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BRIEF COMMUNICATION

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Detection of human papillomavirus type 57 in a case of inverted nasal papillomatosis in Japan

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Abstract Human papillomavirus (HPV) type 57 DNA was detected in recurrent nasal inverted papillomatosis, in a 60-year-old Japanese male, using Southern blot hybridization. HPV types 6, 11, 16 and 18 were not detectable in the papilloma. Previous studies have shown that HPV types 6, 11, 16 and 18 were mainly found in nasal papillomatosis in other laboratories but the actual detection rate of virus was low. We speculate that this low detection rate may be due to the lack of a method for detecting HPV type 57.

Key words Inverted nasal papilloma · Human papillomavirus type 57 · Southern blot hybridization

Introduction

Nasal papillomatosis has been shown by immunological methods to contain human papillomavirus (HPV) structural proteins [13]. The presence of HPV DNA in lesions has been demonstrated by DNA hybridization (for review, see [2]). Thus far, HPV types 6 [2, 7] and/or 11 [1, 2, 10, 11, 14, 17] have been mainly detected in nasal papillomatosis, and HPV type 16/18 in cancer-associated cases [1, 5, 11, 14]. However, the detection rate of HPV DNA in nasal papillomatosis has varied widely (19–76%) even in recurrent cases suggestive of HPV infection [1, 5, 7, 14, 17]. In contrast, the prevalence of HPV types 6 and 11 in juvenile and adult multiple laryngeal papillomas was almost 100% [3, 6, 15, 16].

It is probable that HPV types other than 6 and 11 also play a role in the pathogenesis of nasal papillomatosis. In unpublished studies we detected hybrid signals in DNA from a case of recurrent inverted nasal papillomatosis, using Southern blot hybridization under non-stringent conditions with HPV type 2a probe. Since the presence of HPV type 57, related to HPV type 2, has recently been reported in nasal papillomatosis [4, 18], a trial was undertaken to detect HPV type 57 in nasal papillomatosis.

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Materials and methods

Papilloma specimens

Papilloma samples were obtained surgically from the sphenoid sinus of a 60-year-old male with a fourth recurrence of inverted papilloma. A part of the resected tissue was fixed and processed for histopathological examination and the remaining portion was kept frozen at –80°C until DNA extraction.

Extraction and digestion of DNA

DNA was extracted from tumor homogenates and purified as described previously [8]. Restriction enzymes were purchased from Takara-shuzo (Kyoto, Japan). Digestion with these enzymes was performed according to the manuals provided by the manufacturer.

DNA labeling and Southern blot hybridization

HPV types 2a and 11 DNA was cloned in the Okayama University Medical School Laboratory. HPV types 6b, 16, 18 and 57 were obtained from Drs. E.-M. de Villiers and H. zur Hausen (Heidelberg, Germany). These HPV DNAs were separated from vector plasmids and labeled with digoxigenin-deoxyuridine triphosphate, using the kit prepared by Boehringer Mannheim, Germany. Electrophoresis of DNA (6 μg) after digestion with restriction enzymes was done in 0.8% agarose in E buffer (40 mM TRIS-acetate, pH 8.0, 2 mM Na_2EDTA). After gel electrophoresis, DNA was transferred to a Nytran membrane (Schleicher and Schuell, Dassel, Germany) by Southern blotting [12]. After hybridization under non-stringent conditions in 20% formamide at 42°C overnight and final washing in 0.1 × SSC, 0.1% SDS at 48°C or under stringent con-

ditions in 50% formamide at 42°C overnight and final washing at 68°C, hybrids were detected by enzyme-linked immunoassay [9].

Results

Histopathology

The excised papillomatous tissues demonstrated an inverted growth into underlying stroma (Fig. 1a). Squamous dysplasia was observed in hyperplastic epithelia (Fig. 1b). Koilocytosis was seen as pyknotic nuclei with perinuclear haloes but was present only in certain places in the upper layer (Fig. 1b). There was no evidence for carcinoma.

Detection of HPV type 57 DNA

Sample DNA extracted from papilloma specimens hybridized with HPV type 57 DNA probe under non-stringent conditions is shown in Fig. 2 while that hybridized under stringent conditions is shown in Fig. 3. Under non-stringent conditions, both HPV type 2a DNA (100 pg) and positive control HPV type 57 DNA (10 pg) hybridized with the HPV 57 DNA probe (Fig. 1, lanes 8 and 6, respectively). The sample DNA did not hybridize with HPV types 6b, 11, 16, and 18 probes, even under non-stringent conditions (not shown).

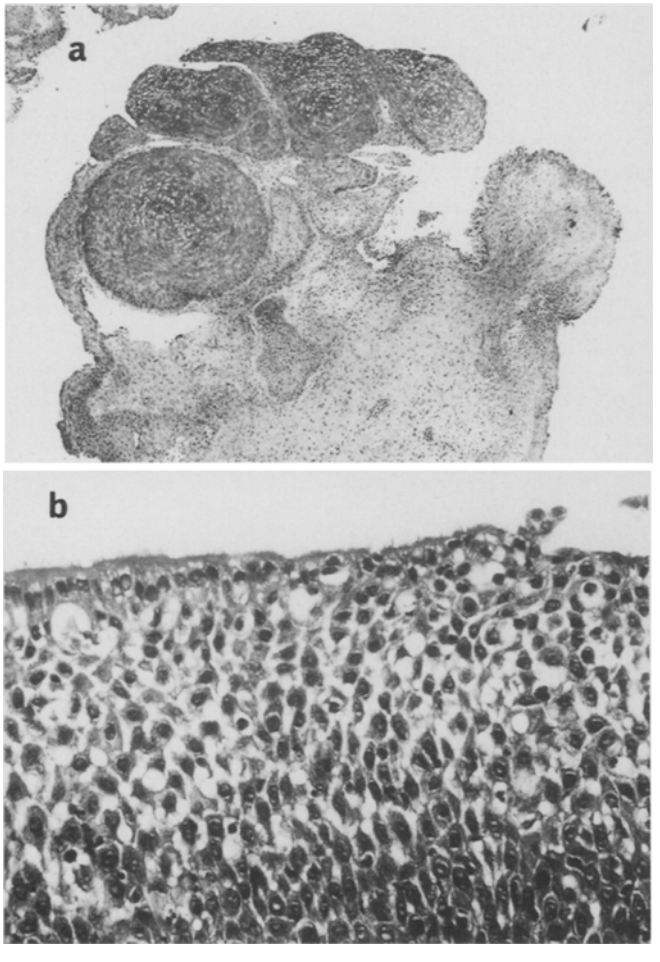

Fig. 1 **a** Histology of inverted nasal papilloma showing typical growth pattern. H&E stain, × 10. **b** Nasal papilloma under higher magnification demonstrating squamous dysplasia in hyperplastic epithelia. Some koilocytes as defined by pyknotic nuclei with perinuclear haloes are present in the upper layer. H&E stain, × 100

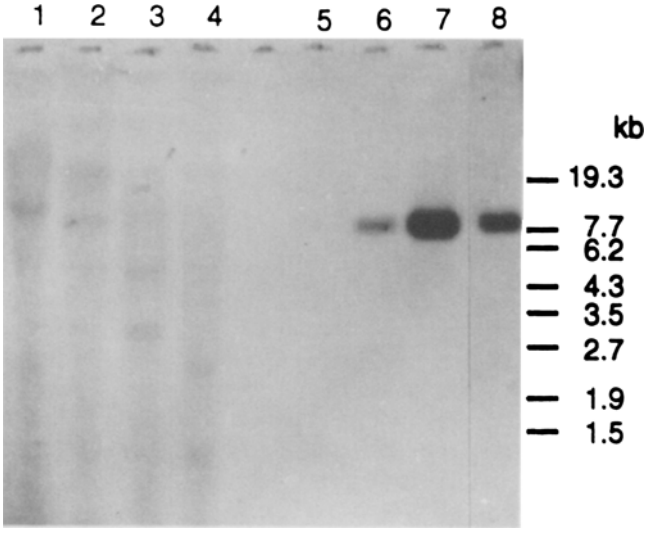

Fig. 2 Detection of HPV DNA by Southern blot hybridization under non-stringent conditions. *Lane 1* DNA digested with *Bam*HI, *lane 2 Eco*RI, *lane 3 Hinc*II, *lane 4 Pst*I, *lane 5* HPV type 57 DNA 1 pg, *lane 6* HPV type 57 10 pg, *lane 7* HPV type 57 DNA 100 pg, *lane 8* HPV type 2a DNA 100 pg

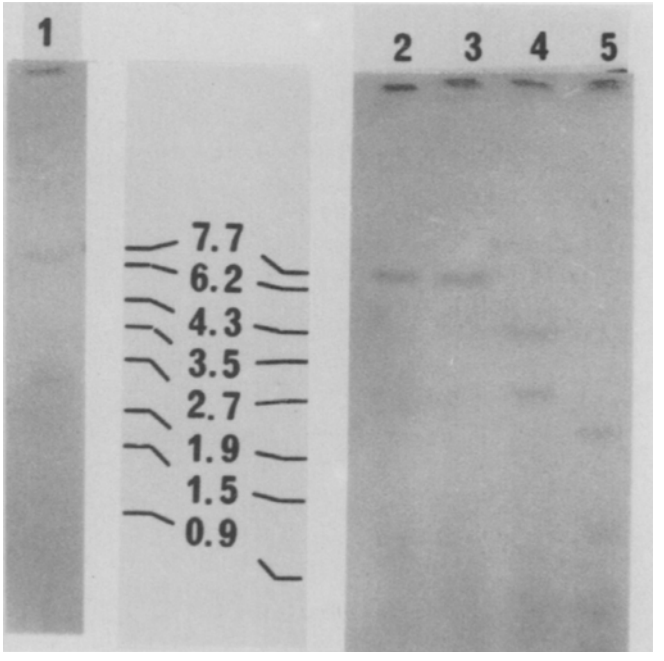

Fig. 3 Cleavage patterns of HPV 57 DNA detected in the papilloma. DNA after endonuclease digestions was hybridized with HPV DNA probe under stringent conditions. DNA was digested with following endonucleases: *lane 1 Bgl*II, *lane 2 Bam*HI, *lane 3 Eco*RI, *lane 4 Hinc*II, *lane 5 Pst*I

The cleavage patterns of the HPV 57 DNA by endonucleases *Bgl*II, *Bam*HI, *Eco*RI, *Hinc*II and *Pst*I are shown in Fig. 2. After *Bgl*II cleavage, two bands were detectable in the DNA and demonstrated the presence of two cutting sites (Fig. 3, lane 1). *Bam*HI had no cleavage site and a band corresponding to the form II DNA was demonstrable (Fig. 2, lane 1; Fig. 3, lane 2). Restriction enzymes, *Eco*RI, *Hinc*II and *Pst*I had single, double and several cleavage sites in the HPV DNA, respectively (Fig. 2, lanes 2–4; Fig. 3, lanes 3–5). At least four bands were visible after *Pst*I digestion (Fig. 2, lane 4; Fig. 3, lane 5).

Discussion

Histopathological examination of the inverted papilloma tissue specimens revealed the presence of a moderate koilocytosis, which is regarded to be characteristic for HPV infection. Southern blot hybridization under stringent conditions demonstrated that DNA isolated from the inverted papilloma hybridized with HPV type 57 DNA, which is closely related to HPV type 2 DNA. When digested with *Bgl*II, *Bam*HI, *Eco*RI, *Hinc*II and *Pst*I, the DNA yielded fragments characteristic of HPV type 57 [4]. The hybrid signal was rather weak, suggesting that the HPV type 57 DNA copy number was small in amount in the sample studied. This finding corresponded with the presence of koilocytes only in certain places in the upper layer of the papilloma studied.

In previous reports, the presence of HPV types 6 and 11 was believed to be responsible for the induction of multiple laryngeal papillomas [3, 6, 15, 16]. However, the situation in nasal papillomatosis seems to be different. To date the detection rate for HPV types 6 and 11 in nasal papillomatosis has been low. However, an association of HPV type 57 has now been demonstrated in nasal papillomatosis in Germany [4], the United States [18] and in Japan (our study). Thus, it should be understood that not only HPV types 6 and 11 but also HPV type 57 may play a role in the induction of nasal papillomatosis. Studies on the prevalence of HPV type 57 in nasal papillomatosis in Japan are now in progress.

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