	試験相	奏効率(%)	無増悪生存期間(月)	全生存期間(月)	文献
CDDP 50mg/m² 100mg/m² 20mg/m²×5days	3	21 ⌒ p=0.015 ⌒ ns 31 ⌒ ns ⌒ 25	3.7 ns 4.6	6.1 ns 7.1	Bonomi P, et al. J Clin Oncol 1985; 3: 1079-85.
CDDP単剤 vs IFM/CDDP(IP療法)	3	18 vs 31 p=0.004	3.2 vs 4.6 p=0.003	8.0 vs 8.3 ns	Omura GA, et al. J Clin Oncol 1997; 15: 165-71.
IP±BLM	3	32 vs 31 ns	ns	ns	Bloss JD, et al. J Clin Oncol 2002; 20: 1832-7.
PTX単剤	2	17%(9/52)			McGuire WP, et al. J Clin Oncol 1996; 14: 792-5.
TP療法(GOG)	2	46.3%(19/41)	5.4+ (0.3 ~22+)	10.0+ (0.9 ~22.2)	Rose PG, et al. J Clin Oncol 1999; 17: 2676-80.
TP療法	2	47%(16/34) 95%CI 30~65%	5	9	Papadimitriou CA, et al: J Clin Oncol 1999; 17: 761-6.
CDDP(n=134) vs TP(n=130) (GOG 169 trial)	3	19 vs 36 p=0.002	2.8 vs 4.8 p<0.001	8.8 vs 9.7 ns	本論文

表1 進行・再発子宮頸部扁平上皮癌に対する化学療法の臨床試験

CDDP用量増加は毒性増強だけで予後は改善せず、分割投与でも毒性は軽減されず、CDDPの50mg/m²の3週1回投与が標準となった。CDDPに次ぐ奏効率(22%)のIFM追加はPFSを向上させたが、白血球減少や腎毒性、上部消化管症状、神経毒性といった毒性も増強し、総合的な患者のメリットが疑問視された。BLMの追加も予後改善には結びつかなかった。そこで、PTXに期待が持たれたが、CDDP 75mg/m²と併用された2つの臨床第Ⅱ相試験では、血液毒性が強くG-CSF併用頻度が高くなった。そこで本研究では、CDDPの投与量として単剤群との比較可能性向上も含め、50mg/m²を用いることで安全性向上が図られた。
CDDP：シスプラチン、IFM：イフォスファミド、BLM：ブレオマイシン、PTX：パクリタキセル、TP：PTX/CDDP併用療法。

		奏効率(%)		無増悪生存期間(月)		全生存期間(月)	
		P群	TP群	P群	TP群	P群	TP群
cCRTの既往	(+)	5 (2/40)	32 (10/31)				
	(−)	26 (24/94)	37 (37/99)	3.0	4.9	8.9	9.9
				p<0.02		有意差なし	
病巣部位	骨盤内限局	21 (14/66)	33 (17/52)				
	遠隔転移を有する	18 (12/68)	38 (30/78)				
合計		19 (26/134)	36 (47/130)	2.8	4.8	8.8	9.7
				p<0.001		有意差なし	

表2 本研究結果のサブグループ解析

本試験のなかで、各治療群別の奏効率を、cCRTの既往の有無、病巣が骨盤内に限局するかどうかでサブグループに分け、解析された。特に、cCRTの既往がないものでは無増悪生存期間と全生存期間に関しても解析された。cCRTにCDDPを含む化学療法を用いることが多いことから、cCRTの既往がある患者ではCDDP単剤の奏効率が低い傾向が伺えた。しかし、それを差し引いても生存期間に及ぼす影響はほとんどないようであった。また、従来より放射線照射の既往を有する病巣は薬剤の血流移行の悪さなどから奏効率が低下するといわれており、骨盤内に限局する病巣のほとんどがそれにあたるはずだが、本研究結果からはその差がみられなかった。
P群：CDDP単剤治療群、TP群：PTX/CDDP併用療法群、cCRT：放射線同時併用化学療法。

シスプラチン(CDDP)は20～30%という比較的高く安定した奏効率を示し[1]、20年以上にわたりKey-drugとして用いられてきた。**表1**のように、CDDP単剤投与における投与量と投与スケジュールの検討から50mg/m²の3週1回投与が標準とされた[2]が、奏効期間は短く、全生存期間の中央値は6ヵ月ほどであり、多剤併用療法に打開策を求めた臨床試験が行われてきた。**表1**のようにIP療法(IFM＋CDDP)は有効であったが毒性が強く、ブレオマイシン(BLM)追加効果も否定され、パクリタキセル(PTX)が着目され本研究に至った。PTXは175mg/m²の3時間投与と135mg/m²の24時間投与で効果が変わらず、神経毒性は後者で軽減する[3]。CDDPも神経毒性を有し、PTXと併用するTP療法での神経毒性増強と患者のQOL低下を最小限とするため、PTX投与法には臨床第Ⅱ相試験から24時間投与が用いられている(Papadimitriouらの第Ⅱ相試験では3時間投与を用い、grade 2以上の神経障害が21%以上も認められたことが問題となった)。

本研究はCDDP単剤療法に対する

TP療法の優越性を検証する前向き臨床第Ⅲ相試験であり、ITT解析されたRCTである。この生存期間と毒性に関する結果は、すでに2001年のASCO annual meetingにおいて報告された。やはり骨髄抑制はTP群に多くみられたが、感染症を含め生命に危険を及ぼす毒性はほとんどなく、嘔気・嘔吐は両群でほぼ差がなかった。軽度の神経毒性はTP群に若干多かったものの、重篤なものはみられなかった。表1のように全生存期間（OS）こそ有意差を認めなかったが、CDDP単剤群を上回る奏効率（RR）・無増悪生存期間（PFS）における有用性に加え、同じ併用療法でもIP療法と異なる毒性の低さから非常にインパクトが強く、子宮頸癌の新たな標準化学療法として認識されるに至っている。さらに本文献ではQOLデータの解析がなされたうえで報告されたが、完全集積率が高いとはいえず、後述の疑問点が残る。

本試験結果の特徴と注目すべき点

子宮頸癌の治療体系にcCRTが加わり、前治療歴を有する症例が多くなっていることから、本研究では表2のようなサブグループ解析も行われたが、生存期間の結果はcCRTの既往がなくても変わらなかった。また、放射線治療の既往を有することが多い骨盤内病巣は化学療法の感受性が悪いというのが通説であるが、本研究結果からは両治療群ともに差がみられなかった。しかし、これらの結果に関する考察は本文中にみられない。

また、化学療法により本試験対象の生存期間が多少延長しても予後が厳しいことに変わりはないため、治療によりQOLが損なわれてはならない。本研究では患者の主観による毒性と効果のバランスをQOLスコアにより包括的に評価しようとした点が、今までの試験にはみられず意義深い。QOL調査票は患者自身により、治療前と治療開始3・6・9週後の計4回集積された。しかし、そのすべてに答えた患者（完遂者）は60%にすぎず、完遂者のQOLスコアは両群で差がなかった。この完遂者は治療が奏効し治療継続できている患者に多かったことから、TP療法の無増悪生存期間延長はQOLを著しく損ねて得られたものではない、という結論であった。しかし、QOL評価において欠損データの意味するものは大きく、その解釈にバイアスが入ることは避けられず、解析を困難にする。今後も本研究対象のような予後不良患者からQOLデータを集積することには疑問点が残る。

今後の世界の方向性と日本での動き

最近では、トポテカン・ビノレルビン・ゲムシタビンといった新規抗癌剤が注目され、それらとCDDPの併用療法による有効性も高く評価された。よって、現在GOGはそれら3つのCDDP併用療法と、標準治療としてのTP療法を比較する、計4群間でのRCTを行っている（GOG 0204 trial）。また、それと同時に分子標的療法薬の有効性も評価されつつある。一方、日本では毒性が全般に軽いカルボプラチンに着目し、それとパクリタキセルとの併用療法（TJ療法）による臨床第Ⅱ相試験を行って58.6%（95%CI＝40.7～74.5%）の奏効率に加え、無増悪生存期間の中央値が5.9ヵ月以上、と良好な有効性を証明した[4]。この成績を基に、TP療法とTJ療法を比較するRCTをJCOG（Japan Clinical Oncology Group）において2005年春より開始する計画を進めている。この試験は、子宮頸癌に対しシスプラチンとカルボプラチンを比較する世界初のRCTとなり、大きな意義を有すると期待される。

文献

1) Thigpen JT, Vance R, Puneky L, Khansur T. Chemotherapy as a palliative treatment in carcinoma of the uterine cervix. Semin Oncol 1995; 22 (2 Suppl 3): 16-24.
2) Bonomi P, Blessing JA, Stehman FB, DiSaia PJ, Walton L, Major FJ. Randomized trial of three cisplatin dose schedules in squamous-cell carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol 1985; 3: 1079-85.
3) Eisenhauer EA, ten Bokkel Huinink WW, Swenerton KD, Gianni L, Myles J, Van der Burg ME, et al. European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: High-dose versus low-dose and long versus short infusion. J Clin Oncol 1994; 12: 2654-66.
4) Kitagawa R, Katsumata N, Watanabe T, et al. Phase Ⅱ trial of paclitaxel and carboplatin in patients with recurrent or metastatic cervical carcinoma. Proc Am Soc Clin Oncol 2004; 23: 5048.

Identification of human papillomavirus 16-E6 protein-derived peptides with the potential to generate cytotoxic T-lymphocytes toward human leukocyte antigen-A24$^+$ cervical cancer

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Received May 31, 2005; Accepted July 4, 2005

Abstract. Human papillomavirus 16 (HPV16)-E6 and -E7 proteins are considered to be appropriate targets in specific immunotherapy for cervical cancer. In this study, we attempted to identify epitope peptides from the HPV16-E6 protein that have the potential to generate cytotoxic T-lymphocytes (CTLs) toward human leukocyte antigen (HLA)-A24$^+$ cervical cancer. Two HPV16-E6 peptides at positions 75-83 and 91-100 effectively induced peptide-specific CTLs from peripheral blood mononuclear cells of HLA-A24$^+$ cervical cancer patients. These HPV16-E6 peptide-induced CTLs showed cytotoxicity against HLA-A24$^+$ and HPV16-E6 protein-expressing cervical cancer cells. Experiments with blocking antibodies and cold inhibition targets revealed that the cytotoxicity was mainly dependent on peptide-specific and CD8$^+$ T cells. In addition, based on our observation that induction of immunoglobulin G (IgG) reactive to administered CTL-directed peptides is correlated with clinical responses, we attempted to detect IgG reactive to HPV16-E6 peptides in the plasma of cancer patients. As a result, IgGs reactive to the HPV16-E6$_{91\text{-}100}$ peptide were detected in 4 of 12 cervical cancer patients. These results indicate that these HPV16-E6-derived peptides are good candidates in peptide-based immunotherapy for HLA-A24$^+$ cervical cancer patients.

Introduction

The vast majority of cervical carcinomas are associated with infection by malignant human papillomavirus (HPV) serotypes, particularly HPV16 and HPV18 (1,2). Because the HPV *E6* and *E7* genes are selectively retained and expressed in cervical carcinomas (3-5), they are attractive targets for specific immunotherapy. Indeed, HPV16-specific cytotoxic T-lymphocytes (CTLs) have been detected in both peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating lymphocytes after antigen-specific *in vitro* restimulation (6,7). In addition, several HPV16-E7-derived epitope peptides with the potential to generate cervical cancer-reactive CTLs in human leukocyte antigen (HLA)-A2$^+$ patients have been identified (8-10) and applied in specific immunotherapy to cervical cancer patients (11-14). However, there is no information regarding HPV16-E6- or -E7-derived peptides applicable for HLA-A24$^+$ cervical cancer patients.

We identified a panel of antigenic peptides having the potential to induce peptide-specific and tumor-reactive CTLs in patients (15). We utilized some of them as peptide vaccinations for human leukocyte antigen (HLA)-A24$^+$ or HLA-A2$^+$ cervical cancer patients and found that the clinical response was unsatisfactory, although a major tumor regression was observed in several cases (16). In this study, to develop a clinically effective peptide vaccination for cervical cancer patients, we have attempted to identify HPV16-E6-derived peptides that have the potential to generate cervical cancer-reactive CTLs in HLA-A24$^+$ cervical cancer patients. As a consequence, we identified two new CTL-directed HPV16-E6-derived peptides that are applicable to specific immunotherapy for HLA-A24$^+$ cervical cancer patients.

Materials and methods

Patients. Informed consent was obtained from all of the HLA-A24$^+$ cervical cancer patients and healthy volunteers who were enrolled in this study. None of the participants was infected with HIV. Twenty milliliters of peripheral blood was obtained and the peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll-Conray density gradient centrifugation. The expression of HLA-A24 molecules on the PBMCs of the cancer patients and healthy donors was determined by flow cytometry.

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Key words: cervical cancer, HPV16, cytotoxic T-lymphocytes, peptide, HLA-A24

Table I. Reactivity of HPV-E6 peptide-stimulated PBMCs from HLA-A24$^+$ cervical cancer patients and healthy donors.

Patients and donors	Name Sequence Score[a]	HPV-E6$_{42-50}$ VYDFAFQDL 288	HPV-E6$_{75-83}$ EYRHYCYSL 200	HPV-E6$_{91-99}$ QYNKPLCDL 300	HPV-E6$_{91-100}$ QYNKPLCDLL 360	EBV TYGPVFMCL	Flu RFYIQMCYEL
Patient				IFN-γ production (pg/ml)[b]			
1		0	827/101/85	0	165	0	566
2		0	564/1175	0	311	0	107
3		0	150/318	0	273/173	98	0
4		0	0	0	0	123	0
5		0	0	0	0	0	97
6		0	1373/96/62	2417	0	174/166	171
7		0	77/453	0	428	0	0
8		0	0	610	0	0	0
9		0	0	0	0	0	0
10		0	0	0	0	0	0
11		83	41	66	48	0	548
12		0	282	0	0	504	73
13		0	0	0	233	0	176
Total		1/13	6/13	3/13	5/13	4/13	7/13
Healthy donor							
1		144	54/28	0	0	292/274/1157	193/170
2		198/163	494/177	150	0	92	102/62
3		0	0	0	0	103	0
4		0	0	0	0	220	0
5		0	0	0	0	0	0
6		0	488	0	0	304	353/147
Total		2/6	2/6	1/6	0/6	5/6	3/6

[a]The peptide-binding score was calculated based on the predicted half-time of disscoation. [b]The PBMCs of HLA-A24$^+$ cervical cancer patients and healthy donors were stimulated *in vitro* with the indicated peptide, as described in Materials and methods. On day 15, the cultured PBMCs were tested for their reactivity to C1R-A24 cells, which were pre-pulsed with a corresponding peptide or an HIV peptide. The values represent the mean of 2-wells, and the backgroud IFN-γ production in response to the HIV peptide was subtracted. Significant values ($p<0.05$ by Student's t-test) are underlined.

Cell lines. OMC-1, SKG-I and SKG-IIIb are cervical squamous cell carcinomas. OMC-4 and SKG-IIIa are cervical adenocarcinomas. OMC-3 is ovarian carcinoma; all of them were maintained in F-12 Nutrient Mixture medium (Gibco BRL, Grand Island, NY) supplemented with 10% FCS. KE-4 is an esophageal cancer cell line. C1R-A24 is an HLA-A*2402-expressing subline of C1R lymphoma (Dr M. Takiguchi, Kumamoto University, Japan). These three cell lines were maintained in RPMI-1640 medium (Gibco BRL) supplemented with 10% FCS.

Peptides. Four HPV16-E6-derived peptides (listed in Table I) were prepared based on the HLA-A24 binding motif (17). All peptides were of >90% purity and were purchased from Biologica Co., Nagoya, Japan. Influenza (Flu) virus-derived (RFYIQMCYEL), EBV-derived (TYGPVFMCL) and HIV-derived (RYLRQQLLGI) peptides with the HLA-A24 binding motif were used as controls. All peptides were dissolved with dimethyl sulforoxide at a dose of 10 mg/ml.

Assay for peptide-specific CTLs in PBMCs. The assay for the detection of peptide-specific CTLs in PBMCs was performed according to a previously reported method (18). In brief, PBMCs ($1x10^5$ cells/well) were incubated with 10 μg/ml of each peptide in a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) in a volume of 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640, 45% AIM-V medium (Gibco BRL), 10% FCS, 100 U/ml of interleukin (IL)-2 and 0.1 mM MEM non-essential amino acid solution (Gibco BRL). Half of the culture medium was removed and replaced with new medium containing a corresponding peptide (20 μg/ml) every 3 days. On the 15th day of culture, the cultured cells in 1 well were separated into 4 wells; 2 wells were used for the HPV16-E6 peptide-pulsed

C1R-A24 cells and the other 2 wells were used for the HIV peptide-pulsed C1R-A24 cells. After an 18-h incubation period, the levels of interferon (IFN)-γ in the supernatants were determined by ELISA.

RT-PCR. Total RNA was isolated from cells using RNAzol B (Tel-Test, Friendswood, TX). The cDNA was prepared using the SuperScript Preamplification System for First Strand cDNA Synthesis (Invitrogen, CA) and it was amplified using the primer pairs 5'-GTGTGTACTGCAAGCAACAG-3' (forward) and 5'-GCAATGTAGGTGTATCTCCA-3' (reverse) for HPV16, and 5'-ACAACAGCCTCAAGATCATCAG-3' (forward) and 5'-GGTCCACCACTGACACGTTG-3' (reverse) for GAPDH.

The PCR was performed using TaqDNA polymerase in a DNA thermal cycler (iCycler, Bio-Rad Laboratories, Hercules, CA) for 30 cycles (45 sec at 54°C and 60°C for HPV16 and GAPDH, respectively).

Immunoblotting. The expression of HPV16-E6 protein in cell lines was detected by Western blot analysis. In brief, the samples were lysed with a buffer consisting of 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 0.2 mM PMSF (Sigma, St. Louis, MO) and 0.03 trypsin-inhibitory units/ml aprotinin (Sigma), and sonicated and centrifuged at 14,000 rpm for 20 min, and the supernatant was used as the cytoplasm fraction. The supernatant was separated by polyacrylamide gel (PAG Mini 15/25: Daiichi Pure Chemicals Co., Ltd. Tokyo, Japan). The proteins in the polyacrylamide gel were blotted onto a Hybond-polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ) and were incubated with the appropriate antibodies for 3 h at room temperature. For the blotting of HPV16-E6 protein, anti-HPV16-E6 mouse monoclonal antibody (mAb) (X300) (Chemicon International, Temecula, CA) was followed by horseradish peroxidase-linked anti-mouse immunoglobulin (Ig) (NA931V; Amersham Biosciences). The development was performed using Western Lightning Chemiluminescence Reagent (Perkin-Elmer Life Sciences, Boston, MA).

Flow cytometry. The expression of HLA-A24 molecules on tumor cells was examined by flow cytometry. The tumor cells were stained with anti-HLA-A24 mAb (0041HA; One Lambda Inc., Canoga Park, CA), followed by FITC-conjugated anti-mouse IgG.

Cytotoxicity assay. After *in vitro* stimulation with HPV16-E6 peptides, the peptide-stimulated PBMCs were additionally cultured with 100 U/ml IL-2 for approximately 10 days for use in a cytotoxicity assay. These cells were then tested for cytotoxicity against SKG-IIIa, OMC-1 and phytohemagglutinin (PHA) blasts by a 6-h ^{51}Cr-release assay. Two-thousand ^{51}Cr-labeled cells per well were cultured with effector cells in 96-round-well plates at the indicated effector/target ratios. In some experiments, either anti-HLA class I (W6/32: mouse IgG2a), anti-HLA class II (HLA-DR) (L243: mouse IgG2a), anti-CD4 (NU-Th/i: mouse IgG1), anti-CD8 (NU-Ts/c: mouse IgG2a), or anti-CD14 (H14: mouse IgG2a) mAb was added to the wells at a dose of 10 μg/ml at the initiation of the assay.

Cold inhibition assay. The specificity of the HPV16-E6 peptide-stimulated PBMCs was confirmed by a cold inhibition assay. In brief, ^{51}Cr-labeled target cells ($2x10^3$ cells/well) were cultured with the peptide-stimulated PBMCs ($4x10^4$ cells/well) in 96-round-well plates with $2x10^4$ cold target cells. C1R-A24 cells that had been pre-pulsed with either the HIV peptide or a corresponding HPV16-E6 peptide, were used as cold targets.

Measurement of anti-peptide antibody. The levels of anti-peptide IgG were measured using the Luminex™ system as previously reported (19). In brief, plasma was incubated with 25 μl of the peptide-coupled color-coded beads for 2 h at room temperature on a plate shaker. After incubation, the mixture was washed using vacuum manifold apparatus and incubated with 100 μl of biotinylated goat anti-human IgG (γ chain-specific) for 1 h at room temperature. The plate was then washed, followed by addition of 100 μl of streptavidin-PE to each of the wells and incubation for 30 min at room temperature on a plate shaker. The bound beads were washed three times followed by addition of 100 μl of Tween-PBS into each well. Sample (50 μl) was detected using the Luminex system. To confirm the specificity of IgGs to the HPV16-E6$_{91-100}$ peptide, sample plasma was cultured in plates coated with or without the HPV16-E6$_{91-100}$ peptide or the HPV16-E6$_{75-83}$ peptide. Thereafter, the levels of HPV16-E6$_{91-100}$ peptide-specific IgG in the resultant supernatant were determined using the Luminex system.

Statistics. The statistical significance of the data was determined using a two-tailed Student's t-test. A p-value of <0.05 was considered to be statistically significant.

Results

HPV16-E6 peptides capable of inducing peptide-specific CTLs from HLA-A24$^+$ cervical cancer patients. We first searched peptide candidates derived from the HPV16-E6 or -E7 protein based on the binding affinity to HLA-A24 molecules, and four HPV16-E6-derived peptides with a moderate level were prepared (Table I). No appropriate candidate was found in peptides derived from the HPV16-E7 protein. Next, to examine the immunogenicity of four HPV16-E6 peptides, the PBMCs of 13 HLA-A24$^+$ cervical cancer patients and 6 female healthy donors were stimulated *in vitro* with each of the four HPV16-E6 peptides and were then examined for their IFN-γ production in response to C1R-A24 cells, which were pre-pulsed with a corresponding HPV16-E6 peptide. Flu- and EBV-derived peptides were used as controls. The assay was carried out in quadruplicate and the cultured cells in one of the 4 wells were independently separated into 4 wells; 2 wells were used for the HPV16-E6 peptide-pulsed C1R-A24 cells and the other 2 wells for the HIV peptide-pulsed C1R-A24 cells. The background IFN-γ production in response to the HIV peptide was subtracted and the successful induction of peptide-specific CTLs was judged to be positive when significant values ($p<0.05$ by two-tailed Student's t-test) were observed. The results showed that the HPV16-E6$_{42-50}$, HPV16-E6$_{75-83}$, HPV16-E6$_{91-99}$ and HPV16-E6$_{91-100}$ peptides induced peptide-specific CTLs in 1, 6, 3 and

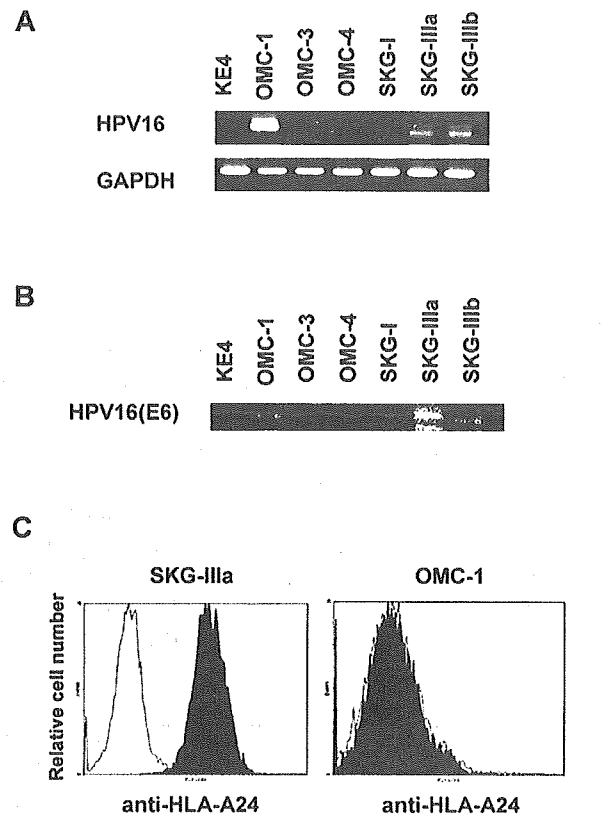

Figure 1. Characteristics of cervical cancer cells. (A) The expression of HPV16 mRNA in seven tumor cell lines was examined by RT-PCR. GAPDH was used as a control. (B) The expression of HPV16-E6 protein in seven tumor cell lines was examined by Western blotting. (C) Flow cytometric analysis was performed on the SKG-IIIa and OMC-1 cells. These cells were stained with anti-HLA-A24 mAb, followed by FITC-conjugated anti-mouse IgG mAb. The open background indicates staining without a second antibody.

5 of 13 HLA-A24$^+$ cervical cancer patients and in 2, 2, 1 and 0 of 6 HLA-A24$^+$ healthy donors, respectively. The efficacy of the HPV16-E6$_{75\text{-}83}$ or HPV16-E6$_{91\text{-}100}$ peptide to induce peptide-specific CTLs was relatively high in cancer patients, although that of the HPV16-E6$_{75\text{-}83}$ peptide was also observed in healthy donors. These findings indicate that both the HPV16-E6$_{75\text{-}83}$ and HPV16-E6$_{91\text{-}100}$ peptides are promising peptide candidates for generation of peptide-specific CTLs in HLA-A24$^+$ cervical cancer patients. Therefore, these two peptides were further studied in this study.

Characteristics of cervical cancer cell lines. Before investigating the cervical cancer-reactive cytotoxicity of peptide-stimulated PBMCs, characteristics of the cervical cancer cell lines were investigated. KE4 was used as a negative control. RT-PCR revealed that OMC-1, SKG-IIIa and SKG-IIIb were clearly positive for HPV16 mRNA (Fig. 1A). The expression of the HPV16-E6 protein was detected in these 3 cell lines, too (Fig. 1B). Cervical cancer has been reported to frequently lose HLA class I expression, as a result of a loss or decrease in the TAP expression (20-22). Therefore, we also investigated the expression of these molecules in cervical cancer cell lines. SKG-IIIa and OMC-1 were genotypically positive and negative for the *HLA-A*2402* gene, respectively (data not shown). The results showed that SKG-IIIa cells clearly expressed HLA-A24 molecules on their surfaces, while OMC-1 cells were negative for these molecules (Fig. 1C). In addition, the expression levels of TAP1 and TAP2 proteins in SKG-IIIa were comparable to that in KE-4, which is an HLA-class I-expression esophageal cancer cell line (data not shown). Based on these findings, we employed the following three target cells in the cytotoxicity assay: HLA-A24$^+$

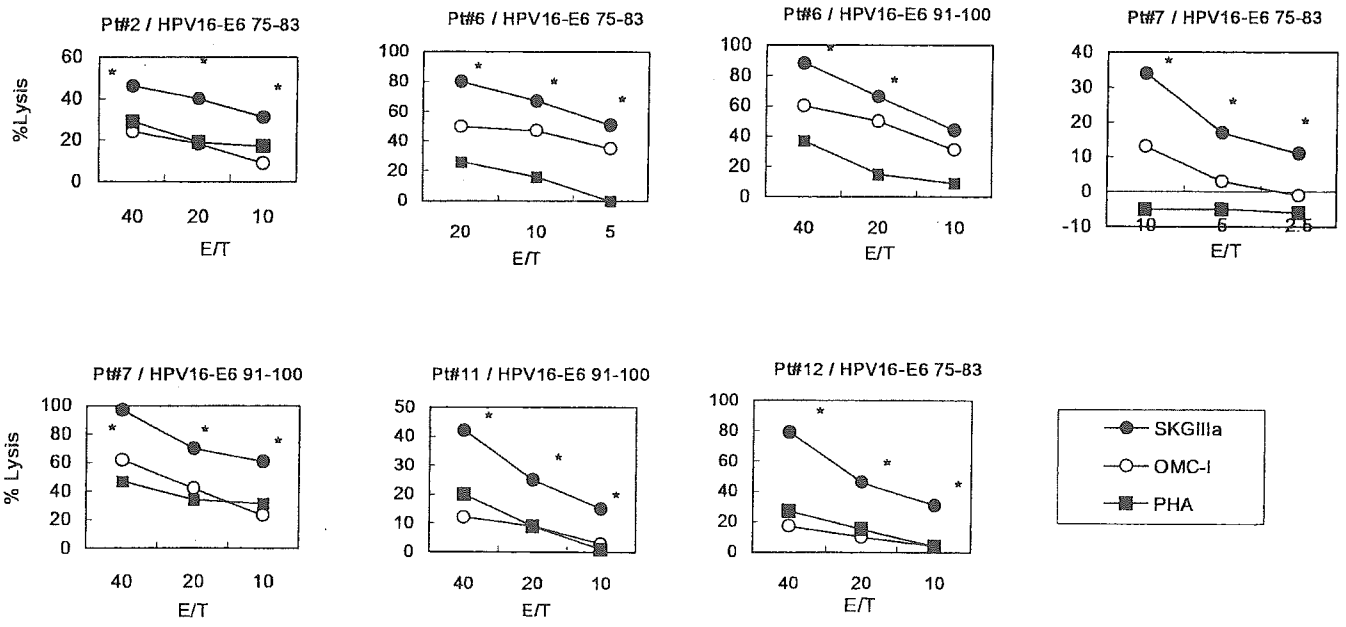

Figure 2. Induction of cancer-reactive CTLs from the PBMCs of HLA-A24$^+$ cervical cancer patients. PBMCs from 5 HLA-A24$^+$ cervical cancer patients were stimulated *in vitro* with the indicated HPV16-E6 peptides, as described in Materials and methods. Thereafter, these cells were examined for their cytotoxicity against three targets: HLA-A24$^+$ HPV16-E6$^+$ SKG-IIIa, HLA-A24$^-$ HPV16-E6$^+$ OMC-1 and HLA-A24$^+$ HPV16-E6$^-$ PHA blasts. A 6-h ^{51}Cr-release assay was performed. Values represent the mean of triplicate assays. *Statistically significant at $p<0.05$.

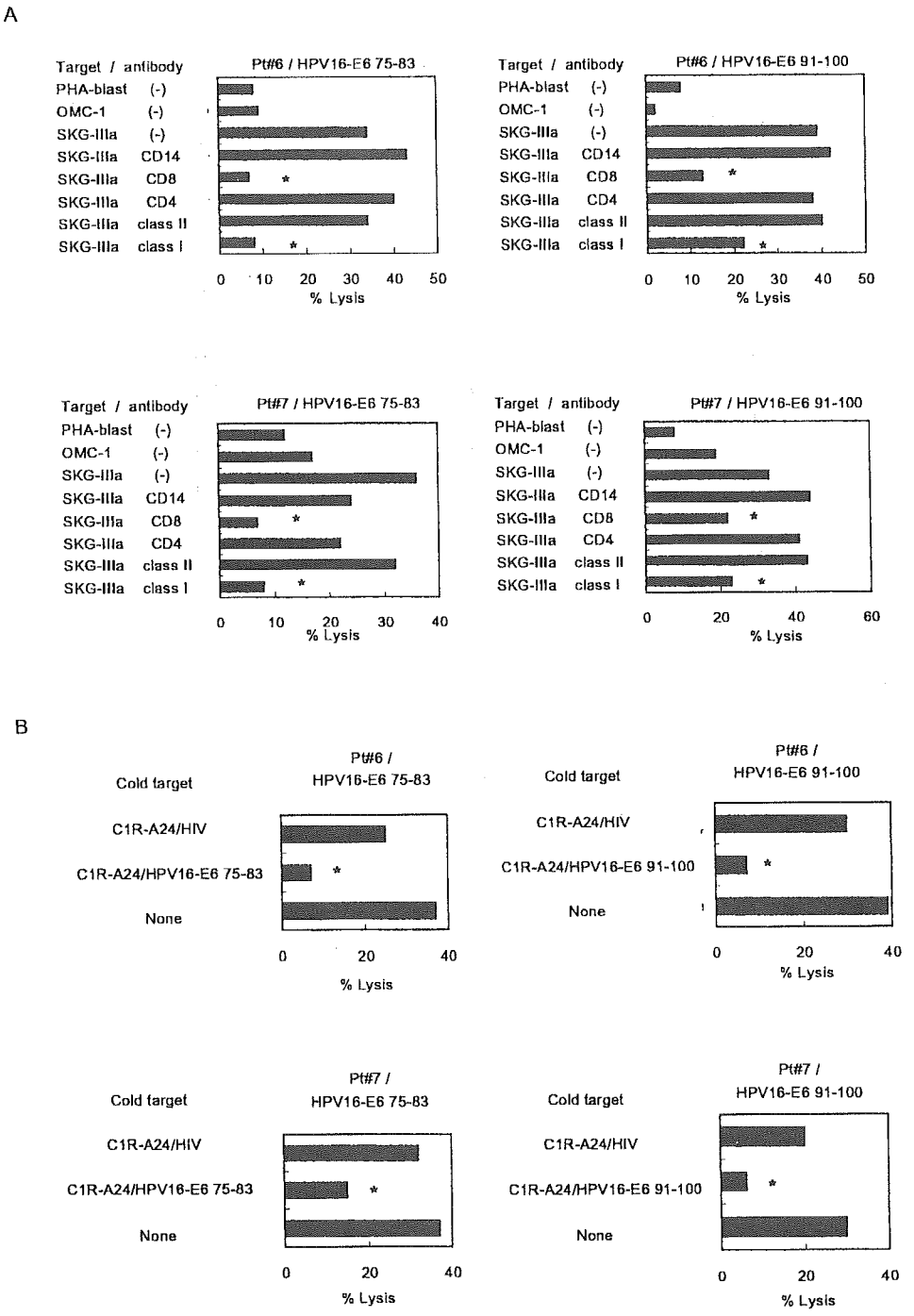

Figure 3. $CD8^+$ T cell-dependent and peptide-specific cytotoxicity of CTLs against SKG-IIIa cells. (A) The HPV16-E6 peptide-stimulated PBMCs were examined for their cytotoxicity against SKG-IIIa, with or without anti-HLA class I, anti-HLA class II, anti-CD4, anti-CD8 or anti-CD14 mAb at a dose of 10 μg/ml. The values represent the mean of triplicate assays. *Statistically significant at $p<0.05$. (B) The cytotoxicity against SKG-IIIa cells ($2x10^3$ cells/well) was also examined in the presence of unlabeled C1R-A24 cells ($2x10^4$ cells/well), which were pre-pulsed with the HIV peptide or a corresponding PHV16-E6 peptide. The values represent the mean of triplicate assays. *Statistically significant at $p<0.05$.

HPV16-$E6^+$ SKG-IIIa, HLA-$A24^-$ HPV16-$E6^+$ OMC-1 and HLA-$A24^+$ HPV16-$E6^-$ PHA blasts.

Induction of cervical cancer-reactive CTLs from HLA-$A24^+$ cervical cancer patients. We next investigated whether or not PBMCs stimulated by the HPV16-$E6_{75-83}$ or HPV16-$E6_{91-100}$ peptide showed cytotoxicity toward SKG-IIIa. PBMCs from 5 HLA-$A24^+$ cervical cancer patients (patients 2, 6, 7, 11 and 12) were repeatedly stimulated with either the HPV16-$E6_{75-83}$ or HPV-$E6_{91-100}$ peptide, based on the culture protocol described in Materials and methods. After confirming their specificity to a corresponding HPV16-E6-derived peptide by ELISA, the peptide-stimulated PBMCs were examined for their cytotoxicity against SKG-IIIa, OMC-1 and HLA-$A24^+$ PHA blasts. Only the positive results are shown in Fig. 2. As can be seen, the HPV16-E6 peptide-stimulated PBMCs showed higher levels of cytotoxicity against the SKG-IIIa than against the OMC-1 and HLA-$A24^+$ PHA blasts.

Table II. IgG reactive to HPV16-E6-derived peptides in the plasma of cervical cancer patients and healthy female donors.

Patients and donors	Peptides			
	HPV16-E6$_{42-50}$	HPV16-E6$_{75-83}$	HPV16-E6$_{91-99}$	HPV16-E6$_{91-100}$
Patients				
3	886	0	0	0
9	11	0	123	337
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	255	0	2724	3808
18	0	0	0	1670
19	0	0	0	0
20	0	0	0	10
21	0	0	0	0
22	972	0	1891	4222
23	0	0	5	59
Total	3/12	0/12	3/12	4/12
Healthy donors				
3	20	0	140	408
4	269	82	336	1009
5	0	0	601	5718
6	0	0	37	771
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	287
13	0	0	0	0
14	0	0	0	0
15	0	0	0	429
16	0	0	259	1372
Total	1/14	0/14	4/14	7/14

The levels of peptide-specific IgG were measured by the Luminex system, as described in the Materials and methods. The number indicates the fluorescence intensity and significant values (>100) are underlined.

The cytotoxicity of peptide-stimulated PBMCs from two patients (patients 6 and 7) was further investigated. Their cytotoxicity against SKG-IIIa cells was significantly inhibited by the addition of anti-HLA-class I and anti-CD8 mAbs, but not by the addition of other anti-HLA-class II, anti-CD4 or anti-CD14 mAbs (Fig. 3A). Furthermore, their cytotoxicity against SKG-IIIa cells was significantly suppressed by the addition of corresponding HPV16-E6 peptide-pulsed C1R-A24 cells as a cold target, but this suppression was not observed with the addition of HIV peptide-pulsed C1R-A24 cells (Fig. 3B). These results indicate that both the HPV16-E6$_{75-83}$ and HPV16-E6$_{91-100}$ peptides have the potential to generate cervical cancer-reactive CTLs from HLA-A24$^+$ cervical cancer patients and that their cytotoxicity against cervical cancer was dependent on HPV16-E6 peptide-specific CD8$^+$ T cells.

Detection of IgGs reactive to the HPV16-E6 peptides. We previously reported that IgGs reactive to CTL-directed peptides were detected in patients with several types of cancer (16,23-25). In the present study, therefore, we attempted to determine whether or not IgGs reactive to four HPV16-E6-derived peptides could be detected in the plasma of cervical cancer patients and healthy donors. The results showed that an IgG reactive to the HPV16-E6$_{91-100}$ peptide was present in 4 of 12 cancer patients and in 7 of 14 healthy donors (Table II). In contrast, no IgG reactive to the HPV16-E6$_{75-83}$ peptide was detected in any of the subjects. IgGs reactive to either the

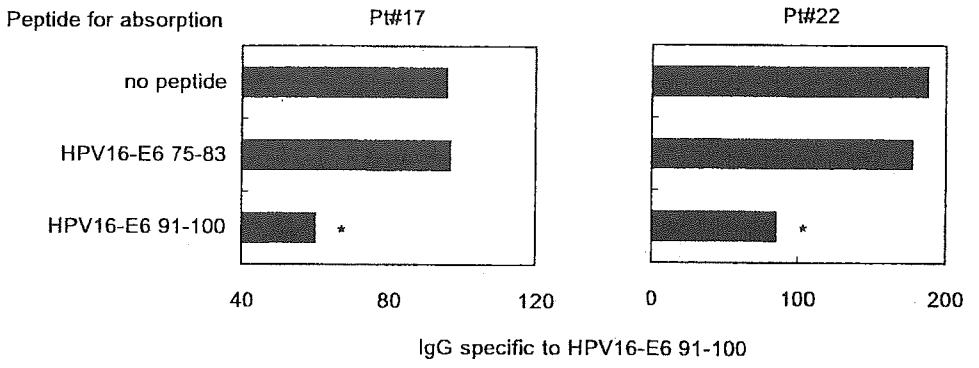

Figure 4. Specificity of IgG to the HPV16-$E6_{91-100}$ peptide. To confirm the specificity of IgG to the HPV16-$E6_{91-100}$ peptide, 100 μl of sample plasma from either patient 17 or patient 22 was cultured in a plate pre-coated with or without the HPV16-$E6_{75-83}$ or HPV16-$E6_{91-100}$ peptide. Thereafter, the levels of IgG reactive to the HPV16-$E6_{91-100}$ peptide in the resultant samples were determined using the Luminex system.

HPV16-$E6_{42-50}$ or HPV16-$E6_{91-99}$ peptide were detected in 3 of 12 cancer patients. As shown in Fig. 4, the levels of the IgG reactive to the HPV16-$E6_{91-100}$ peptide were significantly diminished by culturing the plasma in the corresponding HPV16-E6 peptide-coated wells, indicating the validity of the method used for measuring peptide-reactive IgG.

Discussion

Recent studies on tumor antigens revealed that most tumor antigens recognized by CTLs are non-mutated self-antigens (26). Therefore, it is necessary to consider the risk of autoimmune responses and to overcome immunological tolerance to self-antigens when applying such antigens to immunotherapy for cancer patients. On the other hand, the infection with particular types of HPV has been causally associated with the development of cervical cancer, making them attractive targets for CTL-directed immunotherapy. Indeed, several HPV16-E7-derived peptides have been used in specific immunotherapy for HLA-$A2^+$ cervical cancer patients (11-14). In this study, we identified two new HPV16-E6-derived peptides that have the potential to generate cancer-reactive CTLs in HLA-$A24^+$ cervical cancer patients. PBMCs from HLA-$A24^+$ cervical cancer patients showed peptide-specific IFN-γ production in 6 or 5 of 13 patients when stimulated with the HPV16-$E6_{75-83}$ and HPV16-$E6_{91-100}$ peptides, respectively. More importantly, these peptide-stimulated PBMCs showed cytotoxicity against HLA-$A24^+$ and HPV16-$E6^+$ cervical cancer cells. These results indicate that these two HPV16-E6 peptides are potentially useful in specific immunotherapy for HLA-$A24^+$ cervical cancer patients and extend the possibility of peptide-based immunotherapy against cervical cancer.

Both the HPV-derived E6 and E7 oncoproteins are responsible for the onset and maintenance of malignant transformation through inactivation of the *p53* and *retinoblastoma* tumor suppressor genes, respectively (27,28). E6 and E7 are constitutively expressed in cervical cancer cells and their continuous expression is regarded as necessary for the maintenance of the transformed phenotype (29,30). Although both of them are considered to be appropriate targets, E7-derived peptides have been preferentially identified as CTL-directed epitope peptides. This is probably because the expression of the E6 protein in cervical cancer cells appears to be lower than that of the E7 protein (31,32). In this study, we demonstrated that the HPV16-E6 protein was detected in some cervical cancer cell lines.

Although the HPV16-E6 and -E7 proteins are suggested to be appropriate targets in specific immunotherapy, antigen processing defects in cervical carcinoma are a major obstacle. Cervical cancer cells are reported to escape CTL killing by down-regulating MHC class I expression, and can evade immune surveillance by down-regulating TAP and thereby inhibiting peptide transport (20-22). However, several studies have shown that the HPV-specific CTL directed against HLA-A^*0201-restricted HPV16-E6 epitopes can kill an HLA-$A2^+$ cervical cancer cell line (33,34). In this study, we confirmed the protein expression of HLA-A24, TAP1 and TAP2 in SKG-IIIa cells before the assay of cytotoxicity. These cells expressed HLA-A24 molecules on their surfaces and their expression of TAP1 and TAP2 was at levels comparable to those of an esophageal cancer cell line. Although the loss of HLA class I expression is critical for CTL-based immunotherapy, the treatment with IFN-γ can effectively up-regulate the TAP proteins in cervical cancer cells (20). These lines of evidence may suggest that a therapy aimed at local production of cytokines can augment the efficacy of CTL-based immunotherapy against cervical cancer.

Here, we investigated whether IgGs against HPV16-E6 peptides are detectable in plasma from HLA-$A24^+$ cervical cancer patients and healthy donors because the antibodies against CTL-directed peptides had already been observed in certain cancer patients and healthy donors (25,35). In this study, an IgG reactive to the HPV16-$E6_{91-100}$ peptide was detected in 4 of 12 cervical cancer patients. This indicates that the HPV16-$E6_{91-100}$ peptide was recognized by both the cellular and humoral immune systems. Our clinical trials revealed that a peptide vaccination frequently resulted in the induction of an IgG reactive to the CTL epitope peptides that were administered (23,24) and that the augmentation of peptide-specific IgGs after peptide vaccination could be a laboratory marker for the prediction of prolonged survival in vaccinated cancer patients compared to the induction of peptide-specific CTLs or the delayed-type hypersensitivity test (36). Furthermore, we recently observed that peptide vaccination with a

CTL-directed peptide could induce peptide-specific and HLA-DR-restricted $CD4^+$ T cells *in vivo* (37). Because these findings belong to circumstantial evidence, further clinical study is needed to elucidate the role and meaning of peptide-specific IgGs in anticancer immunotherapy.

IgGs reactive to the HPV16-$E6_{91-100}$ peptide were also observed in the plasma of 7 of the 14 healthy donors tested, while PBMCs from 2 of 6 healthy donors had CTL precursors reactive to the HPV16-$E6_{75-83}$ peptide. This might be plausible because substantial numbers of healthy females are expected to be infected with HPV, although we have not intensively investigated this issue in this study.

In conclusion, we identified two HPV16-E6-derived peptides that are immunogenic in HLA-$A24^+$ cervical cancer patients. The frequencies of the HLA-A24 allele are relatively high throughout the world (38). The information provided here might increase the possibility of treating HLA-$A24^+$ cervical cancer patients by targeting HPV16.

Acknowledgements

This study was supported in part by Grants-in-Aid for Cancer Research (15-17) from the Ministry of Health, Labor and Welfare of Japan, and Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Walboomers JM and Meijer CJ: Do HPV-negative cervical carcinoma exist? J Pathol 181: 253-254, 1997.
2. Munoz N, Bosch FX, De Sanjose S, *et al*: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518-527, 2003.
3. Munger K, Phelps WC, Bubb V, *et al*: The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J Virol 63: 4417-4421, 1989.
4. Von Knebel DM, Rittmuller C, Zur HH and Durst M: Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6-E7 anti-sense RNA. Int J Cancer 51: 831-834, 1992.
5. Borysiewicz LK, Fiander A, Nimako M, *et al*: A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 347: 1523-1527, 1996.
6. Ressing ME, van Driel J, Celis E, *et al*: Occasional memory cytotoxic T-cell responses of patients with human papillomavirus type 16-positive cervical lesions against a human leukocyte antigen-A*0201-restricted E7-encoded epitope. Cancer Res 56: 582-588, 1996.
7. Evans EM, Man S, Evans AS and Borysiewicz LK: Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. Cancer Res 57: 2943-2950, 1997.
8. Nijman HW, Houbiers JG, van der Burg, *et al*: Characterization of cytotoxic T lymphocyte epitopes of a self-protein, p53 and a non-self-protein, influenza matrix: relationship between major histocompatibility complex peptide binding affinity and immune responsiveness to peptides. J Immunother 14: 121-126, 1993.
9. Kast WM, Brabdt RM, Sidney J, *et al*: Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins. J Immunol 152: 3904-3912, 1994.
10. Ressing ME, Sette A, Bradt RM, *et al*: Human CTL epitopes encoded by human papollomavirus type 16 E6 and E7 identified through *in vivo* and *in vitro* immunogenicity studies of HLA-A*0201-binding peptides. J Immunol 154: 5934-5943, 1995.
11. Muderspach L, Wilczynski S, Roman L, *et al*: A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. Clinical Cancer Res 6: 3406-3416, 2000.
12. Murakami M, Gurski KJ, Marincola FM, Ackland J and Steller MA: Induction of specific-$CD8^+$ T-lymphocyte responses using a human papillomavirus-16 E6/E7 fusion protein and autologous dendritic cells. Cancer Res 59: 1184-1187, 1999.
13. Nimak M, Fiander AN, Wilkinson GW, Borysiewicz LK and Man S: Human papillomavirus-specific cytotoxic T lymphocytes in patients with cervical intraepithelial neoplasia grade III. Cancer Res 57: 4855-4861, 1997.
14. Steller MA, Gurski KJ, Murakami M, *et al*: Cell-mediated immunological responses in cervical and vaginal cancer patients immunized with a lapidated epitope of human papillomavirus type 16 E7. Clin Cancer Res 4: 2103-2109, 1998.
15. Itoh K, Shichijo S, Yamada A, *et al*: Squamous cell and adeno cancer antigens recognized by cytotoxic T lymphocytes. In: Tumor Immunology Series. Stauss H, Kawakami Y and Parmiani G (eds). Taylor & Francis, pp75-96, 2003.
16. Tsuda N, Mochizuki K, Harada M, *et al*: Vaccination with predesignated or evidence-based peptides for patients with recurrent gynecologic cancers. J Immunother 27: 60-72, 2004.
17. Parker KC, Bednarek MA and Coligan JE: Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 152: 163-175, 1994.
18. Hida N, Maeda Y, Katagiri K, *et al*: A new culture protocol to detect peptide-specific cytotixic T lymphocyte precursors in the circulation. Cancer Immunol Immunother 51: 219-228, 2002.
19. Komatsu N, Shichijo S, Nakagawa M and Itoh K: New multiple flow cytometric assay to measure anti-peptide antibody: a novel tool for monitoring immune responses to peptides used for immunization. Scand J Clin Lab Invest 64: 1-11, 2004.
20. Evans M, Borysiewicz LK, Evans AS, *et al*: Antigen processing defects in cervical carcinomas limits the presentation of a CTL epitope from human papillomavirus 16 E6. J Immunol 167: 5420-5428, 2001.
21. Cromme FV, Airey J, Heemels MT, *et al*: Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. J Exp Med 179: 335-340, 1994.
22. Koopman LA, Corver WE, van der Slik AR, Giphart MJ and Fleuren GJ: Multiple genetic alterations cause frequent and heterogeneous human histocompatibility leukocyte antigen class I loss in cervical cancer. J Exp Med 191: 961-975, 2000.
23. Mine T, Gouhara R, Hida N, *et al*: Immunological evaluation of CTL precursor-oriented vaccines for advanced lung cancer patients. Cancer Science 94: 548-556, 2003.
24. Sato Y, Shomura H, Maeda Y, *et al*: Immunogical evaluation of peptide vaccination for patients with gastric cancer based on pre-existing cellular response to peptide. Cancer Science 94: 802-808, 2003.
25. Nakatsura T, Senju S, Ito M, Nishimura Y and Itoh K: Cellular and humoral immune responses to a human pancreatic cancer antigen, coactosin-like protein, originally defined by the SEREX method. Eur J Immunol 32: 826-836, 2002.
26. Renkvist N, Castelli C, Robbins PF and Parmiani G: A listing of human tumor antigens recognized by T cells. Cancer Immunol Immunother 50: 3-15, 2001.
27. Scheffner M, Wemess BA, Huigbreste JM, Levine AJ and Howley PM: The E6 oncoprotein encoded by human papillomavirus type 16 and 18 promotes the degradiation of p53. Cell 63: 1129-1136, 1990.
28. Dyson N, Howley PM, Munger K and Harlow E: The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243: 934-937, 1989.
29. Von Knebel-Doeberitz M, Oltersdorf M, Schwarz E and Gissmann L: Correlation of modified human papillomavirus early gene expression with altered growth properties in C4-1 cervical carcinoma cells. Cancer Res 48: 3780-3786, 1998.
30. Von Knebel-Doeberitz M, Rittmuller C, Zur Hausen H and Durst M: Inhibition of tumorigenicity of C4-1 cervical cancer cells in nude mice by HPB18 E6-E7 antisense RNA. Int J Cancer 51: 4706-4714, 1992.
31. Seedorf K, Alterdorf T, Krammer G and Rowekamp W: Identification of early proteins of the human papillomavirus type 16 (HPV16) and type 18 (HPV18) in cervical carcinoma cells. EMBO J 6: 139-144, 1987.
32. Androphy EJ, Hubbert NL, Schiller JT and Lowy DR: Identification of the HPV-16 E6 protein from transformed mouse cells and human cervical carcinoma cell lines. EMBO J 6: 989-992, 1987.

33. Youde SJ, Dunbar PR, Evans EM, *et al*: Use of fluorogenic histocompatibility leukocyte antigen-A*0201/HPV 16 E7 peptide complexes to isolate rare human cytotoxic T-lymphocyte-recognizing endogenous human papillomavirus antigens. Cancer Res 60: 365-371, 2000.
34. Evans C, Bauer S, Grubert T, *et al*: HLA-A2-restricted peripheral blood cytolytic T lymphocyte response to HPV type 16 proteins E6 and E7 from patients with neoplastic cervical lesions. Cancer Immunol Immunother 42: 151-160, 1996.
35. Ohkouchi S, Yamada A, Imai N, *et al*: Non-mutated tumor rejection antigen peptides elicit type-I allergy in the majority of healthy individuals. Tissue Antigens 59: 259-272, 2002.
36. Mine T, Sato Y, Noguchi M, *et al*: Humoral responses to peptides correlated with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing, peptide-specific cellular responses. Clin Cancer Res 10: 929-937, 2004.
37. Harada M, Gohara R, Matsueda S, *et al*: *In vivo* evidence that peptide vaccination can induce HLA-DR-restricted $CD4^+$ T cells reactive to a class I tumor peptide. J Immunol 172: 2659-2667, 2004.
38. Imanishi T, Akazawa T, Kimura A, *et al*: Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: HLA 1991. Tsuji K, Aizawa M and Sasazuki T (eds). Oxford: Oxford Scientific Publications, pp1065-220, 1992.

5022 **Poster Discussion, Sat, 1:00 PM - 4:00 PM**

Fertility-sparing treatment by high dose oral medroxyprogesterone acetate for endometrial cancer and atypical hyperplasia in young women: A multicentric phase II study. *K. Ushijima, H. Yoshikawa, T. Hirakawa, T. Yasugi, T. Saito, M. Yasuda, K. Kuzuya, T. Fujii, M. Hatae, T. Kamura; Kurume Univ Sch of Medicine, Kurume, Japan; Univ of Tsukuba, Tsukuba, Japan; Kyushu Univ, Fukuoka, Japan; Univ of Tokyo, Tokyo, Japan; National Kyushu Cancer Ctr, Fukuoka, Japan; Kashiwa Hosp, Jikei Univ, Kashiwa, Japan; Aichi Cancer Ctr Hosp, Nagoya, Japan; National Kure Medcl Ctr, Kure, Japan; Kagosima City Hosp, Kagoshima, Japan*

Background: Standard treatment for endometrioid adenocarcinoma, stage Ia (EC) and atypical endometrial hyperplasia (AH) is hysterectomy even in young women. High-dose of Medroxyprogesterone Acetate (MPA) is one of the options for patients who desire preserving childbearing potential. Nevertheless, the optimal dose or duration and curative rate of MPA therapy for in EC and AH in young women are still uncertain. **Methods:** Multicentric prospective study was carried out by 16 institutions in Japan. Twenty-five EC patients with no myometrial or cervical invasion and 17 AH patients under 40 years of age were enrolled. All patients were given MPA 600mg with low dose aspirin orally daily, and the treatment were continued for 26weeks as far as the lesions responded to it. Endometrial tissue was collected and histologically assessed at 8, 16, 26 weeks of the treatment. The diagnoses were confirmed by central pathological review board. Estrogen-progesterone therapy or assisted reproductive technologies were provided for the responders after MPA therapy. Complete response (CR) rate was the primary endpoint and toxicity, rate of pregnancy and progression free interval (PFI) were the secondary endpoints. **Results:** CR was found in 44% in EC and 82% in AH. Overall CR rate was 60%. Any grade 3 toxicities except body weight increase in two patients or therapeutic death were not observed. So far 9 pregnancies and 4 normal deliveries have been recorded after MPA therapy. Twelve recurrences were found in 30 CR patients (40%) between 7 to 22 months, they rechallenged MPA or underwent hysterectomy. No patients died of disease. PFI of EC and AH were 20.1months and 28.5 months respectively. **Conclusions:** The efficacy of fertility-sparing treatment by MPA for EC and AH was proved by this first prospective trial. Even in the CR patients, close follow-up is required because of their high recurrence rate.

International Journal of Gynecology and Obstetrics (2005) 89, 39–44

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CLINICAL ARTICLE

Clinical aspects and prognosis of pelvic recurrence of cervical carcinoma

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Received 28 September 2004; received in revised form 28 December 2004; accepted 28 December 2004

KEYWORDS
Cervical carcinoma;
Local recurrence;
Pelvic exenteration;
Radiotherapy

Abstract

Objective: To identify which patients with locally recurrent cervical carcinoma are potentially curable. *Method:* A total of 664 stage IB-IVA patients were examined following surgery or radiotherapy. *Result:* Among the 664 patients, 193 (29%) developed recurrence. Sixty-seven (35%) of these recurrences were located in the pelvis alone. Among these 67 recurrences, 24 (35%) were central recurrences and the remaining 43 (65%) were pelvic side-wall recurrences. Of the 24 patients with central recurrences, 8 were salvaged. Of these 8 patients, 3 underwent pelvic exenteration, and 5 received optimal radiotherapy. The recurrent tumor in these 5 survivors who received radiotherapy had consisted of a small (<2 cm) tumor. All 43 patients with pelvic wall recurrence developed progressive disease. *Conclusion:* The following patients are potentially curable: patients with a resectable, centrally located tumor who are candidates for pelvic exenteration, and patients with a small central recurrence for whom complete radiation therapy is feasible.

1. Introduction

Over the past 20 years, there has been remarkable progress in diagnostic imaging and in the identification of serial tumor markers that can detect tumor recurrence at an early stage, and advances in new chemotherapeutic agents and new surgical and radiotherapeutic approaches have been introduced. Despite these advances, the overall prognosis of patients with locally recurrent cervical cancer is very poor and optimal treatment for recurrent disease is still problematic [1]. In nearly all cases, treatment for local recurrence should be considered palliative, and a very small proportion of these patients is cured. In fact, 10–15% of patients with stage IB-IIA disease who undergo radical hysterectomy will develop recurrence, and 50–60% of these recurrences will be located in the

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0020-7292/$ - see front matter
doi:10.1016/j.ijgo.2004.12.020

pelvis alone [2,3]. Also, 20–50% of patients with stage II–III disease who undergo radiotherapy will relapse locally [4,5]. This retrospective study was undertaken to identify which patients with locally recurrent cervical carcinoma are potentially curable, to design new therapeutic strategies for dealing with these locally recurrent cervical carcinoma cases in the future, and to improve their prognosis.

2. Patients and methods

2.1. Patients

The medical records of 664 patients with stage IB-IVA cervical carcinoma who were treated at the Gynecology Division of National Cancer Center Hospital in Tokyo, between 1989 and 2000 were reviewed. Four patients who were lost to follow-up and 14 patients with persistent disease were excluded. All of the patients were staged according to the FIGO staging system, and histological typing was performed according to the criteria of the WHO International Histological Classification of Tumors. Follow-up continued through September 2003. Survival curves were obtained by the Kaplan–Meier method, and patients who died of other causes were included as deaths in the survival analysis.

2.2. Treatment

Our standard treatment for primary invasive cervical carcinoma was as follows. Patients with stage IB, IIA or IIB cervical carcinoma were appropriately treated with either radical hysterectomy or radiotherapy with equivalent results. In patients with lymph node metastasis or parametrial invasion (pT2b), following surgery, adjuvant radiotherapy to the whole pelvis was administered. The daily dose ranged from 1.8 Gy to 2 Gy, 5 fractions/week, and the dose for the whole pelvis was 50 Gy with opposed anterior and posterior fields. Primary radiotherapy was composed of external-beam plus high-dose-rate intracavitary irradiation. A remote afterloading system of 192Ir with Tandem and Ovoid applicators was employed. Extra-beam radiotherapy with a total dose of 50 Gy was administered over 5 weeks in daily fractions to the pelvis, and the applicator (6 Gy/A-point) was applied with 4–5 insertions at weekly intervals. Various combinations of external and intracavitary irradiation were tailored based on the size and distribution of the tumor. The choice of treatment modality depended on the age of the patient, presence of comorbid conditions, and histologic subtype. For patients with advanced stage carcinoma of FIGO IIIA, IIIB, or IVA, primary radiotherapy using external-beam radiation and high-dose-rate brachytherapy was employed. Following the primary treatment, asymptomatic patients underwent pelvic examination, Pap smear, chest radiograph, and determination of serial tumor markers every 4–6 months. Symptomatic patients underwent appropriate examinations where indicated by ultrasonography, computed tomography, and/or magnetic resonance imaging.

Our management of local recurrence of cervical carcinoma was generally as follows: (1) patients with recurrent disease arising in the previously irradiated pelvis received chemotherapy or palliative care, except for patients with central recurrence who were candidates for pelvic exenteration; (2) radiotherapy was used to treat recurrent disease in patients who had no prior history of receiving radiotherapy.

3. Results

3.1. Patient characteristics

The clinical characteristics of the 664 patients with primary cervical carcinoma are summarized in Table 1. Among the 353 patients with stage IB disease, surgery was performed in 326 patients (92%), of whom 89 (27%) subsequently received postoperative adjuvant radiotherapy. The remaining 27 patients (8%) with stage IB disease received primary radiotherapy. Of the 65 patients with stage IIA disease, surgery was performed in 45 patients (69%), of whom 18 (40%) subsequently received

Table 1 Patients characteristics, n=664

Mean age	52 years	(range, 22–86)
Histologic subtypes	Squamous	493 (74%)
	Adeno	117 (18%)
	Adenosquamous	38 (6%)
	Others	16 (2%)
FIGO stage	IB	353 (53%)
	IIA	65 (10%)
	IIB	108 (16%)
	IIIA	3 (0.5%)
	IIIB	119 (18%)
	IVA	16 (2.5%)
Treatment modalities	Surgery	471 (71%)
	Radical hysterectomy	419
	Simple hysterectomy	46
	Pelvic exenteration	6
	Postoperative adjuvant radiotherapy	156
	Radiotherapy	193 (29%)

postoperative adjuvant radiotherapy, and primary radiotherapy was performed in 20 patients (31%). Of the 108 patients with stage IIB disease, surgery was performed in 79 patients (73%), of whom 43 (54%) subsequently received postoperative adjuvant radiotherapy, and primary radiotherapy was performed in 29 patients (27%). Among the 117 patients with stage IIIA, IIIB, and IVA disease, 96 patients were treated with primary radiotherapy using external-beam radiation and high-dose-rate brachytherapy as described above, and 21 patients received the surgical approach. The 664 patients were followed for 1–166 months, and the median follow-up time was 68 months.

3.2. Survival and prognosis

The cumulative 5-year survival rate among patients with stage IB, IIA, IIB, IIIA, IIIB, or IVA primary cervical carcinoma was 84%, 78%, 65%, 67%, 54%, and 38%, respectively. Among the 664 patients, 193 patients (29%) suffered tumor recurrence. Of these 193 recurrences, 67 (35%) were located in the pelvis alone, 109 (56%) outside the pelvis, 13 (7%) intra- and extra-pelvis, and the remaining 4 in an unknown location.

Of the 67 patients with pelvic recurrence, 26 patients had FIGO stage IB disease, 6 had stage IIA, 13 had stage IIB, 1 had stage IIIA, 18 had stage IIIB, and 3 had stage IVA. Thirty-nine patients underwent radical hysterectomy, 1 underwent simple hysterectomy, and 2 underwent total pelvic exenteration. In total, 42 patients (63%) received surgical treatment. Among them, adjuvant external beam radiotherapy to the whole pelvis (total dose of 50 Gy) was administered postoperatively to the 12 patients (29%) who had parametrial invasion and/or lymph node metastasis. The remaining 25 patients (37%) received primary radiotherapy composed of external-beam plus high-dose-rate intracavitary irradiation. The histologic subtypes were squamous cell carcinoma in 46 cases (67%), adenocarcinoma in 14 cases (21%), the adenosquamous type in 4 cases (6%), the glassy cell type in 2 cases (3%), and the undifferentiated type in one case (1%).

Table 2 summarizes the location of the 67 pelvic recurrences, whether the recurrent tumor was located inside or outside of the previously irradiated field among patients who had undergone radiotherapy for treatment of primary cervical carcinoma, the treatment modality for the recurrence, and outcome. The 24 patients with central recurrence had undergone the following treatments for their primary cervical carcinoma: 11 patients had undergone surgery alone, 2 patients had undergone surgery followed by postoperative adjuvant radiotherapy, and the remaining 11 patients had undergone primary radiotherapy alone. Therefore, 13 patients with central recur-

Table 2 Clinical states of patients with pelvic recurrence, n=67

Location of tumor	n (%)	Prior radiotherapy	n	Treatment modality for recurrence	n	Status[a]	n (mo)[b]
Central pelvis	24 (35%)	Outside the irradiated field[c]	4	Radiotherapy	4	NED[d]	1 (71)
		Inside the irradiated field[e]	9	Radiotherapy	3	NED	1 (62)
				Pelvic exenteration	2	NED	1 (142)
				Palliative surgery	2		
				Not done	2		
		Not done[f]	11	Radiotherapy	8	NED	3 (70, 78, 86)
				Pelvic exenteration	3	NED	2 (61, 24)
Pelvic wall involvement	43 (65%)	Inside the irradiated field	25	Radiotherapy	5		
				Pelvic exenteration	1		
				Palliative surgery	1	AWD[g]	1 (34)
				Chemotherapy	6	AWD	2 (17, 23)
				Not done	12		
		Not done	18	Radiotherapy	9		
				Pelvic exenteration	2		
				Chemotherapy	4		
				Not done	3		

[a] Blank represents dead of disease.
[b] Survival after initial treatment.
[c] The recurrent tumor was located outside the field of irradiation that had been performed for the initial treatment.
[d] No evidence of disease.
[e] The recurrent tumor was located inside the field of irradiation that had been performed for the initial treatment.
[f] Patients who did not receive radiotherapy for treatment of initial therapy.
[g] Alive with disease.

rence had previously undergone radiotherapy for treatment of their primary carcinoma. Nine of the centrally recurrent tumors arose in the previously irradiated field, while 4 arose outside the irradiated field. These 4 tumors were located in the lower vagina outside the previously irradiated field. Among the 24 patients with central recurrence, 8 patients (33%) were alive without disease after salvage therapy and the remaining 16 patients died of disease.

The 43 patients with recurrence with pelvic wall involvement had undergone the following treatments for their primary cervical carcinoma: 18 patients had undergone surgery alone, 11 patients had undergone surgery followed by postoperative adjuvant radiotherapy, and 14 patients had undergone primary radiotherapy alone. All 43 patients with recurrent tumor involving the pelvic side-wall developed tumor progression. Forty patients died of disease, and 3 were alive with disease and were receiving palliative care at the end of the follow-up period. After tumor recurrence in the pelvic-side-wall, 10 patients were treated with one of the following chemotherapy regimens: cisplatin (Briplatin; Bristol-Myers Squibb, NY, USA) + 5-fluorouracil(5-FU; Kyowa Hakko Kogyo, Tokyo, Japan), bleomycin hydrochloride (Bleomycin; Nippon Kayaku, Tokyo, Japan) + vincristine sulfate (Oncovin; Eli Lilly Japan, Kobe, Japan) + mitomycin c (Mitomycin; Kyowa Hakko Kogyo, Tokyo, Japan) + cisplatin, carboplatin (Paraplatin; Bristol-Myers Squibb, NY, USA) + irinotecan hydrochloride (Campto; Yakult Honsya, Tokyo, Japan), or paclitaxel (Taxol; Bristol-Myers Squibb, NY, USA) + carboplatin. Among the 67 patients with pelvic recurrence, the 5-year survival rate after the development of pelvic recurrence was 15% and the median survival time was 12 months.

Table 3 shows details of the clinicopathological states of the 8 survivors who suffered central recurrence and were alive without disease after salvage therapy. Four patients could receive complete radiation therapy composed of external beam plus brachytherapy; 3 of the 4 patients had no prior history of radiotherapy (Case 4, 5, and 6) and one had a recurrent tumor originating outside the previously irradiated field (Case 2). These 4 tumors were less than approximately 2 cm in diameter and/or spread on the surface of the vaginal wall. Three of the 8 survivors had undergone pelvic exenteration for treatment of the recurrent tumor. Two of them had not received prior radiotherapy, and their histologic subtypes were glassy cell type and endometrioid adenocarcinoma (Case 7 and 8). The remaining one patient who was a candidate for pelvic exenteration, received intracavitary brachytherapy even though she had a history of prior radiotherapy because she refused surgery (Case 2). Although she was alive without disease for 50 months at the end of follow-up, she suffered severe radiation cystitis as a late complication of irradiation at 12 months.

4. Discussion

Our review demonstrated that patients with locally recurrent cervical carcinoma have a poor outcome, similar to previous studies. Only 13% of the recurrent patients with IB-IIB disease and 11% of the recurrent patients with IIIB disease were cured. According to the results of our study, patients who are potentially curable are limited to the following recurrence patterns.

First, a recurrent tumor that is located centrally in the pelvis, is associated with better

Table 3 Clinical states of patients with no evidence of disease after salvage therapy for cervical carcinoma

Patient no.	FIGO stage	Histological subtype	Primary therapy	Recurrent site	Recurrence free interval (mo)	Salvage therapy	Survival after recurrence (mo)
1	IIA	Squamous	Radiotherapy	Cervix, Bladder	7	Anterior exenteration	135
2	IIIB	Squamous	Radiotherapy	Vaginal wall	21	Radiotherapy	50
3	IB	Adenosquamous	Radical hysterectomy+ radiotherapy	Vaginal stump	12	Radiotherapy	50
4	IB	Squamous	Radical hysterectomy	Vaginal stump	7	Radiotherapy	79
5	IB	Squamous	Radical hysterectomy	Vaginal wall	2	Radiotherapy	76
6	IB	Adenosquamous	Radical hysterectomy	Vaginal wall	18	Radiotherapy	52
7	IB	Adenocarcinoma	Radical hysterectomy	Rectum, Bladder	26	Total exenteration	35
8	IIIb	Glassy cell	Anterior exenteration+ chemotherapy	Rectum	44	Posterior exenteration	80

prognosis. All of our patients in whom the tumor was fixed to the pelvic side-wall developed progressive disease. Although the patients who had not previously received radiotherapy could receive optimal doses of radiation, there were no long-term survivors. As pelvic exenteration is not indicated for tumors involving the pelvic wall, and as chemotherapy for recurrent cervical cancer should be considered as a palliative treatment, no curative approach exists for recurrence in the pelvic side-wall. It has been discussed that pelvic side-wall recurrence which indicates metastatic systemic disease, is biologically different from central recurrence. On the other hand, the efficacy of combined surgery and radiotherapy on small-size lateral recurrences was reported, and was equivalent to its efficacy on central recurrences [6,7]. In this method, maximum tumor resection and intraoperative radiotherapy using the applicator of high-dose-rate brachytherapy are performed. This method is not widely accepted, and further studies are needed.

Secondly, based on the results of the present study, patients with central recurrence who are either (1) a candidate for pelvic exenteration or (2) a candidate for complete radiotherapy, are potentially curable. Pelvic exenteration is the only potentially curative approach for central recurrence in patients who had previously undergone radiotherapy. The overall 5-year survival rate after exenteration varied between approximately 20 and 60%, and the operative mortality was less than 10% in the literature [8–11]. In our 27 years' experience in performing pelvic exenteration in patients with recurrent cervical carcinoma (between 1973 and 2000) including the patients in the present study, the 5-year survival rate was 36% and the procedure-related mortality was 6%. The preoperative status of the patient is related to survival. Leg edema, sciatic pain, and urethral obstruction in patients with recurrent cervical carcinoma almost always indicate pelvic side-wall involvement and lead to abortion of extensively planned procedures. A short interval from primary therapy to recurrence (within one year) and nodal metastasis are also poor prognostic factors [1,12]. As for histologic subtypes, Crozier et al. [13] reported that central recurrence of cervical adenocarcinoma could be successfully treated with pelvic exenteration and was associated with a survival rate similar to that of squamous carcinoma. In the present study, 2 out of the 3 patients who were successfully treated with exenteration had non-squamous cell carcinoma. Pelvic exenteration is a traumatic operation for patients, and selection of patients for this procedure should be carefully performed in preoperative assessment and at the time of laparotomy [14]. On the other hand, since this exenterative procedure offers the only possibility for cure in patients with central recurrence after optimal radiotherapy, gynecologic oncologists should not miss this only chance for saving the patient's life.

For patients who develop central recurrence outside the initial irradiated field, radiotherapy can provide long-term local control. Above all, under ideal conditions, curable treatment can be achieved. Survival is greatly influenced by tumor size, and a small tumor originating on the surface of the vagina is highly curable. Ito et al. [15] reported that among patients with recurrent cervical cancer of the vaginal stump that was treated with high-dose-rate intracavitary brachytherapy with or without external irradiation, the 10-year survival rate of patients who had a small (no palpable tumor), medium (less than 3 cm), or large (3 cm or more) tumor was 72, 48, and 0%, respectively. In the present study, all survivors who received optimal radiotherapy for their centrally recurrent tumor satisfied the ideal conditions, that is, they had either an unpalpable tumor or a small tumor of the vagina.

Patients who do not meet the criteria for curable treatment mentioned above were compelled to receive palliative treatment such as chemotherapy, palliative surgery, palliative radiotherapy, or palliative care. Although various agents have been investigated as a single-drug regimen or in combined regimens, chemotherapy for the treatment of local recurrence is considered to be palliative at present [16]. Although cisplatin is the most effective single agent, only 25% of patients show a clinical response. Several phase II studies have been performed to assess the effectiveness of gemcitabine, paclitaxel, vinorelvine, and camptothecines, and the overall response rate to these new drugs ranged between 8 and 25% [17]. Several recent phase II studies on combination chemotherapy with cisplatin and these new drugs demonstrated an overall response rate of 41 to 64% [17]. Nevertheless, it is uncertain whether chemotherapy has a palliative effect and whether it prolongs the survival of patients with locally recurrent cervical carcinoma, and oncologists should assess the potential benefit to each patient before administration.

The prognosis of patients with locally recurrent cervical carcinoma is very poor; however, the following patients are potentially curable: patients with a resectable, centrally located tumor who are candidates for pelvic exenteration; and patients with a small central recurrence for whom complete

radiation therapy is feasible. Gynecologic oncologists should not miss the only chance for life in these patients.

References

[1] Friedlander M. Guidelines for the treatment of recurrent and metastatic cervical cancer. Oncologist 2002;7:342-7.

[2] Samlal RA, Van Der Velden J, Van Eerden T, Schilthuis MS, Gonzalez Gonzalez D, Lammes FB. Recurrent cervical carcinoma after radical hysterectomy: an analysis of clinical aspects and prognosis. Int J Gynecol Cancer 1998 (Jan);8:78-84.

[3] Lanciano R. Radiotherapy for the treatment of locally recurrent cervical cancer. J Natl Cancer Inst Monographs 1996:113-5.

[4] Perez CA, Grigsby PW, Camel HM, Galakatos AE, Mutch D, Lockett MA. Irradiation alone or combined with surgery in stage IB, IIA, and IIB carcinoma of uterine cervix: update of a nonrandomized comparison. Int J Radiat Oncol Biol Phys 1995 (Feb 15);31:703-16.

[5] Montana GS, Martz KL, Hanks GE. Patterns and sites of failure in cervix cancer treated in the U.S.A. in 1978. Int J Radiat Oncol Biol Phys 1991 (Jan);20:87-93.

[6] Hockel M, Sclenger K, Hamm H, Knapstein PG, Hohenfellner R, Rosler HP. Five-year experience with combined operative and radiotherapeutic treatment of recurrent gynecologic tumors infiltrating the pelvic wall. Cancer 1996 (May 1); 77:1918-33.

[7] Hockel M, Baussmann E, Mitze M, Knapstein PG. Are pelvic side-wall recurrences of cervical cancer biologically different from central relapses? Cancer 1994 (Jul 15);74: 648-55.

[8] Shingleton HM, Soong SJ, Gelder MS, Hatch KD, Baker VV, Austin Jr JM. Clinical and histopathologic factors predicting recurrence and survival after pelvic exenteration for cancer of the cervix. Obstet Gynecol 1989 (Jun);73:1027-34.

[9] Soper JT, Berchuck A, Creasman WT, Clarke-Pearson DL. Pelvic exenteration: factors associated with major surgical morbidity. Gynecol Oncol 1989 (Oct);35:93-8.

[10] Lawhead Jr RA, Clark DG, Smith DH, Pierce VK, Lewis Jr JL. Pelvic exenteration for recurrent or persistent gynecologic malignancies: a 10-year review of the Memorial Sloan-Kettering Cancer Center experience (1972–1981). Gynecol Oncol 1989 (Jun);33:279-82.

[11] Averette HE, Lichtinger M, Sevin BU, Girtanner RE. Pelvic exenteration: a 15-year experience in a general metropolitan hospital. Am J Obstet Gynecol 1984 (Sep 15);150: 179-84.

[12] Stanhope CR, Webb MJ, Podratz KC. Pelvic exenteration for recurrent cervical cancer. Clin Obstet Gynecol 1990 (Dec); 33:897-909.

[13] Crozier M, Morris M, Levenback C, Lucas KR, Atkinson EN, Wharton JT. Pelvic exenteration for adenocarcinoma of the uterine cervix. Gynecol Oncol 1995 (Jul);58:74-8.

[14] Miller B, Morris M, Rutledge F, Mitchll MF, Atokinson EN, Burke TW, et al. Aborted exenterative procedures in recurrent cervical cancer. Gynecol Oncol 1993 (Jul);50: 94-9.

[15] Ito H, Shigematsu N, Kawada T, Kubo A, Isobe K, Hara R, et al. Radiotherapy for centrally recurrent cervical cancer of the vaginal stump following hysterectomy. Gynecol Oncol 1997 (Nov);67:154-61.

[16] Leitao Jr MM, Chi DS. Recurrent cervical cancer. Curr Treatm Opt Oncol 2002 (Apr);3:105-11.

[17] Savarese A, Cognetti F. New drugs in the treatment of recurrent or metastatic cervical cancer. Crit Rev Oncol Hematol 2003 (Dec);48:323-7.

Int J Gynecol Cancer 2005, **15**, 995–1001

REVIEW ARTICLES

Radical hysterectomy for stage IIB cervical cancer: a review

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Abstract. Suprasert P, Srisomboon J, Kasamatsu T. Radical hysterectomy for stage IIB cervical cancer: a review. *Int J Gynecol Cancer* 2005; 15:995–1001.

Patients with stage IIB cervical cancer in some countries in Europe and Asia especially in Japan are usually treated with radical hysterectomy and pelvic lymphadenectomy. Extrauterine diseases, ie, nodal metastases, parametrial invasion, and intraperitoneal spread, can be readily identified. We present the literature review of radical hysterectomy in stage IIB cervical cancer by searching data since 1980 from Medline, and we found that the parametrial involvement of patients in this stage was only 21–55%, the incidence of pelvic node metastases was about 35–45%, and 5-year survival rate was between 55% and 77%. Lymph node metastases and the number of positive nodes were significant prognostic factors of patients in this stage.

KEYWORDS: cervical cancer, radical hysterectomy, stage IIB.

According to the National Comprehensive Cancer Network guideline version 1, 2004, the treatment of choice for stage IIB cervical cancer is concurrent cisplatin-based chemoradiation therapy. However, in some countries in Europe and Asia especially in Japan, these patients are generally treated with radical hysterectomy and pelvic lymphadenectomy using the Okabayashi or Tokyo technique. In 1983, the Japan Society of Obstetrics and Gynecology reported that 62.7% of stage IIB cervical cancer patients were treated with radical hysterectomy[1]. The advantages of this approach are avoiding the long-term complications of radiation therapy and the morbidity of concurrent chemoradiation in patients who did not have high-risk pathologic factors, ie, positive nodes, parametrial invasion, and involved surgical margins. In young patients, ovarian function and vaginal pliability can also be preserved. On the other hand, in case of lymph node metastases, the ovaries may be transposed outside the radiation field. Occult extrauterine diseases such as nodal involvement, parametrial invasion, or intraperitoneal spread can be identified, and removal of bulky positive nodes may improve survival after adjuvant radiation. Additionally, removal of the primary tumor may preclude some radioresistant cervical cancers. The disadvantage of primary surgery for stage IIB cervical cancer is the risk of morbidity associated with receiving combined treatment of chemoradiation following the radical operation.

This article presents the review of primary surgical treatment for stage IIB cervical cancer. We conducted a literature search on Medline using PubMed, from January 1980 to April 2004, using search terms "cervical cancer," "stage IIB," "surgical treatment," and "hysterectomy." Only English articles were included. All

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identified articles that reported on the same aspects of prognostic factors, outcomes, and complications of radical surgery for stage IIB cervical cancer were included in the review. This review will summarize the incidence of parametrial invasion and pelvic node metastases in stage IIB cervical cancer, the relationship between parametrial invasion and pelvic node metastases, the adjuvant treatment after surgery, the prognostic factors, the outcomes, and also the complications of this treatment approach.

The parametrial invasion

Among the seven studies reporting performing radical hysterectomy in patients with stage IIB cervical cancer, shown in Table 1, parametrial involvement was pathologically confirmed in approximately 21–55%[2–8]. These findings imply that approximately one half to three fourths of patients with clinical stage IIB cervical cancer are overstaged. The discrepancy between the clinical and the pathologic stages is most likely caused by difficulty in discriminating parametrial extension from inflammatory change, endometriosis, adhesion, and irregular shape of large cervical tumor[4,7]. Most authors[2,5,6,8] except Kamura *et al.*[7] did not mention whether the clinical staging procedure for parametrial evaluation was carried out under anesthesia or not. Kamura *et al.*[7] studied the histopathologic prognostic factors in stage IIB cervical cancer patients treated at Kyushu University Hospital, Japan. There were 133 stage IIB patients. The clinical staging procedures consisted of the pelvic examination under anesthesia, cystoscopy, colposcopy, proctoscopy, chest X-ray, and intravenous pyelogram. Of these patients, radical surgery was not performed in 26 patients. Among the remaining 107 patients, radical hysterectomy was abandoned in 25 patients because of the following reasons: suspicious para-aortic node metastasis (5), fixation of pelvic lymph nodes with iliac vessels (7), and unable to separate the cervix and vagina from the bladder (14). Consequently, there were 82 patients undergoing radical hysterectomy. Parametrial invasion was identified as high as 45% from this series.

Matsuyama *et al.*[2], from the same institute as Kamura, divided the parametrial invasion into two groups according to the site of involvement, ie, the inner half and the outer half; the 5-year survival rate of these two groups were not significantly different, at 62.8% and 65.7%, respectively.

Burghardt *et al.*[5], from the University Hospital of Graz, Austria, classified the parametrial involvement into four patterns, ie, the continuous, the discontinuous, the involvement of parametrial nodes, and the involvement of parametrial vessels. The most common pattern was the involvement of parametrial nodes (21.6%), followed by the continuous pattern (8.7%), the involvement of parametrial vessels (6.7%), and the discontinuous pattern (3.6%). Evaluation of the parametrium shows that there are many lymph nodes and lymphatic vessels in both the inner and the outer part of the parametrium; these areas can be the potential sites of tumor spreading. The tumor mainly spreads to the adjacent parametrium by tumor cell emboli and lymph node involvement[6]. Girardi *et al.*[6] from the same institute as Burghardt demonstrated that parametrial involvement was related to pelvic node metastasis. Approximately 80% of patients with parametrial node metastasis had positive pelvic nodes, while 74% of those with negative parametrial nodes would have had negative pelvic nodes.

From these studies, it can be concluded that the incidence of parametrial invasion in stage IIB cervical cancer is at most 55%. This means that nearly one half of the patients are overstaged.

Table 1. Parametrial invasion and node metastases in stage IIB cervical cancer patients treated with radical hysterectomy

Authors	Study period	Year	Number of patients	Parametrial invasion (%)	Pelvic node metastases (%)	Para-aortic node metastases (%)
Matsuyama *et al.*[2]	1973–1977	1984	99	42	—	—
Inoue and Okumura[3]	1965–1977	1984	223	34	38.6	—
Inoue and Morita[4]	1965–1986	1990	295	51.2	43	—
Noguchi *et al.*[9]	1950–1984	1987	239	—	33.9	—
Burghardt *et al.*[5]	1971–1985	1987	195	29.7	44.1	—
Girardi *et al.*[6]	1971–1986	1989	219	21.5	40.6	—
Kamura *et al.*[7]	1979–1988	1993	82	45	35	—
Okada *et al.*[10]	1988–1994	1998	30	—	36.7	—
Sakuragi *et al.*[11]	1982–1995	1999	97	—	39.2	7.2
Kawagoe *et al.*[8]	1984–1996	1999	24	55	45.8	—
Takeda *et al.*[12]	1982–1995	2002	88	—	36.4	4.5