Cisplatin was administered intravenously at 30 mg/m^2 once per week for four (n=3), five (n=5), or six weeks (n=5).

Estimation of TRR

MR imaging was performed with a 1.5-Tesla system. Pre-RT MR images were defined as images obtained from 34 days before to 3 days after the start of RT (median, 14 days before RT), and mid-RT MR images were defined as images obtained from 25 to 57 days (median, 38 days) after the start of RT. Mid-RT MR images were obtained before starting intracavitary RT (n=12), during intracavitary RT (n=39), and immediately after the cessation of RT (n=7). Tumors identified as high-intensity lesions on T2-weighted MR images were measured three-dimensionally, by lateral width (W), dorsoventral thickness (T), and craniocaudal length (L). Tumor volume (V) was estimated according to the following equation on the assumption that the tumor mass was ellipsoid:

$$V = (W \times T \times L)\,\pi / 6 \qquad \text{[Eq. 1]}$$

Calculated pre-RT volume and mid-RT tumor volumes were plotted on a semilogarithmic graph, with the start of RT set as day 0. A tumor that responded completely or was recognizable only as a trace (complete response) was regarded as 0.1 cm^3 in volume, and a tumor visible as a small high-intensity "scar" that was difficult to measure (near complete response) was regarded as 0.2 cm^3. TRR was defined as the slope of the tumor regression curve shown by the exponential regression equation as follows:

$$Y = A \times 10^{-B \times X} \qquad \text{[Eq. 2]}$$

where Y is the post-RT volume X number of days after the start of RT, A is the pre-RT volume, and B (day^{-1}) is TRR.

Table 1. Age of patients, disease stage, pre-RT tumor volume, and TRR according to treatment group

	CRT group (n=13)	RT-alone group (n=45)	P value
Age range (median) in years	34-65 (44)	34-95 (72)	<0.001
FIGO stage			0.089*
IB1	0	2	
IB2	0	3	
IIB	1	9	
IIIA	2	3	
IIIB	9	23	
IVA	1	5	
Pre-RT volume range (median) in cm^3	4.9-159.1 (46.2)	2.0-301.6 (35.2)	0.830
TRR range (median) 10^{-2} in day^{-1}			
All tumors	1.0-8.1 (3.2)	0.4-9.0 (2.5)	0.361
Tumors > 33.5 cm^3 (n=7, 24): (CRT, RT-alone)	1.4-8.1 (3.5)	0.6-5.6 (2.0)	0.094
Tumors > 65.4 cm^3 (n=5, 12): (CRT, RT-alone)	1.5-8.1 (3.7)	0.6-5.6 (1.7)	0.065

*For stage IB1-IIB vs. stages III-IV

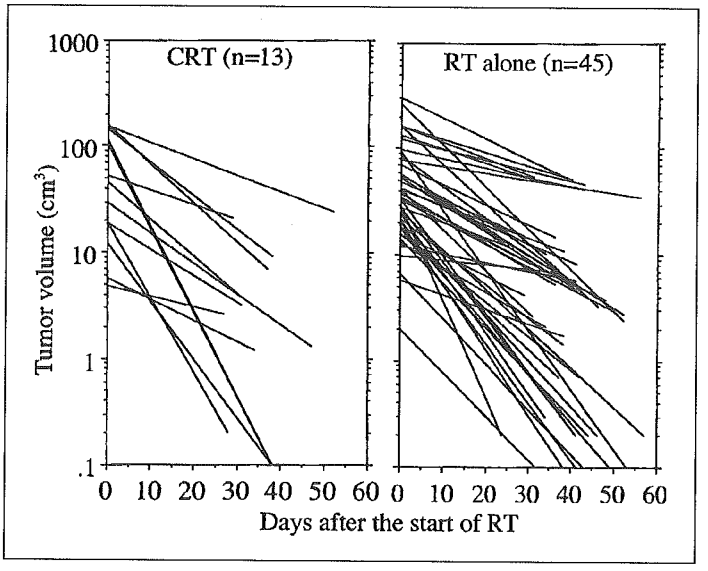

Fig. 1. Tumor regression curves of cervical squamous cell carcinomas according to type of treatment: chemoradiotherapy (CRT) or radiotherapy (RT) alone.

Statistical analysis

Differences in patient age, clinical stage, pre-RT tumor volume, and TRR between the CRT group and the RT-alone group were analyzed. Statistical difference was tested by the Mann-Whitney U-test. In addition, the chi-square test or Fisher's exact test was used in the contingency table analysis of difference in the frequency of cases. StatView 5.0 (SAS Institute Inc., Cary, NC) was used for all statistical analyses. P values of less than 0.05 were considered statistically significant.

Results

Patient age was significantly greater in the RT-alone group (range, 34-95 years; median, 72 years) than in the CRT group (range, 34-65 years; median, 44 years) (p<0.001), as shown the Table 1. There were more cases of stage III-IV disease in the CRT group (92.3%, n=12) than in the RT-alone group (68.9%, n=31), but the difference was not significant (p=0.089). Pre-RT tumor volume ranged from 4.9 cm^3 to 159.1 cm^3 (median, 46.2 cm^3) in the CRT group and from 2.0 cm^3 to 301.6 cm^3 (median, 35.2 cm^3) in the RT-alone group (p=0.830).

Tumor regression curves for patients in each treatment group are shown in Fig. 1. The period between the pre-RT MR imaging and the start of RT did not differ between the CRT group (range, 5-25 days; median, 14 days) and the RT-alone group (range, −3-34 days;

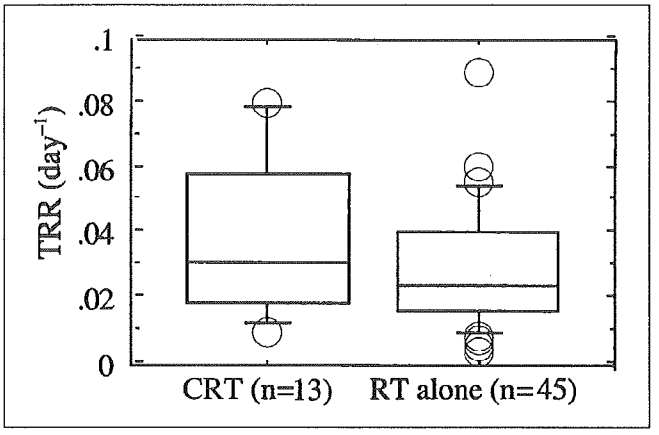

Fig. 2. Box plots of the tumor regression rate (TRR) of cervical squamous cell carcinomas according to type of treatment: chemoradiotherapy (CRT) or radiotherapy (RT) alone. The box indicates the 25th to 75th percentile with the median value, and bars indicate 10th and 90th percentiles.

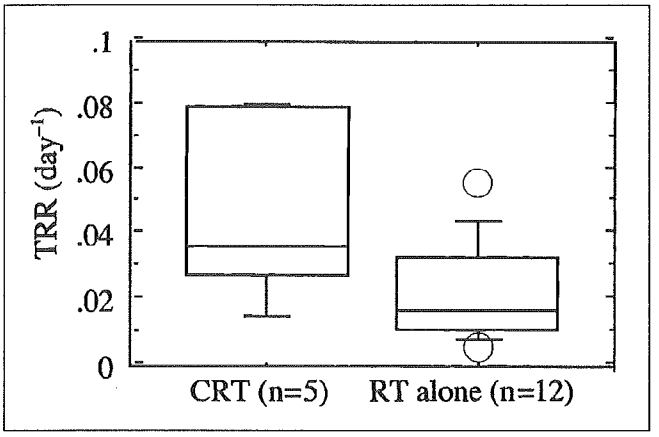

Fig. 3. Box plots of the tumor regression rate (TRR) of cervical squamous cell carcinomas greater than 65.4 cm³ in volume (5.0 cm in diameter), according to type of treatment: chemoradiotherapy (CRT) or radiotherapy (RT) alone. The box indicates the 25th to 75th percentile with the median value, and bars indicate 10th and 90th percentiles.

median, 15 days) (p=0.508). Mid-RT MR images were obtained earlier after the start of RT in the CRT group (range, 27-52 days; median, 37 days) than in the RT-alone group (range, 24-57 days; median, 41 days), but the difference did not reach a significant level (p=0.053). Complete response or near complete response was observed in four tumors (30.8%) in the CRT group and in 12 tumors (26.7%) in the RT-alone group. TRR did not correlate with the pre-RT volume in the entire study group (r=–0.101, p=0.450), the CRT group (r=0.149, p=0.627), or the RT-alone group (r=–0.206, p=0.176).

TRR ranged widely in both treatment groups (Table 1). TRR did not differ between the CRT group (range, 0.010-0.081 day^{-1}; median, 0.032 day^{-1}) and the RT-alone group (range, 0.004-0.090 day^{-1}; median, 0.025 day^{-1}) (p=0.361, Fig. 2). TRR was greater than 0.05 day^{-1} in four tumors of the CRT group (30.8%) and six tumors of the RT-alone group (15.0%) (p=0.207). Conversely, TRR was less than 0.01 day^{-1} in no tumor of the CRT group (0.0%) and five tumors of the RT-alone group (11.1%) (p=0.180). The tumors were divided into two groups according to the pre-RT volume, with a voluntary cut-off point of 33.5 cm^3 (4.0 cm in diameter) or of 65.4 cm^3 (5.0 cm in diameter). For tumors measuring 33.5 cm^3 or more, the median of TRR was 0.035 day^{-1} for the CRT group (n=7; range, 0.014-0.081 day^{-1}) and 0.020 day^{-1} for the RT-alone group (n=24; range, 0.006-0.056 day^{-1}) (p=0.094). The difference in TRR became conspicuous for tumors measuring 65.4 cm^3 or more; TRR tended to be greater in the CRT group (n=5; range, 0.015-0.081 day^{-1}; median, 0.037 day^{-1}) than in the RT-alone group (n=12; range, 0.006-0.056 day^{-1}; median, 0.017 day^{-1}) (p=0.065, Fig. 3).

DISCUSSION

Patterns of tumor regression during the course of RT are not well studied. We determined TRR on the assumption that cervical tumors regress exponentially through the course of RT. Gong *et al.* determined TRR by using serial MR imaging for 11 cervical cancers (10 squamous cell carcinomas and one adenocarcinoma) during external RT.[3] They measured tumor size weekly and five times per tumor on average, and found that

the shrinkage of all tumors fitted well to a simple exponential equation and that TRR of their tumors ranged widely, like that of our tumors. Although their findings are preliminary due to the small number of cases, patterns of tumor shrinkage during external RT are highly likely to be exponential. Further study is necessary to confirm patterns of tumor shrinkage, particularly in relation to the application of intracavitary RT, whose impact on tumors could differ from that of external RT.

If patterns of tumor shrinkage are anticipated with an equation, TRR is considered an index superior to the response rate usually used. Response rate is normally defined as the ratio of the decreased volume to the pretreatment volume, i.e., the extent of shrinkage, and the time of assessment is not defined. Therefore, tumor response differs according to the time of assessment: early assessment yields a low rate, and late assessment yields a high rate. In contrast, TRR is independent of the time of assessment and specific to individual tumors because TRR is a function of time. Therefore, TRR is useful in early estimation and comparison of radioresponsiveness of individual tumors.

An increase in TRR implies not only the sensitization of tumors to the effect of RT but also an increase in the minimum target dose delivered by intracavitary RT.[4] With its specific steep dose fall-off, intracavitary RT delivers high target doses effectively while suppressing delivery to the surrounding normal tissues. This specific dose distribution, however, reduces the dose at the periphery of the target volume. This dose corresponds to the minimum target dose; the larger the target volume, the smaller the minimum target dose per insertion. Target underdosing is critical in achieving local disease control. Therefore, to achieve effective treatment, it is important to let a tumor shrink substantially before performing intracavitary RT.

Our results did not show that tumors responded significantly more rapidly with CRT than with RT alone, although TRR could be underestimated in cases that had achieved complete response. Kim *et al.*, however, showed more rapid response with CRT than with RT alone, although they estimated tumor regression by means of a response rate determined on computed tomograms.[5] From the standpoint of effective intracavitary RT, it is of particular importance that tumors shrink more rapidly with CRT than with RT alone before performing intracavitary RT. The difference in TRR between the treatment groups was not statistically significant, most likely due to the small sample size. A well-designed prospective study will be necessary to validate the significant level of difference.

The difference in TRR for tumors greater than 65.4 cm^3 was greater with CRT than with RT alone, approaching a statistically significant level. CRT could be particularly beneficial for such large tumors; it may not be as beneficial for small tumors. Small tumors can receive high target doses of intracavitary RT even if they have not regressed much in advance. In fact, traditional RT treatment has provided substantial local disease control in stage IB1-IIA disease without concurrent chemotherapy. The effect of intracavitary RT is considered strong enough for small tumors to overcome a wide variation in TRR.

Further study is needed to confirm whether high TRR is associated with improved local disease control and survival. The next steps in increasing local disease control by CRT will be to differentiate patients who benefit from CRT from those who are treatable by RT alone[6] and to intensify the chemotherapy regimen[7] conversely when TRR is not sufficiently increased by single-agent chemotherapy. In conclusion, on the assumption that tumors regressed exponentially during the course of RT, cervical squamous cell carcinomas greater than 65.4 cm^3 appeared to regress more rapidly with CRT than with RT alone.

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EARLY DETERMINATION OF UTERINE CERVICAL SQUAMOUS CELL CARCINOMA RADIORESPONSE IDENTIFIES HIGH- AND LOW-RESPONSE TUMORS

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Purpose: To investigate whether early-assessed radioresponse of tumors corresponds with late-assessed radioresponse, which is associated with local disease control in radiotherapy (RT) for cervical cancer.
Methods and Materials: This prospective study included 12 patients with cervical squamous cell carcinoma treated by RT with or without concurrent cisplatin. Tumor volume was estimated by scheduled magnetic resonance imaging before (preRT), 3 to 4 weeks after (early assessment), and 6 to 7 weeks after (late assessment) RT initiation. Radioresponse was assessed with tumor shrinkage curves based on these volumes. Radioresponse for each tumor was calculated as the slope (day^{-1}) of the shrinkage curve by fitting to an exponential equation.
Results: Early-assessed radioresponse ranged from 0.001 to 0.106 day^{-1} (median, 0.021 day^{-1}) and late-assessed radioresponse from 0.009 to 0.091 day^{-1} (median, 0.021 day^{-1}), with no significant difference between them ($p = 0.1191$). The early-assessed radioresponse correlated with the late-assessed radioresponse ($R^2 = 0.714$, $p = 0.0005$).
Conclusions: Correspondence between early- and late-assessed radioresponse in a series of tumors showing a wide range of radioresponse was not particularly close overall. However, early assessment of radioresponsiveness did seem to be useful for characterizing those tumors with high or low radioresponsiveness. © 2006 Elsevier Inc.

Radiosensitivity, Intracavitary radiotherapy, Minimum target dose, Chemoradiotherapy.

INTRODUCTION

In radiotherapy (RT) for uterine cervical cancer, significant predictors of local disease control include not only clinical stage but also pretreatment tumor size and tumor radioresponse (1–5). Of the latter two, radioresponse is of greater practical importance because whereas pretreatment tumor size is deterministic, radioresponse is subject to modification, for example by concurrent chemotherapy. The degree of tumor shrinkage is commonly used as an index of radioresponse (6, 7)—for example, complete response (disappearance, 100% decrease in volume), partial response (≥65% decrease), and stable disease (<65% decrease). A complete response at the end of RT, which is assessed by subjective pelvic examination, is usually associated with local disease control (3–5). It would therefore be valuable to be able to predict early in the course of RT whether a tumor is to achieve a complete response; if not, intensification of treatment or the use of additional treatment could be considered earlier than otherwise possible. However, because the degree of tumor shrinkage is categorical and independent of time, it is not suitable as an index for the early estimation of radioresponse. In contrast, the speed of tumor shrinkage, another expression of radioresponse, is continuous and a function of time and pretreatment tumor size and should therefore serve as a useful index for prediction of posttreatment size.

Here, we prospectively investigated whether the speed of tumor shrinkage as assessed in the early phase of RT corresponds with that assessed in the late phase of RT, under conditions of standard clinical practice for concurrent chemoradiotherapy as proposed by the U.S. National Cancer Institute (8).

METHODS AND MATERIALS

Patients

The study group consisted of 12 patients with cervical squamous cell carcinoma selected from 19 consecutive cervical squamous cell carcinoma patients treated primarily by RT with or without concurrent cisplatin chemotherapy between December 2003 and

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December 2004. Following normal clinical practice, patients were scheduled to undergo magnetic resonance imaging (MRI) of the pelvis in three phases of RT, namely before and at 3 to 4 weeks (early phase) and 6 to 7 weeks (late phase) after the start of RT. The accuracy and clarity of MRI in demonstrating cervical tumors has been confirmed (9, 10). Seven patients were excluded from the study because not all MR images were available or because the images did not clearly identify the tumor. Clinical disease stages according to the International Federation of Gynecology and Obstetrics staging system were IB1 (n = 1), IIB (n = 1), and IIIB (n = 10). Patients ranged in age from 37 to 81 years (median, 51 years).

Treatment

Radiotherapy consisted of external and intracavitary RT. External RT was performed with a 10-MV X-ray in 1.8-Gy fractions at 5 fractions per week. Clinical target volume was the pelvis (n = 5) or the pelvis plus para-aortic nodes (n = 7), with para-aortic nodes treated prophylactically. A conformal box-field technique was used for all but 1 patient, in whom anterior–posterior opposing portals were used. A central block was placed in the pelvic RT field for the start of intracavitary RT after a total dose of 45.0 Gy (stage IIIB) or 36.0 Gy (stages IB1 and IIB) was reached. Total dose to the pelvis ranged from 50.4 to 66.6 Gy (median, 54.0 Gy), including boost doses to parametrial induration or lymphadenopathy, and total dose to the para-aortic nodes was 45.0 Gy. 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 three (n = 10) or four (n = 2) weekly insertions per patient. One patient underwent an interstitial implant after three intracavitary insertions. Thus, overall RT treatment duration ranged from 42 to 63 days (n = 11; median, 50 days) and was 70 days for the patient treated by interstitial implant.

Ten patients were treated by concurrent chemotherapy with cisplatin, and 2 (both aged 81 years) were treated by RT alone. Cisplatin was given by single weekly i.v. administration at 35 mg/m^2 (n = 3), 30 mg/m^2 (n = 6), or 20 mg/m^2 (n = 1, aged 72 years) for 3–6 weeks, starting from the first (n = 5), second (n = 4), or third week (n = 1) of RT. Delayed chemotherapy (n = 5) was due to renal dysfunction caused by hydronephrosis, which was managed by nephrostomy.

Tumor measurement with MR images

Magnetic resonance imaging was performed with 1.5-T units. The preRT images were obtained from 1 to 26 days (median, 11 days) before RT, with early-phase images obtained from 18 to 34 days (median, 24 days) and late-phase images obtained from 36 to 59 days (median, 46 days) after the start of RT, the latter being before (n = 1) or during (n = 11) the intracavitary RT course. Tumors identified as high-intensity lesions on T2-weighted images were measured three-dimensionally by width, thickness, and length for each tumor, and tumor volume was calculated on the assumption that the tumor mass was ellipsoid. The volume of tumors that disappeared or were recognized as only a remnant was regarded as 0.01 cm^3, whereas that of those remaining as a small, high-intensity "scar" that was difficult to measure was regarded as 0.05 cm^3.

Radioresponse assessment

Estimated tumor volumes were plotted on a semilogarithmic graph, with the start of RT set as Day 0. The early-phase shrinkage curve was calculated from the preRT and early-phase volumes, the late-phase shrinkage curve from the early-phase and late-phase volumes, and the through-phase shrinkage curve from the preRT and late-phase volumes. The slope of the curve (day^{-1}) (i.e., the speed of shrinkage per day) was determined by fitting an exponential regression equation to the respective curve. Radioresponse was defined as the speed of shrinkage, with radioresponsive tumors thus characterized by steep slopes. With the equation of the through-phase shrinkage curve, the tumor volume at the end of RT (postRT volume) was duly calculated for each tumor and categorized according to the degree of shrinkage. For this, either shrinkage to $\leq$0.05 cm^3 or to $<$1% of the preRT volume was regarded as complete response, whereas shrinkage to $<$35% of the preRT volume and shrinkage confined to $\geq$35% of the preRT volume were defined as partial response and stable disease, respectively.

Statistical analysis

The early-assessed radioresponse was compared with the late-assessed and with the through-assessed radioresponse. Differences in response between phases were analyzed by the Wilcoxon signed rank test. Correlation between the early-assessed and through-assessed radioresponses was analyzed by regression analysis. Radioresponse was compared between the speed of shrinkage (through-assessed radioresponse) and the degree of shrinkage. StatView 5.0 (SAS Institute, Cary, NC) was used for all analyses. P values of $<$0.05 were considered statistically significant.

RESULTS

The preRT volume ranged from 2.3 to 301.6 cm^3 (median, 95.5 cm^3). Complete response was observed in the early phase in one tumor and in the late phase in two (Fig. 1). Radioresponse ranged from 0.001 to 0.106 day^{-1} (median, 0.021 day^{-1}) in the early phase, from 0.013 to 0.121 day^{-1} (median, 0.025 day^{-1}) in the late phase, and from 0.009 to 0.091 day^{-1} (median, 0.021 day^{-1}) in the through phase. Radioresponse did not differ significantly between the early and late phases or between the early and through phases (p = 0.1361 for both). When the tumor that achieved a complete response in the early phase was excluded, however, the difference in response between the early and late

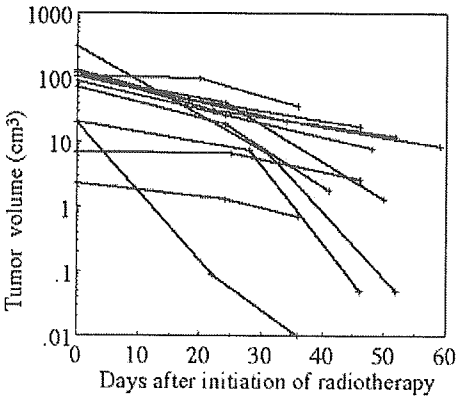

Fig. 1. Tumor shrinkage curves composed of three-phase volumes of preradiotherapy, early phase (3 to 4 weeks), and late phase (6 to 7 weeks) (n = 12).

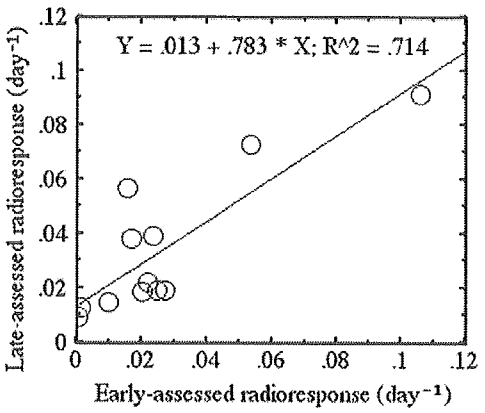

Fig. 2. Correlation between early-assessed and late-assessed radioresponse (n = 12, p = 0.0005).

phases approached significance, with radioresponse greater in the late (range, 0.013–0.121 day^{-1}; median, 0.022 day^{-1}) than in the early phase (range, 0.001–0.054 day^{-1}; median, 0.021 day^{-1}) (n = 11, p = 0.0505).

The early-assessed radioresponse correlated with the late-assessed radioresponse (Fig. 2; R^2 = 0.714, p = 0.0005). This correlation remained significant even when the tumor that achieved a near-complete response was excluded (n = 11, R^2 = 0.496, p = 0.0155).

The postRT volume ranged from 0.01 to 21.95 cm^3 (median, 0.41 cm^3) and was ≤0.05 cm^3 in three tumors. The postRT volume as a percentage ranged from 0 to 17.8% (median, 4.5%) of the preRT volume. Response category was complete response for five tumors and partial response for the remaining seven (Fig. 3). None was categorized as stable disease.

DISCUSSION

Characterization of radioresponse is particularly important for large tumors, from the standpoint of not only radiosensitivity but also dose delivery by intracavitary RT, which is characterized by steep dose fall-off within the tumor. Given that radioresponse normally implies generic radiosensitivity of tumor cells, tumors with low radioresponsiveness require larger doses for local disease control than those with high radioresponsiveness. Nevertheless, large tumors with low radioresponsiveness receive smaller target doses at the tumor periphery (minimum target doses) by intracavitary RT than large tumors with high radioresponsiveness, because the latter undergo significant shrinkage subsequent to the preceding external RT (11). Compared with large tumors, small tumors receive substantially higher minimum target doses irrespective of tumor shrinkage induced by external RT, and these high doses are considered to effectively overcome any radioresistance.

Tumors were categorized by the degree of shrinkage into either complete response or partial response only. Whereas complete response is characterized by shrinkage within a very narrow range (99–100% decrease), partial response is characterized by a wide range of shrinkage (65%–99% decrease) and is therefore not suitable for differentiating tumors at the respective ends of this range. In contrast, the speed of shrinkage is shown as a variable specific to the individual tumor and is therefore useful for differentiating partial response tumors by calculation, if the shrinkage is fitted well by a regression equation.

Our results showed that the early-assessed radioresponse corresponded with the late-assessed radioresponse, although not particularly closely. In contrast, Gong *et al.* (12), who used frequent, rigidly scheduled MRI (four to eight times per patient) and sophisticated tumor measurement methods, reported that the radioresponse of cervical tumors is exponential. Several possible reasons for this apparent discrepancy can be suggested.

First, Gong *et al.* investigated radioresponse during simple treatment with external RT alone, whereas our study involved complex treatment. Second, most of our tumors were treated by concurrent chemotherapy that was nevertheless not always simultaneous with the start of RT and by intracavitary RT that was performed in the late phase. The impact of our treatment might therefore have differed between phases, or even by week. In fact, we previously showed that the use of concurrent chemoradiotherapy tends to increase radioresponse over that achieved with RT alone (13). Further, radioresponse might have been underestimated in our three tumors that achieved a complete response because the response might have occurred before the time of observation. On these bases, we suggest that the lack of a clear exponential radioresponse in the present study was likely due to the complex treatment given, in addition to differences in the accuracy and frequency of tumor measurement.

Although exact correspondence was not obtained, our response assessment, conducted under conditions of stan-

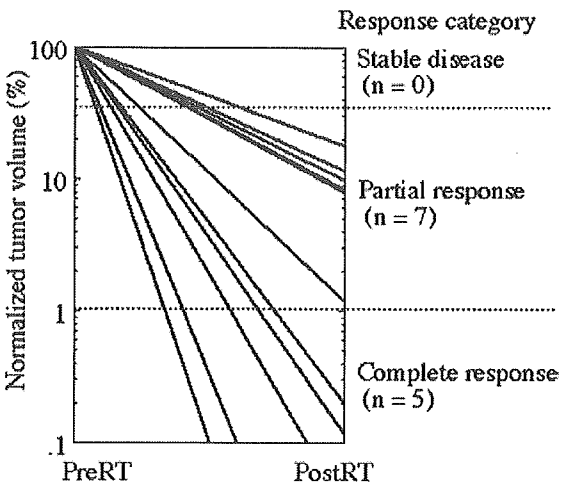

Fig. 3. Comparison of calculated radioresponse at the end of radiotherapy (RT) between the speed of shrinkage (curves) and the degree of shrinkage (response category). The postRT volume was calculated with the regression equation for each tumor at the end of RT for each individual (42–63 days from the start of RT).

dard clinical practice, is considered effective in the differentiation of highly (e.g., >0.05 day^{-1}) and poorly radioresponsive (e.g., <0.02 day^{-1}) tumors, which here represented the upper and lower quartiles of tumors by response, from those moderately radioresponsive, which made up the middle half of tumors. This is because the wide radioresponse seen facilitates the recognition of tumors at the respective ends of radioresponsiveness. Moreover, this finding is consistent between our results and those of Gong *et al.*: radioresponse range from 0.001 to 0.106 day^{-1} (early phase, 106-fold variation) and from 0.009 to 0.091 day^{-1} (through phase, 10-fold variation) in the present study and from 0.007 to 0.182 day^{-1} (26-fold variation, by planimetry) in Gong *et al.* (12).

The U.S. National Cancer Institute has recommended the concurrent use of RT and chemotherapy with cisplatin or cisplatin plus fluorouracil (as radiosensitizers) in place of the conventional use of RT alone to improve survival in patients with locally advanced cervical cancer (8), and the efficacy of this treatment has been confirmed by systematic review and meta-analysis (14). However, this recommendation is based on the assumption that the radioresponse of tumors is unknown. Early knowledge of the radioresponsiveness of tumors during treatment would allow the individualization of treatment. Given that a substantial proportion of patients have been cured by conventional RT treatment alone, those with highly radioresponsive tumors, so-called radiosensitive tumors, might not necessarily require concurrent chemotherapy. Conversely, patients with poorly radioresponsive tumors, so-called radioresistant tumors, might benefit from the intensification of treatment, such as the planned use of interstitial implants and the incorporation of a potent new radiosensitizer (gemcitabine) into concurrent chemotherapy (15).

In conclusion, the early-assessed radioresponse of uterine cervical squamous cell carcinoma corresponded with the late-assessed radioresponse, albeit not particularly strongly. Although it would be premature to incorporate these findings directly into local disease control, early determination might nevertheless be useful for identifying tumors at either extremity of the wide radioresponse range seen here.

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ORIGINAL ARTICLE

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Expression of cyclins, p53, and Ki-67 in cervical squamous cell carcinomas: overexpression of cyclin A is a poor prognostic factor in stage Ib and II disease

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Abstract We previously reported the overexpression of cyclins in uterine cervical carcinoma; however, their clinicopathological significance remained undetermined. In the present study, we examined the immunohistochemical expression of cyclins (D1, E, A, B1), p53 and Ki-67 in squamous cell carcinoma (stage Ib+II; 80 cases, stage III+IV; 23 cases). Correlations between the expression of cyclins and clinicopathological parameters and patient survival were statistically evaluated. The results indicated that in the normal squamous epithelium, the expression of cyclins and Ki-67 was sporadically observed in the parabasal layer. Of the 103 cervical carcinomas, overexpression of cyclins D1, E, A, B1 and p53 was observed in 13 (13%), 23 (22%), 25 (24%), 18 (18%) and 23 (22%) cases, respectively, with a slight predominance in advanced stage tumors. The expression of cyclin D1, E, A and p53 significantly correlated with that of Ki-67 (Spearman's rank correlation). Univariate and multivariate analyses revealed that lymph node metastasis and cyclin A overexpression were independent prognostic factors for unfavorable outcomes in stage Ib+II patients. These findings suggest that the overexpression of various cyclins is involved in the acquisition of the vigorous growth potential of cervical carcinoma cells, and that cyclin A is an independent prognosticator of cervical carcinoma in early stages.

Keywords Cyclin · Ki-67 · p53 · Cervical cancer · Immunohistochemistry

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Introduction

Uterine cervical cancer is the second most common malignancy among women worldwide. Although the prevalence of effective screening systems has reduced the number of cervical carcinoma patients in developed countries, more than 4000 deaths were still reported in the United States in 2002 [23]. It is well known that human papillomavirus (HPV) is the main etiologic factor of cervical carcinoma, and that more than 95% of cervical carcinomas have high-risk HPV types [4]. Two major viral proteins, E6 and E7, are known to bind p53 and retinoblastoma gene products (pRB), abrogating their functions as tumor suppressors, leading to an abnormal cell cycle machinery [5]. Although the loss of the cell "brake" system can be attributed to viral proteins, molecular mechanisms which actually promote cell proliferation are not fully elucidated.

Recent studies have revealed that cell growth is exquisitely controlled by interactions of cell cycle-related molecules such as cyclins, cyclin-dependent kinases (cdks) and tumor suppressor gene products [31, 32]. Cyclins D1, E, A and B1 are expressed in a cell cycle-specific manner, and they form complexes with their respective cdks. These complexes phosphorylate substrates such as pRB, leading to cell growth. On the other hand, tumor suppressor gene products, such as p53 and cdk inhibitors (cdkIs), suppress cell growth by counteracting cyclin/cdk functions [32]. In various malignancies, abnormalities of these cell cycle-related molecules, i.e. overexpression of cyclins/cdks and loss of tumor suppressor functions, have been reported [12]. Some of these abnormalities were shown to have prognostic value [12].

In cervical carcinoma, possible prognostic factors reported to date are overexpression of growth factors and receptors [11], angiogenetic factors [10], and type of HPV [7], in addition to clinicopathological parameters such as age, clinical stage, lymph node and parametrial involvement, and lymph vascular permeation [41]. We previously reported the overexpression of various classes of cell cycle-

related molecules associated with loss of steroid receptor expression in cervical intraepithelial neoplasias and invasive carcinomas [22]. However, the correlation of these factors with clinicopathological parameters and with patient survival remained undetermined. Therefore, we examined the immunohistochemical expression of cyclins (D1, E, A, B1), p53 and the proliferation marker Ki-67 in cervical squamous cell carcinoma to evaluate the relationship between the expression of cell cycle regulators and clinicopathological indicators. In addition, the prognostic significance of the expression of these cell cycle regulators was also analyzed.

Materials and methods

Histological materials

One hundred and three squamous cell carcinoma tissues of the uterine cervix were obtained from women (28–90 years of age) who underwent hysterectomy or biopsy at Shinshu University Hospital during 1990–1997. Each specimen was immediately fixed in 10% phosphate-buffered formalin for 24 h and embedded in paraffin. Each tissue sample was used with the approval of the Ethics Committee of Shinshu University, after obtaining written consent from the patients. Serial sections 3 μm thick were prepared for hematoxylin and eosin (H.E.) staining and immunostaining. The histological diagnosis was performed using an H. E.-stained slide. Of the 103 cervical carcinomas, 56 were stage Ib, eight were stage IIa, 16 were IIb, 18 were stage III and five were stage IV, according to the classification of the International Federation of Gynecology and Obstetrics (FIGO).

With regard to the treatment modality, 80 patients with stage Ib and II carcinoma primarily underwent radical hysterectomy with pelvic lymphadenectomy. Patients with high-risk factors such as parametrial invasion and lymph node metastasis received adjuvant postoperative treatment withwhole pelvic irradiation of 40–50 Gy (13 cases), cisplatin-based chemotherapy (11 cases), or both (22 cases). Clinicopathological factors including tumor size, lymph vascular permeation, parametrial involvement, and lymph node metastasis were evaluated in resected tissue specimens. The tumor size was determined from the longest axis of the tumor. The remaining 23 patients with stage III and IV tumors underwent whole pelvic irradiation (40–50 Gy) and intracavital brachytherapy.

Immunohistochemistry

Staining procedures

Immunostaining was performed using specific antibodies against cyclin D1 (DCS-6; Progen, Heidelberg, Germany). Antibodies against cyclin E (HE1), cyclin A (BF683), and cyclin B1 (GNS-1) were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif., USA). Antibodies against p53 (Do-1) and Ki-67 were purchased from Immunotech (Marseille, France). Indirect immunohistochemical staining was performed by the avidin–biotin–peroxidase complex method using a Histofine SAB–PO detector kit (Nichirei, Tokyo, Japan) with microwave pretreatment as described previously [22].

Interpretation of immunohistochemical staining and statistical analysis

The specific staining of each antibody was identified in the nucleus with or without in the cytoplasm. In the present study, we evaluated only nuclear staining, since most of the cyclins are known to work in the nucleus [31, 32]. Cyclin B1 was reportedly expressed in the cytoplasm, and in this study, the positive staining for cyclin B1 was observed in both the nucleus and cytoplasm. Although the significance of subcellular distribution of cyclin B1 has not fully been elucidated, we have previously reported that the nuclear staining of cyclin B1 topologically correlated well with Ki-67 [34], thus suggesting an importance of nuclear staining. Immunoreactivity was evaluated as the percentage of positive cells among 500 tumor cells in more than three high power fields, and described as the positivity index (PI). In cervical carcinoma, cut-off PI values for the overexpression of cyclin D1, E, A, B1, p53 and Ki-67 were determined in reference to the mean PI values of each staining and previous studies [6, 13, 19, 28, 37], and were 5, 25, 25, 20, 10 and 50, respectively. The significance of the differences in PI between stages IB+II and stages III+IV was examined by Mann–Whitney *U*-test. A tied *P*-value of <0.05 was considered significant. Correlations between the expression of cyclins and Ki-67, and between cyclins and clinicopathological factors were examined using Spearman's rank correlation test. A tied *P*-value of <0.05 was considered significant. A correlation coefficient (ρ) greater than 0.4 or less than -0.4 was considered to represent a "strongly" positive or negative correlation. These analyses were made using the StatView system (Abacus, Berkeley, Calif., USA).

Survival analysis

Of the 103 patients examined in the present study, 29 (13 in the stage Ib+II group, 16 in the stage III+IV group) had died from the disease and the remaining 74 were alive with no evidence of disease at the last follow-up. The mean post-treatment follow-up period was 65.1±45.9 months. The prognostic factors used in the survival analysis were as follows: tumor size ($\geq$30 mm versus $<$30 mm), lymph vascular permeation (positive versus negative), stromal invasion ($\geq$1/2 versus $<$1/2), parametrial involvement (positive versus negative), pelvic lymph node metastasis (positive versus negative), overexpression of cyclins (D1, E, A, B1), p53, and Ki-67 (positive versus negative). The prognostic value of these parameters was evaluated first with a univariate analysis using Cox's proportional hazard model,

and then with a multivariate analysis. A P-value of <0.05 was considered significant.

Results

Expression of cyclins and p53

Positive staining for cyclin D1, E, A and B1 in the normal ectocervical epithelia was sporadically observed in parabasal cells (Fig. 1), with a PI of less than 5 for cyclin D1, E and A, and 10 for cyclin B1. The expression of p53 in the normal ectocervical epithelia was negative. In cervical carcinoma, the expression of cyclins (Fig. 2) and p53 was increased compared to that in the normal squamous epithelium. Of the 80 stage Ib+II cervical carcinomas, the number of cases with cyclin D1, E, A, B1, and p53 overexpression was 10 (13%), 14 (18%), 12 (15%), 8 (10%), and 17 (21%), respectively. Of the 23 stage III+IV carcinomas, the numbers of cases with cyclin D1, E, A, B1, and p53 overexpression were 3 (13%), 9 (39%), 13 (57%), 10 (44%), and 6 (26%), respectively. The PIs of each staining are summarized in Table 1. The mean PI for cyclin A in total cases was 16.0. The PI for cyclin A was higher in stage III+IV (25.1) than stage Ib+II (13.3) (P=0.0128). The mean PI of cyclin D1, E, B1, and p53 in total cases was 2.0, 12.3, 12.2, and 8.3, respectively. There was no significant difference in these factors between those with stage Ib+II and stage III+IV tumors. The mean PI of Ki-67 for all cases was 46.2, and there was no significant difference between stage I+II (46.1) and stage III+IV (46.9).

Correlation of cyclin expression and Ki-67

Correlations between the immunohistochemical expression of cyclins and Ki-67 are summarized in Table 2. In stage Ib+II tumors, the expression of cyclin D1, E, A, and p53 positively correlated with the expression of Ki-67 (P=0.0379 for cyclin D1, P=0.0053 for cyclin E, P=0.0065 for cyclin A, and P=0.0284 for p53). In stage III+IV tumors, the expression of cyclin D1, E, A, B1, and p53 strongly and positively correlated with the Ki-67 expression. The P-value for cyclin E, A, B1 and p53 was 0.0102, 0.0002, 0.0002 and 0.0423, respectively.

Correlation between the expression of cyclins and clinicopathological parameters

Correlations between the expression of cell cycle regulators and clinicopathological factors in stage Ib+II tumors are summarized in Table 3. Lymph vascular permeation was correlated positively with the expression of cyclin D1 and negatively with Ki-67 (P=0.0424 for cyclin D1, P=0.0140 for Ki-67). Stromal invasion was correlated negatively with the expression of p53 (P=0.0058). Lymph node metastasis was correlated negatively with the expres-

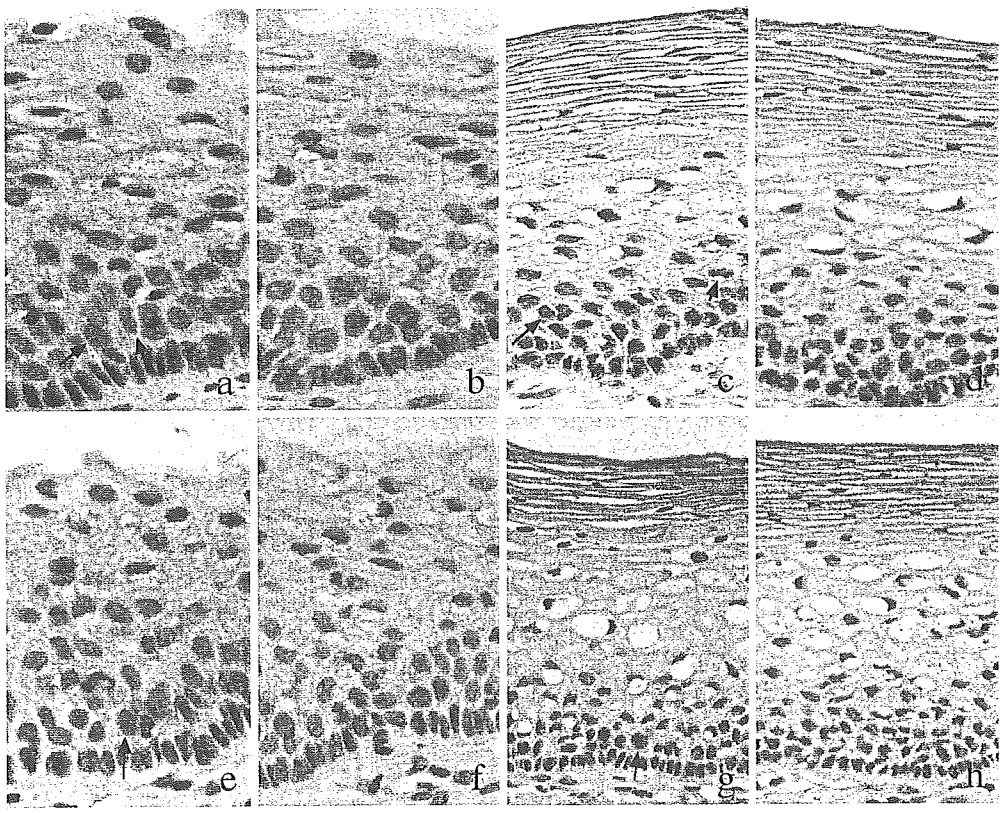

Fig. 1 Immunostaining of cyclin D1 (**a**, **b**), cyclin E (**c**, **d**), cyclin A (**e**, **f**) and cyclin B1 (**g**, **h**) in the normal squamous epithelium. b, d, f and h show negative control of the same sites. Positive cells (arrows) are sporadically observed in the parabasal layer. (**a**, **b**, **e**, **f**; ×200, **c**, **d**, **g**, **h**; ×175)

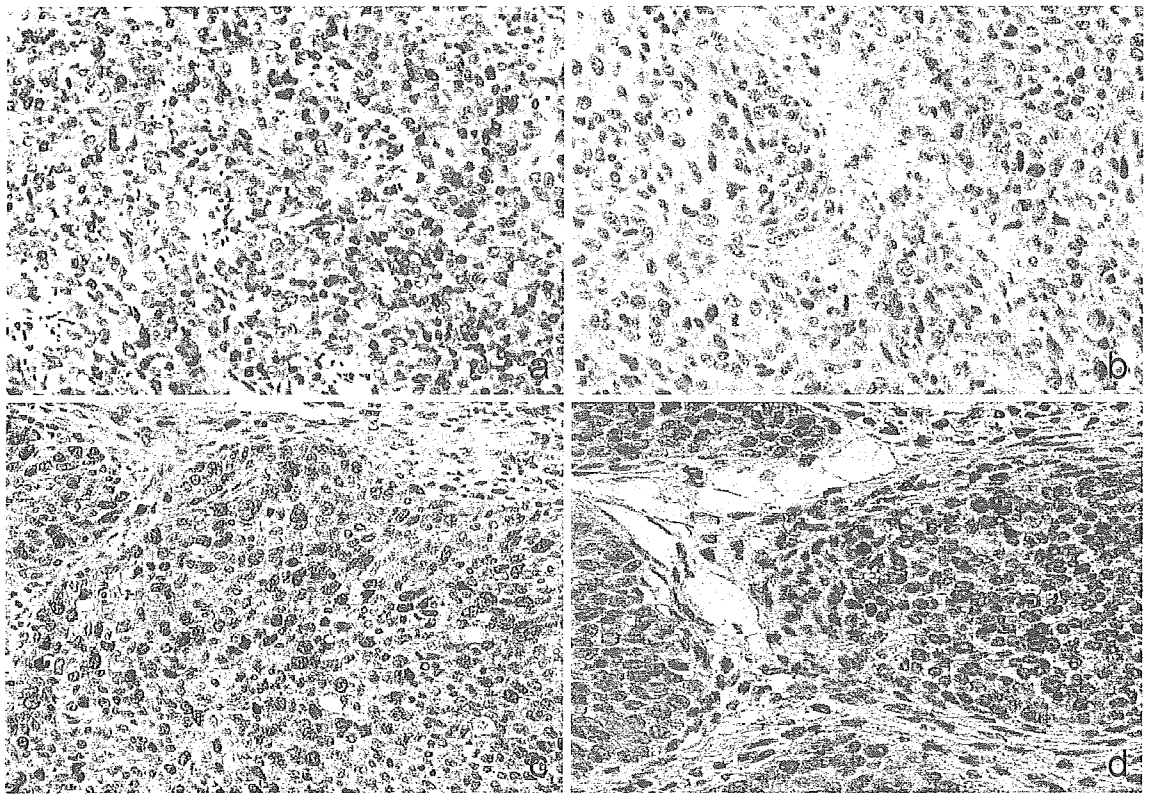

Fig. 2 Immunostaining of cyclin D1 (**a**), cyclin E (**b**), cyclin A (**c**) and cyclin B1 (**d**) in squamous cell carcinoma. **a**– **c**: nuclear-positive tumor cells are observed with a considerable heterogeneity among weak, cytoplasmic-positive cells (**a**; stage Ib, **b**; stage IIa, **c**; stage IIa, ×225). **d**: nuclear-positive cells with weak cytoplasmic staining are diffusely observed (stage Ib, ×250)

sion of Ki-67 (P=0.0170). Tumor size greater than 30 mm was negatively correlated with the expression of p53 (P= 0.0044). There were no significant correlations between cell cycle-related molecules and parametrial involvement.

Prognostic significance of the expression of cyclins, and clinicopathological factors in cervical carcinoma patients

Correlations between the expression of cell cycle regulators, Ki-67, clinicopathological parameters and survival in patients with stage Ib+II cervical carcinoma were analyzed by univariate and multivariate analyses (Table 4). The univariate analysis demonstrated that the overall survival was significantly shorter in patients with lymph node metastasis (P=0.0261, RR; 3.46) and those with cyclin A overexpression (P=0.0109, RR; 4.33) (Fig. 3). Of the 58 patients without lymph node metastasis, six (10.3%) died of the disease during the follow-up, whereas seven (31.8%) of 22 patients with lymph node metastasis died. Of the 68 patients without cyclin A overexpression, eight (11.8%) died of the disease, whereas five (41.7%) of 12 patients with cyclin A overexpression died. Other parameters did not show significant differences. We then examined whether there are strong correlations among each clinopathological and immunohistochemical parameter or not using a Spearman's rank correlation analysis. Since no "strong" correlations (i.e. ρ-value of more than 0.4 or less than −0.4)

Table 1 Result of immunostaining for cyclins and Ki-67

Factor	Stage Ib+II (80 cases)	Stage III+IV (23 cases)	Total	P value
Cyclin D1	2.0±5.9	1.9±5.4	2.0±5.8	0.5951
Cyclin E	9.8±17.7	20.9±27.8	12.3±20.7	0.1395
Cyclin A	13.3±12.5	25.1±19.9	16.0±5.2	0.0128
Cyclin B1	8.4±18.5	25.2±30.9	12.2±22.8	0.3264
p53	6.6±12.1	14.3±23.0	8.3±15.4	0.5903
Ki-67	46.1±19.8	46.9±25.8	46.2±21.0	0.4376

Each figure indicates the mean positivity index (PI)±SD
P-value of less than 0.05 is considered significant

Table 2 Correlation between the expression of cell cycle regulators and Ki-67 (Spearman's rank correlation)

Factors	Stage Ib+II		Stage III+IV		Total	
	P	ρ	P	ρ	P	ρ
Cyclin D1	0.0379	0.234	0.0526	0.423	0.0069	0.269
Cyclin E	0.0053	0.313	0.0102	0.548	0.0003	0.356
Cyclin A	0.0065	0.306	0.0002	0.806	0.0001	0.415
Cyclin B1	0.1609	0.158	0.0002	0.807	0.0010	0.326
p53	0.0284	0.247	0.0423	0.432	0.0011	0.322

A P-value of <0.05 was considered significant. A ρ-value (correlation coefficient) greater than 0.4 or less than −0.4 was considered to represent a "strongly" positive or negative correlation

Table 3 Correlation between the expression of cell cycle regulators and clinicopathological factors in stage Ib and II cervical carcinoma (Spearman's rank correlation)

	lymph vascular permeation	Stromal invasion (≧1/2)	Parametrial involvement	lymph node metastasis	Tumor size (≧30mm)
Cyclin D1					
P-value	0.0424	0.1763	0.8838	0.9087	0.7460
ρ-value	0.228	NE	NE	NE	NE
Cyclin E					
P-value	0.6147	0.5424	0.9683	0.6699	0.7323
ρ-value	NE	NE	NE	NE	NE
Cyclin A					
P-value	0.3290	0.1492	0.6999	0.9785	0.1784
ρ-value	NE	NE	NE	NE	NE
Cyclin B1					
P-value	0.9451	0.1822	0.4451	0.7708	0.8712
ρ-value	NE	NE	NE	NE	NE
p53					
P-value	0.0610	0.0058	0.0594	0.0881	0.0044
ρ-value	NE	−0.310	NE	NE	−0.321
Ki-67					
P-value	0.0140	0.2058	0.9688	0.0170	0.0564
ρ-value	−0.276	NE	NE	−0.269	NE

A *P*-value of less than 0.05 was considered significant. A ρ-value (correlation coefficient) greater than 0.4 or less than −0.4 was considered to represent a "strongly" positive or negative correlation. When the *P*-value was greater then 0.05, the respective ρ value was not examined (NE)

among the parameters were noted as shown in Table 3, we then performed a multivariate analysis using the same variables as used in the univariate analysis. The results again indicated that lymph node metastasis (P=0.0174, RR; 13.36) and cyclin A overexpression (P=0.0355, RR; 5.29) showed significant correlations. These findings indicated that the lymph node metastasis and cyclin A overexpression are independent prognostic factors for shorter survival in patients with surgically treated stage Ib+II cervical carcinoma. With regard to survival analysis in 23 patients with stage III+ IV carcinoma, the *P*-values determined by the univariate analysis for the overexpression of cyclin D1, E, A, B1, p53, and Ki-67 were 0.7608, 0.3878, 0.8236, 0.3650, 0.0997, and 0.7571, respectively. These results indicated that the overexpression of cell cycle-related molecules did not have prognostic significance in stage III+IV cervical carcinoma patients.

Discussion

The present study demonstrated that various cell cycle-related molecules were overexpressed in squamous cell carcinomas. Among the cyclins examined, the expression of cyclin A showed a significant positive correlation with that of Ki-67. Since cyclin A accelerates the cell cycle progression by binding to cdk2 in late G1 phase and to cdc2 in the G2-M phases [31, 32], the correlation between cyclin A and Ki-67 seems reasonable. Although cyclin A overexpression is reportedly caused by an increase in transcription [42], the molecular mechanisms involved in cyclin A transcription are not fully elucidated. One of the candidates of such an enhancer is p53 protein, because we previously showed a close topological correlation between the expression levels of cyclin A and p53 in endometrial

Table 4 Prognostic value of the clinicopathological parameters, and expression of cell cycle regulators and Ki-67 in patients with stage Ib and II cervical cancer

Cox's proportional hazard model				
Factors (No. of cases)	Univariate		Multivariate	
	P-value	RR (95%C I)	*P*-value	RR (95% CI)
LVP: positive (38)	0.2474	1.87 (0.61–5.71)	0.1875	0.28 (0.04–1.85)
StI: ≧1/2 (57)	0.6350	1.37 (0.38–4.97)	0.5416	0.58 (0.10–3.32)
PMI: positive (13)	0.4387	1.67 (0.46–6.07)	0.4875	2.17 (0.24–19.26)
LNM: positive (22)	0.0261	3.46 (1.16–10.35)	0.0174	13.36 (1.56–113.07)
TS: ≧30mm (38)	0.1104	2.61 (0.80–8.50)	0.3328	3.09 (0.32 –30.26)
Cyc D1: PI≧5 (10)	0.1301	2.74 (0.74–8.19)	0.1708	3.77 (0.57–25.11)
Cyc E: PI≧25 (14)	0.6019	1.41 (0.39–5.15)	0.8234	1.28 (0.14–11.53)
Cyc A: PI≧25 (12)	0.0109	4.33 (1.40–13.37)	0.0355	5.29 (1.12–25.02)
Cyc B1: PI≧20 (8)	0.4965	1.69 (0.37–7.67)	0.8854	0.84 (0.08–8.75)
p53: PI≧10 (17)	0.3465	1.76 (0.54–5.72)	0.7440	1.27 (0.30–5.45)
Ki-67: PI≧50 (35)	0.4193	1.57 (0.53–4.66)	0.6352	1.44 (0.32–6.39)

LVP lymph vascular permeation, *StI* stromal invasion, *PMI* parametrial involvement *LNM* lymph node metastasis, *TS* tumor size, *Cyc* cyclin, *RR* relative risk, *CI* confidence interval
P<0.05 was regarded as significant

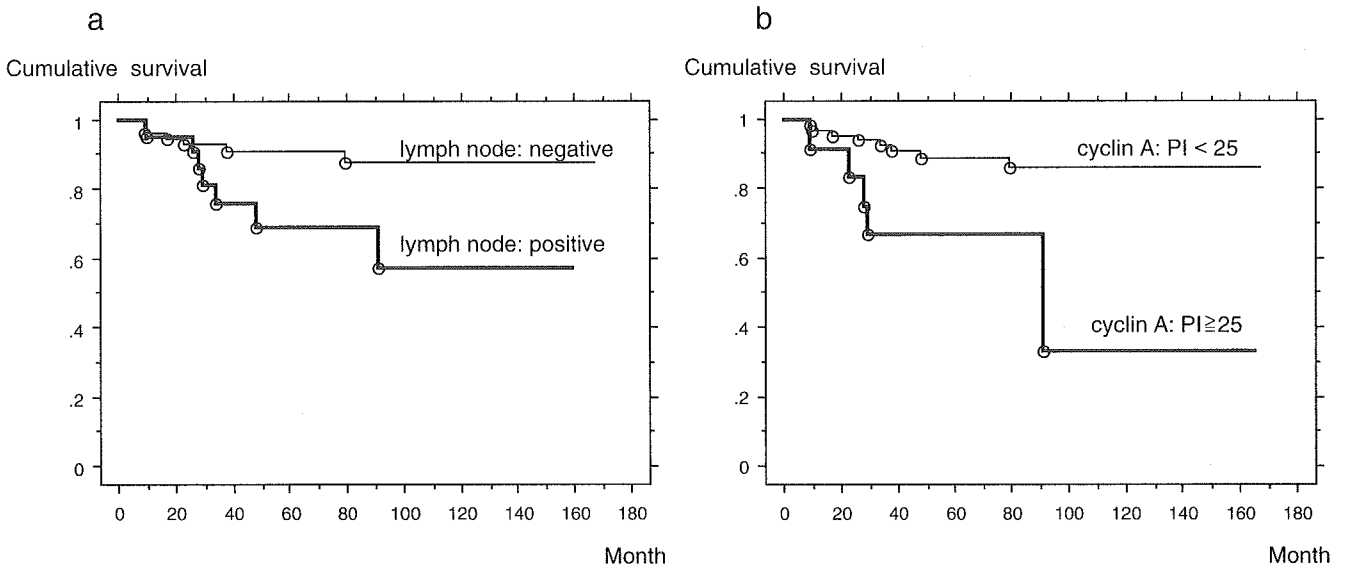

Fig. 3 Kaplan–Meier analysis of patients survival, with respect to lymph node metastasis (**a**) and cyclin A overexpression (**b**). The patients with positive lymph node metastasis and with cyclin A overexpression showed significantly poorer outcomes. The *P*-values are described in the text

carcinoma [33] and leiomyosarcoma [44]. However, no such spatial correlation between p53 and cyclin A was observed in the present study. Therefore, other mechanisms boosting cyclin A expression can be expected in cervical carcinoma. In this regard, HPV-E7 protein is reported to increase the expression of a transcription factor, E2F, which can activate the E2F-dependent promoter of the cyclin A gene [43].

In the present study, stage Ib+II patients with cyclin A overexpression exhibited a significantly shorter overall survival than those without cyclin A overexpression by multivariate analysis, and therefore cyclin A is an independent prognostic factor in early stage cervical carcinoma. In carcinomas of the breast and lung, overexpression of cyclin A has been reported as a prognostic factor for poor outcome [27, 40]. We have also reported that cyclin A is a prognosticator of uterine endometrial carcinoma [33] and uterine leiomyosarcoma [44]. The present result is in line with these reports. In contrast, a study by Van de Putte et al. reported that there was no prognostic significance of cyclin A overexpression in stage Ib cervical squamous cell carcinoma [39]. This difference may be due to the different selection of disease stages and different cut off values. To our knowledge, this is the first report of prognostic significance of cyclin A in stage Ib+II cervical carcinoma. The poor prognosis of patients with cyclin A-positive carcinoma may be attributed to the higher proliferative activity of the tumor, since the expression of cyclin A significantly correlated with Ki-67 expression. However, other cell cycle markers, such as cyclin D1, cyclin E and p53, did not show prognostic significance, although they also correlated with Ki-67. It is known that forced expression of cyclin A confers on non-neoplastic cells the anchorage-independency, that is an important hallmark of malignant transformation [16]. A recent study demonstrated that overexpression of cyclin A can suppress ultraviolet-induced, p53-mediated apoptosis [18]. Our preliminary experiments have shown that the forced expression of cyclin A in endometrial carcinoma cell lines resulted in the induction of cisplatin-resistance (unpublished observation). Accordingly, the tumor cells may acquire the specific biological characteristics via cyclin A overexpression, which may be related with the poor prognosis. On the other hand, we could not detect prognostic significance of cyclin A overexpression in stage III and IV tumors. Although the reason for this is unknown, it may be related to the limited number of cases examined.

In our series of cervical carcinoma, the clinicopathological parameters such as lymph node metastasis and lymph vascular permeation were not statistically correlated with the expression of cyclin A, but were correlated negatively with Ki-67. The results suggest that tumors with invasive or metastatic potentials have less growth activities. Recent research has indicated that tumor cells during invasion or metastasis are usually in the quiescent status of cell cycle [24, 25], such as G0 or early G1 phases in which Ki-67 is negative [14]. Therefore, negative correlations of Ki-67 with lymph node metastasis and lymph vascular permeation may represent that the presence of many quiescent tumor cells involved in the invasion or metastasis. However, further researches are needed to clarify the cell cycle status of cervical carcinoma cells in lymph node metastasis or vascular space invasion.

Cyclin D1 is expressed in the G1 phase of the cell cycle, forms a complex with cdk4 and 6 which phosphorylate pRB, resulting in cell cycle progression [31]. Therefore, cyclin D1 is regarded as an important G1 phase regulator. In the carcinoma tissues examined in the present study, although cyclin D1 expression increased compared to that in the normal epithelium, the mean PI (2.0) was relatively low. This result was consistent with a report by Skomedal et al. [35]. Cyclin D1 overexpression is reported in various

human malignancies. In breast carcinomas, overexpression of cyclin D1 has been reported to be a prognostic maker for favorable outcomes [3]. Conversely, in squamous cell carcinoma of the head and neck [26] and esophagus [38], overexpression of cyclin D1 has been reported to be a prognostic factor for poor outcomes. In addition, Bae et al. reported that the overexpression of cyclin D1 has prognostic significance in squamous cell carcinoma of the uterine cervix [2]. However, contrary to these reports, no prognostic value was detected in the present study, being similar with that of a previous report [35]. With regard to the expression mechanism of cyclin D1, Cattani et al. reported that the cyclin D1 gene amplification was associated with HPV infection, as oncoproteins E6 and E7 induce gene instability in laryngeal squamous cell carcinoma [8]. However, Southern et al. reported that cyclin D1 was frequently expressed in low risk HPV-infected low grade intra-epithelial lesions but not in high risk HPV-infected lesions [36].

The present study indicated that the p53, one of the most important transcription factors to control cell cycle progress and apoptosis, was nearly absent in the normal squamous epithelium, but was overexpressed in cervical squamous cell carcinoma. This result was consistent with the previous report [22]. It has been shown that the p53 is bound and inactivated by E6 oncoproteins in HPV-positive squamous cell carcinoma of the uterine cervix [5]. However, genetic analyses of p53 have demonstrated that p53 mutation is rare in cervical carcinoma [1]. In addition, Akasofu et al. reported that more than 50% of the tumor cells showed strong p53 staining in cases with p53 mutation [1]. In the present study, the mean PI of the p53 was 8.3 and the PI of most of p53-positive cases was less than 50. Therefore, though we have not performed the sequencing of p53 gene, the expression of p53 in the present study may represent an accumulation of wild-type p53 protein. Although, the significance of p53 protein accumulation in cervical tissues needs further investigation, a study reported that disease progression in ectocervical epithelia is associated with an increased incidence of apoptosis [21]. In this regard, the immunoreactive p53 could be involved in p53-mediated apoptosis. The prognostic significance of p53 protein accumulation is controversial. Chen and others claimed that p53 overexpression has prognostic value [9], while other reports stated it did not [20]. In the present study, we could not detect any prognostic significance of p53 in surgically treated stage Ib+II patients (P=0.3396) or in stage III+IV patients (P=0.0997), being consistent with the latter group of studies.

Overexpression of cyclin B1 and cyclin E was also observed in cervical carcinoma in the present study. Overexpression of cyclin E is an important prognostic factor of breast carcinoma [30], and cyclin B1 has been reported to have a prognostic impact in squamous cell carcinoma of the esophagus [37]. However, we did not find prognostic implications of cyclin E and B1 in the present study. Although this reason is not understood, a previous study has reported "unscheduled" expression of cyclin E and B1 in solid tumors, i.e. these cyclins were overexpressed irrespective of cell cycles [15].

Ki-67 staining has been evaluated extensively as a prognostic parameter in squamous cell carcinoma. Ho et al. [17] reported that those with increased Ki-67 labeling had shorter survival in stage I cervical carcinoma patients. In contrast, the increased labeling of Ki-67 was not associated with poor outcomes in cervical carcinoma patients with stage I [39] and stage III+IV [29] tumors. Although the exact reason for this discrepancy is unknown, our results are similar to those in the latter group.

In summary, the present study demonstrated various cyclins are overexpressed in squamous cell carcinomas, and considered to be involved in the gaining of the active growth potential of squamous cell carcinoma. In addition, cyclin A overexpression, as well as lymph node metastasis, was an independent prognosticator for unfavorable outcomes in patients with surgically treated stage Ib and II cervical cancer. Further studies are needed to clarify the biological roles of cyclin A overexpression other than growth stimulation.

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SURGEON'S CORNER

A systematic nerve-sparing radical hysterectomy technique in invasive cervical cancer for preserving postsurgical bladder function

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Abstract. Sakuragi N, Todo Y, Kudo M, Yamamoto R, Sato T. A systematic nerve-sparing radical hysterectomy technique in invasive cervical cancer for preserving postsurgical bladder function. *Int J Gynecol Cancer* 2005;**15**:389–397.

The objective of this study is to describe a technique for preserving the autonomic nerve systematically, including the hypogastric nerves, pelvic splanchnic nerves, and pelvic plexus and its vesical branches, based on anatomic considerations for the autonomic nerves innervating the urinary bladder, in radical hysterectomies and to assess postsurgical bladder function. A nerve-sparing radical hysterectomy was carried out on 27 consecutive patients with uterine cervical cancer treated between 2000 and 2002. The FIGO stages of the disease consisted of 10 stage Ib1, 6 stage Ib2, 3 stage IIa, and 8 stage IIb. The nerve-sparing procedure was successfully completed in 22 of the 27 patients (81.5%) in the study. At 1 year after the operation, bladder symptoms were significantly improved in the nerve-sparing group compared to the non–nerve-sparing group. Urinary incontinence and abnormal (diminished) bladder sensation were observed in three of the five patients (two patients had both symptoms), for whom the nerve-sparing procedure could not be performed, but none of the 22 patients for whom the nerve-sparing procedure was performed had incontinence, and only two patients had abnormal (increased) bladder sensation ($P = 0.0034$ for incontinence and $P = 0.030$ for abnormal bladder sensation). The patients' survival was not adversely affected by the nerve-sparing procedure. Although it is still preliminary, the surgical technique described in this report is thought to be effective for preserving bladder function, and thus, the quality of life could be improved for patients with cervical cancer who are treated with a radical hysterectomy. For further evaluation of the efficacy of nerve-sparing radical hysterectomy, a prospective randomized trial needs to be performed.

KEYWORDS: autonomic nerve, bladder function, cervical cancer, nerve sparing, quality of life (QOL), radical hysterectomy.

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