Detection of Human Papillomavirus Type 16 DNA and Evidence for Integration into the Cell DNA in Cervical Dysplasia

By HIROSHI SHIRASAWA, 1 YOSHIMI TOMITA, 1 KOICHI KUBOTA, 2 TOKUZO KASAI, 2 SOUEI SEKIYA, 3 HIROYOSHI TAKAMIZAWA 3 AND BUNSITI SIMIZU 1*

1 Department of Microbiology, School of Medicine, Chiba University, Chiba 280, 2 Kaihin Hospital, Isobe, Chiba 260 and 3 Department of Obstetrics and Gynecology, School of Medicine, Chiba University, Chiba 280, Japan

(Accepted 20 May 1986)

SUMMARY

The presence of human papillomavirus (HPV) type 16 DNA in biopsies from precancerous lesions and from early lesions of human cervical cancer, and the integration of virus DNA into host cell DNA were analysed by dot blot and Southern blot hybridizations. HPV 16 DNA was detected in 23% of mild dysplasias, 32% of moderate dysplasias, 55% of severe dysplasias and 62% of carcinomas in situ by dot blot hybridization. Digestion of the DNA with restriction enzymes PstI and BamHI followed by Southern blot analysis revealed the presence of some typical restriction fragments of HPV 16 DNA in most virus-positive samples. In addition, we detected submolar fragments which might represent virus-cell junction sequences in 86% of dysplasias, suggesting that the integration of HPV 16 DNA could occur in the precancerous stage.

Human papillomavirus (HPV) type 16 and type 18 DNAs are frequently found in biopsies from precancerous and malignant cervical lesions (Boshart et al., 1984; Crum et al., 1985; Lehn et al., 1985; Tomita et al., 1986), with HPV 16 DNA being more common than HPV 18 DNA in these lesions (Dürst et al., 1983; Boshart et al., 1984; Yoshikawa et al., 1985). HPV 16 DNA has been cloned from an invasive cervical carcinoma (Dürst et al., 1983) and the complete nucleotide sequence was determined (Seedorf et al., 1985). Recently, Dürst et al. (1985) reported the physical state of HPV 16 DNA in some malignant tumours and showed that the integration of HPV 16 genome into the host cell DNA occurs with a head-to-tail viral genome arrangement. Their report includes a discussion of HPV integration in connection with the causative event in malignant transformation.

Dysplasias are considered to be precancerous lesions and are classified as mild, moderate and severe (Koss, 1978). In order to see whether the integration of HPV 16 DNA into host cell DNA occurs in precancerous lesions, biopsy samples from mild, moderate and severe dysplasias and from cervical cancers were screened for the presence of HPV 16 DNA by dot blot hybridization. Virus-specific restriction fragments were analysed by Southern blot hybridization. We report here the detection of submolar fragments, which might be virus-cell junction sequences, in most dysplasias and carcinomas in situ that harbour HPV 16 DNA.

Biopsy samples were collected under colposcopy and kept at −70 °C. High molecular weight DNA was extracted and purified from samples that had been histopathologically confirmed (Tomita et al., 1986). For dot blot hybridization, about 7.5 μg of the purified DNA was denatured, neutralized, then spotted onto a nitrocellulose filter and hybridized with 32P-labelled cloned HPV 16 DNA in 6 × SSC at 68 °C for 24 h. The specific activity of the HPV 16 DNA was 10^8 to 2 × 10^8 c.p.m. μg. The filter was washed extensively with 0.1 × SSC containing 0.5% SDS at 68 °C and autoradiographed (Tomita et al., 1986). For Southern blotting about 10 μg
Table 1. Occurrence and integration of HPV 16 genome in biopsies from cervical lesions

<table>
<thead>
<tr>
<th>Cervical lesion</th>
<th>Occurrence of HPV 16 (%)</th>
<th>Integration in HPV 16-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild dysplasia</td>
<td>3/13 (23%)</td>
<td>3/3</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>8/25 (32%)</td>
<td>4/5</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>12/22 (55%)</td>
<td>5/6</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>8/13 (62%)</td>
<td>5/5</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>3/6 (50%)</td>
<td>3/3</td>
</tr>
</tbody>
</table>

DNA was digested with PstI or BamHI, then subjected to agarose gel electrophoresis (0·8% or 1·0%). After transfer onto a nitrocellulose filter, hybridization was done using 32P-labelled HPV 16 DNA, from which the vector DNA had been removed, under conditions of high stringency (Tomita et al., 1986). For low stringency hybridization the filter was washed with 1·1 × SSC at 52 °C.

The occurrence of HPV 16 DNA in biopsies was determined by dot blot hybridization and the results are shown in Table 1. Virus DNA was detected in about 38% of precancerous dysplasias, while in the case of malignant lesions (carcinoma in situ and invasive carcinoma) the virus DNA was found at a higher percentage (58%). The restriction endonuclease PstI cleaves cloned HPV 16 DNA at six sites generating fragments A (2·8 kb), B (1·8 kb), C (1·5 kb), D (1·1 kb), E (0·5 kb) and F (0·2 kb). These fragments are good markers for detecting typical HPV 16 fragments by agarose gel electrophoresis. As shown in Fig. 1(a) and Fig. 2(a), typical HPV 16-specific 2·8 kb, 1·8 kb, 1·5 kb and 1·1 kb fragments were easily detected in most HPV 16-positive samples from moderate and severe dysplasias as well as from carcinomas in situ and invasive carcinomas. In cases of mild dysplasia, the patterns of restriction fragments were not typical (Fig. 1a; 12, 15), but each sample contained fragments that hybridized with 32P-labelled HPV 16 DNA. In addition to these fragments, some faint fragments which migrated higher than the 2·8 kb fragment or between the 2·8 kb and the 1·5 kb fragments were found in most samples from moderate and severe dysplasias, although the pattern varied from sample to sample. Even under low stringency conditions these additional fragments hybridized as faint bands, indicating that they did not result from cross-hybridization with other types of HPV (data not shown). Dürst et al. (1985) also observed similar faint fragments in a cervical cancer biopsy and they showed that these represent the virus–cell DNA junction fragments. To confirm these integrations more firmly, DNA samples were completely digested with BamHI which cuts HPV 16 DNA at one site. As shown in Fig. 1 (b) and Fig. 2 (b), one or two submolar fragments which migrated higher or lower than the 7·9 kb fragment were found in biopsies from dysplasia, carcinoma in situ and invasive carcinoma. A dense 7-9 kb fragment which is the same size as the linear form of the complete HPV 16 genome was also found (Fig. 1b; S1,O24 and Fig. 2b; C18,C5).

On the basis of these observations, we assume that the detection of submolar fragments, especially of those which migrate higher than the 7·9 kb band, shows the existence of virus genomes integrated into cell DNA, because the HPV 16 genome and other HPV genomes (HPV types 6,11,18) are 7·9 kb or less in size and have at least one BamHI site. We then surveyed the possible integration of viral genome in other biopsy samples. As shown in Table 1, we found that integrated viral DNA occurs even in a high percentage of dysplasias (86%).

It has been reported that in some cervical cancers the full-length HPV 16 genome exists as high molecular weight oligomeric episomes and integrated in the host cell DNA in the form of head-to-tail tandem repeats (Dürst et al., 1985). The state of the HPV 16 DNA in our samples C18 from invasive carcinoma and S1 from severe dysplasia seems to be similar to this, because the 7-9 kb band is much more dense than the submolar bands. We further analysed S1 DNA by isopycnic centrifugation in a CsCl gradient followed by Southern blotting and detected the major virus DNA at a density of 1·58 to 1·59 g/ml; this DNA migrated to the 9 kb position during gel electrophoresis (data not shown). Therefore, the integrated viral DNA seems to be a
Fig. 1. Detection of HPV 16-specific DNA fragments in severe, moderate and mild dysplasias. Purified DNA was digested with PstI (a) or BamHI (b), then analysed by Southern blotting. (a) Samples S1, S20, S21 and S22 from severe dysplasia, samples O3, O24 and O25 from moderate dysplasia and samples 12 and I5 from mild dysplasia. (b) Samples S1, S20, S24 and 15. A (2.8 kb), B (1.8 kb), C (1.5 kb), D (1.1 kb) and E (0.5 kb) indicate PstI-specific HPV 16 fragments and arrowheads $\triangleright$ indicate submolar fragments which might represent virus-cell DNA junction sequences.
Fig. 2. Detection of HPV 16-specific DNA fragments in an invasive carcinoma and carcinomas in situ.
Purified DNA was digested with PstI (a) or BamHI (b), then analysed by Southern blotting. (a) Sample C18 from invasive carcinoma and samples C5 and C8 from carcinoma in situ. (b) Samples C18 and C5. A to E and arrowheads are as in Fig. 1.

A minor proportion of the total viral DNA associated with this sample, and the major part exists as oligomeric episomes. In samples S20 from severe dysplasia and S5 from mild dysplasia, no typical 7.9 kb band was observed after BamHI digestion. These results suggest that recombination and deletion within the HPV genome occur frequently before or after integration even in dysplasia.

Lehn et al. (1985) observed identical cleavage patterns of integrated HPV 16 DNAs extracted from different sections of the same specimens, suggesting a monoclonal origin for tumours in some cervical carcinomas. Dürst et al. (1985) clearly demonstrated that the integration of HPV 16 DNA occurs at different sites in the host genome, by isolating three different clones of the virus–cell junction fragment from one invasive carcinoma. The latter is the case in sample S22 from severe dysplasia (Fig. 1a) and sample C8 from carcinoma in situ (Fig. 2a), since three or more submolar fragments which are larger than the 2.8 kb fragment were observed after PstI digestion. From our results, it seems very likely that integrated HPV 16 DNA exists in dysplasias as well as in carcinomas in situ and in invasive carcinomas. By Southern blotting the fragments representing the virus–cell junctions are recognized as bands. Therefore, it seems probable that the lesion might originate from one clone in which viral integration has occurred, perhaps in a basal cell since cell division takes place in the basal cell layer. However, the contribution of the viral genome integrated in dysplasia to the progression of the lesion to carcinoma in situ and to invasive carcinoma is still not clear.

We thank Dr. H. zur Hausen and Dr. L. Gissmann, Deutsches Krebsforschungszentrum, Heidelberg, F.R.G. for kindly supplying the cloned HPV 16 DNA.
REFERENCES


(Received 20 March 1986)