A putative human papillomavirus type 57 new subtype isolated from plantar epidermoid cysts without intracytoplasmic inclusion bodies

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Human papillomavirus type 60 (HPV-60) is the only virus type that has been identified in epidermoid cysts. In this study, HPV-57 DNA was found in three out of 18 plantar epidermoid cysts with different histological features from HPV-60-associated cysts, using PCR and Southern hybridization. The HPV-57-associated cysts had features resembling an HPV-2-specific cytopathic effect. The sequences of two HPV-57 DNA clones isolated from two patients were identical, but differed at some positions from those of two reported HPV-57 subtypes, HPV-57a and HPV-57b. This putative new subtype was tentatively designated as HPV-57c, and may be associated with plantar epidermoid cysts showing histological features resembling the HPV-2 cytopathic effect.

Human papillomavirus (HPV) has recently been identified frequently in palmar plantar epidermoid cysts (Egawa et al., 1987, 1990, 1995; Kimura et al., 1987; Matsukura et al., 1992), suggesting that HPV may play a role in the pathogenesis of these cysts, and HPV-60 is the only HPV type that has been identified in epidermoid cysts so far (Matsukura et al., 1992; Egawa et al., 1994, 1995; Kawase et al., 1994). It has also been well-established that homogeneous intracytoplasmic inclusion bodies in the cyst wall and vacuolar structures in the cyst cavity are pathognomonic for HPV-60 infection in the cysts (Matsukura et al., 1992; Egawa et al., 1993a, 1994, 1995).

Here we have isolated a putative new HPV-57 subtype from plantar epidermoid cysts using PCR and Southern hybridization. Instead of intracytoplasmic inclusion bodies and vacuolar structures, the histological features of the HPV-57-associated cysts were like those of the HPV-2-specific cytopathic effect (Gross et al., 1982; Jablonska et al., 1985). We have previously found a plantar epidermoid cyst with inverted growth, which histologically showed HPV-2-related features (Egawa et al., 1993b), and we identified an HPV-57 DNA fragment using PCR (Egawa et al., 1998). To establish whether that was an isolated finding or a reflection of a close relationship between HPV-57 and plantar epidermoid cysts, we searched for HPV-57 in 18 plantar epidermoid cysts with histological features similar to those of the first HPV-57-related case, using PCR and Southern hybridization. Sequencing analysis of the amplified HPV-57 DNA fragments was also performed and sequences of cysts were compared with each other and with those of two reported HPV-57 subtypes, HPV-57a and HPV-57b.

Sections (4 µm) were serially prepared from formalin-fixed and paraffin-embedded specimens of the 18 epidermoid cysts and were stained with haematoxylin–eosin for conventional histological studies. On microscopy, instead of intracytoplasmic homogeneous inclusion bodies in the cyst wall or vacuolar structures within the keratinous mass, which are pathognomonic for HPV-60-related epidermoid cysts, the characteristic histological features of these cysts were vacuolated cells or condensed keratohyalin granules in the granular cell layer, or parakeratotic nuclei in the keratinous mass within the cyst cavity. These findings most closely resembled the HPV-2-specific cytopathic effects, but the extent of the histological changes varied among the lesions. We therefore performed an HPV type-specific PCR, as described by Saiki et al. (1988), using a thermal cycler (Perkin Elmer PTC 200) to detect HPV-2-related viruses (HPV-2, -27 and -57) and to check whether HPV-60 was present as a dual infection in the cysts. The HPV type-specific primer pairs from the L1 region of each virus and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown in Table 1. We designed the primer pairs to amplify approximately 300 bp, because the PCR product...
Table 1. Sequences and positions of the PCR primers

<table>
<thead>
<tr>
<th>Primer*</th>
<th>Sequence (5’ → 3’)</th>
<th>Position (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDHF</td>
<td>TCCCCTGCCAGCTAGTGACC</td>
<td>2278–2301</td>
</tr>
<tr>
<td>GAPDHB</td>
<td>GCCCAGGCCCACTACAGAAG</td>
<td>2474–2511</td>
</tr>
<tr>
<td>HPV-2F</td>
<td>GTGGGCGTGGGGACTAGAGA</td>
<td>6083–6103</td>
</tr>
<tr>
<td>HPV-2B</td>
<td>GGGCAGTCACCAGCAGCAGA</td>
<td>6334–6313</td>
</tr>
<tr>
<td>HPV-27F</td>
<td>GTGGGCGTGGGGACTAGAGA</td>
<td>6060–6081</td>
</tr>
<tr>
<td>HPV-27B</td>
<td>GGGCAGTCACCAGCAGCAGA</td>
<td>6305–6282</td>
</tr>
<tr>
<td>HPV-57F1</td>
<td>GCCGAGGAGCAATGTTC</td>
<td>6491–6509</td>
</tr>
<tr>
<td>HPV-57B1</td>
<td>GCCCTCCGGAGGCTCTTA</td>
<td>6605–6609</td>
</tr>
<tr>
<td>HPV-57F2</td>
<td>GGGGTCTCGGCTGAGGGG</td>
<td>6271–6289</td>
</tr>
<tr>
<td>HPV-57B2</td>
<td>ATCCGAGGCTGCTGAGGG</td>
<td>6561–6543</td>
</tr>
<tr>
<td>HPV-57F3</td>
<td>CAGCCTCTGGGTGTAGGG</td>
<td>6081–6098</td>
</tr>
<tr>
<td>HPV-57B3</td>
<td>AAAATCCAGGCGGCGCCAAC</td>
<td>6371–6352</td>
</tr>
<tr>
<td>HPV-60F</td>
<td>TAGCCCAAAACTGATAAAC</td>
<td>6233–6253</td>
</tr>
<tr>
<td>HPV-60B</td>
<td>ATGGCGTAAATACTGCTAAA</td>
<td>6519–6499</td>
</tr>
</tbody>
</table>

* F, forward primer; B, backward primer.

DNA was extracted from the 18 paraffin-embedded specimens of plantar epidermoid cysts using a DEXPAT kit (Takara), and was first subjected to GAPDH PCR to confirm the presence of suitable DNA. The positive controls consisted of DNAs extracted from formalin-fixed, paraffin-embedded specimens of HPV-2- and HPV-60-positive warts (683-2, 798-1 and V-1280) using the same kit.

GAPDH PCR-positive samples (data not shown) were then subjected to specific PCRs for HPV-2, -27, -57 and -60 (primers are shown in Table 1). Each PCR was performed in a total volume of 50 µl with 1 µM primer, 1 U Taq polymerase (Perkin Elmer), and the following buffer: 1·5 mM MgCl₂, 50 mM KCl, 10 mM Tris–HCl (pH 8·3), 0·001% (w/v) gelatin, and 200 µM

Fig. 1. HPV-2, -27, -57 and -60 PCR Southern hybridization. 683-2, 798-1 and V-1280 were used as positive controls for HPV-2, -27 and -60 PCR, respectively.
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Fig. 2. L1 nucleotide and amino acid sequences of three HPV-57 subtypes (nucleotide positions 6081–6684). Only differing amino acids are shown for HPV-57b and -57c in comparison with HPV-57a.

of each dNTP (Perkin Elmer). Amplification consisted of 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C (HPV-2, -27 and -57) or 45 °C (HPV-60) for 2 min, and extension at 72 °C for 2 min (the initial denaturation step was 5 min and the final extension step was 10 min). The PCR products were separated on 2% agarose gel and stained with ethidium bromide, revealing that approximately 300 bp fragments were amplified in the KE-1 sample by HPV-57 primers but not by HPV-2, HPV-27 or HPV-60 primers (data not shown). Amplified PCR product was then transferred to a nylon membrane (Boehringer Mannheim) under alkaline conditions. The membrane was hybridized with 32P-labelled specific DNA probes, which were made using a Rediprime labelling system (Amersham), [α-32P]dCTP and HPV plasmid DNAs, in 6× SSC, 5× Denhardt’s solution, 0.1% SDS, 100 µg/ml sonicated salmon sperm DNA at 65 °C overnight, then washed in stringent conditions. HPV-2 plasmid DNA was kindly provided by E.-M. de Villiers (German Cancer Research Center, Heidelberg, Germany), and HPV-27, HPV-57 and HPV-60 DNAs were cloned from warts using L1 degenerate primers (Shamanin et al., 1994) in our laboratory.

Three samples (KE-1, 96645 and V-1249) were positive by HPV-57 type-specific PCR Southern hybridization, while no sample was positive for HPV-2, -27 or -60 (Fig. 1). HPV-57
type-specific PCR products obtained from two samples (KE-1 and 96645) were further cloned into the pBlueScript vector (Stratagene) using the TA cloning technique. The cloning was not successful for sample V-1249 because of the low amount of PCR product. In order to get further sequence data, KE-1 and 96645 DNAs were amplified with HPV-57-2 and HPV-57-3 primer pairs (Table 1), respectively, and the resulting approximately 300 bp fragments were subcloned into pBlueScript using same method. In order to exclude the sequencing errors, not only both strands of the cloned fragments were sequenced but also several colonies were picked up for each cloning and those DNAs were subjected to sequence reactions. The sequences were determined with a Butast sequencing kit (Takara) using T7 and universal primers. The sequence data were combined in each sample, then the 603 bp sequences were compared with each other and aligned with data on HPV-57a (nucleotide position, 6081–6684; GenBank no., X55965; Hirsh-Beham et al., 1990) and HPV-57b (GenBank no., U37537; Trujillo et al., 1996) using a Blastn program through the Internet and those amino acids were also aligned (Fig. 2).

Interestingly, not only were the HPV-57 sequences obtained from the two different epidermoid cysts identical to each other, but they also differed in some positions from the sequences of HPV-57a and HPV-57b. Therefore, we tentatively designated the virus as HPV-57c. For amino acid alignment in the sequenced 603 bp, divergence was found in eight amino acids between HPV-57c and HPV-57a/b: in these eight amino acids, HPV-57c was identical to HPV-57b in five amino acids and identical to HPV-57a in two amino acids, but different from both in one amino acid. The rest of the sequence was identical among the three subtypes (Fig. 2).

HPV-57 is closely related to HPV-2 and HPV-27 (Hirsh-Beham et al., 1990; Delius & Hofmann, 1994). Although HPV-57 was initially isolated from a mucosal inverted papilloma, it was subsequently found in an oral wart, a genital condyloma acuminatum, and common skin warts (Rübben et al., 1993). Thus, HPV-57 is considered to be a papillomavirus associated with mucosal tumours that can also infect cutaneous epithelium. In the present study, it was suggested that HPV-57c is associated with plantar epidermoid cysts. HPV-57a was originally detected in an isolated case of inverted papilloma (de Villiers et al., 1989), which shows endophytic growth in the mucosa and commonly occurs in the nasal cavity and paranasal sinus in humans (Christensen & Smith, 1986), and subsequently HPV-57b was frequently found in inverted papillomas (Wu et al., 1993). Interestingly, one HPV-57c-associated case studied here showed extreme endophytic growth of acanthotic epidermis, creating an epidermoid cyst. The growth pattern was very similar to that of mucosal inverted papillomas.

The histological features of the cyst in which HPV-57c was identified were very similar to those of HPV-2-associated warts, suggesting that HPV-57c may be associated with plantar epidermoid cysts with histological features resembling the HPV-2-related cytopathic effect. Interestingly, the sequence variant of the non-coding region of the HPV-57-associated wart has also recently been reported (GenBank nos, AF026960-AF026967; Chan et al., 1997).

Although further studies, including whole sequencing of the HPV-57 subtype associated with the epidermoid cyst using frozen materials obtained from much larger number of cases, are required to clarify whether a specific HPV-57 subtype is associated with the epidermoid cyst with the specific cytopathic effect, on the basis of our results, it seems likely that HPV-57 may be associated with the inverted growth of not only mucosal but also cutaneous epithelium, and may then play a role in the pathogenesis of the plantar epidermoid cysts.

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References


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