Presence of Human Papillomavirus Type 18 DNA in a Pharyngeal and a Laryngeal Carcinoma

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We examined the presence of human papillomavirus (HPV) genome in pharyngeal and laryngeal squamous cell carcinomas by employing the polymerase chain reaction. We detected HPV 16 DNA in one of 11 pharyngeal carcinomas and in 3 of 28 laryngeal carcinomas, and HPV 18 DNA in a pharyngeal and a laryngeal carcinoma. In one of the laryngeal carcinomas, DNA of both HPV types 16 and 18 were detected. To our knowledge, this is the first report of detection of HPV type 18 in head and neck carcinomas.

Key words: HPV-18 — Laryngeal carcinoma — Pharyngeal carcinoma — Polymerase chain reaction — Slot blot hybridization

Recently much attention has been paid to the possible association of human papillomavirus (HPV) with head and neck carcinomas. HPV has been detected in head and neck carcinomas by using an immunohistochemical method, *in situ* hybridization, dot blot hybridization and Southern blot hybridization.^{1–6} More recently, use of the polymerase chain reaction (PCR) has made it possible to detect a small copy number of the HPV genome in lesions.^{7–10} We have applied this method to study the association of HPV with head and neck carcinomas, and we detected DNA specific to HPV types 16 and 18 in some cases.

Squamous cell carcinoma samples were obtained from 11 patients with pharyngeal carcinoma (one female and 10 males, 36–80 years of age), and from 28 patients with laryngeal carcinomas (3 females and 25 males, 48–82 years of age). These patients were treated at the Department of Otolaryngology, Okayama University Medical School from 1981 to 1990. The clinical diagnosis of tumors was confirmed by histopathological examination.

DNA was extracted from tumors and purified as described previously.¹¹ The sequences of PCR primers and probes for hybridization were the same as those reported by Shimada *et al.*⁹ The primers and the probe for the E6 of HPV type 16 were 5'-AAGGGCGTAACCGAAATCGGT-3' (base positions 26–46 as sense primer), 5'-GTTTGCAGCTCTGTGCATA-3' (base positions 147–165 as antisense primer) and 5'-CATTTT-ATGCACCAAAGAGAACTGCAATG-3' (base positions 77–106 as probe). The sense primer for HPV type 16 was also used as that for HPV type 18, because the sequence is only two bases different from that of base positions 33–53 of the E6 of HPV 18. The antisense

primer and the probe for the E6 of HPV 18 were 5'-GTGTTTCAGTTCCGTGCACA-3' (base positions 154–172 as antisense primer) and 5'-TGAGAAACACACCAACAATACTATGGCGCGC-3' (base positions 84–113 as probe). These synthetic oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer. Amplification of DNA by PCR was done in 50 μ l reaction volumes containing 0.5 μ g of template DNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 μ M of each primer, 200 μ M each of deoxyribonucleotide triphosphate, 0.01% gelatin and 1.25 unit of Taq polymerase (Perkin Elmer Cetus, USA). The reaction was carried out on a DNA Thermal Cycler (Astec, Fukuoka) for 30 cycles of 1 min at 94°C, 2 min at 55°C and 3 min at 72°C. A part of the PCR products (10 μ l) was electrophoresed through a 3% agarose gel and visualized by ethidium bromide staining, and the remaining PCR products were denatured in 0.2 N NaOH and slot-blotted onto nitrocellulose filters (Bethesda Res. Lab., USA). The DNA on the filters was hybridized under stringent conditions with probes labeled with digoxigenin. The labeling of probes was performed with a terminal transferase kit (Boehringer Mannheim, Germany). The detection of the hybrids was performed by enzyme-linked immunoassay according to the instructions of the manufacturer.

PCR products using HPV 18 specific primers of a case of pharyngeal carcinoma DNA (Fig. 1a, lane 4), and a case of laryngeal carcinoma DNA (Fig. 1b, lane 2) showed an amplified DNA band of 140 base pairs (bp) which corresponded to those detected in positive controls, cloned HPV type 18 DNA and HeLa cell DNA containing HPV 18 genome (Fig. 1a, lanes 1 and 2). Each of these amplified DNAs hybridized with HPV 18 spe-

Fig. 1. a and b. Electrophoretic detection of HPV 18 DNA amplified by PCR. After PCR using HPV 18 specific primers, 10 μ l of the reaction mixture was electrophoresed through 3% agarose gel, and the resulting bands of 140 bp were visualized by ethidium bromide staining. Lanes in a: M, Phix DNA digested by *Hae* III as size markers; 1, HPV 18 DNA; 2, HeLa cell DNA; 3–5, DNA samples from pharyngeal carcinomas. Lanes in b: M, marker DNA; 1–4, DNA samples from laryngeal carcinomas; 5, HEC DNA; 6, without template DNA. c and d. Slot blot hybridization of PCR products with HPV 18 specific oligonucleotide probe. The sample numbers in slot blot hybridization correspond to those of lanes in a and b.

Table I. Detection of HPV DNA in Pharyngeal and Laryngeal Carcinomas by PCR^{a)}

Case	Carcinoma in	Age	Sex ^{b)}	Type of HPV
1	Pharynx	54	M	16
2	Pharynx	69	M	18
3	Larynx	60	M	16
4	Larynx	63	M	16
5	Larynx	79	M	16 and 18

a) Total cases examined were 39 (35 males of 36–82 years of age, and 4 females of 48–68 years of age).

b) M; male.

cific probe (Fig. 1c and 1d). On the other hand, the PCR products of human embryonic cell (HEC) DNA and the template DNA-free reaction mixture did not show any amplified DNA band (Fig. 1b, lanes 5 and 6), nor did they hybridize with any probe (Fig. 1d).

Similarly, using HPV 16 specific primers and probe, HPV 16 DNA was detected in one of 11 pharyngeal carcinomas and in 3 of 28 laryngeal carcinomas (Table I). Case 5 was positive for both HPV types 16 and 18 (Table I).

Association of HPV types 16^{1, 2, 4–6, 8, 10)} and 30³⁾ with head and neck carcinomas has been reported by using several HPV detection methods, but HPV 18 DNA has not previously been detected. In the present study, we detected HPV 18 DNA in a pharyngeal and a laryngeal carcinoma. The patient with pharyngeal carcinoma was a 69-year-old man, who was a non-smoker. The nature of the giant tumor was well differentiated squamous cell carcinoma located on the pharynx. The carcinoma cells had invaded to the mesopharynx and epiglottis. The patient with laryngeal carcinoma was a 79-year-old man, also a non-smoker. The nature of the tumor was well differentiated squamous cell carcinoma transglottically located on the larynx. The carcinoma cells had extensively invaded deeper layers.

Our results raise the possibility that HPV type 18 is also related to invasive squamous cell carcinomas of the head and neck. Thus, the presence of HPVs with oncogenic potential in head and neck carcinomas^{1–6, 8, 10)} suggests strongly that HPV is one of the etiological factors of head and neck carcinomas.

Kiyabu *et al.*⁸⁾ and Hoshikawa *et al.*¹⁰⁾ used PCR to detect HPV DNA in laryngeal carcinoma, and detected HPV 16 DNA in 40% and 44.4% of specimens, respectively. We also applied the PCR method and detected HPV DNA in 2 of 11 pharyngeal carcinomas (18%) and 3 of 28 laryngeal carcinomas (10.7%). Although the number of cases in this study is small, these data may suggest that the difference of HPV types and detection rate compared to other groups reflects the regional epidemic status of HPV infection.

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