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Characterization of a Novel Human Papillomavirus DNA in the Cervical Carcinoma Cell Line ME180

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The human cervical carcinoma cell line ME180 was examined for human papillomavirus (HPV) DNA and RNA. The integrated DNA of a presumably new HPV type showing a relationship closer to HPV39 than to HPV18 was cloned and sequenced. HPV sequences from the E6-E7-E1 region are expressed as poly(A)$^+$ RNAs.

Several human papillomavirus (HPV) types are suspected to contribute to the development and growth of cervical carcinomas, since they have been found to be associated with these cancers and with precursor lesions that have a high risk of malignant progression (for reviews, see references 26 and 29). The subgroup of cancer-associated genital HPVs is represented in particular by HPV types 16 (HPV16) and 18, and by several additional HPV types (HPV31, 33, 35, 39, 45, 51, 52, 56, and 58) (6). For the analysis of the viral components and the virus-host cell interactions involved in carcinogenesis, cell lines established from human cervical carcinomas have turned out to provide valuable model systems. HPV16 or HPV18 DNA has been found as a constituent of the cell genome in several cell lines, and the E6 and E7 genes are selectively expressed (1, 8, 11, 17, 18, 20). An integrated state of HPV DNA and the expression of the E6 and E7 genes are also properties of many HPV-positive cervical carcinomas, indicating their functional importance for cancer cell growth both in tissue culture and in the organism. Indeed, the E6 and E7 genes exhibit transforming activities in rodent and human cells (for a review, see reference 26) and are important for cell proliferation in vitro (25) and apparently also in vivo (2). The E7 and E6 gene products seem to act, at least in part, via complex formation with the pRB and p53 tumor suppressor proteins (7, 27). In cell lines and tumors, the integrated HPV DNA is usually disrupted in the region covering the open reading frames (ORFs) E1 and E2 (3, 18). The consequent inactivation of E2 is thought to contribute to dysregulation of E6 and E7 gene expression. Furthermore, the flanking cellular sequences may influence the expression of the E6 and E7 oncogenes (24).

The cell line ME180 was established from an omental metastasis of a rapidly spreading cervical carcinoma (22). On the basis of DNA and RNA filter hybridization data, ME180 cells have been assumed to contain HPV18 DNA (14, 28). In contrast to all other HPV16- or 18-positive cell lines analyzed so far, only RNAs containing ORF E1 sequences, but no E6 and E7 transcripts, had been found in ME180 cells (15).

In this study, we have analyzed the HPV sequences in ME180 cells by molecular cloning, DNA sequence analysis, and Northern (RNA) blot hybridization. ME180 cells were obtained from the American Type Culture Collection. First, ME180 genomic DNA was examined for the presence of HPV18 sequences by Southern blot analysis (Fig. 1). Under stringent conditions, no hybridization was detected with a radiolabeled HPV18 probe that encompassed the early and late coding regions (Fig. 1A). However, when hybridization was performed under conditions of reduced stringency, distinct fragments hybridized to the three subgenomic HPV18 restriction fragments used as probes (Fig. 1B). These results indicated that ME180 cells do not contain HPV18 DNA but rather the DNA of a papillomavirus related to HPV18.

For molecular cloning of the HPV18-related papillomavirus DNA, ME180 DNA was digested with *Sac*I and the phage vector lambda 2001 (DNA was obtained from Stratagene, Heidelberg, Germany) (12) was used for construction of a genomic library. *Sac*I cleavage was chosen since it produced a fragment of about 20 kb that hybridized to all three HPV18 subgenomic probes (shown only for probe XB1200; Fig. 1B, lane 4) and thus should cover all of the HPV sequences present in ME180 cells. Plaque hybridization of replica filters was performed under reduced stringency conditions with the two HPV18 probes, XX1412 and XB1200. Screening of 2×10^6 recombinant phages led to the identification of a single positive recombinant that carried a DNA insert of 13.1 kb, which was designated AA13.1. Restriction enzymes *Bam*HI and *Spe*I were employed for construction of a restriction map of fragment AA13.1 (Fig. 2A). Different subfragments were then examined for the presence of viral and/or cellular DNA sequences by using them as radiolabeled probes in genomic Southern blot analysis. Fragment EE4.0 hybridized to nucleotide sequences specific for ME180 DNA (Fig. 3). With regard to the reports on HPV18 DNA in ME180 cells (14, 28), it should be noted here that DNA of ME180 cells from two other laboratories gave identical hybridization patterns with the EE4.0 probe, thus confirming the common origin of the cells (ME180 DNA and cells, respectively, were kindly provided by Peter M. Howley and Rudolf Schmits). In contrast to fragment EE4.0, fragment AA13.1 as well as the terminal subfragments AE6.0 and EA3.1 recognized sequences present in both ME180 DNA and in DNA of other cell lines (data not shown). These results indicated that fragment AA13.1 is composed of ME180-specific papillomavirus sequences together with flanking cellular sequences at both ends.

For DNA sequence analysis, the *Bam*HI and *Spe*I subfragments of AA13.1 were subcloned into the plasmid vector pBluescript KS$^-$, and sequence analysis was performed by

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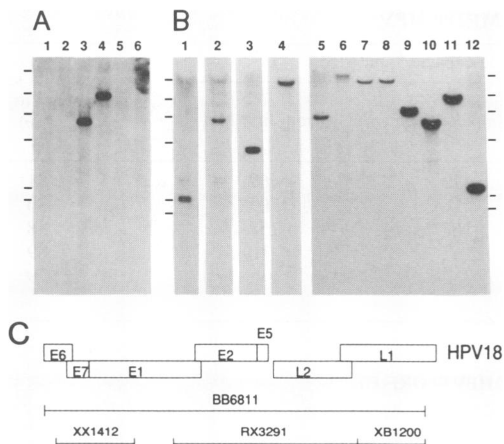

FIG. 1. Southern blot analysis of ME180 DNA with subgenomic HPV18 hybridization probes. DNA was isolated from cells of the cervical carcinoma cell lines ME180, C4-I (HPV18-positive), and CaSki (HPV16-positive) by proteinase K treatment and phenol extraction. After restriction endonuclease digestion and electrophoretic separation in 0.8% agarose gels, DNA was transferred to GeneScreen nylon membranes (Dupont NEN, Dreieich, Germany). HPV18 restriction fragments were radiolabeled by the random priming method (9) with the Stratagene T7 DNA polymerase protocol (21). (A) DNA was cleaved with *Eco*RI (lanes 1, 3, and 5) or *Hin*dIII (lanes 2, 4, and 6). Lanes 1 and 2, ME180 DNA; lanes 3 and 4, C4-I DNA; lanes 5 and 6, CaSki DNA. Hybridization was performed with probe HPV18 BB6811 (see panel C) under high-stringency conditions (50% formamide, 5× SSC at 42°C), and the filters were washed in 2× SSC–0.1% sodium dodecyl sulfate (SDS) at 68°C. (B) DNA was digested with *Bam*HI (lanes 1, 2, 5, and 9), *Spe*I (lane 3), *Sac*I (lane 4), *Eco*RI (lanes 6 and 10), *Hin*dIII (lanes 7 and 11), or *Xba*I (lanes 8 and 12). Lanes 1 through 8, ME180 DNA; lanes 9 through 12, C4-I DNA. Hybridization at reduced stringency was performed either in 30% formamide–5× SSC at 42°C with HPV18 probes XX1412 (lane 1) and XB1200 (lanes 3 through 12) or in 20% formamide–5× SSC at 42°C with probe RX3291 (lane 2). Filters were washed in 2× SSC–0.1% SDS at 56 and 48°C, respectively. The bars indicate the positions of the λ *Hin*dIII size marker fragments (23,130, 9,419, 6,557, 4,371, 2,322, and 2,028 bp). (C) Distribution of open reading frames in HPV18 DNA and localization of restriction fragments used as hybridization probes. Fragments are designated according to the restriction enzymes used (B, *Bam*HI; R, *Eco*RI; X, *Xba*I), and the fragment size in base pairs is indicated.

the dideoxy method (16) by using universal primers (Pharmacia, Freiburg, Germany) and synthetic oligonucleotide primers corresponding to parts of the newly established sequence. The nucleotide sequence of a 10,079-bp segment was determined, and the sequence data were analyzed by use of the HUSAR computer program package (10).

DNA sequence comparison with HPV18 (5) revealed that the 10,079-bp sequence includes two portions of 5,993-bp and 867-bp length, respectively, of an HPV genome that is closely related to, but not identical to, HPV18. This virus will subsequently be referred to as ME180-HPV. By alignment of the 5,993-bp sequence to HPV18, HPV39 (23), and HPV16 (19), nucleotide sequence homologies of 82% to HPV39, 69% to HPV18, and 60% to HPV16 were determined. Among the sequenced HPVs, ME180-HPV is thus most closely related to HPV39. Corresponding results were obtained by Southern blot analysis of the cloned DNAs of HPV types 1 through 53 by using the ME180-HPV-specific fragment EE4.0 (Fig. 2) as a radiolabeled probe (hybridization was performed in 5× SSC [1× SSC is 0.15 M NaCl plus

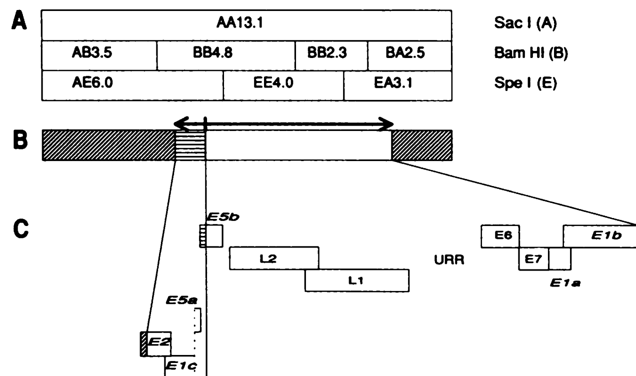

FIG. 2. Physical map of restriction fragment AA13.1 isolated from a ME180 genomic DNA library and location of viral and cellular sequences. (A) The positions of cleavage sites for restriction enzymes *Sac*I (A), *Bam*HI (B), and *Spe*I (E) are shown. Fragments are designated according to the restriction enzymes used and the fragment size (in kilobase pairs). (B) Cellular sequences are indicated by regions with diagonal hatching. The 5,993- and 867-bp segments of HPV sequences are given as open and horizontally hatched regions, respectively. The arrows indicate the 5′-to-3′ polarity of the mRNA-analogous DNA strand. (C) Positions of open reading frames in the 5,993-bp and 867-bp segments. Names of truncated ORFs that include only partial information of the respective full-length ORFs are given in italic letters. The continuation of ORFs E1b and E2 across the virus-cell junction into cellular sequences is indicated by the hatched regions. The two truncated ORFs, E5a and E5b, both contain a 25-bp sequence that allows the assembly of the complete ORF E5 from the two partial sequences. The amino acid sequence homology to HPV39 E5 is 86%. The dotted line represents a 13-bp sequence without homology to HPV39.

0.015 M sodium citrate] at 68°C). Probe EE4.0 hybridized strongly with HPV39 DNA, to a lesser extent with HPV18, and weakly with HPV26, whereas it did not react with any other HPV DNA.

The 5,993-bp segment contains ME180-HPV sequences

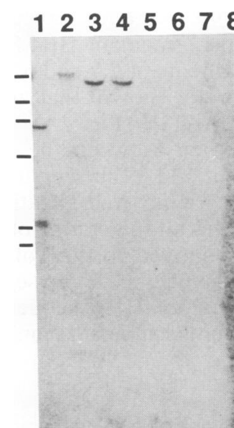

FIG. 3. Comparative Southern blot analysis of cervical carcinoma cell lines for the presence of AA13.1 DNA sequences. DNA of cell lines ME180 (lanes 1 through 4) and C33A (lanes 5 through 8) cleaved with *Bam*HI (lanes 1 and 5), *Eco*RI (lanes 2 and 6), *Hin*dIII (lanes 3 and 7), or *Xba*I (lanes 4 and 8) was separated on a 0.8% agarose gel and transferred to a nylon membrane. Hybridization was performed at 68°C with the AA13.1 subfragment EE4.0 as a radiolabeled probe. The bars indicate the λ *Hin*dIII size marker fragments.

TABLE 1. Features of ORFs in ME180-HPV

ORF	Nucleotide position[a]			ORF coding capacity[b] and size			Nucleotide sequence homology to HPV39 (%)
	First nucleotide	First ATG	Nucleotide preceding stop codon	ORF size (bp)	Complete ORF	From first Met	
L2	221	227	1633	1,413	471	469	81
L1	1584	1617	3131	1,548	516	505	80
E6	3925	3946	4419	495	165	158	88
E7	4337	4430	4759	423	141	110	89
E1a	4763	4769	5116	354			79[c]
E1b	4960		6039[d]	1,080[d]			

[a] According to the nucleotide sequence in Fig. 4.
[b] Number of amino acid residues.
[c] % Homology of nucleotide sequence position 4769 to 5993 to the corresponding part of HPV39 ORF E1.
[d] The 3′-terminal 46 nucleotides are probably cellular sequences.

that extend from within ORF E5 up to E1 and include an 811-bp upstream regulatory region and the complete ORFs L2, L1, E6, and E7 (Fig. 2). Their features and the sequence homologies to the corresponding ORFs of HPV39, HPV18, and HPV16 are summarized in Tables 1 and 2. The nucleotide sequence of the 5,993-bp segment is given in Fig. 4. ME180-HPV sequences with homology to ORF E1 of HPV39 and HPV18 are split into two ORFs, E1a and E1b (Fig. 2). This is similar to the situation in the HPV16 prototype DNA, in which ORF E1 is disrupted by a frameshift mutation (1, 13, 19). Within ME180 ORF E1b at nucleotide position 5994, the DNA sequence starts to diverge significantly from HPV39 and HPV18 E1. This transition point probably represents the junction between the integrated HPV sequences and the 3′-flanking cellular sequences.

The 867-bp segment contains additional ME180-HPV sequences, however, in opposite orientation. These sequences include the 5′ end of ORF E5, the 3′ end of ORF E1 (462 bp), and the 5′ end of ORF E2 (356 bp from the first ATG) (Fig. 2). These truncated HPV sequences are located within a region of the cloned AA13.1 restriction fragment for which structural differences to the corresponding part of the ME180 genomic DNA were observed in Southern blot analysis. For example, fragment BB4.8 hybridized to two genomic *Bam*HI fragments of 2.7 and 6.0 kb and to a 1.4-kb *Spe*I fragments (data not shown) that are all not present in the cloned fragment AA13.1 (Fig. 2A). Furthermore, a size difference was observed between the cloned and the genomic *Sac*I fragment (13.1 kb versus about 20 kb, as estimated from Southern blot hybridization; Fig. 1B, lane 4). These data indicate that in the course of molecular cloning, a deletion event has removed portions of the integrated HPV sequences, thereby leaving the 867-bp segment as a remnant. The exact arrangement of HPV sequences in the ME180 genome is currently under investigation.

TABLE 2. Comparison of amino acid sequences

ME180-HPV ORF	Amino acid sequence homology (% identical amino acids) to corresponding ORF in:		
	HPV39	HPV18	HPV16
L2	85	69	48
L1	88	68	59
E6	86	63	54
E7	82	55	35

Expression of ME180-HPV DNA was examined by Northern blot hybridization of ME180 poly(A)$^+$ RNA with radiolabeled AA13.1 subfragment probes (Fig. 5). Each of the probes BE1.7, EB0.6, and BA2.5 that together cover the ME180-HPV upstream regulatory region and ORFs E6, E7, and E1 hybridized to at least two major RNA species. In contrast, no hybridization was detected with probe EB2.2, containing ORF L2 and L1 sequences. These data indicate that expression of HPV sequences in ME180 cells is confined to ORFs E6, E7, and E1.

This study has shown that cells of the human cervical carcinoma cell line ME180 contain the DNA of a human papillomavirus (provisionally designated here as ME180-HPV) that is more closely related to HPV39 than to HPV18. The previous assumption that ME180 cells harbor HPV18 DNA (14, 28) might have been caused by cross-hybridization between the endogenous ME180-HPV DNA and the HPV18 hybridization probe because of the close sequence relationship. The examination of whether ME180-HPV represents a new HPV type according to the current classification criterion (4) will require the availability of the complete genome in order to determine the degree of homology to HPV39 in liquid phase hybridization.

As an exception among analyzed HPV-positive cervical carcinoma cell lines, ME180 cells had been reported to express HPV sequences only from ORF E1, but not from E6 and E7, supposedly because of a deletion of the upstream enhancer-promoter region (15). Our data, however, show that sequences from ORFs E6 and E7 (and in addition from ORF E1) are transcribed into poly(A)$^+$ RNA; thus, the situation is strictly comparable with that of HPV16 and HPV18 in other cervical carcinoma cell lines. Furthermore, the nucleotide sequence of the 5,993-bp segment clearly shows that the integrated ME180-HPV DNA includes a complete upstream regulatory region (position 3135 to 3924) with a TATA box sequence (position 3912), corresponding to that of the HPV18 early promoter, thus strongly suggesting that transcription of ORF E6 and E7 sequences is initiated at this site.

The analysis of HPV DNA in ME180 cells has provided evidence that established human cervical carcinoma cell lines may harbor the DNA of HPV types different from HPV18 and HPV16 and furthermore that these cell lines provide a potential source for the isolation of new HPV DNAs associated with cervical carcinoma.

Nucleotide sequence accession number. The nucleotide sequence data presented in Fig. 4 have been deposited under GenBank accession number M73258.

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6001 TGCAGAATTT ACTACTTGTG TTTATTACAG GCGATTTCCT GA   6042
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FIG. 4. Nucleotide sequence of the sense strand of the ME180-HPV 5,993-bp segment. Nucleotides 5994 through 6042 are of presumed cellular origin and constitute the 3′-part of ORF E1b. For the structural organization of the 5,993-bp segment, see Fig. 2.

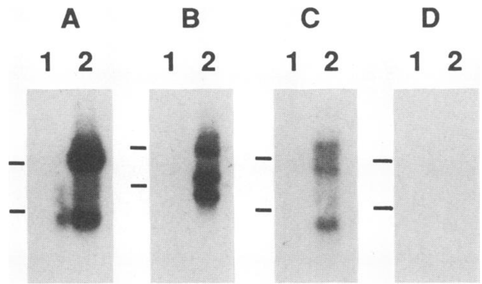

FIG. 5. Expression of HPV sequences in ME180 cells. Northern blot analysis was performed with poly(A)$^+$ RNA isolated from HPV18-positive SW756 cells (lanes 1) and from ME180 cells (lanes 2). The following AA13.1 subfragments were used as radiolabeled probes for hybridization (in 5× SSC at 68°C). (A) Fragment BE1.7 (position 2934 to 4660) containing the 3′-terminal 200 bp of ORF L1, the upstream regulatory region, ORF E6, and the 5′-terminal two-thirds of ORF E7. (B) Fragment EB0.7 (position 4661 to 5229) containing the 3′-terminal third of ORF E7 and the 5′-terminal 466 bp of ORF E1. (C) Fragment BA2.5, composed of 765 bp of ME180-HPV E1 sequences (position 5230 to 5993) and 1,768 bp of 3′-flanking cellular sequences. (D) Fragment EB2.2 (position 690 to 2933) containing the 3′-terminal 954 bp of ORF L2 and the 5′-terminal 1,300 bp of ORF L1. Filters were exposed to X-ray films (Kodak X-Omat XR5) with intensifying screens for 13 h (A and B) and 27 h (C and D). The bars indicate the positions of the 28S and 18S rRNA.

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