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A Novel Genital Human Papillomavirus (HPV), HPV Type 74, Found in Immunosuppressed Patients

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The genome of a novel human papillomavirus (HPV) type, HPV74, was cloned from an iatrogenically immunosuppressed woman with persisting low-grade vaginal intraepithelial neoplasia. HPV74 was found to be phylogenetically related to the low-risk HPV types 6, 11, 44, and 55. HPV74 or a variant of this type was found in specimens from three additional immunosuppressed women but not in about 3,000 anogenital specimens from immunocompetent patients.

Genital human papillomaviruses (HPVs) are responsible for the most commonly diagnosed, viral sexually transmitted diseases (10). They cause genital warts and are associated with the cytological abnormalities found in cervicovaginal smears and diagnosed as squamous intraepithelial lesions. Squamous intraepithelial lesions may reflect the presence of cervical intraepithelial neoplasia, which includes the precursors of invasive cervical carcinoma (15, 16). More than 35 HPV types infecting specifically genital mucous membranes have been characterized to date (7). Sequence alignments have allowed the construction of phylogenetic trees that reflect the biological properties of genital HPVs (5, 23). In spite of the great multiplicity of previously recognized HPVs, HPV DNA sequences different from those of known types are detected in genital specimens by Southern blot hybridization experiments performed under nonstringent conditions or by PCR analyses conducted with consensus or degenerate primers (3, 14). The characterization of such HPVs is required to fully understand the epidemiology and the natural history of genital HPV infections. We report here the molecular cloning and the characterization of a novel genital HPV type, HPV74, phylogenetically related to HPV types associated with a low risk of cancer development.

The genome of HPV74 was cloned from persisting vaginal lesions of low-grade intraepithelial neoplasia of an immunosuppressed woman who had undergone renal transplant. Total DNA was extracted from vaginal scrapes, treated with *Pst*I endonuclease, and analyzed by Southern blot hybridization with a mixture of ^{32}P-labeled HPV6, -11, and -42 DNAs at low stringency (40°C below transition temperature [T_m − 40°C]) (2). Three DNA fragments were detected, a one-genome length fragment (7.9 kb) and two fragments of 5.6 and 2.2 kb (Fig. 1, lane 1). After the membrane was washed under more stringent conditions (T_m − 20°C) (2), the two smaller fragments were very faint while the 7.9-kb fragment still yielded a significant signal (Fig. 1, lane 2). The 5.6- and 2.2-kb fragments correspond to HPV61 DNA sequences, as demonstrated by hybridization with an HPV61 DNA probe under stringent conditions (data not shown). The 7.9-kb fragment could correspond to a new HPV, since none of the known genital HPV types yields a one-genome length fragment after cleavage with *Pst*I endonuclease (14).

The viral DNA was cloned after insertion into the bacteriophage λ ZAPII DNA (Stratagene, La Jolla, Calif.) (19). Of the multiple cloning sites of this vector, *Spe*I was the only one to allow insertion of the full-length HPV DNA. The pBluescript SK$^-$ phagemid containing the cloned viral DNA was excised from the recombinant bacteriophages (19). The cloned HPV DNA used as a probe detected only the 7.9-kb *Pst*I fragment after hybridization under stringent conditions (Fig. 1, lane 3). The degrees of similarity between the cloned DNA and the DNAs of 62 HPV types were analyzed by Southern blot hybridization at high stringency (60% formamide; T_m − 10°C)

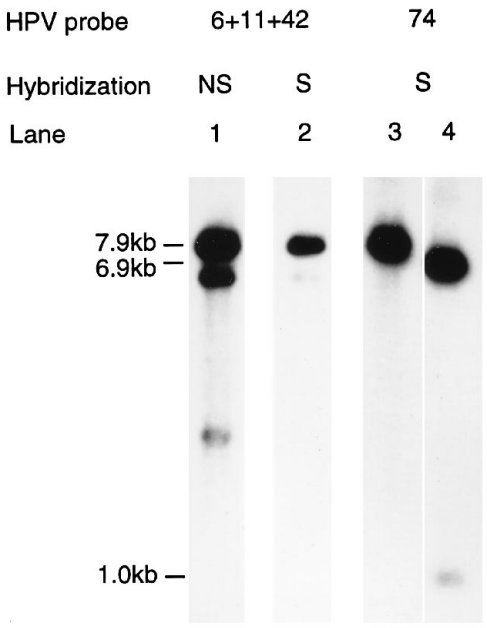

FIG. 1. Southern blot hybridization analysis of HPV DNA sequences in vaginal and cervical specimens. Total cellular DNA preparations (1.5 μg) extracted from vaginal (lanes 1 to 3) and cervicovaginal (lane 4) scrapings obtained from two patients were cleaved with endonuclease *Pst*I. The fragments were separated by electrophoresis in 1% agarose gels, denatured in situ, and transferred to nylon membranes (Amersham, Les Ullis, France). One membrane was hybridized under nonstringent conditions (T_m − 40°C) with a mixture of ^{32}P-labeled HPV6, -11, and -42 DNAs (lane 1) and washed under more stringent conditions (T_m − 20°C) (lane 2). The other membrane was hybridized under stringent conditions (T_m − 10°C) with an HPV74 DNA probe (lanes 3 and 4). The sizes of the HPV74 DNA fragments are indicated.

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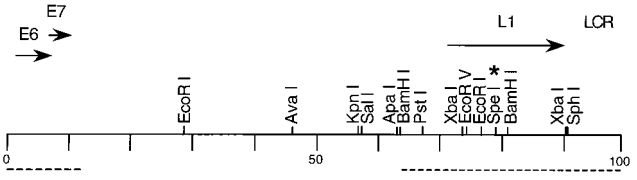

FIG. 2. Physical map of HPV74 DNA. Dashes underline the sequenced segments of HPV74 DNA. The positions of the L1, E6, and E7 ORFs and of the long control region (LCR) are shown at the top of the figure. The origin of the map has been deduced from alignment with HPV13 genome (22). The cloning *Spe*I site is indicated by a star. The enzymes *Bgl*II, *Cla*I, *Hin*dIII, *Not*I, *Sac*I, *Sac*II, *Sma*I, and *Xho*I have no cleavage site in HPV74 DNA.

(2). Substantial cross-hybridization was observed with HPV13, -44, and -55 DNAs, and a weak cross-hybridization was observed with HPV6 and -11 DNAs (data not shown). All these HPV types are phylogenetically related (5, 23) and are associated with benign lesions of oral (HPV13) and genital (HPV6, -11, -44, and -55) mucous membranes. A physical map of the cloned DNA was constructed by using 19 restriction endonucleases (Fig. 2).

To further characterize the cloned DNA, we sequenced a 3,892-bp DNA region encompassing the 3′ end of the L2 open reading frame (ORF), the L1 ORF, the long control region, the E6 and E7 ORFs, and the 5′ end of the E1 ORF (Fig. 3). After subcloning of the appropriate restriction fragments into pBluescript II vector (Stratagene) was carried out, sequence determination was performed in both orientations by the dideoxy method, first with primers selected from the vector sequence and then with synthetic oligonucleotides (Genset, Paris, France) chosen from newly established sequences. The sequences were analyzed and compared with available HPV sequences by using the Genetics Computer Group (Madison, Wis.) sequence analysis package and the FASTA program. Pairwise alignment of the nucleotide and the deduced amino acid sequences of the E6, E7, and L1 ORFs disclosed that HPV74 was related to HPV6 (17), HPV11 (6), HPV13 (22), HPV44, and HPV55 (6a) and to pygmy chimpanzee papillomavirus type 1, a simian virus closely related to HPV13 (22). HPV74 was found to be most closely related to HPV44 and HPV55 (Table 1). It has been accepted until recently that, to be recognized as a novel type, a newly cloned HPV DNA should have less than 90% nucleotide sequence identity with the E6, E7, and L1 ORFs of known types (7, 15). According to the revised definition adopted at the 14th International Papillomavirus Conference held in Quebec City in 1995, a DNA sequence identity with the L1 ORF of less than 90% is sufficient to define a novel type. The percentages of nucleotide identity between HPV74 and HPV types 44 and 55 are less than 90%, supporting the argument that HPV74 is a novel HPV type.

The long control region of HPV74 is 783 bp long, and its organization (Fig. 3) is typical of the regulatory region of

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   1  GGATCCTGCT TTTTTATCCA GTCCTCAACG CCTTATAACC TTTGATAATC CAGTATATGA AGGTGAGGAT GTTAGTTTGC AATTTCAGCA CGATACAATA
 101  CATAATCCTC CTGATGATGC CTTTATGGAT ATTATTCGCT TACACAGACC TGCTATAACG TCACGGCGTG GTCTTGTACG GTTTAGTAGG ATTGGGCAAC
 201  GGGGGTCTAT GCATACACGC AGTGTAAAGC ATATAGGTGC ACGGGTGCAT TTTTTTCAAG ACATATCTCC TATATCTGCT GCAGAAGAAA TAGAACTGCA
 301  CCCTCTTGTG GCTCATGCAC AGGATAGCAG TGGCTTATTT GATGTTTATG CAGAACCTGA CCTTGAGGTT ATGGAAGAAC CTGTTTCATT GTCTTTTCCA
 401  ACATCCACAC CCTTTCAGCG GTCGGCTGTG TCAGCCACAT GGGGCAATAC TACTGTCCCT CTTTCATTGC CAGGTGACAT GTTTATACAG CCTGGTCCTG
 501  ACATAATGTT TCCAACTGCA TCTTCTACAA CACCCTATAA TCCTGTTACT CCTGCTTTAC CTACAGGTCC TGTTGTTATA CATGGTTCTA CATTTTATAT
 601  ATATCCATCA TGGTATTTTG CACGCAAACG CCGTAAACGT GTTCCCTTGT TTTTTACAGA TGTGGCGGCC TAGTGACAAC CAGGTTTATG TGCCTCCTCC
 701  CGCCCCTGTA TCCAAAGTCA TTTCCACGGA TGCCTATGTT ACACGCACCA ACATCTTTTA TCATGCTAGC AGTTCTAGAC TACTTGCTGT AGGAAATCCC
 801  TATTTTCCCA TAAGGCAAAG CAACAAAACA GTTGTTCCTA AGGTGTCTGG ATATCAATTT AGAGTGTTTA AGGTGGTTTT GCCAGATCCC AATAAATTTG
 901  CTTTACCTGA CACTTCTATA TTTGACCCCT CCAGTCAACG CCTGGTATGG GCCTGTACGG GTTTGGAGGT AGGTAGGGGA CAGCCTTTAG GTATTGGCAT
1001  TAGTGGTCAC CCTTTATTAA ATAAATTAGA TGATGTGGAG AATTCAGCTA GTTATGCAGC TAATCCTGGT CAGGATAACA GGGTTAATGT TGCTATGGAC
1101  TATAAGCAGA CACAGTTATG CATGGTAGGC TGTGCTCCCC CGTTAGGTGA ACACTGGGGA AAAGGCAAGC AATGTTCTAA TGTTAATGTA AAACCAGGCG
1201  ATTGCCCTGC ATTAGAATTA ATTACTAGTG TAATTCAGGA TGGTGATATG GTAGATACAG GCTTTGGTGC CATGAATTTT GCTGACTTGC AGCCTATTAA
1301  ATCTGATGTT CCATTGGACA TATGCAACAC TACATGCAAA TATCCTGATT ATTTACAAAT GGCTGCGGAT CCATATGGTG ATAGATTGTT TTTTTACTTA
1401  CGCAAGGAAC AAATGTTTGC AAGACACTTT TTTAATAGGG CTGGAACAGT GGGTGAGGAC ATTCCCCAGG ACCTATATAT TAAAGGTACT ACACGCAGTA
1501  CGCTTGCCAA TGCTATTTAT TTTAATACTC CCAGTGGCTC TCTTGTATCT TCTGAAACAC AGTTGTTTAA TAAGCCGTTT TGGCTGCAAA GGGCGCAAGG
1601  CCACAATAAT GGTATTTGTT GGGGTAATCA ATTATTTGTT ACAGTTGTGG ATACCACACG CAGTACTAAC ATGACTGTGT GTGCTCCTAC CTCACAATCG
1701  CCTTCTGCTA CATATAATAG TTCAGACTAC AAACAATACA TGCGACATGT GGAGGAATTT GATTTGCAAT TTATTTTTCA ATTATGTAGT ATTAAGTTAA
1801  CTGCTGAGGT TATGGCCTAT ATTCATACTA TGAATCCTAC AGTTTTAGAA GAGTGGAACT TTGGGCTAAC GCCTCCCCCC AATGGTACTT TAGAAGACAC
1901  CTACAGATAT GTGCAGTCCC AGGCTATTAC ATGTCAAAAA CCTACGCCTG ATAAAGCAAA GCCCAATCCC TATGCAAATT TAAGTTTTTG GGAAGTTAAT
2001  CTTAAGGAAA AGTTTTCTAG TGAATTAGAT CAATACCCCC TTGGTAGAAA GTTTTTATTG CAGACAGGCG TGCAGGCACG CTCTTCCGTT CGTGTAAGTA
2101  AAAAACGGTC GGCCCCTACA GCCCCCTCCT CAGCCACTAA ACAAAAACGC TCTAGAAAAC GATAGCATGC TGTTGTTGTG TGTGTAATAT GTTGTTGCAT
2201  GGTATATGTG TGTTGTATTA TATTGTGTGT CATATGTTGT TGTTGTGGTA TATATGTTGT ATGTTCCTGT GTGTCATATT GTATGTGTCA TGCTGTGTAA
2301  TGTTGTATGT ACTGCATGTA TGTGTTTATT GTTGTATGTA CTGCATGTAT GTGTTTGTTG TTTTCAATAA ACTTTATTTT TGTGTGTTGT GTACCCTGTA
2401  CGTTTGCATG GTATTTTATA CTTTTATATA CCCATTTTAC CTTTTTATAA CCCTTTCCCC TCCATTTTTT GTGCAACCGT TTTCGGTTAT TACTCATCTC
2501  TCCACATCCT GTAACCAATT TGTTGCAGCA AGCATTTTAT GTAATCCTAT ATACTGTTTG CCAAGGTCAT ACTGTGTTGC CAAGTACACA CCAGGTGCAC
2601  AAATAGGCGC GGTTTGGCAT CCACATAATA CATTCCTAAT CTATATACCA CTCCTGTCTG CCTGTTTAAC AATTGGTTGC TTTTCTAATA CATTTTTGTA
2701  GTTGGTGGCC AACTCTTAAA AGCATTTTTG GCTGCCAGTA CAACATTTTT GTACAGTTAC TGTTAGGTTT ATATAATGAG TAACCTAGGG TTACACACCG
2801  CAACCGGTAT CGTTAACACA CACCCTGTGC CCTTCTTATT ATACATAATA ACAATTTAAA CTTTGTAAAA AATAGGAGGA ACCGAAAACG GTTCCAACCG
2901  AAAACGGTTA TATATAAACC AGCCCAAAAA TTAAGCAAGC GGGGCATAAT GGAAAGTGCA AATGCCTCCA CGTCTGCAAA AAGTATAGAC CAGTTGTGCA
3001  AGGACTGCAA CATTCCTATG CACAATTTGC AAATTTCATG CGTGTTTTGC AGGAAAACCT TGTCTGTTCC AGAGGTATAT TCATTTGCCT ATAAAAATTT
3101  GTATATAGTA TACCGAGGAA ACTTTCCATT TGCAGCGTGT GCCATTTGTT TAGAAGTACA AGGTAAAGTA AATCAGTTTA GACACTTTAA CTACGCGGGA
3201  TATGCGTTAA CAGTGGAAGA AGAAACAAAA AAGTCAATTT TGGATGTGCT AATACGCTGC TACCTGTGCC ATAAGCCGTT GTGCCACGTG GAAAAAGTAC
3301  GGCACATTGT GGAAAGGGCA AGATTTATTA AACTAGGCAA CACCTGGAAA GGACGCTGCT TTCATTGCTG GACAACATGC ATGGAAAATA TTCTACCTTA
3401  AAGGAAATTG TGTTAGAGCT ACAACCTGAC CCTGTAGGCC TACATTGCAA TGAGCAATTA GACAGCTCAG AAGAGGAGGT GGATGAACTA GCCACGCAAG
3501  CCACGCAACA ACTGACACAG GCGTACCAAA TAGTAACCTG CTGTGGTGTA TGTAATCGCA GCCTTCGGCT GGTTGTGCAG TGTACAGGAC CTGACATCAA
3601  CAATCTACAT ACGCTTCTAC TGGGTACACT GAACCTAGTG TGTCCTCTGT GTGCGCCCAA AACGTAACGA CAATGGCGGA AAATTCAGGT ACAGAGGGAA
3701  CGGGGTGCTC AGGGTGGTTT TTAGTAGAAG CAATAGTAGA ACATACAACA GGGCAGCAGA TTTCAGAGGA TGAGGATGAA GCAGTAGAGG ATAATGGGTC
3801  TGACATGGTA GACTTTATAG ATGACAGGCC GATTACACAC AATTCCTTGG AAGCACAGGC ATTATTAAAC GAGCAGGAGG CGATCTCATT AT
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FIG. 3. Partial nucleotide sequence of the HPV74 genome. The four bona fide binding sites for the viral E2 regulatory protein ($ACCGN_4CGGT$) and the putative binding site for the E1 replication protein (9) containing nucleotide 2844 homologous to nucleotide 1 of the HPV6, -11, and -13 sequences (6, 17, 22) (arrowhead) are shaded. Both the first ATG of the L1 ORF and the second ATG, corresponding to the initiation codon of the HPV6, -11, or -13 L1 gene, and the initiation codons of E6 and E7 ORFs are underlined. The stop codons of L1, E6, and E7 ORFs are indicated by dotted lines below the sequence.

TABLE 1. Percentages of nucleotide and deduced amino acid sequence identity between HPV74 and related types

Virus	% Nucleotide sequence identity with HPV74 at ORF:			% Amino acid sequence identity with HPV74 at ORF:		
	E6	E7	L1	E6	E7	L1
HPV44	85.7	86.0	84.1	88.7	76.3	86.1
HPV55	85.7	86.0	83.5	86.0	79.2	87.3
HPV13	81.5	77.8	82.2	78.0	73.3	85.0
PCPV[a]	80.6	80.1	81.8	77.3	76.5	85.1
HPV6b	71.3	74.4	77.4	71.3	66.3	81.1
HPV11	74.4	71.4	76.5	70.7	63.0	81.1

[a] PCPV, pygmy chimpanzee papillomavirus.

genital HPVs (9). On the basis of deduced amino acid sequences, the HPV74 E6 and E7 oncoproteins are 150 and 96 amino acids long, respectively. The L1 major capsid protein may be 501 amino acids long, a size close to that of HPV6, -11, and -13 L1 proteins, or 527 amino acids long as a result of the presence of another ATG located upstream (Fig. 3), a feature shared by HPV44 and HPV55 (6a). Amino acid sequences of the E6 and L1 proteins of HPV74 and related HPV types were aligned with the CLUSTAL W program (21), and phylogenetic trees were generated with the phylogenetic inference package (PHYLIP 3.5) (20). Similar trees were obtained for the two proteins, either by the maximum sequence parsimony analysis or the distance matrix analysis. As illustrated for the E6 protein (Fig. 4), the phylogenetic analysis confirmed the close phylogenetic relationship of HPV74 with HPV44 and HPV55.

HPV74 or a variant with an additional *Pst*I cleavage site (Fig. 1, lane 4) was detected in cervicovaginal scrapings obtained from three other patients of the same follow-up study involving 81 renal allograft recipients (5a). Two of these patients had normal cervical cytologies, and the third one presented with a low-grade cervical intraepithelial neoplasia. In two of these specimens, HPV74 was found to be associated with another HPV type (HPV16 or HPV55). In contrast, we did not find any evidence for HPV74-like DNA sequences, i.e., a high-molecular-weight *Pst*I DNA fragment cross-hybridizing with HPV6 and -11, in the course of screening about 3,000 anogenital specimens from the general population.

HPV74 is phylogenetically related to HPV types that are not significantly involved in the development of invasive cervical carcinoma of the uterine cervix (4, 13, 14). HPV44 and -55 were cloned from genital warts (8, 12) and were found in cervical specimens of women with normal cytologies or low-grade squamous intraepithelial lesions (5a, 13). HPV13 causes oral focal epithelial hyperplasia that never progresses towards malignancy (15, 22). HPV6 and -11 are the most common agents of anogenital warts and laryngeal papillomatosis and are associated with low-grade cervical lesions. HPV6 and -11 seldom express an oncogenic potential, if one excepts the rare anogenital Büschke-Löwenstein tumors and single case reports of carcinoma of the respiratory tract (10, 15). The lack of detection of HPV74 in a large series of immunocompetent patients may indicate that it is a very infrequent HPV type. Immunosuppressed women are known to be at risk for genital HPV infection and cervical intraepithelial neoplasia (1, 11). The relatively high detection rate (5%) of HPV74 in our series of renal transplant patients suggests that this HPV type is rather ubiquitous but causes infections that are efficiently controlled by the host immune defenses.

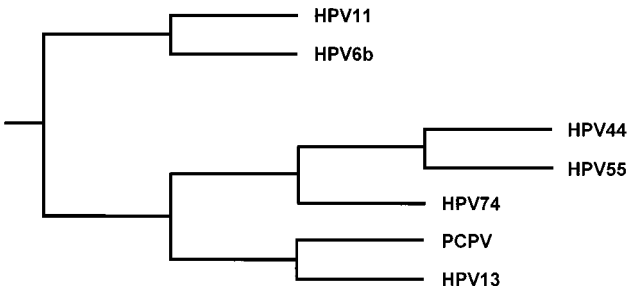

FIG. 4. Phylogenetic relationships among HPV74-related types. A tree was constructed from the comparison of aligned E6 amino acid sequences by using distance matrix algorithms in the PHYLIP 3.5. package (20). The tree was rooted by taking HPV16 (18) as an out-group.

Nucleotide sequence accession number. The nucleotide sequence accession number U40822 has been assigned to HPV74.

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