

人類乳突病毒相關的頸部多發性波文氏病

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Human Papillomavirus - Associated Bowen's Diseases of the Neck

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Human papillomavirus (HPV) is known to be involved in the pathogenesis of anogenital cancer. However, it has also been demonstrated in some patients with extragenital Bowen's disease, mainly on the hands, and it usually involves a mucosal type of HPV. We report a 73-year-old woman who had multiple Bowen's disease lesions on the nape of her neck. HPV-16 DNA was detected in the lesions by means of PCR and DNA sequence analysis. No anogenital lesions related to HPV infection were present. Periodic examination was recommended. HPV type 16, a high risk mucosal type HPV, has been established as a causative agent of cervical neoplasia, bowenoid papulosis and Bowen's disease in the genital region. HPV-16 DNA is associated with Bowen's disease in this case, suggesting that HPV-related Bowen's disease is not always restricted to the genitalia. (*Dermatol Sinica* 22 : 29-40, 2004)

Key words: Extragenital Bowen's disease, Human papillomavirus, Polymerase chain reaction

人類乳突病毒已知與肛門會陰部的癌症生成有關，也曾被報告出現在其它皮膚的波文氏病，其位置最常在手上面，且都是黏膜型的人類乳突病毒。我們報告一例七十三歲女性，在後頸部發生與人類乳突病毒相關的多發性波文氏病。將病灶利用連鎖聚合酶反應和脫氧核糖核酸序列分析發現人類乳突病毒第十六型。雖然檢查並沒有發現人類乳突病毒引起的肛門會陰部病灶，仍建議她需要接受定期的追蹤檢查。人類乳突病毒第十六型是高危險性黏膜型的乳突病毒，已知是子宮頸癌和會陰部的波文氏病和波文樣丘疹病的致病因子，在本病例後頸部出現與此病毒相關的波文氏病，表示與人類乳突病毒相關的波文氏病並非只侷限在會陰部。(中華皮誌22 : 29-40, 2004)

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Fig. 1
a. Multiple brownish plaques on the back of the neck.
b. Appearance of the neck nine months after biopsy and CO₂ laser treatment. There is scarring but no recurrence of the Bowen's disease.

INTRODUCTION

It is generally agreed that Bowen's disease of the genitalia is associated with human papillomavirus (HPV) infection.^{1, 2} There are sporadic reports of HPV DNA being identified in extragenital Bowen's disease.³⁻⁹ The vast majority of such lesions have been on the hands, particularly the fingers. We report a case of HPV-associated Bowen's disease of the neck.

CASE REPORT

A 73-year-old woman presented with a 2-year history of slowly progressive skin lesions on the nape of her neck. There were no similar lesions at other sites, and except for hypertension, no other specific medical history was noted. On examination, there were several well-

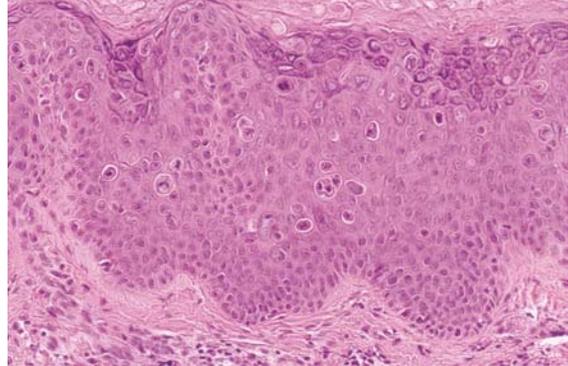


Fig. 2
Full-thickness epidermal atypia with loss of orderly maturation, scattered mitotic figures, and dyskeratotic keratinocytes in all layers of the epidermis. Coarse keratohyalin granules are present in the granular cell layer. There is inflammatory perivascular infiltration in the dermis. (Hematoxylin-eosin stain, x200)

demarcated, slightly elevated, brownish plaques and papules with uneven coloring on the nape of her neck, ranging in size from 0.5 to 2 cm (Fig. 1A). No history of warts was elicited. She had no lesions suspicious for HPV in the anogenital, oral or acral areas. A biopsy specimen and six subsequent shave biopsies from representative lesions all demonstrated similar findings on histologic examination: full-thickness epidermal atypia with loss of orderly maturation, scattered mitotic figures, and dyskeratotic keratinocytes identified in all layers of the epidermis (Fig. 2). Coarse keratohyalin granules were present in the granular cell layer. There were perivascular mononucleated inflammatory cells infiltrating in the dermis. No dermal invasion or koilocytosis was observed. A diagnosis of Bowen's disease was made on the basis of the histologic evaluation.

Following this diagnosis, we investigated her history more thoroughly. She had never lived in areas known to harbor arsenic contamination. No obvious sun damage was noted on her skin and no history of extensive sun exposure was noted. She was a housewife without other specific occupational history or chemical exposure. She was otherwise healthy and was not immunosuppressed. An HIV test was nega-

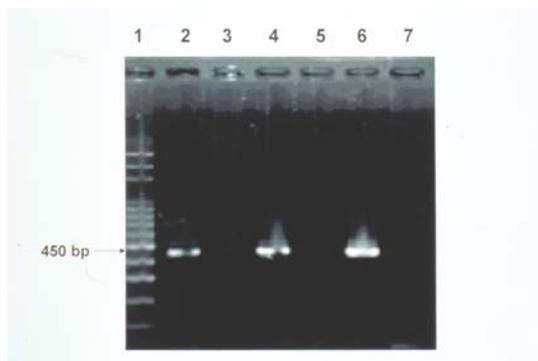


Fig. 3

Amplification of DNA from formalin-fixed tissue of Bowen's disease using MY9 and MY11 as primers to detect human papillomavirus. A positive result is indicated by the appearance of a single band of about 450 bp (arrow). Lane 1: DNA ladder; Lane 2: Bowen's disease-site 1; Lane 3: skin tag; Lane 4 Bowen's disease-site 2; Lane 5: negative control; Lane 6: positive control; Lane 7: water.

tive. Given the increasing evidence of an association between HPVs and malignancies, coupled with the unusual pathological findings similar to bowenoid papulosis, and no history of arsenic or sun exposure, we looked for HPV by using the polymerase chain reaction (PCR) and DNA sequencing. In addition, a skin tag on her neck near the Bowen's lesions was sent for histopathologic examination and PCR analysis as an internal biologic control.

To extract DNA from the tissue block, paraffin sections were transferred directly into PCR tubes and incubated, centrifuged, resuspended, spun down, and lyophilized. The pellets were then processed using a Puregene DNA isolation kit (Gentra, Minneapolis, MN) according to the manufacturer's instructions. Four pairs of oligonucleotide primers were used to amplify exons 3 through 9 of the p53 gene by PCR. The primer pairs to amplify exons 3 and 4 were 3P1 (5'-TTC CTG AAA ACA ACG TTC TGG-3') and 4P2 (5'-GCC AGG CAT TGA AGT CTC AT-3'); for exons 5 and 6, 5P1 (5'-TCT GTT CAC TTG TGC CCT GA-3') and 6P2 (5'-GAC AAC CAC CCT TAA CCC CT-3'), for exon 7, 7P1 (5'-CCT CAT CTT GGG CCT GTG TT-3') and 7P2 (5'-CCA GGG GTC AGC GGC AAG

CA-3'), and for exons 8 and 9, 8P1 (5'-ACT GCC TCT TGC TTC TCT TT-3') and 9P2 (5'-GAA AAC GGC ATT TTG AGT GT-3'). To detect human papillomavirus by PCR, the primers were MY09 (5'-CGT CCM ARR GGA WAC TGA TC-3') and MY11 (5'-GCM CAG GGW CAT AAY AAT GG-3'). PCR was carried out in a DNA thermal cycler (GeneAmp PCR System 9600; Perkin-Elmer; Foster City, CA). The reaction protocol, performed in a microcentrifuge tube, was as follows: 1) denaturation at 95°C for 10 min; 2) amplification for 45 cycles at 94°C for 1 min, 54 ° for 1 min, and 72°C for 1 min; and 3) extension at 72°C for 10 min. A positive result was indicated by the appearance of a single band of about 450 bp in the Bowen's disease specimens. The skin tag (internal biological control) was negative (Fig. 3).

The PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 377 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). Comparison with the published HPV 16R reference sequence revealed that the sequences obtained were from HPV 16. DNA sequencing analysis of p53 tumor suppressor genes in the specimen revealed homozygous proline at codon 72.

The patient was referred to our Gynecological Department for examination. No HPV-related lesions were detected. A Papanicolaou smear of the uterine cervix was negative for intraepithelial lesions or malignancy. Carbon dioxide (CO₂) laser vaporization was performed on the base of the residual lesions on her neck. The patient remains free of disease nine months after treatment (Fig. 1B).

DISCUSSION

Human papillomaviruses (HPVs) have been implicated in the genesis of several human cancers. In the skin, the first evidence suggesting an oncogenic role of HPVs was obtained from studies of epidermodysplasia verruciformis.¹⁰ Characteristically HPV is less associated with squamous cell carcinoma (in situ and

invasive) at sites other than the genitalia, with the exception of patients with epidermodysplasia verruciformis.^{2, 11} HPV type 16, a high risk mucosal type of HPV, has been established as a causative agent of cervical neoplasia, bowenoid papulosis, and Bowen's disease in the genital region.^{1, 2, 12} Cutaneous Bowen's disease associated with HPV occurring a significant distance from the genital region has been less commonly reported. Positive PCR results for HPV have been reported from lesions of Bowen's disease mainly on either periungual area or elsewhere on the hands.^{3-9, 12} Various HPV types (HPV-6, -11, -16, -31, -33, -34, -51, -54, -58, -61, -62, and -73) have been found in extragenital Bowen's disease, with HPV-16 reported most frequently.³⁻⁹ All these HPV types were mucosal types, which are commonly detected in genital neoplasia, such as vaginal intraepithelial neoplasia, cervical intraepithelial neoplasia, and cervical carcinoma.¹² To detect HPV, we use primers MY09 and MY11. MY09/MY11 primer set-mediated PCR is one of the most frequently used amplification systems for the detection of HPV DNA in clinical samples.¹³ The MY09/MY11 primer set is capable of amplifying a wide spectrum of HPV types, including types 6, 11, 16, 18, 26, 31, 32, 33, 34, 42, 45, 51, 52, 53, 56, 58, 61, 66, 68, 69, 70, and 73.¹³

There is some evidence to suggest that an increased incidence of both cutaneous and genital cancers in immunosuppressed individuals, for example renal transplant recipients, may be at least in part related to the presence of certain subtypes of HPV.^{14, 15} With the exception of some women with history of genital dysplasia or cervical carcinoma^{4, 5}, most reports of HPV-related extragenital Bowen's diseases have not mentioned the patients' immune status. Our patient was not immunosuppressed but had clear evidence of HPV-16 DNA in the Bowen's lesions on her neck. The absence of HPV in an adjacent skin tag suggests that the HPV was likely not a coincidental finding but a causative agent in her disease.

There has been an increasing interest in the interaction between a common p53 polymor-

phism and HPV. The p53 tumor suppressor protein functions to signal apoptosis following DNA damage. The ability of the E6 oncoprotein derived from anogenital viruses such as HPV-16 to degrade the p53 protein is a key activity by which the virus promotes cellular immortalization.¹⁶ An arginine substitution for proline at codon 72 of p53 gene results in electrophoretically distinct forms of the protein.¹⁶ Preliminary studies have suggested that the arginine form is preferentially degraded by E6 proteins from both high- and low-risk mucosal HPV types.¹⁷ Consistent with this, some groups have shown an association between arginine homozygosity and susceptibility to cervical cancer.^{16, 18-21} A similar association with HPV-positive non-melanoma skin cancer and arginine homozygosity has also been found by some investigators^{16, 22} but not others.^{23, 24} Samples from our patient's Bowen's lesions were homozygous for proline at p53 codon 72. The extent to which such discrepancies reflect differences in ethnicity, HPV exposure, and inter-lab variation in p53 genotyping remains to be established.²⁰

Multiple lesions of Bowen's disease involving the anogenital skin or multiple bowenoid papulosis lesions have been found to be associated with HPV. In contrast, the occurrence of multiple Bowen's lesions in skin away from the anogenital area is less common, and mainly found in patients with epidermodysplasia verruciformis, arsenic keratosis and solar damage.^{10, 25-26} Our patient had none of these known risk factors. We postulate that her skin lesions may have been due to autoinoculation by digital contact and scratching, although the source of the HPV remains unclear.

Histologically, Bowen's disease usually involves diffuse full-thickness dysplasia and disordered maturation.²⁷ Although histological findings suggestive of HPV infection, such as cytoplasmic vacuolization and koilocytosis, have been found in extragenital Bowen's disease,⁸ Clavel did not find a significant correlation between the presence of one or two HPV types and koilocytosis.⁹ That study also suggested that there was no significant correlation

between the presence of HPV and the location or type of lesions (crusted, erythematous and squamous, etc.).⁹

Some authors have reported that patients with HPV-related Bowen's disease have gynecological lesions, such as cervical intraepithelial neoplasia, vulvar and vaginal dysplasia, associated with HPV infections.^{4, 28} Although mucosal HPV infection is a sexually transmitted disease, the mechanism of HPV inoculation into the skin is yet not clear. Autoinoculation from HPV lesions of the anogenital areas by digital contact is generally accepted.^{4, 6, 9, 11} On the other hand, high-risk mucosal type HPV may be transferred from the skin to the anogenital area. Because the multistage process of oncogenesis starts long after inoculation, the patient may have a previous history of genital warts or genital dysplasia.¹² Forslund *et al.* suggested that in patients with digital squamous cell carcinoma (either *in situ* or invasive), one should consider the possibility of squamous cell dysplasia of the anogenital area.⁴ Our patient had not previously had a Papanicolaou smear or examination of anogenital area until her HPV-induced Bowen's disease was diagnosed. Although she had no anogenital lesions and a normal Papanicolaou smear at that time, periodic Papanicolaou smear analysis is recommended in such patients. The detection of this high-risk mucosal type HPV in extragenital Bowen's disease may lead to better surveillance for anogenital lesions in such patients. The route of infection for HPV-related extragenital Bowen's disease cannot be determined in every patient,³ as is illustrated by our case.

In conclusion, our patient's findings confirm that HPV infection may be related to extragenital Bowen's disease, as demonstrated by PCR and DNA sequencing. This further supports the contention that HPV-associated Bowen's disease is not restricted to the genitalia. Autoinoculation by from one site to another is a possible source of infection in extragenital disease, although it cannot be demonstrated in every case. In patients with HPV-associated Bowen's disease, periodic examinations for squamous cell dysplasia of the anogenital area

are recommended because of the possibility of autoinoculation of HPV to those areas.

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