Human Papillomavirus Type 52: a New Virus Associated with Cervical Neoplasia

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SUMMARY

Analysis of biopsies of cervical intraepithelial neoplasia (CIN) revealed a high percentage with human papillomavirus (HPV) sequences that would hybridize to a mixture of HPV probes only under conditions of relaxed stringency. The DNA sequences of one of these viruses was molecularly cloned and shown to be a new HPV, type 52 (HPV-52). This virus is most closely related to HPV-33. Hybridization analysis with restriction fragments of HPV-52 showed collinearity with the HPV-33 genome. DNA sequencing revealed a high level of conservation between the two viruses within the L1 open reading frame but significant divergence in the non-coding region of the viral genomes. Prevalence studies indicated that HPV-52 sequences were present in three of 137 (2%) CIN and in one of 48 (2%) cervical squamous cell cancers studied in the U.S.A.

The human papillomaviruses (HPVs) represent a heterogeneous family of variably related viruses. HPV infection of the genital tract is now recognized as a sexually transmitted disease implicated in the pathogenesis of cervical cancer and its precursor lesions (zur Hausen, 1986). The HPV types most frequently associated with genital tract infections are HPV type 6 (HPV-6), -11, -16, -18 and -31 (Gissmann et al., 1983; Dürst et al., 1983; Boshart et al., 1984; Lorincz et al., 1986). These viruses were detected in approximately 56% of 93 pre-malignant and malignant cervical lesions in a series of biopsies from patients in the U.S.A. The remaining 44% of lesions either contained viral sequences that could be detected only by relaxed hybridization conditions (50%), or failed to show the presence of HPV DNA (Lorincz et al., 1987). Of these HPV DNA-positive samples, 56% have been shown to contain either HPV-33, -35, -42, -43, -44 or -45 (A. Lorincz, unpublished results). From the remaining group of samples of unknown virus types we have molecularly cloned a new virus associated with benign and malignant cervical lesions occurring at low frequency in the United States.

Cellular DNA from two early cervical intraepithelial neoplasia (CIN) lesions obtained from patients in the Washington D.C. area were shown to contain an HPV-related DNA that yielded an 8 kb fragment after EcoRI digestion. The DNAs were pooled, digested with EcoRI and the HPV-related DNA was cloned into the EcoRI site of lambda L47 as previously described (Lorincz et al., 1986). Three plaques positive by non-stringent hybridization with HPV-16 from 10⁵ plaques were screened by Southern blot analysis. All three plaques contained the same DNA as determined by restriction enzyme analysis. One isolate was subcloned in pUC19 and was designated pCD15.

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This clone was hybridized to a battery of HPV isolates under stringent conditions and was shown to be most related to HPV-33 (E. M. de Villiers, personal communication). Liquid phase hybridization was performed to ascertain whether pCD15 was sufficiently diverse from HPV-33 to be classified as a new virus. The new isolate and HPV-33 were shown to have 28% DNA sequence homology under standard conditions of hybridization (Tm = 25 °C) using hydroxyapatite chromatography to separate single- and double-stranded DNA as previously described (Lancaster & Olson, 1978). Since type designations are based on 50% or less DNA sequence homology under standard conditions of hybridization (Coggins & zur Hausen, 1979), this isolate represents a new HPV. The isolate has been designated HPV-52a (G. Orth, personal communication).

Nucleotide sequence analysis of several papillomaviruses has revealed a common organization of the early and late open reading frames (ORFs) (Cole & Danos, 1987). To determine whether HPV-52a was similarly organized, we constructed a restriction enzyme cleavage map (Fig. 1) for use in comparing its sequence arrangement to that of HPV-33 digested with a variety of restriction enzymes. Most of the fragments of HPV-52a hybridized to discrete fragments of HPV-33 (Fig. 2). Five of the six HPV-52a cross-hybridizing fragments formed stable duplexes with HPV-33 at Tm = 30 °C. Based on these results, the HPV-52a genome appears to be collinear with the HPV-33 genome. HPV-52a restriction fragments failed to cross-hybridize with two regions of the HPV-33 genome. One region encompassed the 3' end of the early region and the other extended from the 3' end of the L10RF through the non-coding region into the E6 ORF. These are the most variable regions on the papillomavirus genome and may reflect important differences in biological properties exhibited by the viruses, such as tissue tropism (Cole & Danos, 1987).

To analyse these non-homologous regions further, we sequenced 1950 nucleotides from 346 nucleotides 5' to the rightward KpnI site on the HPV-52a genome through the 5' BamHI site (see Fig. 1). The equivalent region on the HPV-33 genome would be from amino acid 153 of the L1 ORF through the non-coding region. Comparison of the nucleotide sequences of HPV-33 and HPV-52a revealed 75% overall homology in the L1 ORF and less than 50% homology in the non-coding region. This abrupt change in homology between the L1 ORFs and the non-coding regions is shown in Fig. 3. DNA–DNA heteroduplex mapping has revealed that other closely related HPVs such as HPV-6 and -11 as well as -16 and -31 show a similar drop in homology near the junction of the L1 ORF and the non-coding region (Chow et al., 1987).

Fig. 1. Restriction endonuclease cleavage map of HPV-52a. The map is opened at the unique EcoRI site. Length (in kb) is indicated at the top. The expected direction of transcription is from left to right based on the HPV-33 sequences (Cole & Streeck, 1987). The following enzymes did not cut HPV-52a DNA: BanlI, BclI, HpaI, HindIII, SacI, SphlI, SphI and XhoI.
Fig. 2. Regions of homology between HPV-33 and HPV-52a. Fragments of the EcoRI-linearized HPV-52a clone were generated with the various restriction enzymes indicated, then gel-purified, labelled by nick translation, and used to probe Southern blots containing HPV-33 DNA digested with the indicated restriction enzymes. Solid arrows indicate hybridization at $T_m - 10^\circ C$; the broken arrow indicates hybridization at $T_m - 30^\circ C$. The HPV-52a genome alignment is based on the EcoRI cleavage site; however, from DNA sequence analysis, the end of the L1 ORF of HPV-52a is located downstream of the corresponding site on the HPV-33 genome and is indicated by the large arrowhead. The HPV-33 E5 ORF termination codon is indicated by a broken vertical line $5'$ to the L2 ORF.

Fig. 3. Matrix plotting of DNA sequence homologies between HPV-33 and HPV-52a. The location of the 3' end of the L1 ORF and non-coding region is provided for orientation. A total of 1950 nucleotides of HPV-52a were compared to the corresponding sequence of HPV-33. Sequences were compared using a forward matrix program (Pustell & Kafatos, 1984). Range 10 (21 bp window); minimum value plotted, 80%. The letters on the diagonal indicate the degree of homology within the range decreasing from A (100%) to K (80%).
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HPV-16 and -18 have been detected in 77% of cervical cancers in Germany (Dürst et al., 1983; Boshart et al., 1984), in 71% in South America (Lorincz et al., 1987), but in only 59% in the U.S.A. (Lorincz et al., 1987). There is further evidence to suggest that detection of HPV types in CIN and cervical cancers differ from geographical location, since HPV-16 and -18 are present in only 33 to 39% of CIN and cervical cancers in Japan (Yoshikawa et al., 1985; Saito et al., 1987). Low stringency hybridization on Japanese lesions has revealed that additional virus types are present in 15 to 20% of pre-malignant and malignant cervical lesions. Analysis of restriction enzyme cleavage patterns of the DNA sequences suggest that they do not represent HPV-31 or -33 (Saito et al., 1987). It is interesting to speculate that a low prevalence virus in one geographical location, such as HPV-52, may be a high prevalence virus in another. Thus, the use of a wide variety of HPV types isolated from genital lesions may reveal geographical differences in the world-wide distribution of genital tract HPVs.

REFERENCES


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