

### The pelvic node metastases

There were ten articles reporting the incidence of pelvic node metastases in stage IIB cervical cancer patients undergoing radical hysterectomy as shown in Table 1. The incidence of pelvic node metastases was approximately 35–45.8%<sup>(3–12)</sup>. Para-aortic node metastases were found in 4.5–7.2% of patients in this stage<sup>(11,12)</sup>. The most common sites of pelvic node metastases were the hypogastric<sup>(9)</sup> and the obturator<sup>(12)</sup>. The incidence of node metastases increased with the size of cervical tumor<sup>(5)</sup>.

In 1984, Inoue and Okumura<sup>(3)</sup> from the Aichi Cancer Center in Nagoya, Japan, noted that most of the stage IIB cervical cancer patients had only one positive node (38%), while two, three, and more than four positive nodes were detected in 22%, 8%, and 20%, respectively. Of note is that 12% had unresectable positive nodes. Six years later, these authors reported their experience with extension of the study period from 1965–1977 to 1965–1986, with the number of patients increased from 223 to 295<sup>(4)</sup>. In this later report, they revealed that the incidence of positive pelvic nodes increased from 38% to 43%.

There were two couples of studies from the same institutes, one from the University of Graz, Austria, reported 41–44% incidence of pelvic node metastases in stage IIB cervical cancer<sup>(5,6)</sup>. The other one from the Hokkaido University of Medicine, Japan, showed such incidence of 37–39%<sup>(11,12)</sup>.

In 2002, Winter *et al.*<sup>(13)</sup> from the University of Graz, Austria, evaluated the parametrial spread in patients who had negative pelvic nodes. They performed radical hysterectomy in 556 patients with stage IB, IIA, and IIB cervical cancer. Among 351 patients with negative nodes, 35 were in stage IIB. Of

these 35 patients, parametrial involvement was identified only in 17%.

Benedetti-Panici *et al.*<sup>(14)</sup> evaluated the relation between parametrial and pelvic node involvement in patients with stage IB–IIA cervical cancer undergoing radical hysterectomy. The hysterectomy specimens were processed with the giant section technique to obtain a thorough three-dimensional pathologic assessment of the parametrium. Clinically, undetected parametrial involvement was found by pathologic examination in 31%, 63%, and 58% of stage IB1, IB2, and IIA patients, respectively. Metastases to the pelvic nodes were always associated with parametrial invasion.

In conclusion, the incidence of pelvic node metastases in stage IIB cervical cancer is approximately 35–45%. Pelvic node metastases are always associated with parametrial involvement.

### The relationship between parametrial involvement and pelvic node metastases

Seven studies reported the relationship between the parametrial involvement and the positive pelvic nodes in stage IB–IIIB cervical cancer patients treated with radical surgery<sup>(2–7,9)</sup> as shown in Table 2. Of these, only one study from Kyushu University, Japan, demonstrated the relationship of parametrial status and the incidence of positive pelvic nodes in patients with stage IIB cervical cancer. Pelvic node metastases increased three times from 13.3% to 37.8% when the parametrium was involved with the tumor<sup>(7)</sup>. Invasion of the tumor to the parametrium significantly correlated with the spreading of tumor to the pelvic nodes. Such incidence increased two to six times in patients with positive parametrium.

**Table 2.** The relationship between parametrial status and pelvic node metastases

Authors	Year	Stage	Parametrium	Pelvic node metastases (%)
Noguchi <i>et al.</i> <sup>(9)</sup>	1987	IB–IIIB	Positive	54.8
			Negative	8.5
Burghardt <i>et al.</i> <sup>(5)</sup>	1987	IB–IIIB	Positive	75.3
			Negative	23.4
Inoue and Okumura <sup>(3)</sup>	1984	IB, IIA, IIB	Positive	57.9
			Negative	28.6
Inoue and Morita <sup>(4)</sup>	1990	IB, IIA, IIB	Positive	29.2
			Negative	14.2
Matsuyama <i>et al.</i> <sup>(2)</sup>	1984	IB, IIA, IIB	Positive	42
Giardi <i>et al.</i> <sup>(6)</sup>	1989	IB–IIIB	Positive	81
			Negative	26
Kamura <i>et al.</i> <sup>(7)</sup>	1993	IIB	Positive	37.8
			Negative	13.3

Matsuyama *et al.*<sup>(2)</sup> reported the 42% incidence of pelvic node metastases when the parametrium was involved in patients with stage IB–IIIB cervical cancer who were treated with radical hysterectomy. However, such incidence in cases of negative parametrium was not mentioned. Interestingly, two series, one from Austria, another from Japan, reported the use of surgical treatment for stage IIIB cervical cancer<sup>(5,9)</sup>. From these studies, it can be concluded that parametrial involvement is strongly associated with pelvic node metastases.

### Adjuvant treatment after surgery

After radical operation for stage IIB cervical cancer patients, adjuvant radiations are usually administered if any high-risk pathologic factors, ie, positive pelvic nodes, parametrial invasion, and involved surgical margins, are identified<sup>(2–5)</sup>. Some authors also gave adjuvant radiation to patients who had deep cervical stromal invasion<sup>(2,7,10)</sup> or prominent lymphovascular space invasion (LVSI)<sup>(2,12)</sup>. With various criteria for postoperative radiation therapy, the percentage of stage IIB cervical cancer patients who received adjuvant treatment in one study was surprisingly high at 72%<sup>(7)</sup>. Recently, the Japanese Patterns of Care Study Working Group<sup>(15)</sup> has conducted an extramural survey of 73 institutions in Japan on the postoperative radiation between September 1998 and March 2001. The study revealed that only 33% of stage IIB cervical cancer patients received adjuvant radiation. The summary of the indications for adjuvant radiation after surgery for stage IIB cervical cancer is shown in Table 3.

Currently, concurrent cisplatin-based chemoradiation is recommended in patients who need adjuvant radiation after radical surgery for early-stage cervical cancer<sup>(16,17)</sup>. However, Kawagoe *et al.*<sup>(8)</sup> gave chemo-

therapy alone to patients who had positive surgical margins or lymph node involvement.

Various types of adjuvant treatment have been used for cervical cancer patients who have high-risk pathologic factors after the operation. The Austrian Gynecologic Oncology Group has conducted a prospective, randomized, multicenter study to compare the values of adjuvant radiation, adjuvant chemotherapy, and expectant treatment in IB, IIA, and IIB cervical cancer patients who had high-risk factors identified after radical hysterectomy. Adjuvant chemotherapy or radiation did not improve the survival or reduce the recurrence after the operation when compared with observation alone<sup>(18)</sup>.

In conclusion, most authors advised radiation in patients who had high-risk pathologic factors after the operation. The benefit of adjuvant treatment in these patients is inconclusive and needs further study in a prospective randomized fashion.

### The prognostic factors

There were nine studies using multivariate analysis to determine the significance of various prognostic factors after radical hysterectomy for cervical cancer as shown in Table 4. Kamura *et al.*<sup>(7)</sup> studied only stage IIB cervical cancer, while other authors also included stage IB, IIA, or even IIIB. Lymph node metastases and the number of positive nodes were significant prognostic factors in all studies<sup>(2,4,5,7,8,10–12,19)</sup>, while parametrial involvement affected the patient survival in only four studies<sup>(2,4,10,12)</sup>. The clinical stage of cervical cancer influenced treatment outcomes in two studies<sup>(8,19)</sup>. Kawagoe *et al.*<sup>(8)</sup> noted that the tumor size was also an important prognostic factor, while LVSI and deep stromal invasion were found to affect patient survival in the series of Sakuragi *et al.*<sup>(11)</sup>.

Takeda *et al.*<sup>(12)</sup> had pathologically stratified the cervical cancer patients after treatment with radical operation into three groups, ie, low-risk group, intermediate-risk group, and high-risk group. The low-risk group consisted of patients with tumor confined to the uterus and without LVSI. The intermediate-risk group included patients with tumor confined to the uterus with associated parametrial invasion or pelvic node metastasis. The high-risk group comprised patients with pure adenocarcinoma with associated parametrial involvement or pelvic node metastases and patients with common iliac or para-aortic node metastases. The estimated 5-year survival rates of these three groups were 100%, 85.5%, and 25%, respectively.

Aoki *et al.*<sup>(20)</sup> studied the prognostic factors in 59 cervical cancer patients with positive nodes after

Table 3. Indications for adjuvant radiotherapy

Authors	Positive		Positive margins	Others
	PMI	LN		
Okada <i>et al.</i> <sup>(10)</sup>	+	+	+	DSI
Kawagoe <i>et al.</i> <sup>(8)a</sup>	–	+	+	—
Takeda <i>et al.</i> <sup>(12)</sup>	+	+	–	LVSI, ovarian metastasis
Inoue and Morita <sup>(4)</sup>	+	+	–	—
Matsuyama <i>et al.</i> <sup>(2)</sup>	+	+	+	LVSI, DSI
Kamura <i>et al.</i> <sup>(7)</sup>	+	+	–	DSI
Trattner <i>et al.</i> <sup>(19)</sup>	+	+	+	LVSI

PMI, parametrial involvement; LN, lymph node; DSI, deep stromal invasion; LVSI, lymph vascular space invasion; +, adjuvant radiation; –, no adjuvant radiation.

<sup>a</sup>Adjuvant chemotherapy.

**Table 4.** The significant prognostic factors

Authors	Year	Stage	Total number of patients	Number of stage IIB patients	The prognostic factor
Okada <i>et al.</i> <sup>(10)</sup>	1998	IB–IIB	104	30	+ PM, + LN
Burghardt <i>et al.</i> <sup>(5)</sup>	1987	IB–IIIB	800	195	Number of positive nodes
Inoue and Morita <sup>(4)</sup>	1990	IB, IIA, IIB	875	295	Number of positive nodes, + PM
Kawagoe <i>et al.</i> <sup>(8)</sup>	1999	IB, IIA, IIB	128	24	Stage, tumor size, + LN
Matsuyama <i>et al.</i> <sup>(2)</sup>	1984	IB, IIA, IIB	255	99	Number of positive nodes, + PM
Takeda <i>et al.</i> <sup>(12)</sup>	2002	IB, IIA, IIB	187	88	+ LN, + PM, LVSI, adenocarcinoma
Kamura <i>et al.</i> <sup>(7)</sup>	1993	IIB	107	107	Number of positive nodes
Trattner <i>et al.</i> <sup>(19)</sup>	2001	IB, IIA, IIB	115	35	Stage, + LN
Sakuragi <i>et al.</i> <sup>(11)</sup>	1999	IB, IIA, IIB	208	97	LVSI, DSI, + LN (para-aortic nodes, bilateral pelvic nodes, common iliac nodes)

PM, parametrium; LN, lymph node; LVSI, lymph vascular space invasion; DSI, deep stromal invasion; +, positive.

radical hysterectomy followed by adjuvant pelvic radiation. Of these patients, 37 were in stage IIB cervical cancer. The authors noted that both the number of positive nodes ( $\geq 2$ ) and the parametrial involvement were significantly associated with decreased disease-specific survival. The 5-year recurrence rate in patients with both prognostic factors was as high as 70%, while no recurrence was found in patients who had one positive node and no parametrial invasion.

Among the patients with stage IIB cervical cancer who had negative nodes, tumor size and LVSI were the significant prognostic factors by multivariate analysis in the study of Comerchi *et al.*<sup>(21)</sup>. Winter *et al.*<sup>(13)</sup> showed that parametrial involvement was of no prognostic significance in 149 stage IIB cervical cancer patients who had negative nodes.

It can be concluded that the number of positive nodes is a strongly significant prognostic factor, while the influence of parametrial invasion on patient survival is still controversial in patients with stage IIB cervical cancer.

### The outcomes

There were four studies that reported the overall survival of stage IIB cervical cancer patients treated with radical hysterectomy as shown in Table 5. Matsuyama *et al.*<sup>(2)</sup> found that among 99 patients with stage IIB

cervical cancer, 29 died or had disease recurrence. Three patients died of radiation complications, and one died of intercurrent disease. The overall 5-year survival rate was approximately 71%. Burghardt *et al.*<sup>(5)</sup> reported a 5-year survival rate of 77% in 191 patients with stage IIB cervical cancer treated with radical surgery, higher than that of 63% in the series of Trattner *et al.*<sup>(19)</sup>. The outcome of stage IIB patients was worse in the study of Aoki *et al.*<sup>(20)</sup>, which revealed a 5-year survival rate of 55% in 37 stage IIB cervical patients who had positive nodes.

The nodal status and the number of positive nodes play an important role on the survival outcome of patients with cervical cancer. In the series of Noguchi *et al.*<sup>(9)</sup>, the survival of patients decreased with the increasing number of positive nodes. The 5-year survival rates of patients who had negative nodes was 83%, while those of the patients who had 1, 2–3, and  $\geq 4$  positive nodes were 64.4%, 43.1%, and 30%, respectively. Girardi *et al.*<sup>(6)</sup> studied 219 patients with stage IIB cervical cancer treated with radical hysterectomy. The 5-year survival rate in cases of negative parametrial nodes was high at 81% and dropped to 54% when the parametrial nodes were positive. In the report of Inoue and Morita<sup>(4)</sup>, which included 223 patients with stage IIB cervical cancer, the 5-year survival rate of patients with positive nodes (57%) was significantly lower than that of patients with negative nodes (91%). The 5-year survival rate decreased to 38.5% when the number of positive nodes was  $\geq 4$ . In the study of Okada *et al.*<sup>(10)</sup>, the outcome of patients with parametrial extension was not different from those with positive nodes. The 5-year survival rates of both groups were 76.6% and 76.3%, respectively, while that of patients with only deep stromal invasion was still high at 89.3%.

In conclusion, the 5-year survival rate of stage IIB cervical cancer patients treated with radical

**Table 5.** The 5-year survival rate

Authors	Year	5-year survival rate (%)
Aoki <i>et al.</i> <sup>(20)</sup>	2000	55.2
Trattner <i>et al.</i> <sup>(19)</sup>	2001	62.9
Matsuyama <i>et al.</i> <sup>(2)</sup>	1984	70.7
Burghardt <i>et al.</i> <sup>(5)</sup>	1987	76.9

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<sup>(2,22)</sup>. 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<sup>(2)</sup>.

### 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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## 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<sup>2</sup>, 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<sup>-1</sup>) on the assumption that tumors regressed exponentially with time.

**Results:** TRR ranged widely (0.004–0.090 day<sup>-1</sup>) and did not significantly differ between treatment with chemoradiotherapy (median, 0.032 day<sup>-1</sup>) and with radiotherapy alone (median, 0.024 day<sup>-1</sup>) (p=0.361). TRR > 0.05 day<sup>-1</sup> was seen in four chemoradiotherapy tumors (30.8%) and in six radiotherapy-alone tumors (15.0%) (p=0.207), whereas TRR < 0.01 day<sup>-1</sup> 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

RADIODTHERAPY (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.<sup>1</sup> 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.<sup>2</sup> Measuring tumor size with MR imaging weekly, Gong *et al.* observed that cervical tumors regressed exponentially with time during RT.<sup>3</sup> 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<sup>2</sup> 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:

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

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<sup>3</sup> 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<sup>3</sup>. TRR was defined as the slope of the tumor regression curve shown by the exponential regression equation as follows:

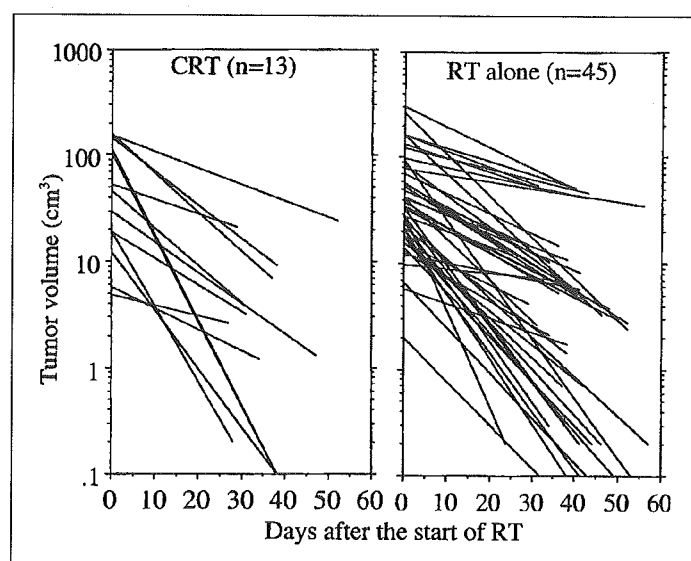
$$Y = A \times 10^{-B \times X} \quad [\text{Eq. 2}]$$

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<sup>-1</sup>) 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 <sup>3</sup>	4.9-159.1 (46.2)	2.0-301.6 (35.2)	0.830
TRR range (median) 10 <sup>-2</sup> in day <sup>-1</sup>			
All tumors	1.0-8.1 (3.2)	0.4-9.0 (2.5)	0.361
Tumors > 33.5 cm <sup>3</sup> (n=7, 24): (CRT, RT-alone)	1.4-8.1 (3.5)	0.6-5.6 (2.0)	0.094
Tumors > 65.4 cm <sup>3</sup> (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<sup>3</sup> to 159.1 cm<sup>3</sup> (median, 46.2 cm<sup>3</sup>) in the CRT group and from 2.0 cm<sup>3</sup> to 301.6 cm<sup>3</sup> (median, 35.2 cm<sup>3</sup>) 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;



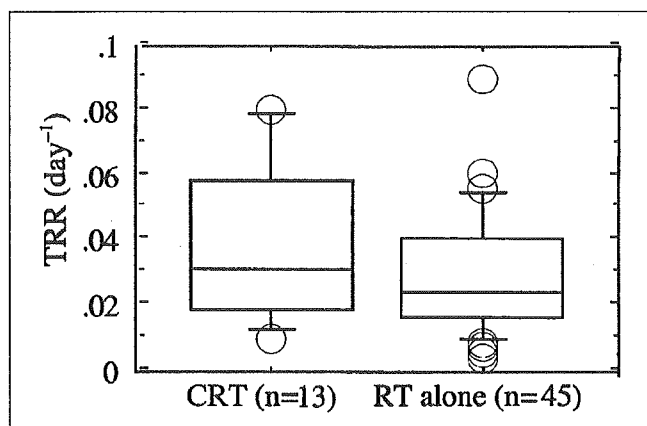


Fig. 2. Box plots of the tumor regression rate (TRR) of cervical squamous cell carcinomas according to type of treatment: chemoradiotherapy (CRT) or radiotherapy (RT) alone. The box indicates the 25th to 75th percentile with the median value, and bars indicate 10th and 90th percentiles.

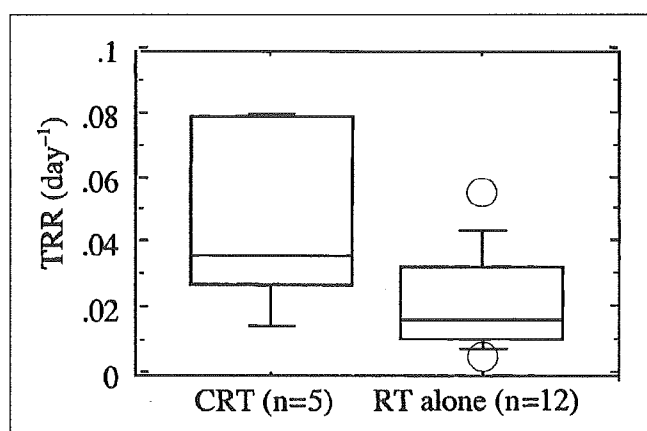


Fig. 3. Box plots of the tumor regression rate (TRR) of cervical squamous cell carcinomas greater than 65.4 cm<sup>3</sup> in volume (5.0 cm in diameter), according to type of treatment: chemoradiotherapy (CRT) or radiotherapy (RT) alone. The box indicates the 25th to 75th percentile with the median 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<sup>-1</sup>; median, 0.032 day<sup>-1</sup>) and the RT-alone group (range, 0.004-0.090 day<sup>-1</sup>; median, 0.025 day<sup>-1</sup>) ( $p=0.361$ , Fig. 2). TRR was greater than 0.05 day<sup>-1</sup> 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<sup>-1</sup> 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<sup>3</sup> (4.0 cm in diameter) or of 65.4 cm<sup>3</sup> (5.0 cm in diameter). For tumors measuring 33.5 cm<sup>3</sup> or more, the median of TRR was 0.035 day<sup>-1</sup> for the CRT group ( $n=7$ ; range, 0.014-0.081 day<sup>-1</sup>) and 0.020 day<sup>-1</sup> for the RT-alone group ( $n=24$ ; range, 0.006-0.056 day<sup>-1</sup>) ( $p=0.094$ ). The difference in TRR became conspicuous for tumors measuring 65.4 cm<sup>3</sup> or more; TRR tended to be greater in the CRT group ( $n=5$ ; range, 0.015-0.081 day<sup>-1</sup>; median, 0.037 day<sup>-1</sup>) than in the RT-alone group ( $n=12$ ; range, 0.006-0.056 day<sup>-1</sup>; median, 0.017 day<sup>-1</sup>) ( $p=0.065$ , 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.<sup>3</sup> 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 radio-responsiveness 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.<sup>4</sup> 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.<sup>5</sup> 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<sup>3</sup> 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<sup>6</sup> and to intensify the chemotherapy regimen<sup>7</sup> 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<sup>3</sup> 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 ( $\text{day}^{-1}$ ) of the shrinkage curve by fitting to an exponential equation.

**Results:** Early-assessed radioresponse ranged from 0.001 to 0.106  $\text{day}^{-1}$  (median, 0.021  $\text{day}^{-1}$ ) and late-assessed radioresponse from 0.009 to 0.091  $\text{day}^{-1}$  (median, 0.021  $\text{day}^{-1}$ ), 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 ( $\geq 65\%$  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<sup>2</sup> ( $n = 3$ ), 30 mg/m<sup>2</sup> ( $n = 6$ ), or 20 mg/m<sup>2</sup> ( $n = 1$ , 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<sup>3</sup>, whereas that of those remaining as a small, high-intensity “scar” that was difficult to measure was regarded as 0.05 cm<sup>3</sup>.

### 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<sup>-1</sup>) (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<sup>3</sup> 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<sup>3</sup> (median, 95.5 cm<sup>3</sup>). 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<sup>-1</sup> (median, 0.021 day<sup>-1</sup>) in the early phase, from 0.013 to 0.121 day<sup>-1</sup> (median, 0.025 day<sup>-1</sup>) in the late phase, and from 0.009 to 0.091 day<sup>-1</sup> (median, 0.021 day<sup>-1</sup>) 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

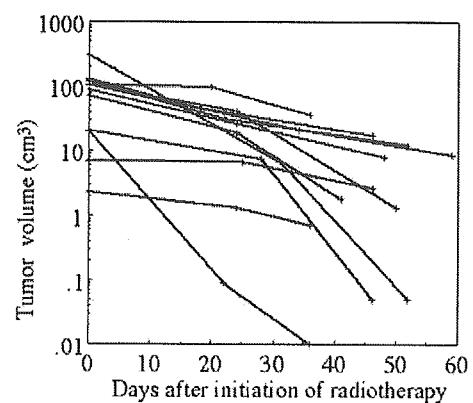


Fig. 1. Tumor shrinkage curves composed of three volumes of preradiotherapy, early phase (3 to 4 weeks), and late phase (6 to 7 weeks) ( $n = 12$ ).

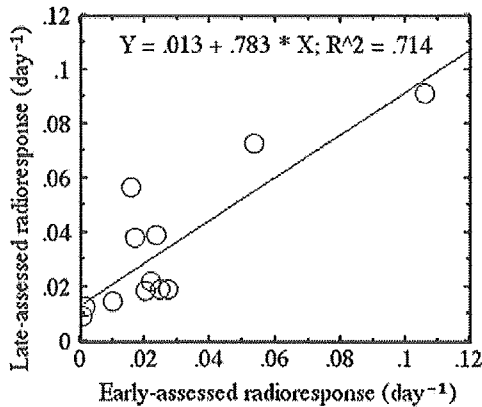


Fig. 2. Correlation between early-assessed and late-assessed radioresponse ( $n = 12$ ,  $p = 0.0005$ ).

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

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  $\text{cm}^3$  (median, 0.41  $\text{cm}^3$ ) and was  $\leq 0.05 \text{ cm}^3$  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-

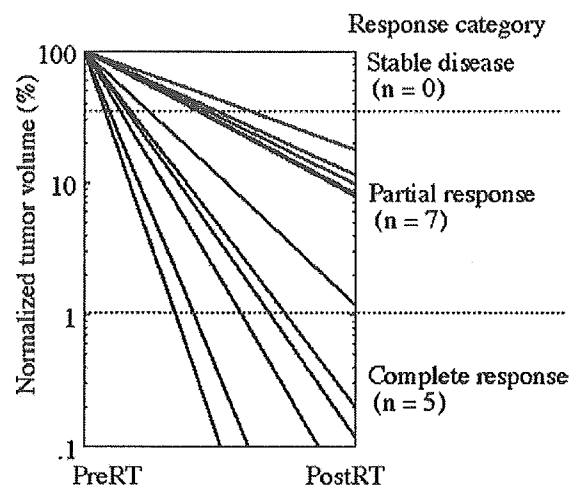


Fig. 3. Comparison of calculated radioresponse at the end of radiotherapy (RT) between the speed of shrinkage (curves) and the degree of shrinkage (response category). The postRT volume was calculated with the regression equation for each tumor at the end of RT for each individual (42–63 days from the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

dard clinical practice, is considered effective in the differentiation of highly (e.g.,  $>0.05 \text{ day}^{-1}$ ) and poorly radioresponsive (e.g.,  $<0.02 \text{ day}^{-1}$ ) 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  $0.106 \text{ day}^{-1}$  (early phase, 106-fold variation) and from 0.009 to  $0.091 \text{ day}^{-1}$  (through phase, 10-fold variation) in the present study and from 0.007 to  $0.182 \text{ day}^{-1}$  (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 recommenda-

tion 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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## 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

### 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-

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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  $\mu$ m 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 with whole 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 intracavitary 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 ( $\rho$ ) 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  $65.1 \pm 45.9$  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 Ib+II (46.1) and stage III+IV (46.9).

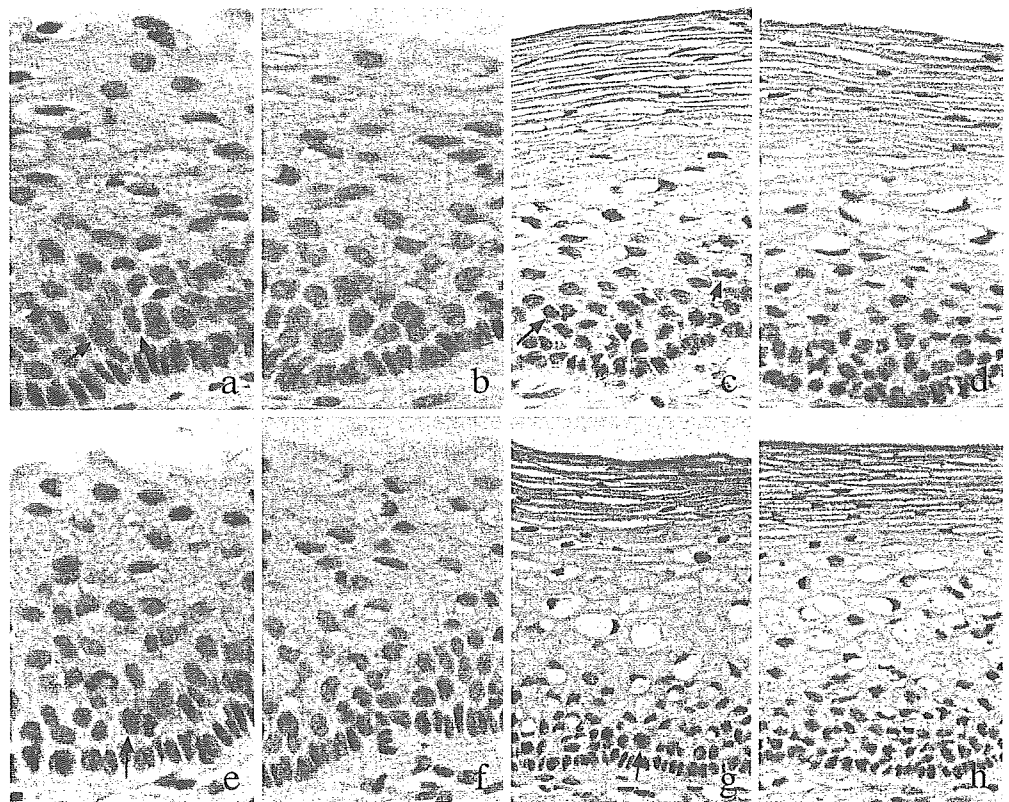
### Correlation of cyclin expression and Ki-67

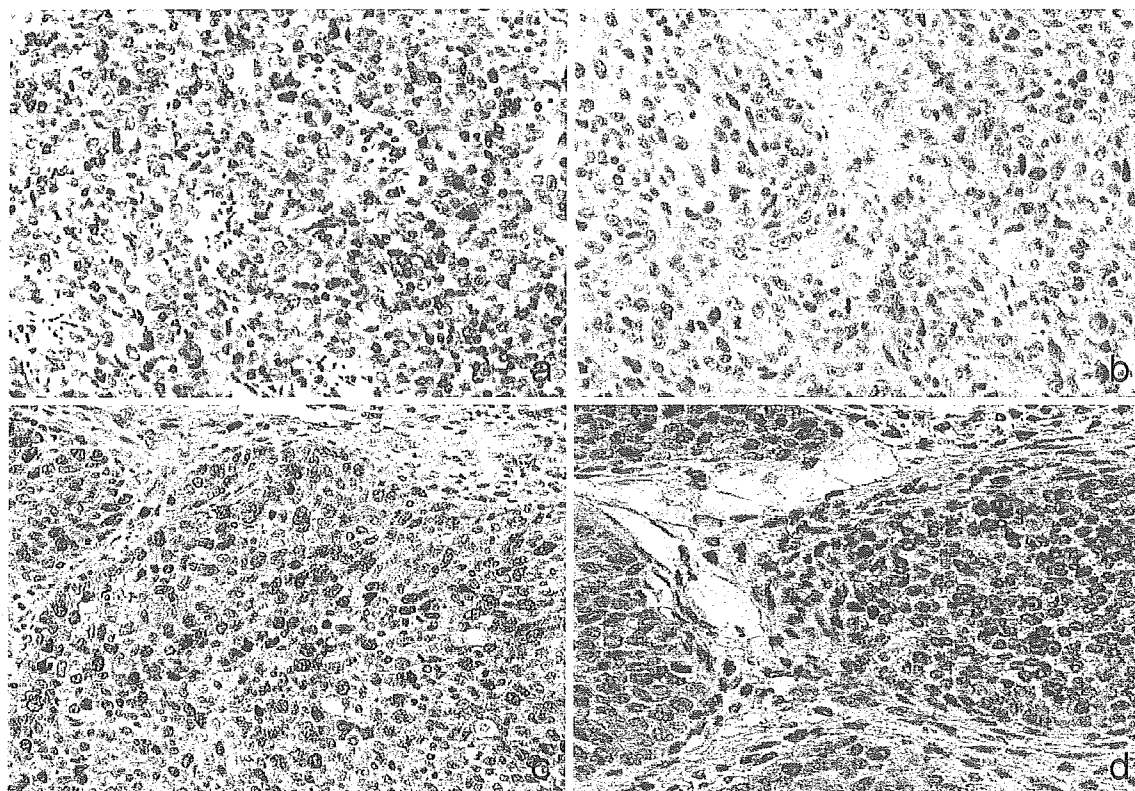
Correlations between the immunohistochemical expression of cyclins and Ki-67 are summarized in Table 2. In stage Ib+II tumors, the expression of cyclin D1, E, A, and p53 positively correlated with the expression of Ki-67 ( $P=0.0379$  for cyclin D1,  $P=0.0053$  for cyclin E,  $P=0.0065$  for cyclin 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;  $\times 200$ , c, d, g, h;  $\times 175$ )





**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,  $\times 225$ ). d: nuclear-positive cells with weak cytoplasmic staining are diffusely observed (stage Ib,  $\times 250$ )

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 clinicopathological and immunohistochemical parameter or not using a Spearman's rank correlation analysis. Since no "strong" correlations (i.e.  $\rho$ -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 $\pm$ 5.9	1.9 $\pm$ 5.4	2.0 $\pm$ 5.8	0.5951
Cyclin E	9.8 $\pm$ 17.7	20.9 $\pm$ 27.8	12.3 $\pm$ 20.7	0.1395
Cyclin A	13.3 $\pm$ 12.5	25.1 $\pm$ 19.9	16.0 $\pm$ 5.2	0.0128
Cyclin B1	8.4 $\pm$ 18.5	25.2 $\pm$ 30.9	12.2 $\pm$ 22.8	0.3264
p53	6.6 $\pm$ 12.1	14.3 $\pm$ 23.0	8.3 $\pm$ 15.4	0.5903
Ki-67	46.1 $\pm$ 19.8	46.9 $\pm$ 25.8	46.2 $\pm$ 21.0	0.4376

Each figure indicates the mean positivity index (PI) $\pm$ 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	$\rho$	P	$\rho$	P	$\rho$
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  $\rho$ -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)

A *P*-value of less than 0.05 was considered significant. A  $\rho$ -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 than 0.05, the respective  $\rho$  value was not examined (NE)

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

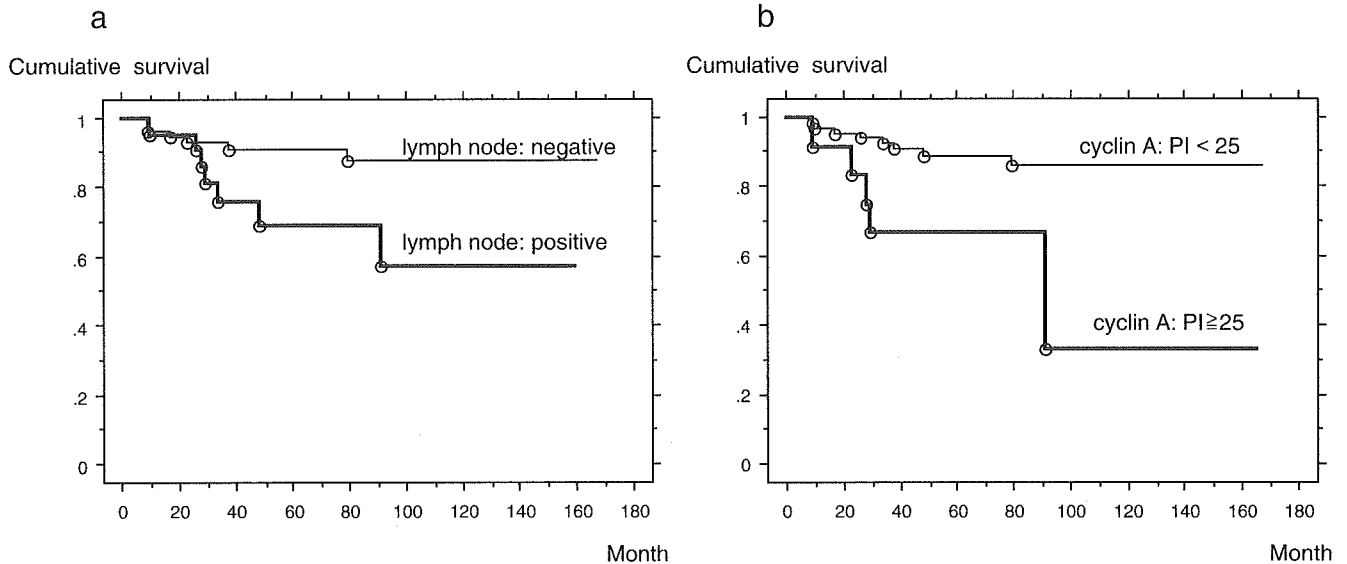
## Discussion

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

*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

Cox's proportional hazard model				
Factors (No. of cases)	Univariate		Multivariate	
	<i>P</i> -value	RR (95%CI)	<i>P</i> -value	RR (95%CI)
LVP: positive (38)	0.2474	1.87 (0.61-5.71)	0.1875	0.28 (0.04-1.85)
StI: $\geq 1/2$ (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: $\geq 30$ mm (38)	0.1104	2.61 (0.80-8.50)	0.3328	3.09 (0.32-30.26)
Cyc D1: $PI \geq 5$ (10)	0.1301	2.74 (0.74-8.19)	0.1708	3.77 (0.57-25.11)
Cyc E: $PI \geq 25$ (14)	0.6019	1.41 (0.39-5.15)	0.8234	1.28 (0.14-11.53)
Cyc A: $PI \geq 25$ (12)	0.0109	4.33 (1.40-13.37)	0.0355	5.29 (1.12-25.02)
Cyc B1: $PI \geq 20$ (8)	0.4965	1.69 (0.37-7.67)	0.8854	0.84 (0.08-8.75)
p53: $PI \geq 10$ (17)	0.3465	1.76 (0.54-5.72)	0.7440	1.27 (0.30-5.45)
Ki-67: $PI \geq 50$ (35)	0.4193	1.57 (0.53-4.66)	0.6352	1.44 (0.32-6.39)



**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 sup-

press 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