Physical State and Biological Activity of Human Papillomavirus Genomes in Precancerous Lesions of the Female Genital Tract

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SUMMARY

The DNA of distinct human papillomaviruses (HPVs) is regularly detected in the majority of human cervical carcinomas. In contrast to benign HPV-induced genital lesions, where the viral genomes are exclusively present as episomes, in cervical carcinomas HPV type 16 (HPV16) DNA was found to be integrated into the host DNA. In order to determine the physical state and expression of HPV DNA sequences at different stages of tumour development, we analysed a series of cervical lesions (mild, moderate and severe dysplasia and carcinoma in situ) that are considered precursors of carcinomas of the cervix. In 66.6% (18 of 27) of the tumours, HPV16 DNA was present. While in mild dysplasias only episomal HPV genomes were found, in all higher grade lesions integration of the viral DNA was detected. There was a close correlation between the episomal state and the expression of the HPV16 genomes: in 15 cases harbouring episomal HPV16 DNA (seven of which also contained integrated genomes) viral transcripts were present. We conclude that integration of HPV genomes takes place very early in cervical cancer development. In addition, the episomal state of the viral DNA depends on viral gene expression. The same conclusion, however, is not applicable in those lesions (three severe dysplasias) containing exclusively integrated HPV16 DNA. Thus, HPV16 DNA can persist in an integrated state without recognizable transcriptional activity. These results point to HPV16 as one potential prerequisite for the first steps in the multistage development of human cervical cancer.

INTRODUCTION

Human papillomaviruses (HPVs) cause benign tumours of the skin and mucosa in humans (for review, see Pfister, 1984). Up to now, more than 40 different genotypes of HPV have been isolated. The DNA of some of these types is regularly detected in specific human cancers, e.g. HPV type 5 (HPV5) and HPV8 in carcinomas of patients suffering from epidermodysplasia verruciformis, and HPV16 and HPV18 in genital cancer (Boshart et al., 1984; Dürst et al., 1983; Lehn et al., 1985; Orth et al., 1980; Villa & Lopes, 1986).

Papillomavirus infections appear to be widespread in the human anogenital region. The viruses are associated with condylomata acuminata and giant condylomas, condylomata and dysplasias of the female genital tract, Bowenoid papulosis and Bowen’s disease, and also with cervical, vulvar and penile cancer (for review, see McCance, 1986; also Kadish et al., 1986; Shirasawa et al., 1986; Villa & Lopes, 1986). A significant number of women (10%) with
clinically, colposcopically and cytologically normal cervices have been shown to be infected with HPV (Schneider et al., 1985; Wickenden et al., 1985).

While it is generally accepted that the viruses are the causative agent for the benign genital lesions, their role, if any, in invasive carcinomas is still unknown. Evidence linking specific HPV types to human genital cancer is derived from epidemiological, cytological, histopathological and serological studies (for review, see Baird, 1985). The strongest argument for the association of HPV infection and genital cancer came from DNA/DNA hybridization studies demonstrating that about 70% of all cervical carcinomas analysed so far and also a number of vulvar and penile cancers harboured HPV16 or HPV18 DNA (Boshart et al., 1984; Dürst et al., 1983; Lehn et al., 1985; Villa & Lopes, 1986). The presence of HPV DNA sequences in tumour cells, however, is not a sufficient reason for attributing an aetiological role to HPV in genital cancer.

It is generally believed that invasive cervical carcinomas are preceded by precursor lesions designated as dysplasia and carcinoma in situ or as cervical intraepithelial neoplasia (CIN). This preinvasive phase of cervical cancer is subdivided into three grades, from mild dysplasia (CIN I), through moderate dysplasia (CIN II) and culminating in severe dysplasia and carcinoma in situ (CIN III) (for review, see Nelson et al., 1984). HPV DNA sequences have been found in the majority of these lesions; however, there are few data available on the important questions of physical state and transcription of these genomes. The genomes of HPV types 6 and 11 are present exclusively as extrachromosomal (episomal) DNA molecules in benign lesions of the genital tract (Gissmann et al., 1982; Lehn et al., 1984), whereas HPV16 genomes were shown to be integrated into the host genome in cervical carcinoma biopsies (Dürst et al., 1985; Lehn et al., 1985; Lehn & Sauer, 1985). Both in benign tumours and in some carcinomas, the HPV genomes were transcriptionally active (Lehn et al., 1984, 1985; Lehn & Sauer, 1985; Schwarz et al., 1985) while in contrast HPV16 DNA was not expressed in some of the cervical carcinoma biopsy specimens screened (Lehn et al., 1985; Lehn & Sauer, 1985; Schwarz et al., 1985).

In order to analyse the physical state (episomal or integrated) and the biological activity of the HPV genomes in greater detail, we examined a series of benign and precancerous lesions of the uterine cervix. A total of 34 biopsy samples (seven cases of benign flat condylomas, 23 of dysplasia and four of carcinoma in situ) were analysed for the presence of HPV DNA and RNA sequences. In this study we were able to demonstrate the presence and determine the physical state of HPV genomes in 24 of the lesions; in addition, RNA analysis revealed a strong correlation between physical state and expression of the HPV DNA. This report will shed further light on the association of papillomavirus infection and human cervical cancer.

METHODS

Biopsy material. Biopsy specimens taken from lesions detected by colposcopy of the female genital tract were provided by the Universitäts-Frauenklinik Freiburg, F.R.G. and by the Hospital A.C. Camargo, São Paulo, Brazil. Biopsies were frozen immediately after surgery and stored at -70 °C. Tissue sections were analysed by the local pathology services.

Extraction of nucleic acids from biopsy specimens. Total DNA and RNA were extracted from biopsy specimens according to the method of Krieg et al. (1983) with some minor modifications. Briefly, frozen tissue samples were homogenized in a dismembrator for 20 to 40 s and transferred frozen into a vial containing 15 to 20 ml extraction buffer composed of 66% equilibrated phenol pH 5.5, 33% 0.3 M-sodium acetate, 5 mM-EDTA and 1% SDS. The mixture was vigorously shaken for 10 min and was then re-extracted twice with chloroform:isoamyl alcohol (24:1). The nucleic acids were precipitated from the upper aqueous phase by the addition of 2 vol. ethanol, left overnight at -20 °C, and after resuspension of the pelleted nucleic acids in double-distilled water, DNA and RNA were separated by precipitation of the RNA with sterile LiCl (2 M final concentration). After centrifugation, RNA-containing pellets were suspended in sterile double-distilled water and treated with DNase I (20 µg/ml DNase I grade I, Boehringer) to remove any DNA contamination. DNA was recovered from the supernatant by another ethanol precipitation.

Owing to the small size of the biopsy specimens, the yield of nucleic acids was 10 to 40 µg of DNA and 3 to 10 µg RNA per tissue sample.

DNA blot hybridization. Depending on the amount of DNA available from each biopsy specimen, the tumour DNAs were digested using various restriction enzymes, separated by electrophoresis on 1% or 1.4% agarose gels (5
to 10 μg DNA per lane), transferred to nitrocellulose filters and hybridized under conditions of different stringency to 32P-labelled, nick-translated (Rigby et al., 1977) cloned HPV DNAs.

Hybridization under stringent conditions (Tm = 28 °C) was as follows. Filters were preincubated overnight at 68 °C in five times the concentration of Denhardt's solution (Denhardt, 1966) and hybridized for 12 to 16 h at 68 °C to 32P-labelled cloned HPV6b, HPV16 and HPV18 DNA (1 × 10⁸ to 3 × 10⁸ c.p.m./μg DNA; 1 × 10⁶ c.p.m./ml) in 1 × Denhardt's solution, 0.5% SDS, 1 mM-EDTA, 6 × SSC (1 × SSC is 150 mM-NaCl, 15 mM-sodium citrate). Filters were subsequently washed six times for 10 min in 3 × SSC supplemented with 1 × Denhardt's solution plus 0.1% SDS, then for 20 min in 0.1 × SSC plus 0.1% SDS once and three times for 10 min in 3 × SSC (all washing procedures at 60 °C). The filters were then dried and exposed to Kodak XAR-5 X-ray films.

Hybridization under relaxed conditions was as follows. Filters were preincubated overnight at 37 °C in 8 × SSC, 20% formamide, and 10% dextran sulphate. Hybridization was performed at 37 °C for 36 h in 8 × SSC, 20% formamide, 10% dextran sulphate plus a mixture of 32P-labelled cloned DNAs of HPV6b, HPV16 and HPV18 (0.5 × 10⁶ to 1 × 10⁶ c.p.m./ml for each HPV DNA), from which the pBR322 moiety had been removed by gel electrophoresis before nick translation. Filters were then washed three times for 20 min in 0.1 × SSC at each of four different temperatures, 23 °C (Tm = 70 °C), 37 °C (Tm = 56 °C), 51 °C (Tm = 42 °C) and 65 °C (Tm = 28 °C). Before washing at higher temperatures, wet filters were analysed by autoradiography for the presence of sequences with homologies to HPV6, HPV16 and HPV18.

Cloned DNAs from HPV6b, HPV16, and HPV18 were kind gifts from Dr L. Gissmann (German Cancer Research Center, Heidelberg).

**RNA analysis.** Owing to the low amount of RNA available (3 to 10 μg total RNA per tumour), RNA analysis was performed by the procedure of synthesis of 32P-labelled cDNAs by reverse transcription of the polyadenylated RNAs present in the tumour RNA samples, as described recently elsewhere (Lehn et al., 1984). Total RNA (3 to 10 μg) was incubated at 37 °C in 200 μg/ml oligo(dT), 30 μM-32P-labelled deoxynucleoside triphosphates (Amersham, 400 Ci/mmole), 100 μg/ml actinomycin D, 50 mM-Tris-HCl pH 8.3, 6 mM-MgCl₂, 140 mM-KCl and 6 to 18 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St Petersburg, Fla., U.S.A.). After 60 min, proteinase K and SDS were added to final concentrations of 0.5 μg/ml and 0.1%, respectively. After incubation for 30 min at 37 °C, the RNA was hydrolysed by NaOH treatment (0.3 M), and the cDNA was purified by chromatography on Sephadex G-100. Hybridization with the 32P-labelled cDNAs was performed under stringent conditions (Tm = 28 °C; see above) with nitrocellulose filters containing restriction fragments of cloned HPV6b, HPV16, HPV18 and pEJ (the cloned ras oncogene of the human bladder carcinoma T24) (1.5 μg plasmid DNA per track). After washing and drying, the filters were exposed for 1 to 3 weeks to XAR-5 X-ray films (Kodak) with intensifying screens.

**RESULTS**

**Strategy**

The detailed analysis of the HPV genomes in precancerous lesions of the uterine cervix is hindered by the size of the specimens, which may comprise only a few milligrams per biopsy. Thus, particular methods for the extraction of nucleic acids are required, such as the pulverization of deep-frozen samples prior to phenol extraction (Krieg et al., 1983). On average, one tumour yielded between 10 and 40 μg DNA and 3 to 10 μg total RNA; this precluded the analysis of the HPV genomes in precancerous lesions of the uterine cervix hindered by the size of the specimens, which may comprise only a few milligrams per biopsy. Thus, particular methods for the extraction of nucleic acids are required, such as the pulverization of deep-frozen samples prior to phenol extraction (Krieg et al., 1983). On average, one tumour yielded between 10 and 40 μg DNA and 3 to 10 μg total RNA; this precluded the analysis of the HPV genomes in precancerous lesions of the uterine cervix.

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Table 1. \textit{HPV DNA and RNA in precancerous cervical lesions}

<table>
<thead>
<tr>
<th>Histology</th>
<th>Case number</th>
<th>DNA/DNA Hybridization</th>
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<tr>
<td></td>
<td></td>
<td>HPV6/11</td>
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<tr>
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<td>CIN III and CIN I</td>
<td>27</td>
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* Owing to the small amount of DNA, we were unable to decide whether this tumour contained integrated HPV16.

In the present study we have analysed seven flat condylomas of the cervix and 27 CINs. HPV DNA sequences were detected in five out of seven (71.4\%) flat condylomas, which were considered to be benign lesions of the uterine cervix. Among the five positive samples, three contained exclusively HPV6/11 DNA; these genomes exhibited the same transcription pattern (data not shown) as described previously for HPV6/11 in biopsies of condylomata acuminata and giant condylomas (Lehn et al., 1984). One of these condylomas also contained DNA of HPV16. The two remaining HPV-positive lesions revealed episomal HPV DNA sequences only under conditions of low hybridization stringency.

The 27 precancerous cervical lesions that were available for analysis are listed in more detail in Table 1. HPV DNA sequences were revealed in 18 out of 27 cases (66.6\%). We found HPV16 DNA exclusively; HPV6/11 and HPV18 sequences were not detected. In all but one of the 18 HPV-positive biopsy specimens, the physical state of the HPV16 genomes was determined by Southern blot analysis. Owing to the low amount of DNA in one carcinoma \textit{in situ} (case 27), we were unable to decide whether integrated HPV16 DNA was present in addition to episomes. Fig. 1 shows the Southern blot analysis of two mild dysplasias (cases 1 and 4). The DNA from these tumours yielded only unintegrated molecules, either undigested (Fig. 1, lanes 1 and 4) or
Fig. 1. Detection of extrachromosomal HPV16 DNA sequences in mild dysplasias of the uterine cervix. DNA samples from two tumours (1 and 4 from Table 1) were separated on a 1% agarose gel, transferred to nitrocellulose, and hybridized under stringent conditions as described in Methods. Lanes 1 to 3, 10 μg DNA/lane from tumour 1; lanes 4 to 6, 6 μg DNA/lane from tumour 4. Lanes 1 and 4, untreated DNAs; lanes 2 and 5, SstI-digested DNAs; lanes 3 and 6, BamHI-digested DNAs. SstI does not cleave HPV16 DNA, but BamHI cleaves it once. HPV16-specific signals were visualized after exposure to X-ray film for 8 days.

Fig. 2. Southern blot analysis of sequences from a moderate dysplasia (tumour 18, Table 1). Untreated or restriction enzyme-digested DNA (10 μg/lane) was hybridized (after gel electrophoresis and transfer to nitrocellulose) under stringent conditions as described in Methods. The filter was exposed for 9 days. Lane 1, untreated tumour DNA; lane 2, SstI-digested tumour DNA; lane 3, BamHI-digested tumour DNA.

Fig. 3. Southern blot analysis of integrated HPV16 DNA from a severe dysplasia (tumour 21, Table 1). Untreated or restriction enzyme-digested DNA (10 μg/lane) was hybridized (after gel electrophoresis and transfer to nitrocellulose) under stringent conditions as described in Methods. The filter was exposed for 12 days. Lane 1, untreated tumour DNA; lane 2, SstI-digested tumour DNA; lane 3, BamHI-digested tumour DNA; lane 4, PstI-digested tumour DNA; lane 5, size markers (numbers refer to fragment size in kilobase pairs).

treated with the enzyme SstI, which does not cleave HPV16 DNA (Fig. 1, lanes 2 and 5). Digestion with the enzyme BamHI, which cleaves HPV16 DNA in one place, revealed the linear form III (FOIII) without any unusual bands (Fig. 1, lanes 3 and 6) showing that the HPV16 DNA in these mild dysplasias was maintained exclusively in the form of extrachromosomal molecules. The episomal state of the HPV16 genomes was also recorded in the other five HPV16-positive CIN I lesions (data not shown) listed in Table 1.

Four of the five moderate dysplasias, one of them diagnosed as CIN I/II, contained HPV16 DNA (Table 1). In all four cases, both extrachromosomal and integrated viral genomes were present. The DNA analysis of one of these tumours (case 18) is shown in Fig. 2. The HPV16-specific signal within the high M, DNA in the untreated sample (lane 1) migrated to another
Fig. 4. Detection of HPV16-specific mRNA in a mild dysplasia by reverse transcription of polyadenylated tumour RNAs. Four μg of total RNA from tumour 4 (Table 1) was used as template for the synthesis of 32P-labelled cDNA, which was hybridized (0-15 × 10^6 c.p.m./ml) to a nitrocellulose filter with different restriction enzyme-treated plasmid DNAs (1-5 μg/lane): lane 1, pEJ digested with SstI; lane 2, pHPV18 digested with HindIII; lane 3, pHPV16 digested with PstI; lane 4, pHPV16 digested with HaeIII; lane 5, pHPV6/21 digested with PstI; lane 6, pHPV6/2 digested with HpalI/PstI. (a) Ethidium bromide-stained 1-4% agarose gel, (b) autoradiogram of the same gel as in (a) after transfer to nitrocellulose and hybridization with the 32P-labelled cDNA from tumour 4 (Table 1). Exposure to X-ray film was for 9 days.

position in the gel after digestion with SstI (lane 2). Treatment with BamHI yielded, in addition to the linear FOIII, several novel bands (lane 3).

A total of nine CIN III lesions (five severe dysplasias and four carcinomas in situ) were analysed for the presence of HPV genomes. In seven of these nine tumours the DNA of HPV16 was detected. In six CIN III lesions the viral genomes were integrated (Table 1); and in one tumour (case 27) unequivocal determination of the physical state of the viral DNA was not possible. As in the less severe cases, in four CIN III lesions extrachromosomal HPV16 DNA was present in addition to the integrated genomes (Table 1); however, we did not detect any episomal molecules in the other three lesions (Table 1). Fig. 3 shows the DNA analysis of one specimen of severe dysplasia (case 21) containing only integrated HPV16 DNA. In the undigested tumour DNA, the HPV16 DNA comigrated with the high M, DNA (lane 1). After digestion with SstI one HPV16-specific signal larger than the linear FOIII was observed (lane 2). Furthermore, altered migration properties were revealed compared with the undigested sample.
Treatment with BamHI, which cleaves HPV16 DNA once, yielded two novel bands (lane 3) while after PstI digestion the authentic PstI fragments became visible, but without the PstI A fragment (lane 4); instead of the PstI A fragment, two novel bands were present. This confines the site of opening within the HPV16 genome present in this tumour biopsy to the PstI A fragment covering the E1 and E2 open reading frames. The exclusive integration of the HPV16 DNA was documented in three other lesions (data not shown); however, owing to the small amount of DNA available, the integration area could not be mapped.

In summary, in early precancerous lesions of the cervix (CIN I) only extrachromosomal HPV16 genomes were detected, while in more advanced lesions (CIN II and III) integrated viral DNA was also present; in some CIN III lesions only integrated HPV16 DNA was found.

**Expression of HPV16 genomes in CIN**

Total RNA preparations from all biopsy specimens were examined for the presence of HPV6/11-, HPV16- and HPV18-specific transcripts using 32P-labelled oligo(dT)-primed cDNAs as probes. These cDNAs were hybridized to filters containing restriction fragments of the cloned genomes of HPV6b, HPV16 and HPV18; in addition, the filters also contained SstI-digested cloned ras oncogene DNA. One example (CIN I, case 4) of this RNA analysis is shown in Fig. 4. Specific signals were obtained with pEJ (Fig. 4b, lane 1) and pHV16 (Fig. 4b, lanes 3 and 4), whereas no transcripts of HPV18 (Fig. 4b, lane 2) and HPV6 (Fig. 4b, lanes 5 and 6) were detectable. It can also be seen that only discrete DNA restriction fragments hybridized with the 32P-labelled cDNA, e.g. only one of three bands in the case of pHV16 digested with HaeIII (Fig. 4a, b; lanes 4). Therefore, it was possible to define the region(s) on the HPV16 genome from which the transcripts were derived; the CIN I lesion in this example contained both early and late transcripts of HPV16.

There were 18 lesions with HPV16 DNA, and 15 were also positive for RNA (Table 1). All these 15 lesions contained extrachromosomal viral genomes; hence, there seems to be a positive correlation between HPV16 DNA replication and viral gene expression. In contrast, RNA analysis of three CIN III lesions containing only integrated HPV16 DNA failed to reveal any viral transcripts. In parallel, we were able to detect HPV16-specific mRNA species in one microinvasive carcinoma, which was classified as stage IA of invasive carcinoma (Nelson et al., 1984), although this tumour contained exclusively integrated HPV16 DNA (data not shown).

**DISCUSSION**

This report contains a preliminary analysis of both the physical state and the biological activity of HPV genomes in those human cervical lesions which are regarded as putative precursors of cervical carcinomas. A total of 27 biopsy samples (13 CIN I lesions, five CIN II lesions and nine CIN III lesions) were analysed for the presence of HPV nucleic acids; in 18 cases DNA of HPV16 was detected. DNA of other genital HPV types, such as HPV6, HPV11 and HPV18, was not found. These HPV DNAs, however, were revealed in a series of other lesions of the human genital tract, such as condylomata acuminata, giant condylomas and flat cervical condylomas, and in biopsies taken from cancerous lesions of cervix and penis. HPV6 and HPV11 are the most prevalent types of papillomavirus found in benign tumours, while in precancerous cervical lesions and in genital carcinomas HPV16 and HPV18 are present (for review, see McCance, 1986; also Kadish et al., 1986; Shirasawa et al., 1986).

Previous studies demonstrated the presence of HPV genomes in a majority of CIN lesions (41% to 83%), HPV16 being the most prevalent type (Crum et al., 1985; Kadish et al., 1986; Scholl et al., 1985; Shirasawa et al., 1986); we detected DNA of HPV16 in 66.6% of the CIN biopsies. In the earliest stages of CIN development the viral genomes were exclusively present as episomes which were actively transcribed. During progression to more severe stages (CIN II, CIN III) integrated HPV16 DNA was observed, as described for cervical carcinomas (Diirst et al., 1985; Lehn et al., 1985; Lehn & Sauer, 1985). In most of these biopsy specimens (eight of 11) episomal viral DNA was also present (Table 1). It is not clear, however, whether extrachromosomal and integrated HPV16 DNA molecules in these lesions co-existed in the
same tumour cell or whether the lesions consisted of different cells containing either exclusively free or exclusively integrated HPV16 genomes.

The different types of CIN lesions are histologically distinguished by the proportion of undifferentiated neoplastic cells present in each: the more advanced the tumour, the more undifferentiated cells can be found (Nelson et al., 1984). Our data suggest a correlation between the increase of neoplastic cells and the detection of integrated HPV16 genomes. This does not by any means exclude the possibility that the integration event took place in CIN I or earlier; integrated genomes might be overlooked at an early stage owing to the level of detection of the method. It is quite conceivable that during progression from CIN I to CIN III only one or a few cells of the primary lesion can proceed to more advanced stages. Integration of HPV16 DNA in some dysplasias (CIN I to CIN III) was reported very recently, and the authors also suggested that the lesion might originate from a single cell clone in which viral integration had occurred (Shirasawa et al., 1986). It seems likely that the higher grade CIN lesions originate from one clone, because the DNA fragments representing the virus–cell junctions of the integrated HPV16 DNA appear as defined bands. The monoclonal nature of human cervical carcinomas has been documented by Lehn et al. (1985) and by Lehn & Sauer (1985).

The integration of HPV genomes in both cervical cancer biopsies and cervical carcinoma cell lines has been shown to occur preferentially within a segment of more than 2000 base pairs (E1/E2 region) of the early region of the viral genome (Dürst et al., 1985; Lehn et al., 1985; Lehn & Sauer, 1985; Schneider-Gädicke & Schwarz, 1986; Schwarz et al., 1985); however, there is no evidence that the integration plays a role in the progression to or in the maintenance of the malignant state. All data available indicate random integration of HPV DNA into the host DNA (Dürst et al., 1985; Lehn et al., 1985; Schneider-Gädicke & Schwarz, 1986). One possible explanation for the fortuitous integration event could be the inability of the undifferentiated neoplastic cells to support episomal replication of the HPV genomes.

In all CIN lesions in which episomal HPV16 genomes were found viral transcripts were also present, while among four cases with exclusively integrated HPV16 DNA, only one, a microinvasive carcinoma, exhibited HPV16 RNA species. The episomal state of papillomavirus genomes in vitro depends on the presence, and probably on the expression, of specific viral DNA sequences; this was shown for bovine papillomavirus (BPV) type 1 in transformed mouse cells (Lusky & Botchan, 1984, 1985; Sarver et al., 1984) and for HPV16 in simian virus 40-transformed monkey cells where HPV16 DNA is able to replicate transiently (Lehn, 1986). We propose on the basis of our data that the expression of such viral DNA sequences is necessary for the maintenance of HPV16 genomes as episomes in vivo, and suggest that all lesions of the genital tract containing episomal HPV16 DNA will reveal viral transcripts. There is no such correlation in tumours where only integrated HPV genomes are present, however. An active role of the virus genomes in tumour maintenance has not yet been demonstrated, although some recent reports suggest an active function of these viruses in carcinomas; it has been demonstrated that some cervical carcinomas and cell lines derived from human cervical cancer contained HPV16 or HPV18 transcripts (Lehn et al., 1985; Schneider-Gädicke & Schwarz, 1986; Schwarz et al., 1985; Smotkin & Wettstein, 1986). However, there are data arguing against this hypothesis, e.g. the lack of HPV16 transcripts in some cervical carcinomas (Lehn et al., 1985; Lehn & Sauer, 1985; Schwarz et al., 1985) and the lack of human T cell lymphotropic virus type I transcripts in adult T cell leukaemia (Franchini et al., 1984) despite the presence of the integrated viral genomes. Furthermore, after the conversion of BPV type 4-induced papillomas in the alimentary tract of cattle into carcinomas, even the viral genomes were absent in 69 of 70 carcinomas, although they were apparently required for induction of the papillomas (Campo et al., 1985). In the present study, we have demonstrated the absence of HPV16 transcripts in a small number of precursor lesions of cervical carcinomas although the tumours contained integrated HPV16 DNA.

The monoclonal nature of cervical carcinomas (Lehn et al., 1985; Lehn & Sauer, 1985) makes the development of such a tumour an extremely rare event. It is known that only a small proportion of CIN I lesions progresses to higher grade lesions and that cervical cancer develops only after a long latency period. On the basis of these observations it is evident that the
papillomavirus infection by itself is not sufficient for malignant conversion, and it is still not clear whether it is even necessary for this event. It is generally accepted that interactions between papillomavirus-infected cells and carcinogens are required for carcinoma development (for review, see Pfister, 1984). The evidence for the very early occurrence of the integration event suggests that the HPV infection may precede the neoplastic change and the virus may contribute to the development of the neoplasia as a carcinogen or tumour promoter; this idea was discussed by Nelson et al. (1984). It was proposed that activated oncogenes also play a part in the process of carcinoma development (Amtmann et al., 1987). It is conceivable that a papillomavirus infection might increase the number of initiated cells, leading to the formation of a papilloma. After exposure to a carcinogen, one single cell might progress to develop a monoclonal carcinoma. Hence, in our view, initiated cells and HPV infection act synergistically to produce a greater risk of CIN development. This model relates the papillomavirus in a formal sense to a tumour promoter. Therefore, the virus is one possible risk factor in the multistage development of human cervical carcinomas.

REFERENCES


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