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Abstract

The presence of high-risk types of human papillomavirus (HPV) 16, 18 and 33 in cell lines established from several malignancies including 5 of cervical cancer and 6 of head and neck cancer was studied. HPV DNA, either type 16 or 18, was detected by polymerase chain reaction, and by Southern blot hybridization in all of the cell lines derived from cervical cancers. The hybridization patterns of HPV DNA after endonuclease digestion differed among cell lines, suggesting that all of these cell lines were independent isolates. Accordingly, high-risk types of HPV DNA seem to be ubiquitous in cervical cancer. HPV DNA was not detected in the cell lines derived from head and neck cancers or from any other malignancies besides cervical cancer in this study.

KEYWORDS: human papillomavirus DNA, polymerase chain reaction, Southern blot hybridization, cell lines, cervical cancer

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Human Papillomavirus DNA in Cell Lines Derived from Malignancies

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The presence of high-risk types of human papillomavirus (HPV) 16, 18 and 33 in cell lines established from several malignancies including 5 of cervical cancer and 6 of head and neck cancer was studied. HPV DNA, either type 16 or 18, was detected by polymerase chain reaction, and by Southern blot hybridization in all of the cell lines derived from cervical cancers. The hybridization patterns of HPV DNA after endonuclease digestion differed among cell lines, suggesting that all of these cell lines were independent isolates. Accordingly, high-risk types of HPV DNA seem to be ubiquitous in cervical cancer. HPV DNA was not detected in the cell lines derived from head and neck cancers or from any other malignancies besides cervical cancer in this study.

Key words: human papillomavirus DNA, polymerase chain reaction, Southern blot hybridization, cell lines, cervical cancer

The role of human papillomavirus (HPV) in the carcinogenesis of cervical cancer has been extensively studied (for a review see ref. 1). The frequency of HPV 16, 18 and 33 genes in cervical cancers and in cell lines established from cervical cancers is high. The HPV DNA is integrated into the cellular genome and the oncogenes E6 and E7 are maintained and expressed in the cell lines (2-8).

While it is agreed that HPV is an important factor in anogenital cancers, much less is known about the role of HPV in malignancies of other organs. In addition to anogenital cancers, HPV DNA has been detected so far in cancers of the head and neck (for a review see ref. 9). We also detected high-risk types of HPV DNA in tissue specimens obtained during surgery of cancer of the head and neck (10, 11). HPV DNA was also demonstrated in cell lines derived from head and neck squamous cell cancer (12).

It seems to be important to perform epidemiological study not only in surgical specimens but also in cell cultures established from malignancies in order to determine the relationship between cancer and HPV. Our intention was to determine the prevalence of high-risk types of HPV DNA in cell lines derived from different malignacies including those of the cervix and of the head and neck.

Materials and Methods

DNA probes and cell lines. HPV 16 (13) and 18 (2) DNA was obtained from Drs. H. zur Hausen and E.-M. de Villiers, Heidelberg, Germany. HPV 33 DNA (14) was supplied by Dr. G. Orth, Paris, France. Cervical cell lines: CaSki (15) was obtained from Dr. M. Chinami, Kurume Japan; QG-H and QG-U (16) from Dr. B. Shimizu, Chiba, Japan; HeLa S3 (17) from Dr. J. Sato, Okayama, Japan; and C4-II (18) from the American Type Culture Collection, Rockville, MD, USA. Cell lines derived from head and neck cancers: HLac79 and 80 DPP4 laryngeal cancer cell lines (19) were obtained from Dr. H. Bier, Duesseldorf, Germany; KOSC-2 and KOSC-3 oral cancer cell lines (20) from Dr. T. Inagaki, Kobe, Japan; SCC-9 and SCC-25 lingual cancer cell lines (21) from the American Type Culture Collection. KMG4 and 118MG glioblastomas, NB-1 and SKNSH neuroblastomas, T24 bladder cancer, W-2 Wilms' tumor, and OS osteosarcoma cell lines were maintained in this laboratory.

DNA extraction. To extract DNA, cultured cells in monolayer were incubated at 37°C for 5h in 10 mM Tris-HCl (pH 8.0) and 1mM ethylenediaminetetraacetate buffer containing 1% sodium dodecyl sulfate and 250μg/ml proteinase K. DNA was isolated

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from lysates by phenol-chloroform extraction followed by RNase digestion (22).

Polymerase chain reaction. Oligomer primers were designed to amplify the E6 open reading frame of HPV 16/18/33 DNA. The sequences were the same as those reported by Shimada *et al.* (23). 5′-AAGGGCGTAACCGAAATCGGT-3′ was the universal sense primer for HPV 16, 18 and 33; 5′-GTTTGCAGCTCTGTGCATA-3′ was the antisense primer for HPV 16; 5′-GTGTTCAGTTCCGTGCACA-3′ was for HPV 18; and 5′-GTCTCCAATGCTTGGCACA-3′ was for HPV 33. These primers were synthesized on an Applied Biosystems DNA synthesizer (Foster City, CA, USA). PCR mixtures containing 0.5 μg template DNA, 0.2 μM of each primer, 200 μM each of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate and thymidine triphosphate, and 1.25 u of *Taq* polymerase were prepared in 50 μl reaction volumes (10) using the GeneAmp DNA amplification reagent kit (Perkin-Elmer Cetus, Norwalk, CT, USA). DNA amplification by polymerase chain reaction (PCR) was performed on a DNA thermal cycler (Perkin-Elmer Cetus) for 30 cycles of denaturation for 1 min at 94°C, annealing for 2 min at 55°C, and extension for 3 min at 72°C. After amplification, 10 μl of the reaction mixture was electrophoresed on 3 % agarose gels, followed by ethidium bromide staining (10).

Southern blot hybridization. The sample DNAs proven to contain HPV 16 or 18 by PCR were examined further by Southern blot hybridization. Probe HPV DNAs were separated from plasmids and labeled with digoxigenin-deoxyuridine triphosphate using a kit from Boehringer (Mannheim, Germany), Sample DNAs were digested with restriction endonucleases (*Bam*HI, *Eco*RI and *Hin*dIII) and electrophoresed in 0.8 % agarose gels. They were then transferred to Nytran membranes (Schleicher and Shuell, Dassel, Germany) by Southern blotting. After hybridizing the sample DNAs with the probes under stringent conditions, hybrid bands were detected by methods described previously (24, 25).

Results

Detection of amplified DNA. Amplified HPV DNA using mixed primers for HPV 16/18/33 was confirmed by the presence of a band at the position of 140 base pairs in 3 % agarose gels after ethidium bromide staining (Fig. 1). All 5 DNA samples from cervical cancer cell lines were positive for HPV DNA but the samples from all other cancer cell lines were negative (Table 1). To confirm type-specific DNA amplification, only one antisense primer was applied for each HPV-positive DNA sample (Fig. 2A, B and C).

DNAs from CaSki, QG-H and QG-U cervical cancer cell lines, a single band corresponding to 140 base paris was observed when HPV 16-specific antisense primer was used, showing a type-specific reaction (Fig. 2A). In the same way, DNAs from HeLa and C4-II were HPV 18-specific (Fig. 2B) and none of them were HPV 33-specific (Fig. 2C). The specificity of the results by PCR was confirmed using positive controls of the cloned

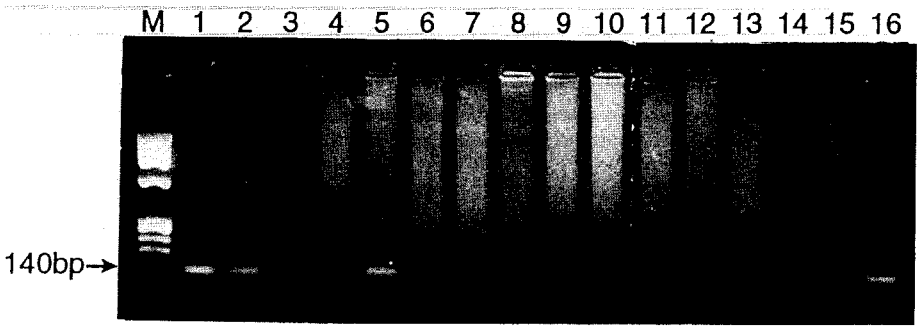

Fig. 1 Electrophoretic detection of HPV 16/18/33 DNA sequences in cellular DNAs amplified by PCR. After PCR using mixtures of universal sense primer and each HPV 16/18/33-specific antisense primers, 10 μl of the reaction mixture was electrophoresed on a 3 % agarose gel, and the resulting bands of 140 bp were stained with ethidium bromide. Lanes: **M**, Phix DNA digested with *Hae*III as a size marker; **1**, CaSki; **2**, QG-H; **3**, QG-U; **4**, HeLa S3; **5**, C4-II; **6**, SCC-9; **7**, SCC-25; **8**, KOSC-2; **9**, KOSC-3; **10**, HLac 79; **11**, 80 DPP4; **12**, 118 MG; **13**, KMG 4; **14**, NB-1; **15**, SKNSH; **16**, HPV 33 DNA.

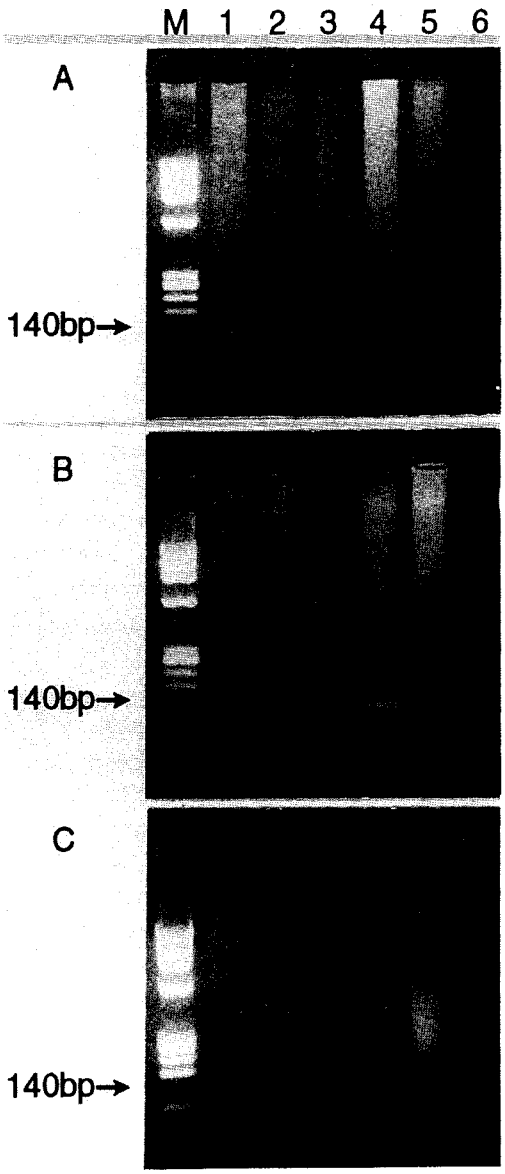

Fig. 2 Electrophoretic detection of **A**) HPV 16-specific, **B**) HPV 18-specific and **C**) HPV 33-specific DNA sequences amplified by PCR. Lanes: **M**, Phix DNA digested with *Hae*III as a size marker; **1**, CaSki; **2**, QG-H; **3**, QG-U; **4**, HeLa S3; **5**, C4-II; **6**, HPV 33 DNA. Methods are the same as in Fig. 1

HPV 16, 18 and 33 DNA (data not shown). No amplified DNA band was observed with the DNA isolated from human embryonic cells or with DNA-free distilled water used as a negative control (data not shown).

Southern blot hybridization. The HPV-positive DNA samples demonstrated by the PCR were hybridized with HPV 16 and 18 DNA probes after

Table 1 Detection of human papilomavirus (HPV) in cell lines

Cell lines	References	Cell origin	HPV (type)
1. CaSki	15	Cervical cancer	(16)
2. QG-H	16	Cervical cancer	(16)
3. QG-U	16	Cervical cancer	(16)
4. HeLa S3	17	Cervical cancer	(18)
5. C4-II	18	Cervical cancer	(18)
6. SCC-9	21	Lingual cancer	—
7. SCC-25	21	Lingual cancer	—
8. KOSC-2	20	Oral cancer	—
9. KOSC-3	20	Oral cancer	—
10. HLac79	19	Laryngeal cancer	—
11. 8ODPP4	19	Laryngeal cancer	—
12. 118MG		BT(Glioblastoma)	—
13. KMG4		BT(Glioblantoma)	—
14. NB-1		BT(Neuroblastoma)	—
15. SKNSH		BT(Neuroblastoma)	—
16. T 24		Bladder cancer	—
17. W-2		Wilms' tumor	—
18. OS		Bone tumor (Osteosarcoma)	—

—: Not detected BT: Brain tumor

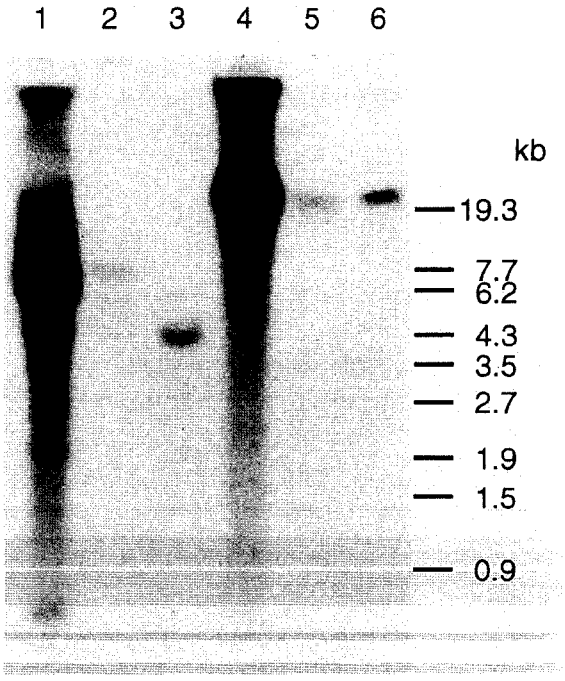

Fig. 3 Detection of HPV 16 DNA sequences in cellular DNAs after digestion with *Eco*RI or *Hin*dIII by Southern blot hybridization with HPV 16 probe. Lanes **1-3**, digested with *Eco*RI; Lanes **4-6**, digested with *Hin*dIII; Lanes **1** and **4**, CaSki; Lanes **2** and **5**, QG-H; Lanes **3** and **6**, QG-U. Molecular size markers in kilobase pairs are on the right.

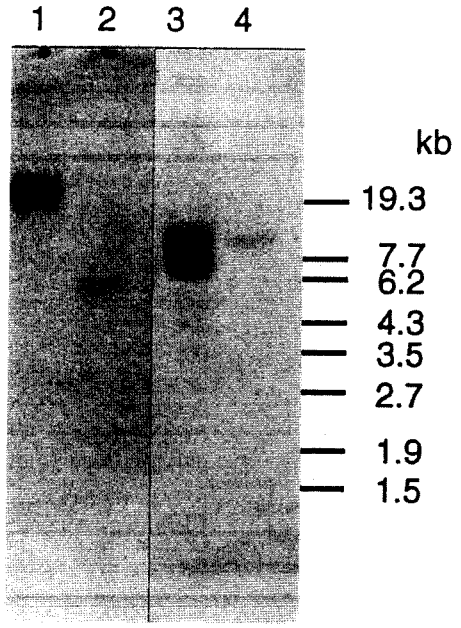

Fig. 4 Detection of HPV 18 DNA sequences in cellular DNAs after digestion with *Eco*RI or *Hin*dIII by Southern blot hybridization with HPV 18 probe. Lanes 1 and 2, digested with *Eco*RI; Lanes 3 and 4, digested with *Hin*dIII; Lanes 1 and 3, HeLa S3; Lanes 2 and 4, C4-II.

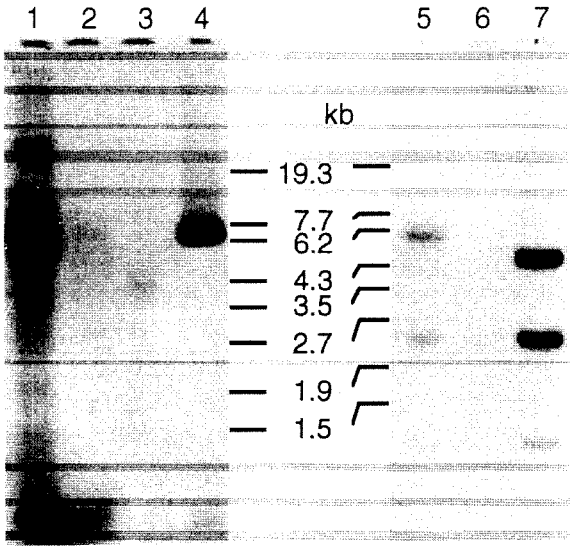

Fig. 5 Hybridization patterns of HPV 16 and 18 DNA in culture cell lines after digestion with *Eco*RI/*Bam*HI detected by Southern blot hybridization. Lanes 1, CaSki; 2, QG-H; 3, QG-U; 4, HPV 16 DNA; 5, HeLa S3; 6 C4-II; 7, HPV 18 DNA. Lanes 1-4, hybridized with HPV 16 probe; Lanes 5-7, hybridized with HPV 18 probe.

digestion with endonucleases and Southern blotting. The three HPV 16-specific DNA samples from CaSki, QG-H and QG-U showed hybrid signal bands with the HPV 16 DNA probe (Figs. 2 and 3). Likewise, DNAs from HeLa and C4-II showed a hybrid signal with the HPV 18 DNA probe (Figs. 2 and 4). Each of the band patterns after digestion with *Eco*RI, *Hin*dIII and *Bam*HI/*Eco*RI, was different (Figs. 3-5), indicating that the integration site of each HPV DNA was different.

Discussion

High-risk types of HPV DNA are found in about 90 % of anogenital cancer (1). The presence of high-risk types of HPV DNA in HeLa cells (2) and other cervical cancer cell lines (3-8) has also been reported. On the other hand, world wide contamination of HeLa cells into established cell lines has been demonstrated (26). Our previous study revealed that some epithelial cell lines like Hep-2, KB and FL are derived from HeLa cells because all these cells contain HPV 18 DNA, integrated into these cellular DNAs, showing the same hybridization patterns after digestion with endonucleases (24). In this study, HPV 16/18 DNA was detected in all cervical cancer cell lines examined by PCR and Southern blot hybridization. The HPV DNA hybridization patterns after digestion with endonucleases in the present study, however, differed among the cell lines, indicating that these cell lines are independent isolates and are not HeLa cell derivatives. Therefore, high-risk types of HPV DNA seem to be ubiquitous in cervical cancer cell lines. These cervical cancer cell lines with HPV DNA might be useful for *in vitro* studies on the carcinogenesis of HPV. For example, viral gene expression in the cells and changes in cancer phenotypes following the introduction of suppresor genes or antisense RNA of viral genes into the cells can be examined. The presence and expression of other oncogenes which may cooperate or interact with HPV oncogenes can be also studied.

HPV DNA has been detected in head and neck cancers (9-11) and in cell lines derived from them (12). The prevalence of HPV DNA in head and neck cancer tissues was 13.2 % in our previous study (11). Although the cell lines from head and neck cancer were all negative for HPV in the presest study, it might be feasible to establish head and neck cancer cell lines with high-risk types of HPV DNA.

HPV DNA was not detected in any cell lines derived

from any of the malignancies of neural or mesodermal origin. However, association of HPV in these cells may not be expected because HPV are highly tropic for epithelial cells of the skin and mucous membranes.

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