or seemed to offer a better prognosis than this standard treatment, it would be quite inappropriate to conduct rigorous RCTs to compare CCRT versus the standard treatment in these patients. In any case, for stage I patients, surgery with or without adjuvant RT can be accepted as the standard treatment, as it has been to date.

Data on therapeutic outcomes according to disease stage are available only from the RTOG 90-01 study.[31] According to a subgroup analysis of this study, CCRT was significantly effective in patients with bulky stage IB/II disease (accounting for 70% of all enrolled patients), but not in patients with stage III/IVA disease. A metaanalysis[36] also found that greater efficacy of CCRT was demonstrated in studies including a high proportion ($\geq$70%) of stage I/II patients. Based on these findings, although it is difficult to determine the best therapeutic approach, due to the lack of data on the comparison between CCRT alone or CCRT combined with surgery versus the standard treatment (surgery with or without RT), patients with IB2 and bulky stage IIA disease should be treated with CCRT, at least, but not with RT alone (GOG 123 trial[30]).

For stage IIB patients, there are no data on the comparison between surgery versus CCRT, because surgery is not indicated for these patients in the United States and Europe. Based on currently available evidence, CCRT, instead of RT alone, will become the new standard for stage IIB patients if surgery is not considered. NAC followed by surgery may also be effective compared with RT alone,[18] but it has not been compared with CCRT in stage IIB patients. RCTs may be required to reach a conclusion about the best therapeutic approach for these patients.

Efficacy of CCRT as adjuvant therapy

According to the results of an RCT (SWOG 8797[32]), the prognosis of stage IA to IIA patients who were found to have poor prognostic factors after surgery was significantly better when they were treated with CCRT than with RT alone postoperatively, with respect to both OS and PFS. This survival benefit of CCRT was also found in a metaanalysis reported by Lukka et al.[37] However, there are no data regarding the efficacy of CCRT as postoperative adjuvant therapy for stage IIB patients, for whom surgery is indicated in Japan. With respect to the indications for postoperative CCRT, further investigation may be required.

Optimal dose and schedule of platinum

In the studies of CCRT reviewed in this article,[28-32,36,37] 5-FU was always administered at 4g/m^{2} over 96h, while the dose and schedule of CDDP varied from 40mg/m^{2} weekly (as a single agent) to 50–75mg/m^{2} every 3 or 4 weeks.

Despite the differences in the doses and schedules of CDDP,[34] or whether CDDP was administered as a single agent or in combination with 5-FU,[29] the survival improvements achieved by these CCRT regimens were similar. On the other hand, significantly more patients treated with a PF regimen compared with a CDDP alone regimen experi-

enced adverse events, especially bone marrow depression. For this reason, weekly CDDP monotherapy is commonly selected as a concurrent CT regimen. In fact, the GOG recommends weekly CDDP (40mg/m^{2}) in this setting and, in the ongoing GOG trial,[49] patients in the control group are being treated with weekly CDDP. In some studies, weekly CDDP was compared with CDDP every 3-weeks[50] or a split dosing regimen of CDDP (20mg/m^{2} daily for 5 days).[51]

In the SWOG 8797 trial,[32] patients received two additional courses of CT as systemic therapy after completion of the CCRT. Also, it is still controversial which is more beneficial, CDDP alone or a CDDP-based multidrug regimen.

It has been reported that CBDCA has a radiation-sensitizing effect,[52] and that, compared with CDDP, the drug is associated with less severe nonhematological toxicities, such as nephrotoxicity, gastrointestinal toxicity, and neurotoxicity, although it has comparable antitumor activity. The reduced toxicity, may be advantageous with respect to both treatment compliance and QOL, especially when treating elderly patients and patients with advanced disease, who sometimes develop hydronephrosis. In this context, the replacement of CDDP by CBDCA is also a subject for future investigation.

As discussed above, an optimal regimen should be comprehensively examined from the perspectives of not only tumor response and long-term survival but also in terms of acute and late-occurring adverse events.

Development of new drugs with greater efficacy

It is also important to develop new chemotherapeutic agents with greater efficacy compared with CDDP monotherapy or CDDP-based combination regimens. Some newer drugs have shown promising results; although the doses varied among studies, paclitaxel (PTX), irinotecan, vinorelbine, and gemcitabine produced response rates of 17%–25%, 13%–21%, 17%–18%, and 8%–9%, respectively, when used as monotherapy.[53]

Several combination CT regimens including these newer drugs have been investigated (Table 6[54-62]).

This section presents the results of phase II studies of these combination regimens, mainly for combinations of paclitaxel (PTX) and platinum, which have been reported most frequently and are associated with higher response rates.

The TP (PTX + CDDP) and TJ (PTX + JM-8 [CBDCA]) regimens investigated to date consist of PTX at a dose of 135mg/m^{2} over 24h in three studies,[54,55,61] or 175mg/m^{2} over 3h in three studies,[56,58,62] in combination with CDDP at a dose of 75mg/m^{2} in three studies,[54-56] or CBDCA at a dose to achieve an AUC of 5 in two studies.[58,62] Except for one study,[55] treatment was repeated every 3 weeks. In another study,[57] patients were treated with a modified TP regimen, consisting of PTX at a dose of 60mg/m^{2} over 3h and CDDP at a dose of 60mg/m^{2}, both repeated every 10 days. Some other studies investigated a triplet CT regimen, consisting of TP combined with IFO (TIP regimen[59]) or Epi (TEP regimen[60]). Although the dose of

Table 6. New combination chemotherapies for cervical cancer

Author	Subjects	Design	CT regimen (mg/m²)	No. of patients	RR (%) (95% CI)	Median survival	Remarks
Rose (GOG),[54] 1999	CT-naive SCC	Phase II	TP: T, 135/24h+P, 75, q 3 Weeks	44	46.3 (30.7–62.6)	PFI:5.4 Months; OS, 10.0 Months	Non-RT site vs RT site: 70% vs 23% ($P = 0.008$)
Piver,[55] 1999	CT-naive SCC + adenoca	Phase II	TP: T, 135/24h+P:75, q 4 Weeks	20	45	PFS:10.5 vs 4 Months ($P = 0.015$) OS.13 vs 6 Months ($P = 0.14$)	RT field: outside vs inside Outside, better response: 60%
Papadimitriou,[56] 1999	Stage IV/Rec. SCC + adenoca	Phase II	TP: T, 175/3h+P:75, q 3 Weeks with G-CSF	34	47 (30–65)	PFI:5.5 Months; TTP, 5 Months, OS:9 Months	G3/4 Neuropathy: 43%
Park,[57] 2004	Stage IB2-IIB SCC + adenoca	Phase II NAC-Surgery	TP: T, 60/3h+P:60, q 10 Days	43	90.7		CR:39.5%; pCR:11.6%; PR: 51.2%, down-staging: 72.1%
Mickiewicz (GOL),[58] 2001	CT-naive	Phase II	TJ: T, 175/3h+J, AUC 5, q 3 Weeks	32	71.9 (56.4–87.4)	PFI:7 Months	
Zanetta,[59] 1999	Rec. SCC	Phase II, Salvage	TIP: T, 175/3h, Day1+ I, 5000, day2+ P, 75, day2, q 3 Weeks	45	67 (51–81)	OS: Non-responders vs responders: 6 vs 13 Months	Non-RT site vs RT site: 75% vs 52% G3/4 Myelotoxicity, 91%
D'Agostino,[60] 2002	IB2-IVA SCC + adenoca	Phase II	TEP:T, 175+E, 100+P, 100, q 3 Weeks	42	78.5 (63.8–93.2)	PFS:47 Months	Surgical rate:76.2%
Moore (GOG),[61] 2001	Stage IVB/rec.	Phase III, P vs TP	P:50 vs TP: T: 135/24h + P, 50, q 3 Weeks	264	19.4% vs 36.2%	PFI:2.8 vs 4.8 Months OS:8.8 vs 9.7 Months	RR: $P = 0.002$; PFI, p < 0.01
Kitagawa,[62] 2004	Rec.	Phase II	T:175/3h+J, AUC 5, q 3 Weeks	28	61 (41–78)	PFS: 5.9 Months	OS: NS Non RT site vs RT site: 57% vs 67%

CT, chemotherapy; RR, response rate; PFI, progression free interval; OS, overall survival; TTP, time to progression; PFS, period free survival; T, paclitaxel; P, cisplatin; J, CBDCA; I, ifosfamide; E, epirubicin; adenoca, aderocarcinoma; rec., recurrent; PCR, pathological complete response

Table 7. Abbreviations

Chemotherapy agents		Terminology	
BOMP	BLM+VCR+MMC+CDDP	CR	Complete response
BOP	BLM+VCR+CDDP	CCRT	Concurrent chemoradiotherapy
FJ	5-FU+CBDCA	CRT	Chemoradiotherapy
PF	CDDP+5-FU	CT	Chemotherapy
PV	CDDP+VCR	DFS	Disease-free survival
PVB	CDDP+VCR+BLM	NAC	Neoadjuvant chemotherapy
TEP	PTX+Epi+CDDP	OS	Overall survival
TIP	PTX+IFS+CDDP	PAN	Paraaortic lymph node
TJ	PTX+JM-8(CBDCA)	PALA	Paraaortic lymph node adenectomy
TP	PTX+CDDP	PFI	Progression-free interval
		PFS	Progression-free survival
BLM	Bleomycin	PLA	Pelvic lymph node adenectomy
CBDCA	Carboplatin	QOL	Quality of life
CDDP	Cisplatin	RCT	Randomized clinical trial
CDGP	Nedaplatin	RR	Response rate
Epi	Epirubicin	RT	Radiotherapy
GEM	Gemcitabine	TTP	Time to progression
HU	Hydroxyurea	95% CI	95% Confidence interval
IFS	Ifosfamide		
MMC	Mitomycin-c		
PTX	Paclitaxel		
VCR	Vincristine		
5-FU	5-Fluorouracil		

each drug varied among studies, the TP regimen produced high response rates: 48%–90% in chemotherapy-naive or untreated patients; 65%–75% in recurrent-disease patients pretreated with RT,[54,55,59] and 79%–91% in untreated patients. These results suggest that the TP regimen is a promising treatment option for cervical cancer.

Response rates to other new CT regimens reported to date were 54% with irinotecan + PTX,[63] 59% and 37% with irinotecan + CDDP,[64,65] 64% with vinorelbine + CDDP,[66] and 41% and 62.5% with gemcitabine + CDDP.[67,68]

The combination of RT and these multidrug CT regimens associated with high response rates is a subject for future investigation. Currently, several studies are being conducted to assess CCRT using the following CT regimens:

1. Weekly CDDP + weekly PTX (phase I/II studies: GOG 9803/9804)
2. Weekly CDDP + oral topotecan, daily (phase I study: GOG 9913)
3. Weekly CDDP + weekly gemcitabine (phase I study)
4. Weekly CDDP + weekly tirapazamine (phase I study)
5. TP (PTX+CDDP), every 3 weeks (phase II study, Korea)

According to an interim report from the Korean phase II study, CCRT using the TP regimen achieved a response rate of 76.1%. The final results of these studies will identify preferred regimens for CCRT.

Future issues in RT treatment

Future issues also include the establishment of the optimal RT treatment (e.g., radiation dose and the use of extended-field radiation). The current clinical practice of RT varies

between Japan and the United States. In the United States, the recommended total dose of radiation is at least 80 Gy (or 85 Gy for bulky tumors), which is higher than that used in Japan.[69] On the other hand, high-dose brachytherapy is more common in Japan than in the United States.[70] The American Brachytherapy Society recommends that concurrent use of high-dose brachytherapy and CT should be avoided, out of concern for increased adverse events. Therefore, investigation should be undertaken to determine whether CCRT with high-dose brachytherapy can be introduced into current Japanese clinical practice.

Management of anemia associated with CT

Significant development of anemia associated with CCRT suggests the influence of the RT effect as a poor prognostic factor. Great emphasis has been placed on this problem. In fact, an ongoing phase III study (GOG 0191) is evaluating the efficacy of erythropoietin in patients receiving CCRT.

Conclusion

Although the mechanism underlying the radiation-sensitizing effect of chemotherapeutic agents is not yet completely understood, the following theoretical advantages of using concurrent CT have been suggested: (1) CT inhibits the repair of RT-induced sublethal damage in tumor cells; (2) CT reduces tumor size directly by exerting its cytotoxic effect; (3) CT enhances the synchronization of tumor cells into a radiosensitive phase of the cell cycle and reduces the proportion of radioresistant hypoxic cells; and (4) CT and RT target different phases of the cell cycle and, therefore,

provide additive efficacy that increases tumor cell death without leading to delay of local therapy or prolongation of RT duration.[23]

It has been demonstrated that CCRT improves the prognosis of cervical cancer patients compared with RT alone. CCRT is currently recommended as standard therapy for advanced cancer (stage III/IVA) in the United States, and is being widely used in Japan. The guidelines published by the National Comprehensive Cancer Network (NCCN) recommend the following therapeutic approaches for cervical cancer patients:

- For patients with stage IB1 or more advanced disease, primary RT should be combined with CDDP-containing CT
- For patients undergoing surgical staging and found to have positive pelvic nodes or positive parametrium, adjuvant CCRT, consisting of pelvic RT and CDDP-containing CT should be considered, regardless of postoperative tumor stage.

However, there remains much controversy and uncertainty regarding the optimal therapeutic approaches for cervical cancer patients, especially for patients with advanced cancer. For example, analyses restricted to advanced cancer did not always find a favorable impact of CCRT on survival. Additional RCTs should be conducted to find the optimal CT regimen and RT treatment for Japanese patients, considering acute and late complications, as well as differences in pelvic anatomy, total radiation doses, and RT procedures between Japan and other countries. Evidence obtained from such studies should establish the optimal CCRT treatment protocol and define the patient population (disease stage) that really benefits from the protocol.

Abbreviations used in this article are summarized in Table 7.

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The tea polyphenol, (−)-epigallocatechin gallate effects on growth, apoptosis, and telomerase activity in cervical cell lines

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Abstract

Objective. To investigate the effect of the major tea polyphenol, (−)-epigallocatechin gallate (EGCG) in cervical carcinogenesis.

Methods. Cell growth rate was examined after treatment for 4, 7, and 10 days with 0–100 μM EGCG in primary human endocervical cells (HEN), human papillomavirus type 18 (HPV 18)-immortalized endocervical cell (HEN-18), ectocervical cell (HEC-18), serum-adapted HEN-18 (HEN-18S), transformed HEC-18 (HEN-18T), and four cervical cancer cell lines. The effect of EGCG treatment was examined on dysplastic epithelium formation in organotypic culture, induction of apoptosis by DNA ladder assay and telomerase activity by PCR telomere extension assay.

Results. EGCG inhibited growth more than 90% in HEN-18 and HEC-18, whereas growth inhibition was less in ME180, TMCC-1, HeLa, SiHa, HEC-18T, and HEN-18S. In organotypic culture, thickness of epithelial multilayers was decreased in all EGCG-treated cells. EGCG resulted in apoptosis of HEN-18 or HEC-18, but not HEN-18S nor HEC-18T and inhibited telomerase activity in HEN-18 and HEC-18, as well as HEN-18S and HEC-18T.

Conclusion. Our data suggest that EGCG prevents the carcinogenesis of cervical cancer, induces apoptosis and inhibited telomerase activity. The effect by EGCG treatment may be associated with the induction of apoptosis and telomerase inhibition in early cervical lesions.
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Keywords: Tea polyphenol; EGCG; cervical carcinogenesis

Introduction

Prevention of carcinogenesis is one of the major strategies for cancer control. Chemoprevention is the term for cancer prevention and cancer control by use of naturally occurring and/or synthetic compounds [1,2]. Cervical cancer is a major health problem worldwide [3]. Cervical cancer develops through a multistep process in which increasingly severe premalignant dysplastic lesions called cervical intraepithelial neoplasia (CIN) I, II, and III progress to invasive cancer [4]. Therefore, the patients with CIN are potential candidates for chemopreventive intervention.

In cervical cancer, some clinical, as well as basic research, studies have focused on chemoprevention with retinoids and/or interferon [5]. Previously, we reported that the treatments with retinoic acid and/or interferon-α may be effective for preventing or treating premalignant cervical lesions [5]. However, retinoids and interferon-α have several negative aspects including severe toxicity, which could result in low compliance [6]. In comparison with these agents, green tea, mainly through its major constituent epigallocatechin gallate (EGCG), has limited toxicity [7,8]. EGCG appears to be potentially an ideal agent for chemoprevention.

Green tea is one of the most common beverages consumed worldwide, and the possible beneficial health effects have received much attention. A number of epidemiological studies have shown that the consumption of green tea may protect against many cancer types [1,9–12] and may inhibit the conversion of pre-malignant lesions to malignancy [13,14]. The inhibitory effects of green tea against experimental carcinogenesis also have been demonstrated in many animal models [15–18]. Green tea contains a variety

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of polyphenols known as cathechins. (−)-Epigallocatechin gallate (EGCG) is a major component of polyphenols in green tea [19]. The antitumor effect of EGCG has been demonstrated [16–21]. However, the mechanisms responsible for these cancer-preventive effects have not been clearly demonstrated. In recent years, some studies suggest that EGCG protect against cancer by causing cell cycle arrest and inducing apoptosis [7,22–25]. It is also reported that telomerase inhibition could be one of the major mechanisms underlying the anticancer effects of EGCG [26,27]. However, little is known regarding the effects and pathways of EGCG in cervical oncogenesis. In present study, we used an in vitro cervical oncogenesis model composed of various HPV 18-immortalized ectocervical, endocervical, and cancerous cervical cells to examine the ability of EGCG to prevent cervical carcinogenesis. Furthermore, we investigated whether the mechanism involves EGCG induction of apoptosis and inhibition of telomerase activity.

Materials and methods

Cells, cell culture, and cell growth assays

Primary human endocervical cells (HEN) were prepared from uterine cervix from hysterectomy performed for benign conditions as described previously [28]. Cervical cancer model cells are summarized in Table 1. Keratinocyte serum-free medium (K-SFM, GIBCO, Grand Island, NY) was used for growth of HEN [28,29], HPV-18-immortalized HEN [28], and HPV-18-immortalized HEC [28]. Non-transformed serum-adapted HPV-18-immortalized human endocervical cells [30], HPV-18-immortalized human ectocervical cells transformed by treatment with cigarette smoke condensate [30], and other cancer cell lines were cultured in Dulbecco's modified Eagle's medium plus 10% fetal calf serum (GIBCO). The uterine cervix adenocarcinoma cell

Table 1
Origin and HPV status of cervical cells and cell lines

Cell line	Origin	HPV DNA type	Tumorigenicity
HEN	Endocervix	None	(−)
HEN-18	Endocervix	HPV-18	(−)
HEN-18-S[a]	Endocervix	HPV-18	(−)
HeLa	Cx, adenocarcinoma	HPV-18	(+)
TMCC-1	Cx, adenocarcinoma	HPV-18	(+)
HEC-18	Ectocervix	HPV-18	(−)
HEC-18-T[b]	Ectocervix	HPV-18	(+)
SiHa	Cx, squamous cell carcinoma	HPV-16	(+)
ME180	Cx, squamous cell carcinoma	HPV-68	(+)

HEN = human endocervical cells; − = negative; + = positive.
[a] Nontumorigenic, adapted to growth in serum.
[b] Tumorigenic, cigarette smoke condensate-transformed.

line, TMCC-1 was kindly obtained from M. Sakamoto (Tokyo Medical College, Tokyo, Japan). For the inhibition assay of cell proliferation, EGCG (Wako Pure Chemical Industries, Osaka, Japan) was diluted 100% ethanol and stored at − 20 C before use. Cells seeded at 2×10^5 cells per 5-cm plate were incubated with 0–100 μM EGCG in medium, which was changed every second day. Cell growth was determined by counting the number of cells with Coulter counter and expressed with the following formula:

$$\text{Growth rate}(\%) = \frac{\text{cell number(experiment)}}{\text{cell number(control)}} \times 100$$

Organotypic raft culture

Raft epithelia formed from cells were examined using histopathology. Rafts were prepared, as described previously [5,31]. Briefly, cells were seeded on a collagen matrix support. When the cells reached confluence, the gel was raised to the air–liquid interface. The rafts were incubated with 100 μM EGCG or without EGCG (control) and the reconstructed epithelia were recovered after 12 days, embedded in paraffin and stained with hematoxilin-eosin for histopathology.

DNA ladder apoptosis assay

Cells were cultured in medium with 0 μM (control) or 100 μM EGCG for 4 days. High molecular weight cellular DNA was extracted, resolved by 1.5% agarose gel electrophoresis, and stained using an Apoptosis Ladder Detection Kit (Wako Pure Chemicals Industries).

Telomerase assay

Telomerase activity was quantified with the overlap extension PCR assay method [32] using TeloChaser (Toyobo Co., Osaka, Japan). Briefly, 2×10^4 cells grown in 0 or 100 μM EGCG for 4 days were obtained and suspended in Lysis solution. Cell extracts were assayed in extension mixture. After a 30-min incubation at 30°C for telomerase extension, the telomerase products were purified by Clean-Up solution, followed by isopropyl alcohol precipitation. Recovered pellets were mixed with 30 μl of PCR mixture, heated at 95°C for 150 s, and then subjected to 30 PCR cycles of 95°C for 30 s, 68°C for 30 s, and 72°C for 45 s. PCR products were resolved by electrophoresis in a 7% polyacrylamide gel and visualized with SYBR Green I nucleic acid stain (Molecular Probes, Inc., Eugene, USA).

Assay of growth was performed in replicates of three. The mean and standard deviation of all samples were calculated and compared with untreated controls. For statistical analysis, all results were compared with two-tailed Student t test. All experiments were repeated, and the results were reproducible.

Results

Inhibition of cell growth was assayed following treatment for 4, 7, and 10 days with 0–100 μM EGCG in various cell types representing a cervical cancer model (Table 1). Cell growth inhibition assays demonstrated that HEN, normal endocervical cells, counterparts of immortalized cells, were less sensitive to EGCG treatment, whereas EGCG treatment resulted in a dose-dependent inhibition of cell growth in immortalized cell lines (90% growth inhibition in HPV 18-immortalized human ectocervical and endocervical cells by 10 μM EGCG) (Table 2). Fifty micromolars EGCG inhibited growth, but 10 μM EGCG treatment caused a lower inhibitory effect in transformed HPV 18-immortalized human ectocervical cells and non-transformed serum-adapted HPV 18-immortalized human endocervical cells. For cervical carcinoma cell lines, growth inhibition was less in ME180, TMCC-1, HeLa, and SiHa (Table 3). The inhibitory effect in cells derived from adenocarcinoma cells was less than that from squamous carcinoma cells.

The effect on EGCG treatment on epithelial dysplastic morphology was examined using organotypic raft culture. Untreated HPV 18-immortalized human ectocervical cells displayed low-grade SIL containing stratified and well-formed cornified layers (Fig. 1a). The reconstructed epithelium of the transformed HPV 18-immortalized human

Table 2
Effect of epigallocatechin gallate (EGCG) on cell growth rate of HPV-18 immortalized cell lines

Cell line	Treatment (μM)	Growth rate (% of untreated control)		
		Day 4	Day 7	Day 10
HEN	100	58.9 ± 4.9**	35.9 ± 4.2**	33.1 ± 4.8**
HEN-18	100	13.8 ± 3.1*	9.8 ± 0.7*	9.4 ± 0.6*
	50	37.3 ± 1.5*	10.5 ± 0.5*	6.8 ± 1.4*
	10	50.4 ± 3.9*	9.1 ± 4.6*	7.2 ± 1.7*
	5	58.6 ± 3.2**	39.3 ± 1.5*	79.7 ± 6.1***
HEN-18S	100	40.8 ± 0.6*	12.6 ± 0.8*	7.6 ± 0.7*
	50	95.2 ± 1.6***	66.0 ± 6.4***	68.6 ± 2.7**
	10	123 ± 13.6****	87.3 ± 1.2***	92.1 ± 4.0****
HEC-18	100	28.4 ± 2.6*	8.9 ± 0.4*	3.9 ± 2.1*
	50	35.6 ± 5.2**	18.6 ± 2.0*	8.3 ± 0.9*
	10	39.8 ± 10.9***	41.6 ± 2.5*	8.2 ± 1.3*
	5	51.6 ± 2.5*	40.8 ± 2.3*	34.4 ± 1.7*
HEC-18T	100	45.2 ± 3.6**	19.9 ± 1.4*	10.0 ± 1.1*
	50	60.9 ± 1.9*	77.5 ± 4.8***	90.9 ± 2.8***
	10	69.4 ± 3.2**	77.2 ± 1.8**	87.7 ± 2.9***

HEN = human endocervical cell; HEN-18 = human papillomavirus type18-immortalized human endocervical cell; HEN-18S = nontransformed, serum adapted HEN-18; HEC-18 = human papillomavirus type18-immortalized human ectocervical cell; HEC-18T = transformed HEC-18; The results represent the mean ± the standard deviation of percent. P is the statistical significance of difference in cell growth rate between each day treated and untreated control cells.
*$P < 0.001$.
**$P < 0.01$.
***$P < 0.05$.
****Not significant.

Table 3
Effect of epigallocatechin gallate (EGCG) on cell growth rate of cervical cancer cell lines

Cell line	Treatment (μM)	Growth rate (% of untreated control)		
		Day 4	Day 7	Day 10
SiHa	100	90.2 ± 13.2****	49.7 ± 3.2**	30.7 ± 1.0*
	50	92.3 ± 2.3***	98.9 ± 3.2****	127.9 ± 1.5***
ME180	100	60.3 ± 7.2*	17.9 ± 5.4*	16.5 ± 6.5*
	50	105.8 ± 5.3****	72.4 ± 2.7**	81.4 ± 2.1**
HeLa	100	75.3 ± 5.1***	94.2 ± 1.3***	62.6 ± 0.9*
	50	88.1 ± 3.0***	102 ± 1.0****	101.6 ± 2.0****
TMCC-1	100	70.8 ± 5.1***	90.7 ± 0.7**	106 ± 1.1***
	50	90.8 ± 4.0****	108.2 ± 1.0**	97.5 ± 2.8****

The results represent the mean ± the standard deviation of percent. P is the statistical significance of difference in cell growth rate between each day treated and untreated control cells.
*$P < 0.001$.
**$P < 0.01$.
***$P < 0.05$.
****Not significant.

ectocervical cells was composed of highly dysplastic cells (high-grade SIL), although most of the thickness was without a cornified layer (Fig. 1c). Figs. 1b and d show the response of HPV 18-immortalized human ectocervical cells and the transformed HPV 18-immortalized human ectocervical cells to 10-day exposure to EGCG, as assessed using hematoxylin and eosin staining. The thickness of the multilayer preparation was decreased in EGCG-treated culture, suggesting an antiproliferative effect. In contrast, HPV 18-immortalized human endocervical cell morphology resembled CIN III (Fig. 2a). The rafts also showed sporadic cells with vacuolated cytoplasm, suggesting glandular cell differentiation. After adaptation to serum, HPV 18-immortalized human endocervical cells showed a more apparent glandular reconstruction with severely dysplastic cells (Fig. 2c). EGCG treatment of HPV 18-immortalized human endocervical cells and serum-adapted HPV 18-immortalized human endocervical cells showed similar results to those of HPV 18-immortalized human ectocervical cells and transformed HPV 18-immortalized human ectocervical cells (Figs. 2b and d, respectively).

To evaluate factors that may be involved in the growth inhibition and dysplastic growth of the cervical cell model, we studied whether EGCG induces apoptosis in cervical carcinogenesis. Cells treated with 0 and 100 μM EGCG for 4 days showed that EGCG treatment resulted in the formation of DNA fragments in HPV 18-immortalized human endocervical and ectocervical cells (Fig. 3, Lanes 2 and 6, respectively). In comparison with these cell lines, EGCG did not induce the formation of DNA fragments in transformed HPV 18-immortalized human ectocervical cells and non-transformed serum-adapted HPV-18 immortalized human endocervical cells (Fig. 3, Lanes 4 and 8), as well as normal endocervical cells (HEN) (data not shown). These results are consistent with those of the antiproliferative effect of EGCG between each cell line (Table 2).
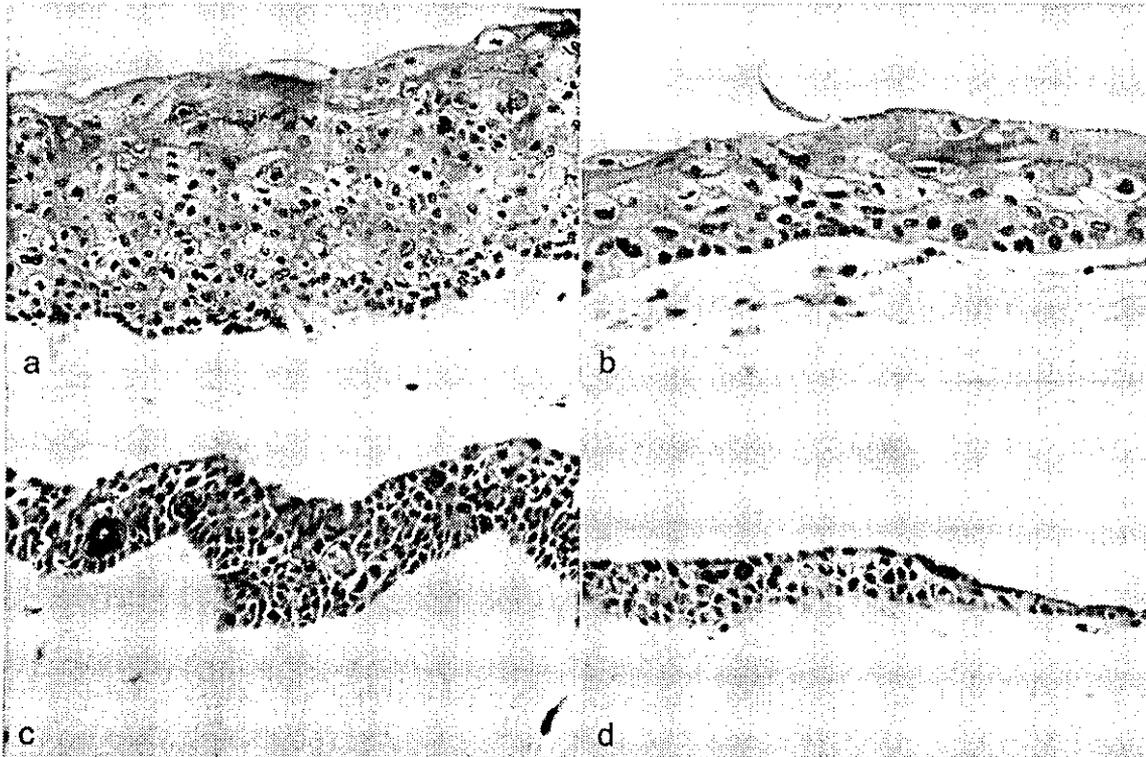
Fig. 1. Effect of epigallocatechin gallate (EGCG) on morphology of epithelium formed from HPV 18-immortalized ectocervical cells (HEC-18) and transformed HPV 18-immortalized human ectocervical cells (HEC-18T). Histology of organotypic culture (rafts) stained by hematoxylin-eosin is shown for HEC-18(a and b) and HEC-18T (c and d) that were untreated (a and c) or treated with 100 μM of EGCG (b and d). (Original magnifications ×200).
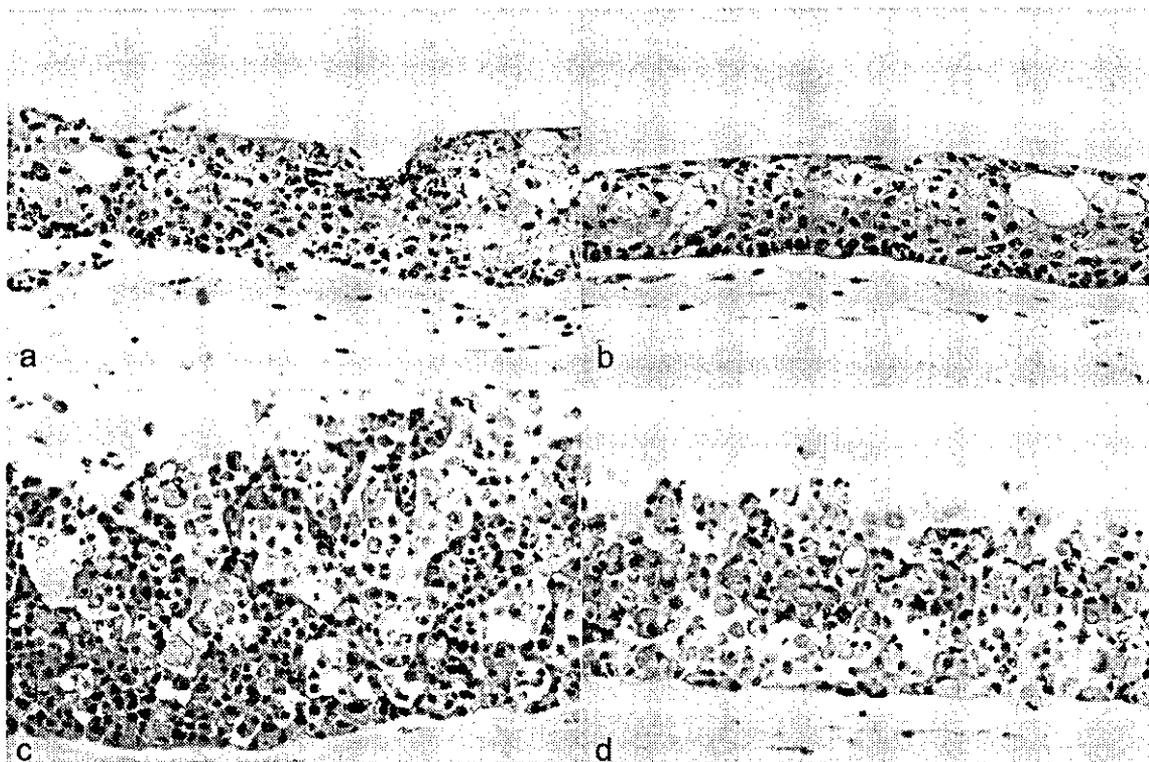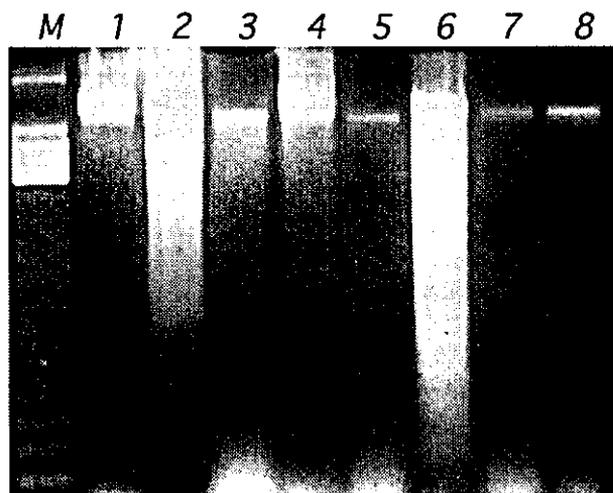

Fig. 2. Effect of epigallocatechin gallate (EGCG) on morphology of epithelium formed from HPV 18-immortalized endocervical cells (HEN-18) and serum-adapted HPV 18-immortalized human endocervical cells (HEN-18S). Histology of organotypic culture (rafts) stained by hematoxylin–eosin is shown for HEN-18(a and b) and HEN-18S (c and d) that were untreated(a and c) or treated with 100 μM of EGCG (b and d). (Original magnifications ×200).

Fig. 3. Effect of epigallocatechin gallate (EGCG) on apoptosis in monolayers of HPV 18-immortalized ectocervical cells (HEC-18), transformed HPV 18-immortalized human ectocervical cells (HEC-18T), HPV 18-immortalized endocervical cells (HEN-18), and serum adapted HPV 18-immortalized human endocervical cells (HEN-18S). Apoptosis was assayed by DNA ladder formation analysis. HEC-18 (lane 1 and 2), HEC-18T (lane 3 and 4), HEN-18 (lane 5 and 6), and HEN-18S (lane 7 and 8) were cultured in medium with or without 100 µM of EGCG. M: 123 bp ladder marker; Lane 1,3,5,7: control medium; Lane 2,4,6,8: 100 µM of EGCG. DNA by ladder formation obtained from cells after EGCG treatment confirmed apoptosis.

Telomerase has been proposed to represent a novel and potentially selective target for cancer therapy. The effect of EGCG treatment on telomerase activity was examined by the stretch PCR assay method. Telomerase product signal

was dramatically decreased in immortalized cell lines treated with EGCG (Fig. 4, Lanes 2 and 4), as well as transformed HPV 18-immortalized human ectocervical cells and non-transformed serum-adapted HPV 18-immortalized human endocervical cells (Fig. 4, Lanes 6 and 8). Telomerase activity was not detected in normal endocervical cell (HEN) (data not shown).

Discussion

Polyphenols derived from green tea, particularly EGCG, have been demonstrated to possess anticarcinogenic and chemopreventive effects both in vitro and in vivo [1,9–13,15–21]. Since most studies of the effects of EGCG have been performed in only cancer cells, it is not clear whether these pharmacological effects of EGCG are specific for cancer cells. Furthermore, little is known about the effects of EGCG in cervical cancer carcinogenesis. In the present study, we used an in vitro cervical oncogenesis model composed of normal endocervical cells, HPV 18-immortalized endocervical cells, HPV 18-immortalized ectocervical cells, non-transformed serum-adapted HPV 18-immortalized endocervical cells, transformed HPV 18-immortalized ectocervical cells [5,28], and various cervical cancer cell lines. Our study showed that EGCG possessed growth inhibitory activities against immortalized cell lines, which represent different CIN premalignant lesions in a cervical oncogenesis model. Immortalized cell lines were much more sensitive to EGCG before than after transformation and serum adaptation, which confer greater cervical
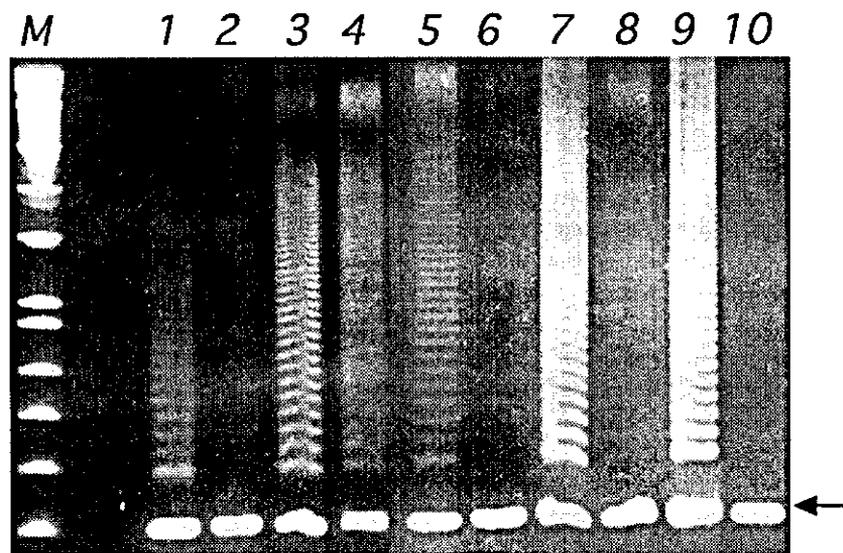

Fig. 4. Effect of epigallocatechin gallate (EGCG) on telomerase activity in HPV 18-immortalized ectocervical cells (HEC-18), transformed HPV 18-immortalized human ectocervical cells (HEC-18T), HPV 18-immortalized endocervical cells (HEN-18), and serum adapted HPV 18-immortalized human endocervical cells (HEN-18S). Semiquantitative telomerase activity was assayed by stretch PCR assay method. HEC-18(lane 1 and 2), HEC-18T (lane 3 and 4), HEN-18 (lane 5 and 6), and HEN-18S (lane 7 and 8) were cultured in medium with or without 100 µM of EGCG. M:φ×174/Hinf I marker; Lane 1,3,5,7: control medium; Lane 2,4,6,8:100 µM of EGCG; Lane 9: positive control(HeLa cell); Lane10: negative control. The arrow indicated the internal standard DNA (65 bp).

cell growth potential and higher grade CIN. We have previously reported greater retinoic acid (RA) sensitivity of HPV 18-immortalized endocervical cells than non-transformed serum-adapted HPV 18-immortalized endocervical cells [5]. Combined with the present findings, these results suggest that the sensitivity to EGCG, as well as RA in cervical cell lines, decreases with the progression of the carcinogenic process. We have also observed here that normal endocervical cells, counterpart of immortalized cells, were less sensitive to EGCG treatment, although the analysis was limited to a single analysis at 100 µM. It is reported that EGCG showed growth inhibitory effect on colon and breast cancer cells but not on their normal counterparts [21]. In cancer cell lines, cell growth rate was inhibited by EGCG, 70–85% in squamous cell carcinoma cell lines, SiHa and ME180, whereas growth inhibition was less in adenocarcinoma cell lines, TMCC-1 and HeLa (30–38%). Thus, growth inhibition by treatment with EGCG may differentially depend on cellular origin in cervical cancer. The combined results support the hypothesis that the target sites of EGCG in premalignant and cancer cells may be different from those of normal cells. The differential effects of EGCG between premalignant and normal cells and the effect in the low concentration in premalignant cells may make EGCG a good model compound for the future design of specific chemoprevention reagents.

The mechanisms of cancer inhibition by EGCG are unclear, although several hypotheses have been proposed. It is known that the regulation of apoptosis and the cell cycle could be important targets for cancer chemoprevention [33–37]. Some studies have shown that EGCG treatment results in an induction of apoptosis in several human carcinoma cells [7,22–24]. We investigated whether EGCG causes apoptosis in a cervical cancer oncogenesis model. Our results demonstrated that EGCG treatment results in the DNA ladder formation of immortalized cells, HPV 18-immortalized endocervical cells, and HPV 18-immortalized ectocervical cells. Non-transformed serum-adapted HPV-18 immortalized endocervical cells and transformed HPV-18 immortalized ectocervical cells, which are less sensitive to EGCG in growth inhibition, were not induced to undergo DNA ladder formation by EGCG. These results suggested that the induction of apoptosis by EGCG treatment is one of the important mechanisms for EGCG-mediated cancer prevention in cervical cancer oncogenesis. It is also reported that EGCG induced apoptosis in carcinoma cells but not in the normal cells [21]. Recently, EGCG was shown to increase apoptosis in skin tumor, but not non-tumor areas of the epidermis in an ultraviolet light-induced mice skin tumor model [38]. The difference between EGCG-sensitive cells and normal counterparts in response to EGCG-induced apoptosis may suggest that components involved in the apoptotic pathway could be the specific target for EGCG in precancerous and some cancer cells, but not normal cells.

The activation of telomerase has been proposed to be a critical event in the immortalization of human cells and is characteristic of most human cancer cell lines and tumors [39–42]. Previously, we reported that telomerase activity was not detected in normal human cervical cells, but HPV-immortalized human cervical cells and transformed cells showed telomerase activity [42]. These results suggest that telomerase activation is a relatively early-stage event in cervical carcinogenesis, and this activation is associated with the initiation and progression of cervical lesions [41,42]. In the present study, we demonstrated that EGCG treatment inhibits telomerase activity in immortalized cervical cell lines, as well as non-transformed serum-adapted HPV 18-immortalized endocervical cells and transformed HPV 18-immortalized ectocervical cells. Naasani et al. [26,27] reported that EGCG treatment reduced life span accompanied with shortening telomeres and inhibiting telomerase activity. These results suggested that telomerase inhibition could be one of the important mechanisms in EGCG treatment. Considering all the results, the targeting of telomerase could therefore be a promising strategy in treatment for cancer, including cervical cancer.

In studies with mice and rats in which inhibition of skin, lung, and esophageal tumorigenesis was found, the effective EGCG levels were lower than were those of in vitro models [43]. There is the disparity between the concentrations needed to achieve the various acute effects observed in vitro and the plasma levels at which significant anticancer and chemopreventive effects were observed in animal and epidemiological studies [44]. Several mechanisms of cancer inhibition by EGCG in vivo have been proposed. EGCG binds strongly to many biological molecules and affects a variety of enzyme activities and signal transduction pathways [45,46]. It was also reported that EGCG suppresses endothelial cell growth in vitro and the formation of new blood vessels in chick chorioallantoic membrane [47]. These effects were associated with vascular endothelial growth factor (VEGF). Angiogenesis is important in the growth of all solid tumors [48]. The inhibition of angiogenesis by EGCG may explain why drinking green tea prevents the growth of a variety of different tumor types at lower serum concentration than those used in vitro.

Our data suggest that EGCG may be effective for preventing or treating premalignant lesion. In vivo studies are ongoing to examine possibly applying EGCG in clinical trials testing its efficacy in interdicting cervical cancer carcinogenesis.

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Pulmonary Metastasectomy for Uterine Cervical Cancer: A Multivariate Analysis

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Background. This study evaluated the results of resection of pulmonary metastases from cervical cancer.

Methods. A total of 7,748 patients with primary stage Ib or II cervical cancer underwent curative initial treatment consisting of radical hysterectomy or radiotherapy in 22 hospitals. Of the 7,748 patients, 29 (0.37%) patients had pulmonary metastases, which were detected after a disease-free period after initial treatment (radical hysterectomy or radiotherapy) and were resected with the intention to cure by June 30, 1998.

Results. The 5-year disease-free survival rate after pulmonary metastasectomy for all patients was 32.9%. Patients with one or two pulmonary metastases had a 5-year disease-free survival rate of 42.2% compared with 0% for patients with three or four metastases ($p = 0.0003$).

Patients with squamous cell cancers had a 5-year disease-free survival rate of 47.4% compared with 0% for patients with adenosquamous cell cancers or adenocarcinoma ($p = 0.0141$). On multivariate analysis, the significant prognostic variables for disease-free survival were two or fewer metastases ($p = 0.0232$) and squamous cell cancer ($p = 0.0168$).

Conclusions. Cervical cancer patients with pulmonary metastases after initial treatment (radical hysterectomy or radiotherapy) could expect to achieve long-term disease-free survival by pulmonary metastasectomy when there are two or fewer metastases diagnosed as squamous cell cancer.

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A lthough the prevalence of invasive cervical cancer among Japanese women has been reported to be gradually decreasing, new cases are still being diagnosed in approximately 7,000 women annually, and 60% of these women have progressive disease [1]. Overall, recurrent disease will develop in 10% to 20% of patients after primary radical surgery. The prognosis of recurrent cervical cancer is dismal. Thus, increasing our understanding of recurrence and treatment remains important.

Material and Methods

The stage of the disease was classified according to the criteria of the International Federation of Gynecology and Obstetrics.

A total of 7,748 patients with primary International Federation of Gynecology and Obstetrics stage Ib or II cervical cancer underwent initial treatment consisting of radical hysterectomy or radiotherapy in 22 hospitals between January 1, 1983, and December 31, 1997. All patients received potentially curative treatment consisting of radical hysterectomy or radiotherapy. Of the 7,748 patients, 29 (0.37%) patients had pulmonary metastases,

which were confirmed by chest radiography or computed tomography after a disease-free period after initial treatment (radical hysterectomy or radiotherapy) and were resected with the intention to cure by June 30, 1998. These patients were examined to analyze the prognostic factors for survival after pulmonary metastasectomy. Their metastatic disease was limited to the lungs.

All thoracotomy specimens were processed according to standard procedures for hematoxylin and eosin–stained histologic preparation and were histologically confirmed to contain cancer consistent with cervical cancer origin. Pulmonary metastases were completely resected in all patients. There were no operative or hospital deaths. Of the 29 patients, 15 patients received cisplatin-based chemotherapy as adjuvant treatment after pulmonary metastasectomy whereas the remainder had no other therapy. The median follow-up period of all patients was 40.1 months, and the median follow-up period of living patients was 51 months (range, 1.4 to 122.3 months).

Clinical data and follow-up information were obtained from the medical records and were further complemented using telephone contacts with patients, family members, and physicians. Disease-free survival (DFS) was defined as the elapsed time from thoracotomy to disease recurrence or death. Death from disease or any recurrent disease, local or distant, was considered an event in DFS calculation. Actuarial survival curves were calculated according to the Kaplan-Meier method [2], and comparisons were made

with the log rank test [3]. For multivariate analysis, we used the Cox proportional hazards model. A *p* value of less than 0.05 was considered significant.

Results

Patients' Characteristics of Primary Cervical Cancer

The median age was 57 years, with a range of 31 to 76 years. There were 12 patients with stage Ib, 5 with stage IIa, and 12 with stage IIb disease. The histologic classification was made in accordance with the World Health Organization classification. Twenty patients had squamous cell cancers, 3 had adenosquamous cell cancers, and 6 had adenocarcinoma. All patients received potentially curative treatment of radical hysterectomy or radiotherapy. As initial treatment, 25 patients underwent radical hysterectomy and the remainder received radiotherapy. Of the 25 patients who underwent surgery, 8 patients had pelvic lymph node involvement. Four patients who had radiotherapy were not assessable.

Patients' Characteristics of Pulmonary Metastatic Lesions

The median age was 60 years, with a range of 32 to 77 years. The median disease-free interval (DFI, interval between initial treatment and onset of pulmonary metastasis) was 42 months (range, 11 to 97 months). A solitary metastatic lesion was found in 17 patients. Multiple metastases were found in the other 12 patients, two metastatic lesions in 6 patients, three metastatic lesions in 3 patients, and four metastatic lesions in 3 patients. In patients with a solitary metastasis, left lung metastasis was found in 5 patients, and right lung metastasis in 12 patients. In patients with multiple metastases, left lung metastases were found in 2 patients, right lung metastases in 3 patients, and metastases to both lungs in 7 patients. Wedge resection was performed in 8 patients (3 with a solitary lesion, 1 with two lesions, 2 with three lesions, and 2 with four lesions), segmentectomy in 2 patients (1 with a solitary lesion and 1 with four lesions), and lobectomy in 19 patients (13 with a solitary lesion, 5 with two lesions, 1 with three lesions). Median sternotomy was performed in 6 patients (3 with two lesions, 1 with three lesions, and 2 with four lesions), and lateral thoracotomy in 23 patients (17 with a solitary lesion, 3 with two lesions, 2 with three lesions, and 1 with four lesions). Sixteen patients underwent either hilar or mediastinal lymph node dissection. Of 11 patients who showed no evidence of any lymph node metastasis, 5 (45.5%) patients had postthoracotomy recurrence. However, of 5 patients who had hilar or mediastinal lymph node metastasis, 4 (80.0%) patients had postthoracotomy recurrence. Pulmonary metastatic tumor size was obtained in only 18 patients. Of 11 patients with pulmonary metastatic lesions less than 3 cm, 5 (45.5%) patients had postthoracotomy recurrence, and of 7 patients with pulmonary metastatic lesions more than 3 cm, 4 (57.1%) patients had postthoracotomy recurrence.

Table 1. Prognostic Factors: Estimation by Univariate Analysis

Factor	Number	5-year DFS Rate (%)	*p* Value
Stage			
Ib, IIa	17	34.1	0.9379
IIb	12	30.0	
Histology			
Squamous	20	47.4	0.0141
Adenosquamous + adeno	9	0	
Lymph node metastasis			
Positive	8	37.5	0.9414
Negative	17	25.3	
Age			
<60	13	15.4	0.0071
≥60	16	50.3	
DFI			
<36 months	10	30.0	0.3728
≥36 months	19	33.9	
Number of metastases			
1, 2	23	42.2	0.0003
3, 4	6	0	
Postthoracotomy chemotherapy			
Done	15	28.6	0.8146
None	14	38.1	

adeno = adenocarcinoma;   DFI = disease-free interval;   DFS = disease-free survival.

Univariate Analysis

The 5-year DFS rate after pulmonary metastasectomy for all patients was 32.9%. Table 1 summarizes the 5-year DFS rate and the results of the univariate analysis of the clinical and pathologic factors using the log rank test. Significant prognostic factors affecting DFS were histology (*p* = 0.0141), age (*p* = 0.0071), and number of metastases (*p* = 0.0003).

Multivariate Analysis

We performed multivariate analysis to identify independent factors affecting DFS. Number of metastases, age, histology, and DFI were included in the model. The results showed that the number of metastases (*p* = 0.0232) and the histology (*p* = 0.0168) were the only independent factors affecting DFS (Table 2). None of the other characteristics were significant on multivariate analysis. The actuarial 5-year DFS rates were 42.2% and 0% for patients with two or fewer metastases and those with three or four, respectively (Fig 1). Regarding histology, the actuarial 5-year DFS rates were 47.4% and 0% for patients with squamous cell cancers and adenosquamous cell cancers or adenocarcinoma, respectively (Fig 2).

Comment

Historically, patients who developed distant metastases from cervical cancer had a poor prognosis and were not considered for resection. Systemic treatment with chemo-

Table 2. Univariate and Multivariate Analysis

Factor	Univariate p Value	Multivariate		
		Hazard Rate	95% CI	p Value
Number of metastasis, 1, 2; 3, 4	0.0003	4.102	1.213-13.869	0.0232
Age, <60 y; ≧60 y	0.0071	0.382	0.126-1.163	0.0903
Histology, squamous; adsq + adeno	0.0141	3.775	1.271-11.212	0.0168
DFI, <36 months; ≧36 months	0.3728	0.662	0.232-1.891	0.4416

adeno = adenocarcinoma;   adsq = adenosquamous cell cancer;   CI = confidence interval;   DFI = disease-free interval.

therapy is the mainstay of treatment for metastatic pulmonary tumors. Various chemotherapy regimens have been used to date. Imachi and associates [4] showed a 45% response rate in patients treated with two or more courses of chemotherapy; however, the mean interval from diagnosis of pulmonary metastasis to death was 7 months (median, 3 months; range, 1 to 59 months). The chemotherapy responses increased with the more frequent inclusion of platinum, but none of these regimens has proven to be useful in significantly prolonging survival [4, 5].

The 5-year DFS rate after pulmonary metastasectomy for the cervical cancer patients in our series was 32.9%, supporting the role of pulmonary resection in selected patients with pulmonary metastases from cervical cancer. The modified indications for pulmonary metastasectomy, ie, (1) the ability to tolerate the procedure, (2) sufficient pulmonary reserve to compensate for the loss of lung capacity, (3) the site of primary must be controlled or controllable, (4) no evidence of extrapulmonary disease, and (5) no better therapy available, are almost universally accepted [6-9]. We also have conformed to these criteria. The reported incidence of pulmonary metastasis from cervical cancer ranges from 2.1% to 9.1% [10-13]. Our 0.37% rate of lung involvement is lower than rates reported previously. This difference may be because we selected patients with stage Ib or II cervical cancer in whom pulmonary metastasis was detected after the disease-free period after initial treatment and surgery was performed in accordance with the indications for surgery described above. Our 65.5% incidence of lobectomy is higher than most pulmonary metastasectomy series. This

was for anatomic reasons, because there were many patients whose pulmonary metastatic lesions were near the hilum of the lung.

Five-year survival after pulmonary metastasectomy for cervical cancer varies greatly, ranging from 0% to 60% in some reports [6, 8, 14-17], because the indications and surgical methods differed. Some authors also have reported various factors affecting the survival after thoracotomy.

In this study, we investigated the stage, histologic type, presence or absence of pelvic lymph node metastasis, age, interval between initial treatment and pulmonary metastasis (DFI), number of metastatic pulmonary foci, and presence or absence of chemotherapy after resection as prognostic factors after resection of metastatic pulmonary foci. Univariate analysis showed that significant prognostic factors included the histologic type, age, and number of metastatic foci. On multivariate analysis, significant prognostic factors included the number of metastatic foci and histologic type.

Concerning histology, squamous cell carcinoma showed a better prognosis than adenosquamous cell carcinoma and adenocarcinoma on both univariate and multivariate analysis in our series. Imachi and colleagues [4, 18] reported that in patients with adenocarcinoma, the incidence of pulmonary metastasis and positive peritoneal cytology were higher than those in patients with squamous cell carcinoma, and that these findings were related to a poor prognosis.

With respect to pelvic lymph node metastasis, in our results, the presence or absence of pelvic lymph node
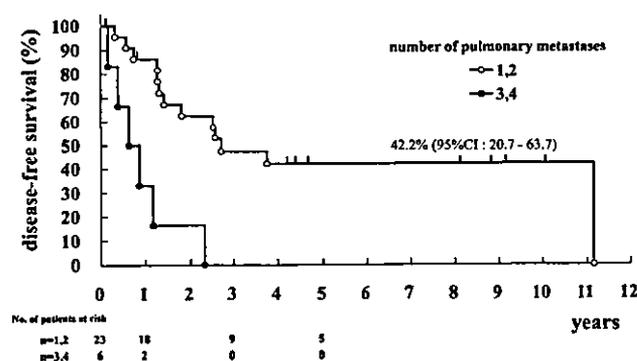

Fig 1. Disease-free survival, patients with one or two pulmonary metastases compared with those with three or four. (CI = confidence interval.)
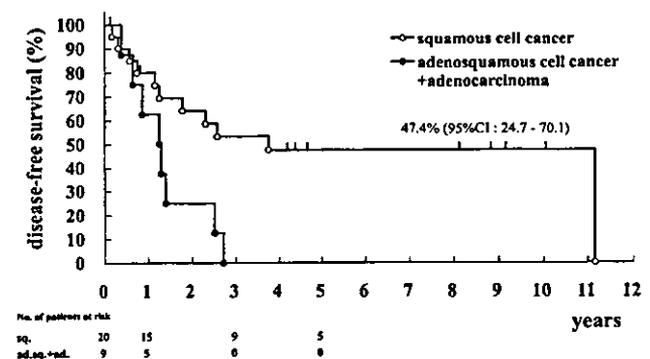

Fig 2. Disease-free survival, patients with squamous cell cancer compared with those with adenosquamous cell cancer or adenocarcinoma. (ad.sq.+ad. = adenosquamous cell cancer + adenocarcinoma; CI = confidence interval; Sq. = squamous cell cancer.)

metastasis did not influence the 5-year DFS. Shiromizu and coworkers [14] reported that patients with pulmonary metastasis alone without pelvic lymph node metastasis showed a good prognosis. However, treatment for metastatic pulmonary foci does not consist of surgery alone, and their subjects included patients with metastatic foci in organs other than the lungs. These factors may have contributed to the difference in results.

Our results showed that DFI did not significantly influence 5-year DFS. Barter and associates [5] reported that the interval between cancer diagnosis and onset of lung metastasis was not prognostic in cervical cancer. Seki and colleagues [16] reported that there were no significant differences between DFIs. However, Anderson and coworkers [10] reported that comparing DFI and survival, there were trends toward increased survival with greater DFI in patients with uterine cancer. Fuller and associates [19] reported that a prolonged time to initial recurrence (latent period) greater than 36 months was associated with improved survival and that there was a 60% survival among patients with latent periods of 60 months or more. Takita and coworkers [20] reported that for many malignancies, the interval between the initial diagnosis and the onset of lung metastasis is prognostic in surgically treated patients. However, that series involved not only cervical cancer but also other sites.

On univariate and multivariate analyses in our series, patients with one or two metastatic pulmonary foci showed a higher 5-year DFS than patients with three or four metastatic pulmonary foci. With respect to overall 5-year survival, Seki and colleagues [16] reported that there were no significant differences in the survival curves between the solitary and the multiple metastasis group.

There are no previous studies that have evaluated surgical therapy only in patients with stage Ib or II cervical cancer in whom pulmonary metastasis was detected after the disease-free period after initial treatment and resection was performed, as determined in this study. The results of this study may provide important information for future surgical therapy for pulmonary metastasis from cervical cancer.

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5029   Poster Discussion, Mon, 8:00 AM - 12:00 PM

Phase III double-blind randomized trial of radiation therapy for stage IIIB cervical cancer in combination with low or high dose Z-100, immunomodulator widely used in Japan. *K. Fujiwara*, Y. Ohashi, H. Nakayama, R. Nishimura, K. Shimizu, N. Mitsuhashi, M. Hatae, K. Ochiai, K. Hatano, K. Noda; Kawasaki Medical School, Kurashiki-City, Japan; University of Tokyo, Tokyo, Japan; Kanagawa Prefectural Cancer Center, Yokohama-City, Japan; Hyogo Medical Center for Adults, Akashi-City, Japan; Toma Hospital, Kumagaya-City, Japan; Tokyo Womens Medical University, Tokyo, Japan; Kagoshima City Hospital, Kagoshima-City, Japan; Jikei University School of Medicine, Tokyo, Japan; Chiba Cancer Center, Chiba-City, Japan; Kinki University, Osaka-sayama-City, Japan

Background: The Specific Substance of Maruyama (SSM) is a well-known immunomodulator that has been used in Japan as an unapproved drug in the treatment of over 240,000 cancer-bearing patients since 1970. Z-100 is the same agent as SSM, used in different concentrations. The aim of this study is to investigate whether Z-100 enhances the efficacy of radiation therapy (RT) for locally advanced cervical cancer of the uterus. **Methods:** Between 1995 and 1999, 221 (217 evaluable) patients with stage IIIb squamous cell carcinoma of the uterine cervix were randomly assigned to treatment with either 0.2 μg Z-100 (Group L: n=109) or 40 μg Z-100 (Group H: n=108) in a double-blind manner in combination with conventional RT. Z-100 was administered twice a week during the RT, and it was continuously administered every two weeks after RT for as long as possible $\geq$ 2 years, or until recurrence or progression was noted. The endpoints were tumor response, progression-free survival (PFS) and overall survival (OS). **Results:** There was no difference in the tumor-regression effect of Z-100 between two groups. The 5-year survival of Group H was 41.5%, (95%CI: 31.7–51.3%), which was equivalent to that of Japanese historical controls treated by RT alone (35–42%), or to the 4-year survival rate of the RT plus hydroxyurea arms of GOG120 study (50%). The 5-year survival of Group L was 58.2% (95% CI: 48.7–67.7%). Compared with Group H, Group L showed a 30% reduction in the death rate (hazard ratio: 0.670 [95% CI: 0.458 - 0.980], log-rank test, p=0.0387). PFS was also significantly improved in favor of Group L (hazard ratio: 0.667 [95% CI: 0.447 - 0.997], p=0.0482). OS and PFS of Group L were equivalent to those of concurrent cisplatin-based chemoradiation arms of the GOG study. **Conclusions:** It was suggested that RT combined with the immunomodulator Z-100 improved the survival of patients with locally advanced cervical cancer. However, more was not better, suggesting that the dose response to immunomodulators is different from the responses to conventional cytotoxic agents. A randomized placebo controlled trial is under planning.

5048   General Poster Session, Sat, 8:00 AM - 12:00 PM

Phase II trial of paclitaxel (T) and carboplatin (C) in patients with recurrent or metastatic cervical carcinoma. *R. Kitagawa*, N. Katsumata, Y. Yamanaka, M. Ando, Y. Fujiwara, T. Kasamatsu, T. Onda, T. Yamada, R. Tsunematsu, T. Watanabe; Shizuoka Cancer Center, Shizuoka, Japan; National Cancer Center Hospital, Tokyo, Japan; Sanno Medical Oncology Center, Tokyo, Japan

Background: For patients (pts) with recurrent or metastatic cervical carcinoma, T plus cisplatin (P) were reported to improve the response rate (RR) and progression-free interval compared to P alone (Moore et al; Proc ASCO 2001) and has been shown as a new appropriate regimen. However, more effective and/or less toxic combinations are needed. C as a single agent has less RR but less overall toxicity than P. In order to evaluate the efficacy and safety of T plus C, we conducted the following phase II trial. **Methods:** Pts with recurrent or metastatic cervical carcinoma no longer amenable to curable surgery or radiatiotherapy (RTx), with measurable lesions, performance status (PS) 0–2, and adequate organ functions, received TC (paclitaxel 175mg/m2 3-hour i.v., carboplatin AUC 5 1-hour i.v.) every 3 weeks. Treatment was continued until progression or completion of 6 courses administration. Tumor response was evaluated every 2 cycles, and toxicities every cycle. **Results:** 28 pts (median age 49; range 29–67) were enrolled and 23 pts were assessable for response and toxicities. 21 pts (91%) had received RTx. The overall RR was 61% (95% CI, 41–78%), especially CR showed in 2 pts (9%). The median progression-free survival was 5.9 months (range 1.0–14.1). RRs according to PS, histological type (Hx), prior RTx for target lesions, and prior chemotherapy (CTx) are given in the table below. As day 1 hematological toxicities, Grade 4 was observed only in Anemia (4 pts; 17%). Non-hematological toxicity included grade 3 neuropathy in 2 pts (8%). Grade 3 febrile neutropenia was observed in 3 pts (13%), but they were managed without hospitalization or G-CSF administration. **Conclusions:** TC is promising and feasible combination in pts with recurrent or metastatic cervical carcinoma and can be safely administered in outpatient clinic. On this basis, phase III study is planed to compare TC with T plus P.

PS		Hx		Prior RTx for target lesions		Prior CTx	
0	1/2	SCC	non-SCC	no	yes	no	yes
7/8(88%)	7/15(47%)	12/18(67%)	2/5(40%)	8/14(57%)	6/9(67%)	8/11(73%)	6/12(50%)

Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a gynecologic oncology group study.

Moore DH, Blessing JA, McQuellon RP, Thaler HT, Cella D, Benda J, Miller DS, Olt G, King S, Boggess JF, Rocereto TF.

子宮頸癌の集学的治療に用いる化学療法として、シスプラチンとパクリタキセル併用療法の高い有用性を示唆する論文

解説：喜多川　亮（久留米大学産科婦人科学教室）
　　　嘉村　敏治（久留米大学産科婦人科学教室教授）

子宮頸癌における化学療法の位置づけと臨床試験の意義

　子宮頸癌に対する治療は手術および放射線療法が中心であったが、過去20年間にわたって予後改善がみられなかった。しかし、1999年に5つの無作為化比較試験（RCT）で予後改善効果を示した放射線同時併用化学療法（cCRT）が標準治療の一角をなしてきているのに続き、術前化学療法の有用性も示されてきている。つまり、子宮頸癌集学的治療における化学療法の果たす役割が重くなりつつある。よって、高い全身制御能力とともに、主治療である手術や放射線療法実施に支障をきたさない高い安全性が求められ

ている。以上のような背景の下に、ここで紹介するような化学療法単独の臨床試験が注目されてきた。

　子宮頸癌では再発しても手術や放射線といった局所治療により病巣制御が可能であれば、長期生存や治癒も期待できる。よって、局所制御でカバーできない病巣を有する進行・再発患者が全身治療である化学療法の適応であり、根治は望めず症状緩和とQOL向上が目的のpalliative therapyとして行われる。1年生存率20％未満の予後不良群だが、延命効果を証明した無治療（best supportive care）との比較試験は存在せず、必ずしも化学療法を行うことが標準的とはされていない。そこ

で、世界的には本研究のような臨床試験のなかで化学療法が行われ、その効果が検証され、前述のごとく初回治療にも活かされてきた。

子宮頸癌化学療法の進展と本研究の概要

　本研究とも関連が深い、代表的な臨床試験結果を表1に記す。手術・放射線治療による根治が期待できない進行・再発症例のうち、子宮頸癌の大多数を占める扁平上皮癌が対象とされてきた。このほとんどは米国婦人科大規模臨床試験グループのGOG（Gynecologic Oncology Group）により行われ、本研究もその1つである。