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Isolation of a Novel Human Papillomavirus (Type 51) from a Cervical Condyloma

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We cloned the DNA from a novel human papillomavirus (HPV) present in a cervical condyloma. When DNA from this isolate was hybridized at high stringency with HPV types 1 through 50 (HPV-1 through HPV-50), it showed weak homology with HPV-6 and -16 and stronger homology with HPV-26. A detailed restriction endonuclease map was prepared which showed marked differences from the maps for other HPVs that have been isolated from the female genital tract. Reassociation kinetic analysis revealed that HPV-26 and this new isolate were less than 10% homologous; hence, the new isolate is a novel strain of HPV. The approximate positions of the open reading frames of the new strain were surmised by hybridization with probes derived from individual open reading frames of HPV-16. In an analysis of 175 genital biopsies from patients with abnormal Papanicolaou smears, sequences hybridizing under highly stringent conditions to probes from this novel HPV type were found in 4.2, 6.1, and 2.4% of biopsies containing normal squamous epithelium, condylomata, and intraepithelial neoplasia, respectively. In addition, sequences homologous to probes from this novel isolate were detected in one of five cervical carcinomas examined.

It is well documented that human papillomavirus (HPV) is frequently associated with lesions of the female genital tract (2, 6, 7, 11, 19, 20). Further, there is a marked segregation of certain virus types with condylomata, which tend to regress, and with cervical intraepithelial neoplasia (CIN), which tends to progress as well as to be associated with an increased incidence of cervical cancer (2, 4, 5, 9, 11, 12). Specifically, HPV types 6 and 11 (HPV-6 and -11) are found in up to 80% of vulvar and cervical condylomata, while they are found in only 3% of CIN lesions (2, 8, 10). Conversely, HPV-16 and -18 DNA sequences are present in up to 85% of CIN lesions and in as few as 5% of condylomata (4, 5, 13). A similar segregation pattern is found in squamous cell carcinoma of the cervix, where DNA sequences from HPV-16 and -18 are routinely found while sequences from HPV-6 or -11 are only rarely identified (9, 10, 14, 19, 20). The basis for these distinctive segregation patterns is the focal point of intensive study.

Over 50 unique types of HPV have been identified (E. M. de Villiers, personal communication). Many of these were isolated from sites other than the female genital tract, such as the larynx, oral mucosa, and skin (3, 15, 19). Until recently, up to 35% of the HPVs found in lesions of the female genital tract were not identified with respect to type (5, 13). Therefore, the isolation and characterization of novel types are important to determine if they segregate within specific classes of lesions, as do HPV-6, -11, -16, and -18. An understanding of virus segregation patterns may make it possible to draw parallels between HPV type, histology, and behavior. Finally, the incorporation of novel types into probes for screening purposes would improve the sensitivity of such assays. Here we report the molecular cloning, partial characterization, and prevalence of a novel type of HPV DNA isolated from a condyloma.

A biopsy from a 25-year-old woman with an atypical Papanicolau smear was diagnosed as a condyloma. Total cellular DNA was extracted (5) and analyzed by low-stringency Southern blot hybridization with a ^{32}P-labeled DNA probe containing various HPV DNA sequences. A hybridization signal was detected by probes containing sequences from either HPV-6 or HPV-16. However, the hybridization signals were lost after a high-stringency wash. Figure 1 shows a comparison of the isolate with HPV-16. An HPV-18 probe did not hybridize with the isolate. These data suggest that this isolate was only distantly related to HPV-16.

Histological analysis of the lesion showed the features typical of a condyloma (Fig. 2); that is, there was koilocytotic atypia which was more marked towards the surface. The basal atypia, increased mitotic index, and atypical mitotic figures characteristic of CIN lesions were not present in the sections examined (13).

To further characterize the HPV DNA, total cellular DNA was digested with numerous restriction endonucleases. A single 8-kilobase fragment was released after *Hin*dIII digestion. Accordingly, we ligated 70 ng of *Hin*dIII-cleaved DNA with 500 ng of *Hin*dIII-digested DNA from the lambda bacteriophage Charon 21. The ligation reaction was packaged in vitro, and about 100,000 plaques from the resultant library were screened under conditions of low-stringency hybridization with a ^{32}P-labeled probe containing DNAs from HPV-6 and -16. Twenty plaques (0.02%) were positive. One plaque was purified and was shown to contain an 8-kilobase insert after *Hin*dIII digestion. This recombinant phage was amplified, and the insert was released from the vector DNA and subcloned into the *Hin*dIII site of pUC13.

Digestion of the subcloned DNA with a variety of restriction endonucleases allowed us to prepare a map which differed markedly from those available for other HPVs (types 6, 11, 16, 18, 31, and 33) (1, 3) isolated from cervical lesions (Fig. 3A). The cleavage sites for enzymes that cut once or twice within the insert were deduced by comparing the fragments released after digestion of the purified insert

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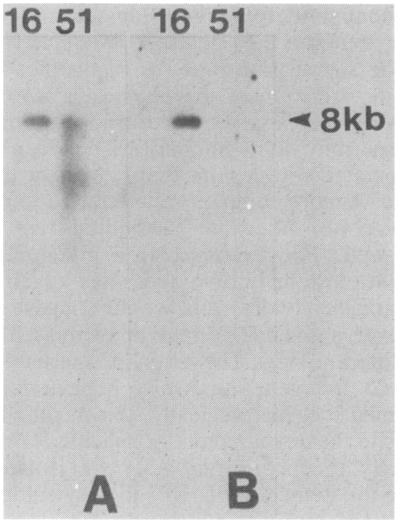

FIG. 1. Relatedness of HPV-51 to HPV-16. A ^{32}P-labeled probe (specific activity, 2×10^8 cpm/μg) was prepared from a clone containing HPV-16 DNA and hybridized at low stringency to a Southern blot containing 5 μg of undigested genomic DNA from the HPV-51-containing lesion. The blot was first washed under conditions of low stringency (A) and then washed at high stringency (B) as previously described (5). kb, Kilobases.

and clone. A modification of the technique of Smith and Birnstiel (18) was used to locate the cleavage sites for those restriction endonucleases which cut the insert several times.

The HPV DNA from the isolate was next analyzed for homology with HPV-1 through -50 by Southern blot hybridization at high stringency. Most of the HPV types showed only weak or no hybridization (data not shown). The strongest hybridization signal was detected with HPV-26. However, reassociation kinetic analysis (8) revealed that HPV-51 and HPV-26 were less than 10% homologous with the isolate. The DNA thus represents a novel isolate, which has been designated HPV-51.

The potential open reading frames of HPV-51 were oriented relative to its map by hybridizing subgenomic probes corresponding to open reading frames E6, E1, and L1 from HPV-16 (17) to Southern blots of HPV-51 DNA digested with either *Hae*II or *Pst*I. On the basis of the highly conserved arrangement of the HPV open reading frames (3, 17), we have tentatively assigned these regions as shown in Fig. 3B.

The incidence and possible segregation patterns of HPV-51 were analyzed by screening DNA from 175 patients with abnormal Papinicolaou smears by dot blot hybridization under high-stringency conditions with a ^{32}P-labeled HPV-51 probe. The biopsies were also analyzed histologically. The results of this analysis are summarized in Table 1.

HPV-associated lesions of the female genital tract fall into two groups histologically and behaviorally (13, 16). Both condylomata and CIN can show superficial koilocytotic cellular atypia, whereas only CIN lesions show the aneuploidy and more severe degree of full-width cellular disorganization typical of carcinomas. Condylomata tend to regress, whereas approximately 30% of CIN lesions progress to cancer over a 10-year period. The HPV types found in the cervix segregate into these two groups. HPV-6 and -11 are common in condylomata but are rare in CIN and very rare in cervical cancer, whereas HPV-16 and -18 are common in CIN and cancer but rare in condylomata. Differences at the

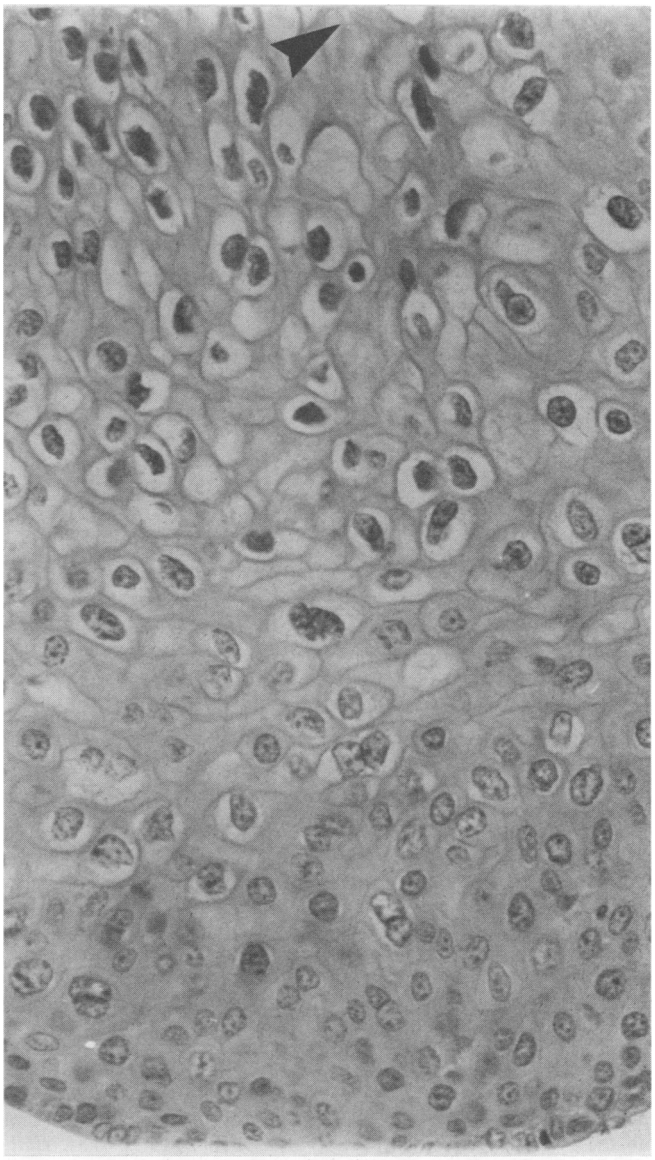

FIG. 2. Histological analysis of the cervical lesion. Hematoxylin-and-eosin-stained section of the condyloma showing the perinuclear halos and nuclear atypia (koilocytotic atypia), which are more pronounced toward the surface (arrow).

TABLE 1. Hybridization analysis of biopsies from patients with abnormal Papanicolaou smears[a]

HPV DNA	No. of samples with the following histology:			
	Normal	Condylomata[b]	Intraepithelial neoplasias[c]	Carcinoma
Positive	2	5	1	1
Negative	48	82	41	4

[a] DNA isolated from biopsy samples was examined for the presence of sequences homologous to HPV-51 by dot blot hybridization with a ^{32}P-labeled purified virus insert after excision from the pUC vector. The blots were hybridized under conditions of high stringency (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]; 42°C) and washed under similarly stringent conditions (5). pUC and purified HPV-16 DNAs were included in each analysis as controls.

[b] Condylomata include cervical, vaginal, and perianal lesions.

[c] Intraepithelial neoplasias include CIN and vulvar lesions.

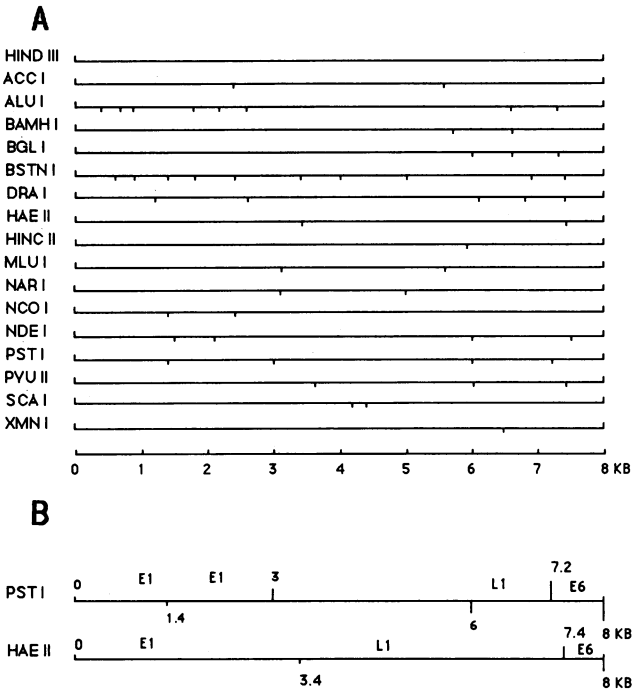

FIG. 3. HPV-51 genome. (A) The cleavage sites for restriction endonucleases were determined and are plotted on a linear map of the virus genome, with the *Hin*dIII site arbitrarily assigned as coordinate 0. A modification of the technique of Smith and Birnstiel (18) was used to locate the sites for those restriction endonucleases that recognized multiple sites within the insert. Briefly, this involved cleaving the recombinant plasmid with *Hin*dIII and labeling the resulting cleavage site by filling in the ends with the Klenow fragment of DNA polymerase I in the presence of ^{32}P-labeled deoxynucleotide triphosphates. The labeled virus fragment was isolated and cleaved asymmetrically with *Hin*cII, releasing two end-labeled fragments. Each of these fragments was subjected to partial digestion with a variety of restriction endonucleases for 1 to 60 min, and the nested family of fragments which resulted was resolved by electrophoresis through agarose gels of various concentrations. In this procedure the ascending order of fragments in the gel corresponds directly to the order of the restriction sites along the DNA. The following restriction endonuclease sites are not present in the HPV-51 genome; *Apa*I, *Ava*I, *Ava*II, *Bgl*II, *Bst*EII, *Cla*I, *Eco*RI, *Eco*RV, *Hpa*I, *Nae*I, *Nhe*I, *Not*I, *Sac*I, *Sal*I, *Sma*I, *Sph*I, *Xba*I, and *Xho*I. KB, Kilobases. (B) The potential reading frames of HPV-51 were assigned after hybridization of subgenomic probes corresponding to the E6, E1, and L1 open reading frames from HPV-16 under low-stringency conditions to blots of HPV-51 digested with either *Hae*II or *Pst*I.

molecular level, such as the physical state of the DNA, transcription and translation patterns, and posttranslational modifications, may help to explain these segregation patterns.

A relatively large proportion of HPV-associated lesions of the female genital tract are thus far uncharacterized with respect to type. We have described the isolation and characterization of a novel HPV from a cervical condyloma. Sequences homologous to those of this strain were present in 6.1% of condylomata and in 2.4% of the intraepithelial neoplasms examined.

The continued isolation and characterization of novel types of HPV will yield important information regarding HPV-associated lesions. It is of interest to determine if these novel types show segregation patterns characteristic of the HPV types commonly found in lesions of the female genital tract. If this occurs, it may be useful in identifying the basis for the strong segregation patterns of distinct HPV types; that is, characterization of the physical state of the DNA (integrated versus episomal) and of transcription and translation patterns may show similarities between HPV types that are associated with lesions that regress or that progress to carcinoma. A more complete characterization of the HPV types found at sites other than the genital tract would be of interest as well. For example, if a given HPV type is associated with lesions at divergent sites which had similar histologies and behaviors, this would suggest that, at the molecular level, a given HPV may be expressed in the same fashion at different sites. However, if a virus is associated with lesions at divergent sites which behave differently and show few similarities histologically, this might suggest a role for cofactors in the development of specific HPV-associated lesions. Finally, the incorporation of novel isolates as probes will improve the detection of HPV in cervical lesions.

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