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## Two human papillomavirus DNAs molecularly cloned from a patient with epidermodysplasia verruciformis: restriction maps.

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## Abstract

Two distinct human papillomavirus (HPV) DNAs (MY-1 and MY-2) were molecularly cloned from the benign skin lesions of a patient with epidermodysplasia verruciformis. The restriction map of MY-1 was the same as that of HPV 3a. The map of MY-2 appeared to be different from those of any HPVs reported in the literature. MY-2 did not cross-hybridize with MY-1 or the DNAs of HPV types 1, 2 and 4 under stringent conditions.

**KEYWORDS:** papillomavirus, viral DNA, molecular cloning, restriction map, epidermodysplasia verruciformis

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## Two Human Papillomavirus DNAs Molecularly Cloned from a Patient with Epidermodysplasia Verruciformis: Restriction Maps

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Two distinct human papillomavirus (HPV) DNAs (MY-1 and MY-2) were molecularly cloned from the benign skin lesions of a patient with epidermodysplasia verruciformis. The restriction map of MY-1 was the same as that of HPV 3a. The map of MY-2 appeared to be different from those of any HPVs reported in the literature. MY-2 did not cross-hybridize with MY-1 or the DNAs of HPV types 1, 2 and 4 under stringent conditions.

**Key words:** papillomavirus, viral DNA, molecular cloning, restriction map, epidermodysplasia verruciformis

The association of many distinct types of human papillomaviruses (HPVs) with epidermodysplasia verruciformis (EV) has been reported (1). We have recently molecularly cloned two distinct HPV DNAs from the benign skin lesions of a patient with EV.

The patient M. Y. was first diagnosed as EV at age 39 and the histological and electron microscopic findings of the skin lesions were reported previously (2, 3). Scrapings were collected from the benign skin lesions on the extremities. Virus particles were extracted from the scrapings and purified as described previously (3). The DNA was extracted from virus particles according to the method by Gissman *et al.* (4).

Restriction endonucleases, T4 DNA ligase and pBR322 DNA were purchased from Boehringer Mannheim Co., West Germany,

or Takara Shuzo Co., Japan. Reactions with these enzymes were carried out according to the methods indicated by the manufacturers.

The electrophoresis was done in 0.8-1.5 % agarose slab gels (14×10×0.4 cm) in TEA·NaCl buffer (50 mM Tris, 20 mM sodium acetate, 2 mM Na<sub>2</sub>EDTA, 18 mM NaCl; pH 8.05) (5) at 10 or 12 mA for 18-20 h at room temperature. The restriction enzyme-digested DNAs of bacteriophage lambda (Boehringer Mannheim Co.) were used as the molecular weight standards.

The preliminary digestion with several restriction endonucleases indicated the presence of at least two distinct HPV DNAs, of which the one-cut enzyme was Bam HI and Sal I respectively. Therefore viral DNA (20 ng) and pBR322 DNA (100 ng) were cleaved with Bam HI or Sal I, ligated with T4 DNA ligase and transfected into *E. coli* K12, strain HB 101, by the calcium chloride procedure (6).

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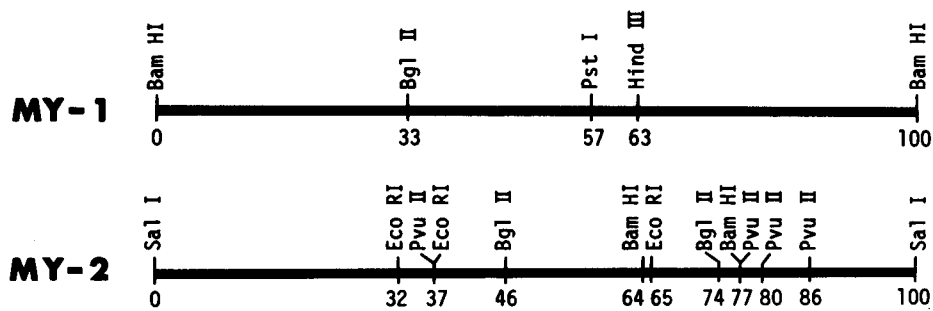


Fig. 1 Restriction maps of MY-1 and MY-2. The distances from the origin (the cloning site) are given in percentages of the molecular weight of viral genomes (MY-1,  $5.24 \times 10^6$ ; MY-2,  $5.00 \times 10^6$ ). MY-1 has no sites for Eco RI and Sal I. MY-2 has no sites for Hind III, Pvu I and Sph I. The map of MY-1 is the same as that of HPV 3a (7).

Transformants were selected on agar plates containing appropriate antibiotics (6). These procedures were carried out under the "Guidelines for Recombinant DNA Research" by the Ministry of Education, Science and Culture of Japan.

The HPV DNAs molecularly cloned by ligation with pBR322 at Bam HI and Sal I site were designated as MY-1 and MY-2 respectively. These cloned HPV DNAs were digested singly and doubly with various restriction endonucleases and electrophoresed. On the basis of the cleavage patterns obtained, their restriction maps were constructed (Fig. 1). The map of MY-1 was the same as that of HPV 3a reported by Kremsdorf *et al.* (7), suggesting its identity with HPV 3a. The map of MY-2 was different from those of any HPVs reported so far in our available literature. It did not cross-hybridize with MY-1 and the DNAs of HPV types 1, 2 and 4 isolated from banal types of cutaneous warts under stringent conditions (50% formamide,  $6 \times$  SSC, 1% sodium dodecylsulfate, 50  $\mu$ g/ml sheared denatured salmon sperm DNA; overnight incubation at  $42^\circ\text{C}$ ).

These results suggest that MY-2 might be another rare type of HPV which is detectable in the patients with EV and rarely in usual persons, though its final classification must await further studies.

On the extremities of the present patient, verrucae planae-like lesions and solar keratoses-like lesions intermingled. HPV 3a has been reported to be associated with verrucae planae-like lesions of EV (1, 7). Therefore, MY-2 might be associated with solar keratoses-like lesions. However, we could not determine this conclusively, because the DNA extracted from the different scrapings of mostly solar keratoses-like lesions was also contaminated with the DNA of MY-1.

*Note added in proof.* Since this communication was submitted for publication, the isolation of a new HPV type designated tentatively as HPV 41 has been reported (Grimmel M, de Villiers E M, Neumann ch, Pawlita M and zur Hausen H; *Int J Cancer*, **41**, 5-9, 1988), and its map markedly resembles that of MY-2 reported in this communication.

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