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Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers

(molecular cloning/blot hybridization/perinatal infection/genital cancer)

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ABSTRACT **Human genital tumors as well as recurrent laryngeal papillomas were analyzed for the presence of human papillomavirus (HPV) 6 and HPV 11 sequences. HPV 11 DNA was found in 7 of 14 laryngeal papillomas; in the 7 other tumors no HPV DNA was demonstrated. HPV 11 DNA was also found in all five atypical condylomata of the cervix included in this study. Condylomata acuminata mainly contained HPV 6 DNA. From 63 biopsy specimens, 41 clearly harbored HPV 6 DNA and 13 harbored HPV 11 DNA. In three tumors accurate typing was impossible, and in six additional ones neither HPV 6 nor HPV 11 DNA could be demonstrated. The data support a genital origin of laryngeal papillomavirus infections. In 4 of 24 malignant tumors, HPV 11 DNA or related sequences were demonstrated; 2 of the 4 were biopsy specimens from invasive cancer, and the other 2 originated from carcinomata *in situ*. A possible role of this or related papillomavirus types in the induction of malignant genital tumors remains to be elucidated.**

An infectious etiology of human genital cancer has been widely discussed (1, 2). Herpes simplex viruses as well as human papillomaviruses (HPVs) have been incriminated as potential causative agents.

Attempts were made in our laboratory to analyze papillomavirus infections of the genital tract and to assess their role in human genital cancer. Because multifocal recurrent laryngeal papilloma is suspected to originate from a perinatal infection of mothers with condylomatous lesions (3), we also included this tumor in our studies.

In previous publications we characterized a papillomavirus in genital warts (condylomata acuminata) (4, 5) and designated it HPV 6. DNA homologous to HPV 6 nucleic acid was found in the majority of genital warts analyzed but not in a number of malignant genital tumors (6). Recently, viral DNA from a laryngeal papilloma was cloned molecularly and subsequently analyzed (7). This DNA shares about 25% of its sequences with HPV 6 DNA. Thus, according to present rules of nomenclature (8), it clearly represents a distinct type of papillomavirus and we have labeled it HPV 11.

The availability of this material permitted studies to test the hypothesis of a genital origin of laryngeal papillomavirus infections and to analyze malignant tumors for the presence of the respective DNAs. Here we report the presence of HPV 11 DNA in atypical condylomata of the cervix (9), in some biopsy specimens from carcinomata *in situ* and invasive cervical cancer, and also in some typical condylomata acuminata.

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MATERIALS AND METHODS

Extraction of Cellular DNA. Biopsy materials were examined histologically and stored at −20°C or −70°C until further processing. Extraction of cellular DNA was done as described (6).

Labeling of HPV DNA. HPV 6 DNA has been cloned into pBR322 in two fragments representing approximately one-third and two-thirds of the total genome, respectively (5). HPV 11 DNA, which has been identified from a genomic library of laryngeal papilloma constructed in λ L47 (7), was subcloned in pBR322 at the single *Bam*HI site.

Both DNAs were prepared as described (10) and labeled with deoxynucleotide [α-^{32}P]triphosphate by the nick-translation procedure to a specific activity of $>10^8$ cpm/μg (6).

Blot Hybridization. About 10 μg of papilloma DNA was cleaved with restriction enzyme; the products were separated on an agarose gel, transferred onto nitrocellulose, and hybridized to the labeled HPV DNA (6). The sensitivity of hybridization enabled the detection of <0.1 genome equivalent of HPV DNA per diploid cell.

RESULTS

The following approaches proved to be suitable to differentiate between HPV 6 and HPV 11 DNA in tumor materials.

(*i*) Cellular DNA was cleaved with *Bam*HI (which cuts HPV 6 and HPV 11 DNA only once), blotted onto nitrocellulose, and hybridized to both labeled DNAs in different tests (Fig. 1). A significantly stronger hybridization was obtained with the identical probe.

(*ii*) Washing of the nitrocellulose filter after hybridization at 2°C below the melting temperature led to loss of activity bound by the heterologous DNA. Hybrids formed with the identical probe were more stable under those conditions (data not shown) (7).

(*iii*) *Pst* I cleaves HPV 6 and HPV 11 DNA several times, resulting in completely different fragment patterns for the two types of genome (Table 1). Cleavage of cellular DNA therefore allows easy differentiation between HPV 6 and HPV 11. Hybridization with the homologous DNA revealed the regular pattern whereas the heterologous probe labeled fragments at unequal molarities (Fig. 2).

The third approach was used to screen a total of 63 typical condylomata acuminata of anogenital origin. HPV 6 or HPV 11 was clearly identified in the majority (86%) of these tumors (Table 2). HPV 6 was three times as frequent as HPV 11. The

Abbreviation: HPV, human papillomavirus.

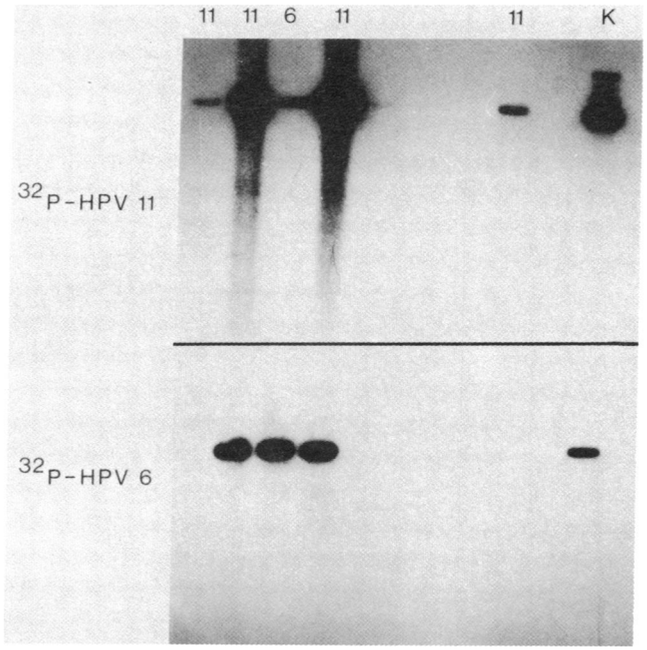

FIG. 1. Blot hybridization of HPV DNA to DNA isolated from genital warts and flat condylomas. Identical amounts of cellular DNA were cleaved by *Bam*HI and hybridized to ^{32}P-labeled HPV 6 (*Upper*) or HPV 11 DNA (*Lower*). Numerals above the lanes indicate the virus type identified in the respective sample. K, HPV 11 DNA cloned in λ. Additional bands in *Upper* are due to contamination with vector DNA.

concentration of viral DNA varied considerably from about 0.1 genome equivalent up to 100 copies per cell. Differentiation was not feasible in three condylomata acuminata. One sample contained viral DNA that was only detected under hybridization conditions of reduced stringency (11), thus representing the genome of a different virus type. In five specimens, no HPV-specific sequences were found.

Five flat or atypical condylomata of the cervix and vagina (9) which were analyzed in this study all harbored HPV 11 DNA in concentrations of 10–100 copies per cell. HPV 11 DNA, which originally was isolated from a laryngeal papilloma, was detected in low concentration in 5 of 12 more cases of laryngeal papillomatosis (Fig. 3) and in 1 case of tracheal papillomatosis. Twenty-four malignant genital carcinomas were tested for the presence of HPV 6 and HPV 11 DNA. HPV 11 DNA showing the typical *Pst* I cleavage pattern was identified in one invasive cervical carcinoma (Fig. 4) and in two carcinomata *in situ*. HPV 11-related sequences of slightly smaller molecular weight compared to regular HPV DNA were identified in one additional carcinoma of the cervix. This DNA requires further characterization.

Table 1. Molecular weights ($\times 10^{-6}$) of the *Pst* I fragments of HPV 6 and HPV 11 DNA

HPV 6a	HPV 6b	HPV 6c	HPV 11a	HPV 11b
2.50	3.45	3.45	1.12	0.98
1.12	0.92	1.12	0.98	0.95
0.92	0.63	0.63	0.95	0.87
0.63			0.60	0.60
			0.50	0.50
			0.50	0.50
			0.30	0.30
				0.30

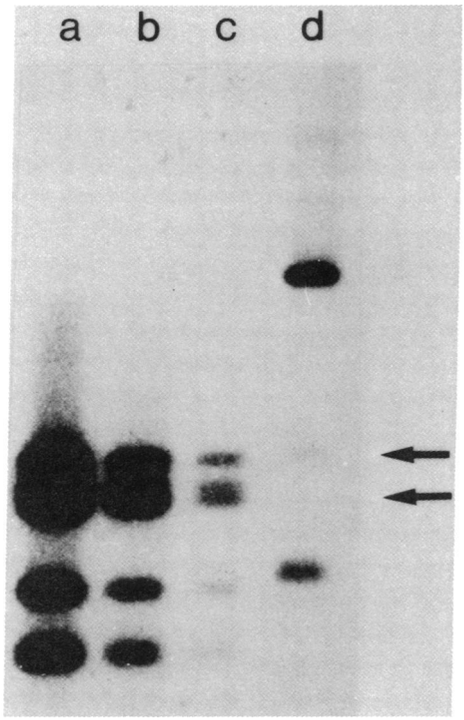

FIG. 2. Blot hybridization of ^{32}P-labeled HPV 11 DNA to DNA isolated from flat condyloma (lanes a–c) and condyloma acuminatum (lane d) and cleaved by *Pst* I. The probe recognized six fragments (molecular weights 1.12, 0.98, 0.95, 0.60, 0.50, and 0.50 × 10^6) that are identical to the *Pst* I fragments listed in Table 1. The smallest fragment was not seen on this gel (1% agarose) but can be detected in Fig. 3 (lanes a–c). The molecular weight 1.12 and 0.92 × 10^6 fragments of HPV 6a (lane d) that were barely labeled by the HPV 11 DNA probe are marked by arrows.

DISCUSSION

The DNA of a new type of HPV was isolated from a biopsy specimen from a patient suffering from recurrent laryngeal papil-

Table 2. Summary of HPV DNA in tumors

	Tumors positive, no.			
	Condyloma acuminatum (n = 63)	Atypical condyloma (n = 5)	Laryngeal papillomas (n = 14)	Genital carcinoma (n= 24)*
HPV 6a	26	—	—	—
6b	6	—	—	—
6c	5	—	—	—
6?†	4	—	—	—
HPV 11a	12	5	7‡	3§
11b	1	—	—	—
HPV 6 or 11¶	3	—	—	—
HPV ?‖	1	—	—	1**
HPV neg.	5	—	7	20

* Consisting of 13 invasively growing in cervix, 6 *in situ* in the cervix, 5 vulval, and 2 penile.
† Subtype of HPV 6 could not be determined.
‡ Including one case of a tracheal papillomatosis.
§ Two *in situ* and one invasively growing carcinoma of the cervix.
¶ Differentiation between HPV 6 and HPV 11 was not possible. It cannot be excluded that another (partially cross-hybridizing) DNA of a new type of HPV is present here.
‖ Positive under hybridization conditions of reduced stringency; determination of the HPV type was not possible.
** Invasively growing carcinoma of the cervix.

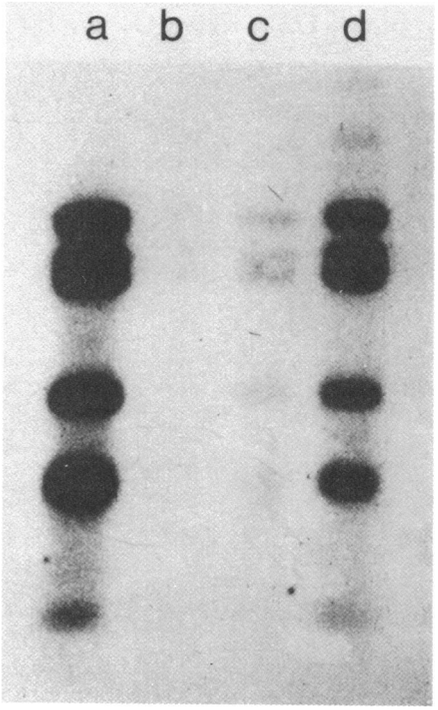

FIG. 3. Blot hybridization of ^{32}P-labeled DNA to *Pst* I fragments of DNA isolated from a laryngeal papilloma (lane a), two carcinomata *in situ* (lanes b and c), and one invasive cervical carcinoma (lane d) (see also Fig. 4). The genome copies per cell were calculated to correspond to 1–2 (lane d), 0.1–0.2 (lane c), and <0.1 (lane b). These values were estimated after blot hybridization of samples in lanes b–d cleaved by *Bam*HI (data not shown) which linearizes the HPV 11 DNA.

lomatosis (7). This virus was designated HPV 11. DNA homologous to this probe was detected in 7 of 14 biopsy specimens from laryngeal and tracheal papillomatoses. In the seven others no HPV DNA could be demonstrated. The amount of DNA available from the latter tumors was extremely small and probably was below the limits of detection of the hybridization procedure. Thus, this study indicates that HPV 11 infections prevail in laryngeal papillomatosis.

It has been shown previously that about 50% of flat condylomatous lesions of the cervix (9) contain typical HPV particles in koilocytotic cells (12) and reveal nuclear labeling after immunoperoxidase staining with antisera reacting with HPV group-specific antigens (13). In view of the suspected genital origin of laryngeal HPV infections (3, 14), particularly considering the young age at onset for the latter lesions (15), it is of interest that typical HPV 11 DNA was present in all of the five flat condylomatous lesions tested in this study. In 21% of the condylomata acuminata specimens, HPV 11 sequences were demonstrated which escaped detection in a previous study (6) due to the cross-hybridization with HPV 6 DNA. It appears therefore that HPV 11 infections are more frequent at intragenital sites, particularly the cervix, although this needs to be substantiated by testing larger numbers of these proliferations.

Three of 24 malignant cervical tumors (one invasive carcinoma and two carcinomata *in situ*) were clearly positive for HPV 11 DNA. In this respect it may be of interest that malignant conversion of multifocal laryngeal papillomas has been reported after x-ray treatment of the tumors (14). Because the majority of these papillomas appears to be caused by HPV 11 infections this may point to a specific potential of this virus in malignant conversion. An additional invasive carcinoma contained slightly

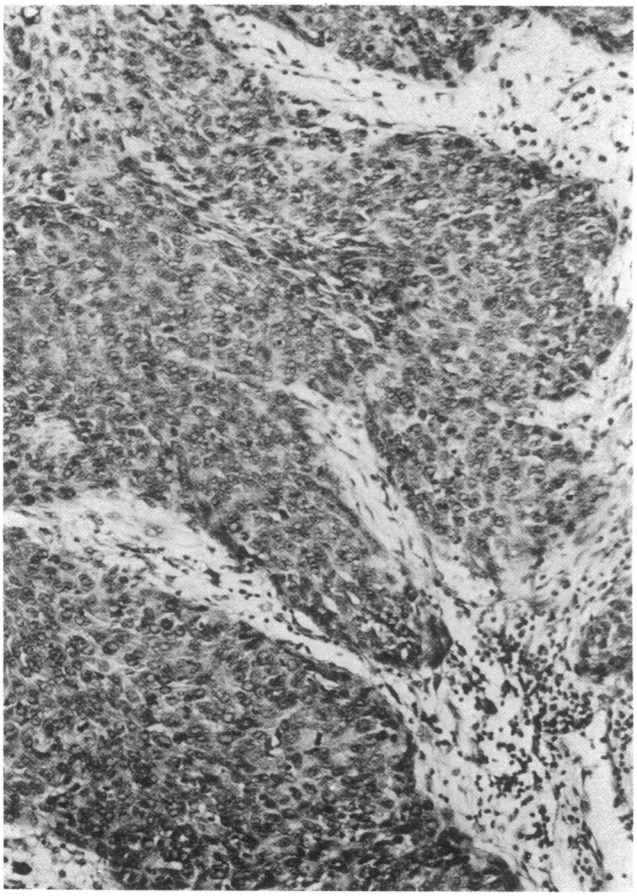

FIG. 4. Invasive squamous cell carcinoma of the cervix found to contain HPV 11 DNA (lane d, Fig. 3). (×325.)

modified HPV DNA, preventing its clear-cut characterization. Nontyped HPV DNA isolated from a patient with epidermodysplasia verruciformis has been found in four carcinomas of vulval and cervical origin (16). HPV 6 or HPV 11 DNA has been found in all four Buschke–Löwenstein tumors (a nonmetastasizing verrucous carcinoma) tested thus far (6).

At present it is hard to assess the significance of these data and additional studies are required to ascribe the specificity of hybridization to the carcinoma cells and to elucidate the role of papillomaviruses in human genital cancer.

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