Molecular cloning and characterization of a new subtype of human papillomavirus 6 DNA

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Summary. A new subtype of human papillomavirus (HPV) 6 was molecularly cloned from a laryngeal papilloma specimen. The DNA of this isolate showed a strong hybridization signal with HPV 6b DNA under stringent conditions. Comparison of restriction enzyme fragment patterns of this HPV DNA with HPV 6b DNA revealed that this DNA is not identical to HPV 6b DNA. Thus, it represents a new subtype of HPV 6 DNA, which we have tentatively designated as HPV 6e DNA. A map of the physical structure of HPV 6e was also established.

Key words: Laryngeal papilloma – Molecular cloning – Human papillomavirus 6

Introduction

Human papillomaviruses (HPVs) are associated with many types of benign and malignant tumors of the skin and mucous membranes. Currently, over 60 types of HPV have been identified [14]. HPV 6 is one of the viruses which has been found in a high percentage of genital condylomas and laryngeal papillomas [3, 4, 7, 8].

Three subtypes of HPV 6 have been molecularly cloned. De Villiers et al. [2] cloned HPV 6 DNA from genital warts and reported the existence of at least two DNA subtypes (HPV 6a and 6b). Rando et al. [10] were able to clone a different HPV 6 subtype (HPV 6c) from a vulvar carcinoma, while Boshart and zur Hausen [1] also cloned another HPV 6 subtype (HPV 6d) from a Buschke-Lowenstein tumor. However, to date an HPV 6 subtype has not been cloned from a laryngeal papilloma. We report here the molecular cloning and characterization of a new subtype of HPV 6 DNA, designated as HPV 6e DNA, isolated from a laryngeal papilloma specimen.

Materials and methods

Extraction of cellular DNA. Papillomatous tissue was removed from a 16-year-old boy who had acquired laryngeal papillomatosis 14 years earlier and had been treated surgically more than 40 times. His mother had no history of condyloma acuminata. Excised tissues were kept frozen at -70°C. Total cellular DNA was extracted by sodium dodecyl sulfate (SDS) - proteinase K treatment, which was followed by repeated phenol and chloroform extractions as described previously [9].

Southern blot hybridization. A DNA sample was digested with BamHI, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose filters, according to the method reported by Southern [12]. The filters were hybridized at high stringency (18°C below the melting temperature, Tm) with HPV 6b, 11, 16 and 18 DNA probes labeled with 32p by nick-translation [6]. Details of the hybridization procedure have also been described previously [13]. After hybridization for 2 days, the filters were washed in 2 × SSC (1 × SSC: 150mM NaCl/15mM sodium citrate, pH 7.0), 0.1% SDS at room temperature, and in 0.1 × SSC, 0.1% SDS at 65°C. The filters were then exposed to Kodak XAR film at -70°C.

Molecular cloning of HPV DNA sequences. Total cellular DNA was digested with BamHI, electrophoresed through a 0.8% agarose gel, and transferred to nitrocellulose filters, according to the method reported by Southern [12]. The filters were hybridized at high stringency (18°C below the melting temperature, Tm) with HPV 6b, 11, 16 and 18 DNA probes labeled with 32p by nick-translation [6]. Details of the hybridization procedure have also been described previously [13]. After hybridization for 2 days, the filters were washed in 2 × SSC (1 × SSC: 150mM NaCl/15mM sodium citrate, pH 7.0), 0.1% SDS at room temperature, and in 0.1 × SSC, 0.1% SDS at 65°C. The filters were then exposed to Kodak XAR film at -70°C.

Molecular cloning of HPV DNA sequences. Total cellular DNA was digested with BamHI and electrophoresed through a 0.8% agarose gel. A portion of the gel corresponding to the position of a 7.9kb linear DNA fragment was excised and the DNA was electro-eluted using a “biotrap” (Schleicher & Schuell, Dassel, FRG). Released DNA was mixed with charomid 9-36 DNA [11], digested with BamHI and ligated for 16h. The ligated DNA was packaged into a lambda phage and introduced into Escherichia coli (HB 101), using an in vitro packaging kit (Amersham International, Amersham, UK).

Infected E. coli were incubated on nitrocellulose membranes layered on agar plates containing 75μg ampicillin/ml. Colony hybridization was performed under stringent conditions with a 32P-labeled HPV 6b DNA probe. An HPV DNA-positive colony was picked up, purified and demonstrated to contain a 7.9kb insert after BamHI digestion. This recombinant charomid clone was subcloned into 2.9kb of the shortened charomid vector without cos or spacer fragments (as described previously [11]). The subcloned DNA was amplified, and the insert was released from the vector DNA after BamHI digestion for further characterization of the clone.

The HPV DNA linearized by BamHI and BamHI-linearized HPV 6b DNA were digested with a variety of restriction enzymes.
and co-electrophoresed through 1% agarose gels. The DNA bands were then visualized by staining with ethidium bromide. The DNA was transferred to a nitrocellulose filter, hybridized to HPV 6b DNA under highly stringent conditions ($T_m - 18^\circ C$).

**Results**

The sample DNA extracted from the laryngeal papilloma showed a strong hybridization signal with HPV 6b DNA, a weak signal with HPV 11 DNA, and no signal with HPV 16 and 18 DNAs. After the DNA had been digested with several restriction enzymes, the filter was hybridized with HPV 6b DNA under stringent conditions ($T_m - 18^\circ C$) (Fig. 1). *BamHI* digestion produced a single band at 7.9 kb, indicating that *BamHI* cut the circular DNA at a single site. An undigested sample and samples digested with *EcoRI* and *PvuII* exhibited bands which migrated at the positions of supercoiled and open circular DNA, indicating that *EcoRI* and *PvuII* did not cut this putative new type of HPV DNA, designated as HPV 6e.

Histological analysis of the lesion showed the typical features of squamous cell papilloma (Fig. 2). There was koilocytotic atypia in the superficial layer, but atypical mitoses or a disturbed polarity of the cells was not seen.

A genetic library was constructed, HPV DNA-positive colonies were identified, and the viral DNA was purified as described above. Restriction enzyme fragment patterns of HPV 6e DNA were then compared with HPV 6b DNA. *BamHI*-linearized HPV 6e DNA and *BamHI*-linearized HPV 6b DNA following digestion and co-electrophoresis allowed visualization of the DNA bands by staining with ethidium bromide (Fig. 3). The DNA fragments were hybridized to HPV 6b DNA under stringent conditions ($T_m - 18^\circ C$). The results were compatible with those of Southern blot hybridization analysis of the genomic DNAs. Figure 4 shows the physical map of HPV 6e DNA. In analyzing the enzyme cleavage patterns with those of HPV 6b DNA, HPV 6e DNA was found to differ in cleavage sites for *PstI*, *KpnI*, *HindIII*, *HpaI*, *AvaI* and *EcoRI*, but was identical to that of HPV 6b DNA in cleavage sites for *BamHI*, *HindII* and *BglII*. Each restriction enzyme fragment of HPV 6e DNA also hybridized at high stringency with HPV 6b DNA (data not shown), indicating that each region of HPV 6e DNA showed a strong homology with HPV 6b DNA.

**Discussion**

The DNA obtained from our patient with juvenile-onset laryngeal papilloma has demonstrated a new subtype of...