Viral DNA Sequences Detected in a Hamster Liposarcoma Induced by Bovine Papillomavirus Type 4

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SUMMARY

Following intradermal inoculation of bovine papillomavirus type 4 (BPV-4) into a Syrian hamster, a liposarcoma developed at the inoculation site 20 months later. The DNA of this tumour contained multiple copies of the BPV-4 genome which existed in a free unintegrated state. Unintegrated viral DNA and viral DNA isolated from virus particles from bovine alimentary tract papillomas revealed identical cleavage patterns with CpG methylation-resistant and -sensitive restriction enzymes: apparently there was no gross methylation of CpG sites in either case. The entire BPV-4 genome appeared to be represented in the tumour DNA.

Several bovine papillomaviruses (BPV) have been described (Campo et al., 1980, 1981; Jarrett et al., 1984; Lancaster & Olson, 1978; Pfister et al., 1979). BPV type 4 (BPV-4) is particularly interesting since BPV-4-induced papillomas are found in cattle with squamous carcinoma of the alimentary tract which is associated with the eating of bracken fern (Pteridium aquilinum) (Campo et al., 1980; Jarrett et al., 1978a, b). The BPV-4 genome is oncogenic since it transforms NIH 3T3 cells in culture and the resulting BPV-4 DNA-containing cell lines are tumourigenic in nude mice (Campo & Spandidos, 1983). So far, however, there are no reports of BPV-4 being directly carcinogenic in the natural or a heterologous host. Here we report, for the first time, the induction of a tumour in a Syrian hamster inoculated with BPV-4 and the analysis of the tumour DNA for BPV-4 DNA sequences.

Six Syrian hamsters were injected with clarified 10% suspensions of BPV-containing bovine alimentary papillomas. One-hundred μl of this preparation, which had been checked for virus by electron microscopy and the viral DNA typed as BPV-4 by restriction cleavage, was inoculated both into the right buccal pouch and intradermally on the skin of the back. Twenty months later one of the hamsters developed a tumour at the inoculation site on the back. This soft tumour (2 × 1 cm) was histologically classified as a liposarcoma. There was no evidence of fibrocytic transformation and no secondary tumours were noted in the lungs, liver or kidneys.

High molecular weight DNA was prepared using guanidinium thiocyanate extraction followed by CsCl density centrifugation (Chirgwin et al., 1979). Approximately 20 μg of DNA was obtained from the small amount of tissue available. To analyse the state and presence of any viral DNA in the liposarcoma DNA, Southern blots (Southern, 1975) of undigested and endonuclease-digested DNAs were prepared and hybridized to a variety of BPV DNAs. Of these DNAs, which included a number of recombinant molecules of pAT153 containing inserts also of BPV-1 (pBV1), BPV-2 (pBV2), BPV-5 (pBV5), only pBV4 hybridized to the tumour DNA. Undigested DNA revealed the presence of viral DNA forms I, II and III representing supercoiled (I) and relaxed (II) circular molecules, and linear molecules (III) (Fig. 1 a). The molecular weight of the form III molecule corresponded to $4.5 \times 10^6$, which is the molecular weight of linear BPV-4 DNA isolated from bovine alimentary tract papillomas (Campo et al., 1980). Cleavage with the restriction enzymes HindIII and EcoRI yielded DNA fragments whose molecular weight corresponded to the DNA fragments produced by these enzymes with BPV-4.
Fig. 1. Autoradiograms of hamster liposarcoma DNA hybridized to pBV4 DNA. Agarose gels (0.7%, 20 × 18 × 0.3 cm) were loaded with individual samples of 2 µg DNA. Blots were hybridized (Southern, 1975; Wahl et al., 1979) with nick-translated (Rigby et al., 1977) 32P-labelled pBV4 DNA (Campo & Coggins, 1982) in 2 × SSC (SSC = 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0) at 60 °C. Molecular weights were calculated using known DNA standards, including HindIII-, BamHI- and EcoRI-digested λ DNA, visualized by staining in ethidium bromide. In some cases this was in addition to internal molecular weight standards (visualized by hybridization) of HindIII- and EcoRI-digested BPV-4 DNA (Campo et al., 1980). (a) to (d) are different gels. Lanes 3 and 4 contain 100 viral genome equivalents; lanes 7 and 8 contain 50 genome equivalents. Lane 1, BamHI/tumour DNA; lane 2, non-digested tumour DNA; lane 3, EcoRI/BPV-4; lane 4, HindIII/BPV-4; lane 5; EcoRI/tumour; lane 6, HindIII/tumour; lane 7, MspI/BPV-4; lane 8, HpaII/BPV-4; lane 9, MspI/tumour; lane 10, HpaII/tumour; lane 11, HhaI/tumour. Arrowheads indicate the top of the gel; viral DNA forms (F) I, II and III are indicated. The high molecular weight (M) bands in lanes 8 to 10 correspond to HpaII C + D and B + D fragments (1.9 × 10^6 and 2.1 × 10^6 mol. wt.).
Table 1. *Molecular weights of restriction enzyme fragments of BPV-4 DNA from virions and hamster liposarcoma*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Fragment mol. wt. (x 10^-6)*</th>
<th>Enzyme</th>
<th>Fragment mol. wt. (x 10^-6)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI and O</td>
<td>Form III 4.5</td>
<td>HpaII/MspI</td>
<td>A 1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 1.25</td>
</tr>
<tr>
<td>HindIII</td>
<td>A 2.13</td>
<td></td>
<td>C 0.98</td>
</tr>
<tr>
<td></td>
<td>B 1.48</td>
<td></td>
<td>D 0.96</td>
</tr>
<tr>
<td></td>
<td>C 0.88</td>
<td></td>
<td>Total 4.54</td>
</tr>
<tr>
<td>Total</td>
<td>4.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EcoRI</td>
<td>A 3.15</td>
<td>HhaI</td>
<td>A 2.1</td>
</tr>
<tr>
<td></td>
<td>B 1.35</td>
<td></td>
<td>B 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C 0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G 0.15</td>
</tr>
<tr>
<td>Total</td>
<td>4.50</td>
<td></td>
<td>Total 4.51</td>
</tr>
</tbody>
</table>

* Mol. wt. values taken from Campo et al. (1980).
† Mol. wt. determined from ethidium bromide-stained gels containing DNA markers such as HindIII- and EcoRI-digested λ DNA and HpaII-digested BPV-2 DNA (Moar et al., 1981) in addition to BPV-4 DNA digested with HindIII and EcoRI.

virion DNA (Fig. 1 b) (Campo et al., 1980). In addition, the tumour DNA was digested with the isochizomers HpaII and MspI, the former being sensitive to methylation at the CCGG cleavage site (McClelland, 1983). In both cases, fragments were obtained, the molecular weights of which corresponded to those obtained by cleaving BPV-4 DNA with these enzymes (Fig. 1 c; Table 1).

The tumour DNA was also digested with the restriction enzyme HhaI which is sensitive to methylation at the CGCG site (McClelland, 1983). Digestion of BPV-4 DNA with this enzyme produced seven DNA fragments with molecular weights ranging from 2.1 × 10^6 (A) to 0.15 × 10^6 (G) (Table 1). Again digestion of tumour DNA produced the same molecular weight fragments, indicating that the viral DNA in the tumour is not methylated at the HhaI sites (Fig. 1 d). In all cases of restriction endonuclease digestion the sum of the molecular weights of the DNA fragments produced equalled the total molecular weight of BPV-4 DNA (Table 1).

Hybridization of pBV4 DNA to hamster liver, kidney or heart DNAs was negative (not shown). Based on the intensity of the autoradiographic signals on the Southern blots compared to control BPV-4 DNA–pBV4 DNA duplexes the number of copies of the BPV-4 genome in the liposarcoma was estimated at approximately 50 per diploid quantity of host DNA (Fig. 1).

The present results are interesting in that they are an addition to the previously reported cases of BPV DNA sequences found in transformed or tumour cells being maintained as unintegrated multiple copies (Danos & Yaniv, 1983). The additional finding of what appears to be an apparent lack of CpG methylation of the viral DNA in the tumour also confirms some other reports concerning BPV DNA methylation (e.g. Pfister et al., 1981; Moar et al., 1981). However, what makes the present report particularly interesting is the demonstration that it is BPV-4 which can exert a direct carcinogenic potential in a heterologous host. So far, there has only been an indication that this might be so, since cloned BPV-4 DNA transfects and transforms mouse NIH 3T3 cells in culture, the resulting cell lines being tumorigenetic in nude mice (Campo & Spandidos, 1983). Previously reported inductions of tumours by BPV in heterologous hosts have almost certainly not been due to BPV-4 since this virus is exclusively found in alimentary tract papillomas (Campo et al., 1980, 1981; Jarrett, 1985) and alimentary tract papillomas were never used for isolation of virus (Olson & Cook, 1951; Gordon & Olson, 1968; Robl & Olson, 1968; Moar et al., 1981). Moreover, BPV-4 is not related to BPV-1 or BPV-2 (Campo et al., 1980) which are most likely, and in those cases analysed in detail definitely are, the viruses responsible for tumour induction in heterologous hosts as previously reported (Lancaster et al., 1979; Moar et al., 1981; Pfister et al., 1981). In fact, BPV-4 is a member of a subgroup of BPVs unrelated to
BPV-1/BPV-2 (Jarrett et al., 1984). In turn, the fact that BPV-4 can directly cause malignant tumours in a heterologous host is an important finding since it is BPV-4 which is associated with malignant cancer in the natural host (Jarrett et al., 1978b).

Finally, it is intriguing that BPV-4 induced a liposarcoma in the hamster. It is usual for BPV to induce fibrosarcomas or fibromas in heterologous hosts. In view of the present result, infection and maintenance of the papillomavirus genome in tumour types in addition to these should be considered.

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REFERENCES

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