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Human papillomavirus DNA in the dermis of condyloma acuminatum

Condyloma acuminatum (CA) has high recurrence rates after local treatments. Why this lesion is difficult to eradicate is unclear. One possible explanation for recurrence after superficial destructive therapy is the presence of residual human papillomavirus (HPV) in the superficial dermis beneath the treated epidermis. Thirteen samples of CA were excised from 13 patients. Thirteen samples of basal cell carcinoma (BCC) were studied for purposes of control. Epidermis was separated from dermis by treatment with sodium bromide. DNA was extracted from both tissues and used sodium bromide solution and amplified for the presence of HPV DNA using the polymerase chain reaction. HPV DNA was detected in the epidermis of 11 samples of CA. HPV type 6 was seen in 7 specimens; HPV type 11, in 4. HPV DNA was found in the dermis of 3 specimens of CA; type 6 in 2 and type 11 in 1. Two samples were excluded because of contamination of the sodium bromide solution by HPV. HPV DNA was not detected in tissue samples from BCC. The presence of HPV DNA in the dermis of some condylomata may explain recurrence in sporadic cases.

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Condyloma acuminatum (CA) is the most common viral sexually transmitted disease; its incidence increased by 459% between the years of 1966 and 1981 (1). CA are frequently refractory to treatment and tend to recur. Podophyllin (2), 5-fluorouracil, trichloracetic acid (3), cryotherapy, electrocautery and laser have all been used as therapies. Recurrence rates in genital warts following local ablative therapies range from 7 to 34% (4). Why condylomata have high recurrence rates is unclear, but possible explanations include reinfection from a sexual partner and extension from adjacent clinically normal skin by HPV (4). HPV infections of squamous epithelia including skin and mucosa are well known. Recently HPV DNA has been detected in other organs including ovary (5–6), uterus (7), endometrial tissue (8), lymph nodes (7), and prostate tissue (9). One cause of treatment failure could be the persistence of HPV in the dermis beneath the epidermal lesion. To investigate this possibility, we assayed for the presence of HPV DNA sequences in the dermis beneath CA.

Material and methods

Epidermal separation and extraction of DNA

Thirteen typical cases of CA and 13 cases of BCC were studied. After removal, the specimens were immediately refrigerated. Specimens were placed in 0.5M sodium bromide for 12–18 hours at 4°C. The epidermis was then manually separated from the dermis. For the CA specimens, DNA and proteinaceous material was precipitated from sodium bromide and removed by low-speed centrifugation ($12000 \times g$, 3 minutes). The supernatant was removed and the precipitated material washed and repelleted. The methodologies for amplification of HPV DNA and detection of the amplified product have been previously described (10). Briefly, epidermis, dermis, used sodium bromide solution and pre-

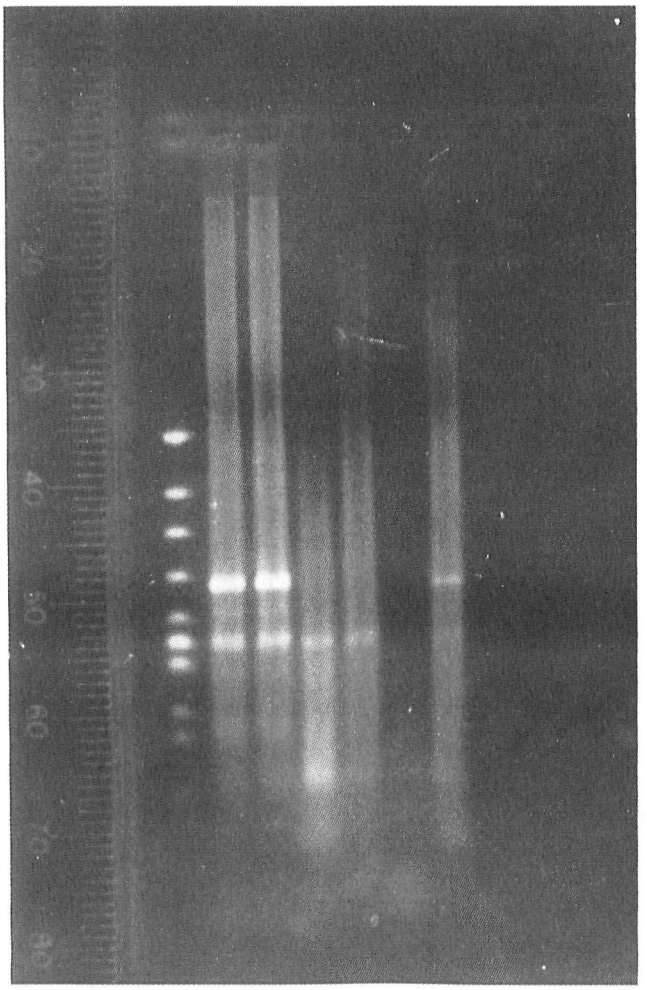

Fig. 1. The products of the polymerase chain reaction in case 5 were electrophoresed on a 2% agarose gel. The contents of the gel lanes are as follows: lane 1: DNA molecular weight markers; Lanes 2 and 3: epidermis and dermis of CA; Lanes 4 and 5: epidermis and dermis of BCC; Lane 6: sodium bromide solution; Lane 7: precipitate from sodium bromide solution from an excluded case.

cipitate were extracted with 1.5 ml of xylene at 55°C for 15 min. The tissue was pelleted at low speed, resuspended in 1 ml of absolute ethanol at room temperature, and repelleted. After lyophilization, the tissue was suspended in 100 µl of digestion buffer (50 mM Tris-HCI, 1 mM EDTA, 0.5% Tween 20, pH 8.5 containing 200 µg/ml proteinase K) and incubated at 55°C for 3 h. The protease was inactivated by heating at 95°C for 10 min. The supernatant was then used as substrate for the polymerase chain reaction.

DNA amplification

To amplify DNA, we used oligonucleotide primers MY11 (5′-GCMCAGGGWCATAAYAATGG-3′) and MY09 (5′-CGTCCMARRGGAWACTGATC-3′) which are specific for a conserved region of the L1 open reading frame of many HPV types (11). To verify the quality of tissue DNA extraction, the oligonucleotide primers GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) and KM38 (5′-TGGTCTCCTTAAACCTGTCTTG-3′) for the human beta-globin gene were used (12). These primers direct the synthesis of a 325 bp fragment of the human beta-globin gene. Each 60 µl reaction mixture contained 2 units of Taq DNA polymerase, 5–10 µl of genomic DNA, 50 pmol of each primer, and 60 nmol of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP) in polymerase chain reaction buffer (50 mM KCi, 4 mM $MgCl_2$, 10 mM Tris hydrochloride pH 8.5). This reaction mixture was overlayed with 100 µl of mineral oil. The polymerase chain reaction was carried out in a DNA thermal cycler (Perkin-

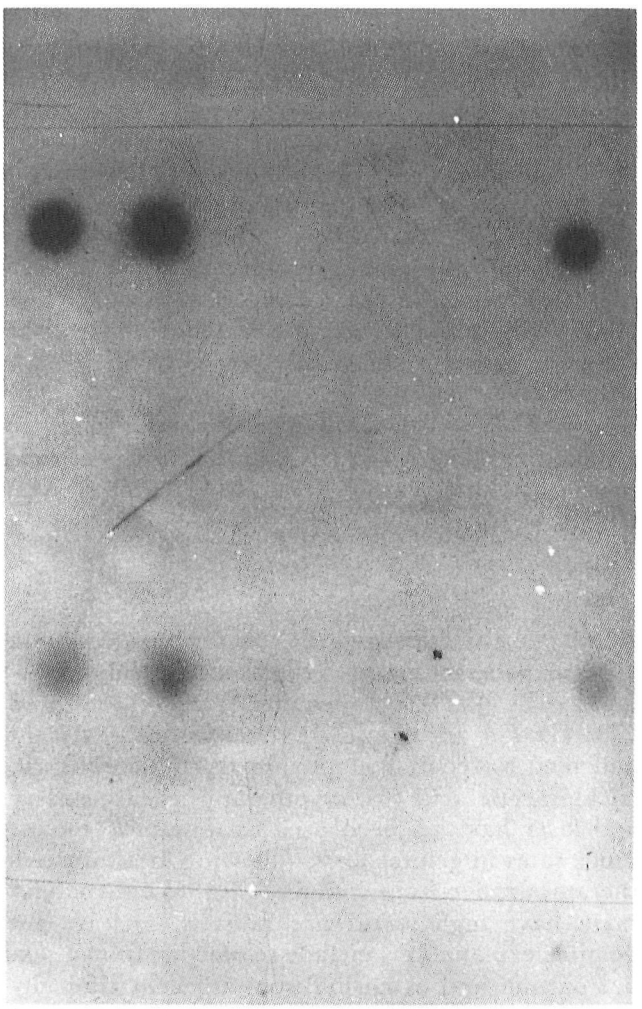

Fig. 2. Dot blot hybridization analysis from case 5. The contents of the vertical lanes are as follows: Lanes 1 and 2: epidermis and dermis of CA; Lanes 3 and 4: epidermis and dermis of BCC; Lane 5: sodium bromide solution; Lane 6: precipitate from sodium bromide solution. The positive blots represent adhesion of radiolabeled probes to HPV type 6 in the upper portion of the figure and to the consensus probe in the lower portion of the figure.

Table 1.

Patient		CA	
1	6	E	D
2	6	+	−
3	6	+	−
4	11	+	+
5	6	+	+
6	6	+	−
7	11	+	−
8	6	+	−
9	6	+	+
10	11	+	−
11	11	+	−

E) Epidermis. D: Dermis.

Elmer Cetus) for 40 cycles. After initial denaturation by heating to 95°C for 2 min, each cycle consisted of denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 2 min. An extra elongation step at 72°C for 5 min was performed after the final cycle. After amplification, 10% of each sample volume was electrophoresed on a 2% agarose gel, stained with ethidium bromide and photographed.

Dot blots

Ten µl of each polymerase chain reaction product was added to 90 µl of denaturation buffer (0.4 M NaOH, 25 mM EDTA), heated at 100°C for 10 min, and filtered through Nytran membrane under low suction. The membranes were rinsed two times with 200 µl of 20X SSPE buffer, and irradiated with ultraviolet light for 2.5 min to cross-link the DNA to the membrane.

Hybridization

The ultraviolet cross-linked membranes were preincubated for 30 min at 56°C in 5 ml of hybridization buffer (5X SSPE, 5X Denhart's solution, and 0.5% SDS). This solution was removed and replaced with fresh hybridization buffer containing a ^{32}P-end-labeled probe specific for either HPV 6 (MY12)(5′-CATCCGTAACTACATCTTCCA-3′), HPV 11 (MY13) (5′-TCTGTGTCTAAATCTGCTACA-3′), HPV 16 (MY14)(5′-CATACACTCCAGCACCTAA-3′), HPV 18 (WD74)(5′-GGATGCTGCACCGGCTGA-3′), or HPV 33 (MY16)(5′-CACACAAGTAACTAGTGACAG-3′)(13). The filters were incubated at 55°C for at least 3 h. The membranes were briefly rinsed at room temperature with 2X SSPE containing 0.1% SDS, and washed in 5X SSPE at 55°C for 15 min. Autoradiography was performed using film (Kodak X-Omat XAR-5) with intensifying screens at -70°C for 12 to 24 h.

Results

Thirteen cases of CA and 13 cases of BCC were studied; however, 2 cases of CA were discarded because of contamination of the sodium bromide solution by HPV, thus invalidating any positive result obtained in dermal tissue. Amplification with the HPV primers was detected in the remaining cases of CA studied. All cases of CA contained HPV amplimers in the epidermal fragments; HPV 6 was found in 7 cases; type 11, in 4. HPV DNA was present in the dermis of 3 of 11 cases of CA; HPV 6 in 2 and HPV 11 in 1. HPV type 16, HPV type 18 and HPV type 33 were not detected. Tissue from BCC did not contain HPV DNA. The results are shown in Table 1.

Discussion

Papillomaviruses are viruses that infect surface epithelia and mucous membranes and often produce warts or epithelial proliferations. Papillomaviruses contain approximately 7800–7900 base pairs of genomic DNA. More that 60 different types of human papillomavirus have been described. Recently HPV DNA has been found in other tissues and organs, including normal ovary and oral mucosa (14). Fuchs and his colleagues (7) studied 28 cases with primary cancers of the cervix; 20 were found to carry viral sequences. HPV 16 sequences were also demonstrated in lymph nodes without any evidence of metastasis. Twenty of 22 diseased prostate tissues contained HPV DNA sequences (9) although an attempt to confirm this finding in prostate resulted in a failure to demonstrate either HPV or herpetic DNA sequences (15). HPV DNA was also detected in endometrial tissue from patients with endometrial cancer (8). Bornstein et al. studied 12 patients with ovarian carcinoma; in 4, HPV DNA type 16 was detected (6). The above findings suggest that HPV can spread to tissues and organs other than the skin and mucosa.

In this study, HPV DNA was detected in the dermal portion of the biopsy specimen in 3 of 11 cases of CA removed from patients with genital warts. Our findings can be explained in a number of ways. First, the dermis may be passively affected by HPV viremia. This is an unlikely possibility since infectious virus has never been detected by immunologic methods in the serum. A second possibility is that the inflammatory response to the presence of HPV infection induces release of HPV DNA. Histologically, condyloma frequently exhibit dilatation of vessels in the papillary dermis, edema and a

lymphohistiocytic infiltrate. Additionally, varying degrees of intraepidermal inflammation are also present and are associated with the presence of a variety of inflammatory cell types. Although virions are only found in the koilocytic and dyskeratotic cells by transmission electron microscopy (1), viral DNA is present in all layers of infected epithelium. HPV may infect the basal cell layer of epithelium (16) and early viral genes are expressed in the lower epithelial layers (17). Hydropic degeneration of basal cells could cause release of virions into the papillary dermis. Furthermore, mobile cells such as mononuclear phagocytes and Langerhans cells could return to the dermis from the epidermis containing HPV DNA sequences. Both are reasonable explanations for the presence of HPV DNA sequences in the dermis. Lastly, the detection of HPV DNA in the dermal component could represent adherence of epidermis, thus contaminating the dermis and resulting in a positive amplification. In this study, all tissues were subsequently digested and were therefore not available for microscopic examination. However, in published works using cold salt-induced cleavage of epidermis and dermis followed by histologic study, the separations occurred cleanly in the lamina lucida, leaving the lamina densa attached to the dermal component (18).

Factors influencing recurrence may vary from one patient to another. Reinfection can occur following intercourse and suggests repeated transmission from sexual partners. Underwear also can serve as a source of reinfection (19). New lesions frequently develop at treated sites, suggesting that latent infection by HPV is present in the untreated adjacent skin or possibly present in the dermis beneath the treated site. If this proves to be true, then extension of the depth of therapy might decrease the rate of recurrence.

It remains to be determined precisely where HPV DNA is located in the dermis. *In situ* hybridization might resolve this problem; however, the sensitivity of *in situ* hybridization is much lower than is that of the polymerase chain reaction. Lastly, recurrence of other types of HPV might also be associated with the presence of dermal HPV DNA sequences. A similar study could be constructed to determine if dermal HPV DNA sequences are found beneath verruca vulgaris at other body sites.

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