Lack of Human Papillomavirus DNA in Carcinoma Cuniculatum

Sir,

Carcinoma cuniculatum is a fungous solitary tumour characterized as a low-grade non-metastasizing squamous cell carcinoma, belonging to the group of verrucous carcinomas (1–3). The tumour shows some affinity to the lower extremity, and the initial lesion is often treated as a benign wart for years (4,5). Besides the planter localization of carcinoma cuniculatum, this tumour has been recorded to occur anywhere on the skin surface (6) (Fig. 1). Virus-like particles were seen by electron microscopy in the superficial epithelium in 4 of 13 cases of carcinoma cuniculatum (1,7,8). Human papillomavirus (HPV) type 11 DNA has been found in verrucous carcinomas of the oral cavity (9), the genitoanal region (10,11) and on the leg (12). Recently HPV type 11 DNA was detected in a case of carcinoma cuniculatum on the left lower leg (13). We examined 13 cases of carcinoma cuniculatum, six carcinoma verrucosum, for the presence of HPV DNA using a sensitive polymerase chain reaction. The 13 tumours, seen in the time period from 1982 to 1991, were all clinically characterized by their warty or cauliflower-like presentation and unpleasant odour, all showing similar histopathology with hyper-parakeratosis and acanthosis in a well-differentiated epithelium with little cell atypia. The tumour invades with broad finger-like epidermal projections, often with keratin-filled cysts, the underlying dermis and subcutis.

Samples were prepared for polymerase chain reaction (PCR) as described by Lungo et al. (14) with minor modifications. From each tissue block three 10 μm sections were cut, placed in a 1.5 ml microfuge tube and 200 μg/ml proteinase K was added. Sections were then incubated for 2 h at 65°C. The tubes were centrifuged and the aqueous phase transferred to a new tube. Proteinase K was inactivated by heating, 95°C for 15 min. PCR was performed as described by Manos et al. (15). Five μl from each sample was added to 45 μl PCR mixture. The final composition of the 50 μl was 50 mM KCl, 4 mM MgCl2, 10 mM Tris (pH 8.5), 200 μM of each dNTP, 20 pmoles of each primer and 1.25 U of Taq polymerase. Samples were overlaid with 100 μl of mineral oil and subjected to 40 cycles in a thermal cycler (Perkin-Elmer/Cetus, model 480). Thermo-cycling conditions were 95°C for 1 min, 55°C for 1 min and 72°C for 1 min. The PCR products were examined by gel electrophoresis (4% 3:1 NuSieve agarose, FMC BioProducts-Europe) in TAE-buffer, and stained with ethidium bromide.

In order to ensure the presence of amplifiable DNA each sample was first amplified with betaglobin-primers, which will amplify a 268 bp fragment of the human betaglobin gene (16).

Only samples showing the expected betaglobin bands were examined for the presence of HPV. Primers for HPV were the degenerated consensus primers MY09 and MY11, described by Manos et al. (15). These primers will amplify 450 base pair fragments from approximately 75% of all known HPV types.

Two of the 13 specimens were not amplified by the betaglobin-primers. However, none of the 11 other specimens showed detectable HPV DNA (Fig. 2).

Many of the hypotheses concerning the aetiology of carcinoma cuniculatum are speculative. Some of the histological features are suggestive of viral infection like a prominent granular cell layer and vacuolated cells (1–3). However, immunohistochemistry with peroxidase-antiperoxidase technique and electron microscopy have failed to prove a viral aetiology (2,17). Using dot-blot hybridization HPV type 11 DNA was detected in 2 of 5 biopsy specimens from one patient with carcinoma cuniculatum on the leg (13). It is known that the type of fixative and fixation time may damage DNA and influence the sensitivity of PCR. In this case all biopsies had been fixed in buffered formaldehyde 10%, which is considered the best fixative for DNA preservation (18,19). The duration of fixation is not known, but it is hardly more than 24–36 h. The blocks are from 1982 to 1991. The described method works well with biopsies from cervical and laryngeal lesions of the same age.

We were not able to disclose HPV DNA in samples from 13 patients with carcinoma cuniculatum, indicating that HPV plays a minor role, if any, in the carcinogenesis of this tumour.

Fig. 1. Carcinoma cuniculatum located on the right first toe.
REFERENCES


Received December 1, 1993.

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