Molecular Cloning of a New Human Papilloma Virus Isolated from Epidermodysplasia Verruciformis Lesions

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SUMMARY

We have recently reported isolation of a new human papilloma virus (HPVNF) from red plaques of a patient (NF) with epidermodysplasia verruciformis. The HPV DNA was molecularly cloned in Escherichia coli χ1776 using plasmid pBR325. We have constructed a detailed physical map of the cloned DNA, which should be useful for examining the relationship of HPV and skin carcinoma.

Epidermodysplasia Verruciformis (EV) is a chronic skin disease caused by human papilloma virus (HPV) (Lewandowsky & Lutz, 1922; Orth et al., 1978). EV converts to squamous cell carcinoma in 30% of the cases (Jablonska et al., 1972; zur Hausen, 1977; Lutzner, 1978; Orth et al., 1979). The HPV DNAs isolated from flat warts or macular lesions of EV patients have been analysed using restriction endonucleases, and several types of HPV, including 3, 5, 8, and 9, have been found in EV lesions (Orth et al., 1978; Pfister et al., 1981; Green et al., 1982; Kremsdorf et al., 1982). HPV-5 DNA has been reported to exist in carcinoma cells as unintegrated, free viral DNA (Orth et al., 1980; Ostrow et al., 1982). Although HPV is strongly suspected of being involved in carcinogenesis, little is known about this virus in comparison with other papovaviruses such as polyoma virus and SV40. This is due in part to the narrow host range of HPV and absence of a system for virus propagation in cultured cells.

Recently, we isolated a new HPV from an EV patient (NF), a 24 year-old man (Yutsudo et al., 1982). The cutaneous lesions of NF were red plaques with pityroid scales on the surface. The molecular weight of isolated HPV DNA was 5.0 x 10^6. The DNA was cleaved at one site with EcoRI or BglI, two sites with HindIII, three sites with BamHI or XbaI, and at more than five sites with HindII. This cleavage pattern differs from those of other types of HPV reported so far. To elucidate the functions of HPV genes and their role in carcinogenesis, a means of efficiently preparing large amounts of DNA is necessary. Unfortunately, the new virus, like other HPVs, does not replicate in cultured cells. We therefore carried out experiments to clone the DNA of this HPV.

HPV DNA used for cloning was extracted from red plaques of patient NF. Form I DNA was purified by caesium chloride–ethidium bromide density gradient centrifugation as described previously (Yutsudo et al., 1982). HPV DNA was cut with EcoRI, producing linear DNA which was then ligated into the EcoRI site of plasmid pBR325 (3.6 x 10^6 daltons). The single EcoRI site in pBR325 lies within the gene coding for chloramphenicol resistance. After digestion with EcoRI, pBR325 DNA was treated with bacterial alkaline phosphatase. The cleaved HPV and plasmid pBR325 DNAs were mixed at a ratio of 2:1 (i.e. 0.2 μg of HPV DNA and 0.1 μg of pBR325 DNA) in a ligation mixture (20 μl) containing 50 mM-Tris–HCl pH 7.8, 10 mM-MgCl2, 20 mM-dithiothreitol, 1 mM-ATP, 50 μg/ml bovine serum albumin, and 3 units of T4 DNA ligase (New England Biolabs, Beverly, Mass., U.S.A.). After incubation for 15 h at 4 °C, the DNA was precipitated, resuspended in 0.1 ml buffer solution (10 mM-Tris–HCl pH 8.0, 10 mM-CaCl2, 10
Short communication

EcoRI

![Restriction map of the cloned HPV DNA](image)

Fig. 1. Restriction map of the cloned HPV DNA. The positions of fragments shown with asterisks could not be determined.

mM-MgCl₂) and used to transfect *Escherichia coli* X1776 that had been treated with 75 mM-CaCl₂, 0.14 mM-NaCl, 20 mM-Tris-Cl pH 8.0. From the colonies resistant to ampicillin and tetracycline and sensitive to chloramphenicol, one clone (pHPVNF) was chosen. The cloned DNA and the original viral DNA were compared by digestion with several endonucleases (EcoRI, BglII, HindIII, BamHI, and XbaI). The number of cleavage sites and the size of each fragment were the same for the cloned DNA and the original DNA.

In order to construct a detailed physical map, the DNA was cleaved with various enzymes, and the products were analysed by 0.8% agarose gel electrophoresis (20 V for about 15 h in 40 mM-Tris, 20 mM-sodium acetate, 18 mM-NaCl, 2 mM-EDTA, pH 8.05), or 5% polyacrylamide gel electrophoresis (100 V for 4 h in 50 mM-Tris, 50 mM-sodium borate, 1 mM-EDTA, pH 8.3). The molecular weights of fragments generated by cleavage with each enzyme are summarized in Table 1. The number of cleavage sites were: *Ava*I, 2; *Kpn*I, 3; *Hpa*II, 5; *Hha*I, 6; *Hind*III, 6. Fig. 1 shows the physical map of the cloned DNA genome deduced from the results of double digestions. The positions of some small fragments generated by *Hha*I and *Hpa*II have not yet been determined.

The cleavage pattern shows no similarities to the physical maps of other HPV types. In addition to the new HPV described here, we have isolated other HPVs from EV; these are considered to be type 3 and 5 on the basis of results from preliminary experiments. No nucleotide sequence homologies were detected between the cloned DNA and DNAs of those types. The physical map of our isolate will be useful for characterizing HPV DNA isolated from EV.
Table 1. Restriction endonuclease digestion of the HPV DNA

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<tr>
<th></th>
<th>EcoRI</th>
<th>BglII</th>
<th>HindIII</th>
<th>AvaI</th>
<th>BamHI</th>
<th>XbaI</th>
<th>KpnI</th>
<th>HpalII</th>
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patients and for analysis of the viral DNA in carcinoma cells. We are also attempting to infect cultured human keratinocytes and athymic nude mice using the cloned DNA.

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REFERENCES


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