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Detection of Human Papillomavirus DNA in Condylomata Acuminata by In Situ Hybridization

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= Abstract =In situ hybridization using biotinylated HPV(Human papillomaviruses) probes was tried in order to detect the presence of HPV DNA in 17 patients with condyloma acuminatum. Fourteen cases responded with positive hybridization signals to HPV 6/11. The specific signals were seen focally in the nuclei of superficial epithelial cells. None of the cases were positive for HPV 16/18. There was no cross hybridization between HPV 6/11 and HPV 16/18 under the stringent conditions used in these experiments. So it could be said that HPV 6/11-related sequences were found in usual condyloma acuminatum.

Key Words: ***In situ hybridization, Human papillomaviruses, Condyloma acuminatum***

INTRODUCTION

Genital warts secondary to HPV infection are usually transmitted by sexual activity (Oriel 1987). For the detection of HPV in clinical lesions, electron microscopy and immunocytochemistry have been employed to demonstrate the virus, but identification of specific types of HPV requires techniques of DNA and RNA hybridization (Schneider 1987). Hybridization of extracted and blotted DNA with radioactively labeled probes has been used with good results, but these techniques do not permit specific localization of HPV within the tissues. Recently, specific HPV types have been localized in fixed paraffin-embedded tissue by radioactively labeled or biotinylated probes (Wells *et al.* 1987).

The present study reports the efficacy of in situ hybridization in detecting the presence of human papillomavirus DNA in genital warts.

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MATERIALS AND METHODS

Materials

A review of pathological records from 1988 to 1989 at our university hospital revealed 17 lesions diagnosed as condyloma acuminatum. The clinical information is summarized in Table 1.

Caski cell line was included for the detection of cross hybridization between HPV 6/11 and HPV 16/18.

In situ hybridization

Sections 4 to 6 μm thick were cut from formalin-fixed, paraffin-embedded tissues and placed on poly-D-lysine coated slides. DNA probes, representing HPV types 6/11, 16/18 provided by Enzo laboratories, were used according to the procedures described by the manufacturers. Briefly, tissue sections were deparaffinized, rehydrated through graded alcohols, digested with proteinase K (50 μg/ml) for 15 minutes at 37°c, treated with hydrogen peroxide, and dehydrated. The hybridization mixture containing a biotinylated probe was applied, and the hybridization was carried out at 37°c for 30 minutes after denaturation at 94°c. The slides were counterstained with hematoxylin and mounted.

Table 1. Distribution of lesions and types of HPV in condyloma acuminatum patients

	Sex/Age	Site	HPV6/11
1.	M/18	coronal sulcus	+
2.	M/21	coronal sulcus	+
3.	M/24	coronal sulcus	−
4.	M/24	coronal sulcus	+
5.	M/27	coronal sulcus	+
6.	M/28	coronal sulcus	−
7.	M/31	urethral orifice	+
8.	M/38	scrotum	−
9.	M/71	anus	−
10.	F/24	vulva	+
11.	F/46	vulva	+
12.	F/58	vulva	+
13.	F/57	vagina	+
14.	F/38	perineum	+
15.	F/40	urethral orifice	+
16.	F/55	cervix	+
17.	F/34	anus	+

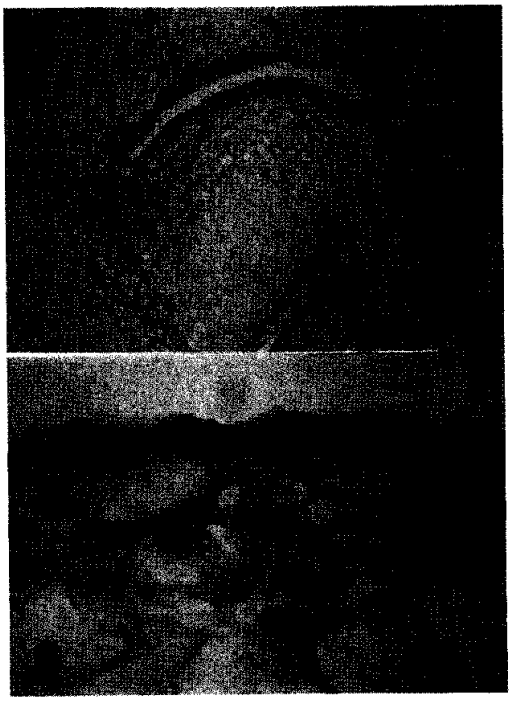

Fig. 1. In situ hybridization using biotinylated HPV6 probe. A : intense staining corresponding to koilocytes in the superficial layers of the epithelium (× 100). B : high magnification (× 400).

RESULTS

The results of the analysis of HPV types are summarized in Table 1. Specimens from 14 cases of condylomata acuminata were positive for HPV 6/11. Caski cell line gave only nonspecific background with HPV 6 although strongly positive for HPV 16 probe. Fig. 1 demonstrated in situ hybridization findings for condyloma acuminatum. Specific hybridization signals were seen focally in the nuclei of superficial squamous epithelial cells, which correlated with koilocytosis, a recognized morphological expression of HPV infection.

None of the lesions were positive for HPV 16/18. There was no cross hybridization between HPV 6/11 and HPV 16/18 under the stringent conditions used in these experiments.

DISCUSSION

In situ hybridization using a biotinylated probe yielded sensitive and reproducible results in this selected series of anogenital lesions. Genital warts from both male and female patients showed HPV 6/11 in 76.5% of the cases. From these results, it is suggested that in situ hybridization is easily applicable to paraffin-embedded tissue sections for the detection of papillomaviruses with high sensitivity. Among the hybridization technigues, Southern or dot hybridization is said to be more sensitive than in situ or filter hybridization for the detection of HPV (Schneider 1987). According to Gissmann *et al.* (1983), HPV were detected in 85.7% of genital warts using Southern blot hybridization. In Korea, Park *et al.* (1989) detected HPV 6/11 in 89.3% of the genital warts found in Korean patients. Croissant *et al.* (1985) suggest that the limit of in situ hybridization is beyond 100 copies per cell. They also showed the existence of a cell population whose terminal differentiation is apparently normal and where the viral DNA is never detected. Although in situ hybridization techniques allow the simultaneous characterization of the types of HPV and the cytopathic effects associated with viral replication, these techniques have the limit of sensitivity. From our experiments, there were 4 cases in which the HPV DNA signals could not be found. These cases were diagnosed as condyloma acuminatum by clinical appearances and histopathologic examination. So biopsy speci-

mens taken in these patients could correspond to noninfected cells or to infected nonpermissive cells.

Because HPV 6 and HPV 11 show a homology of the DNA sequences of about 85% (Dartmann *et al.* 1986), differentiation between two types of HPV was difficult under the stringent conditions used in these experiment. HPV 6/11 is usually found in benign lesions, including genital warts and laryngeal papilloma (Gissmann *et al.* 1983). However, HPV 16/18 is frequently found in cervical carcinoma and cancers of the genital area (Boshart *et al.* 1984; McCance *et al.* 1986). In Korea HPV 16/18 is found in nearly 50% of cervical cancer patients (Park *et al.* 1987). Some authors reported that about 10% of genital warts harbor HPV 16 or HPV 18 which are thought to have oncogenic potential (von Krogh *et al.* 1988). But Sakuma *et al.* (1989) reported HPV 16 was not detected at all in classical condyloma acuminata. In Korea, Park *et al.* (1989) also reported that HPV 16 was not detected in usual condylomata acuminata. Our results also show that oncogenic HPV such as HPV 16 was not detected in usual condylomata acuminata. From these results, it could be said that common cauliflower and papular genital warts do not harbor oncogenic HPV such as HPV 16/18. Recently, polymerase chain reaction was tried in detecting small amounts of DNA by multiplying the specific fragments using Taq polymerase. Although the HPV 6/11-related sequences are discovered in 80% of condylomata acuminata, more sensitive methods, such as polymerase chain reaction, will increase the detection rate of HPV DNA in these lesion.

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REFERENCES

Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer and in cell lines derived from cervical cancer. EMBO J. 1984, 3: 1151-1157

Croissant O, Breitburd F, Orth G. Specificity of cytopathic effect of cutaneous human papillomaviruses. Clinics. in Dermatology. 1985, 3: 43-55

Dartmann K, Schwarz E, Gissmann L, zur Hausen H. The nucleotide sequence and genome organization of human papillomavirus type 11, Virology 1986, 151: 124-130

Gissmann L, Wolnik K, Ikenberg H, Koldovsky U, Schnürch HG, zur Hausen H. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc. Natl. Acad. Sci. USA, 1983: 560-563

McCance DJ, Kalache A, Ashdown K, Andrade L, Menezes F, Smith P, Dol R. Human papillomavirus types 16 and 18 in carcinomas of the penis from Brazil. Int J. Cancer 1986, 37: 55-59

Oriel JD. Genital and anal papillomavirus infections in human males. In Syrnanen K, L. Gissmann, LG Koss (eds) Papillomaviruses and human disease. Springer, Berlin/Heidelberg/New York, 1987: pp 182-196

Park KC, Lee SH, Lee YS, Kim YK, Park HB, Seo JS. Detection of Human papiliomavirus DNA in condylomata acuminata patients using molecular hybridization. Korean J. Dermatol. 1989, 6: 660-665

Park SC, Park JB, Kim SY, Kang SB. Study on human oncogenes-human papilloma viral genes and several oncogenes in Korean cervical cancer tissues. Korean J. Biochem, 1987, 19: 111-118

Sakuma S, Minagawa H, Mori R, Kumazawa J, Sagiyama K, Yanagi K. Human papillomavirus DNA in condyloma acuminatum from Japanese males. Diag, Microbiol Infect Dis. 1988, 10: 23-29

Schneider A. Methods of identification of human papillomaviruses. In Syrnanen K, L. Gissmann, LG Koss (eds) Papillomaviruses and human disease. Springer, Berlin/Heidelberg/New York, 1987: pp. 19-39

von Krogh G, Syrnanen SM, Syrnanen DJ. Advantage of human papillomavirus typing in the clinical evaluation of genitoanal warts. J. Am. Acad. Dermatol. 1988, 18: 495-405

Wells M, Griffiths S, Lewis F, Bird CC. Demonstration of human papillomavirus types in paraffin processed tissue from human anogenital lesions bu in situ hybridization. J. Path. 1987, 152: 77-82

= 국문초록 =

In situ hybridization을 이용한 첨규콘딜롬 조직내 Human papillomavirus DNA의 검색

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첨규콘딜롬으로 진단된 17예의 환자를 대상으로 in situ hybridization을 시행한 결과 다음과 같은 결과를 얻었다. 13예에서 HPV6/11에 양성반응을 보였으며 hybridization 소견은 표피의 표층의 핵에 국소적으로 적색의 반응을 보였다. HPV16/18에 양성반응을 보인 예는 없었으며 HPV6/11과 HPV16/18은 본 실험에 사용된 실험조건에서 교차 hybridization을 보이지 않았다. 따라서 전형적인 첨규콘딜롬에서는 주로 HPV6/11의 염기서열이 발견됨을 보여주었다.