PAPILLOMAVIRUS INFECTION AMONG JAPANESE: AN IMMUNOHISTOCHEMICAL STUDY FOR PAPILLOMAVIRUS GENUS-SPECIFIC ANTIGEN IN HUMAN SURFACE EPITHELIAL LESIONS

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In order to clarify the relationship between human papillomavirus (HPV) and a variety of surface epithelial lesions, the presence of papillomavirus genus-specific common structural antigen (pgs-antigen) was immunohistochemically investigated in 256 cases of various tumors and tumorous lesions. The pgs-antigen was demonstrated in cases of verruca vulgaris (11/23 cases), condyloma acuminatum (13/26), adult laryngeal papilloma (3/12) and Bowenoid papulosis (2/2). No pgs-antigen was observed in ordinary Bowen's disease and other hyperkeratotic skin lesions, such as keratoacanthoma and seborrhoeic keratosis. In uterine cervical lesions, about 15% of cervical dysplasia, most of which later developed into carcinoma in situ, contained pgs-antigen-positive koilocytotic cells. These results suggest that HPV infection is frequently present in human hyperplastic and atypical surface epithelial lesions of Japanese patients and might indicate possible association with neoplastic transformation, especially in the cervix and skin.

Key words: Papillomavirus infection — Human surface epithelium — Japanese cases — Immunohistochemistry

Since the first discovery of papillomavirus in the horned cottontail rabbit in 1933, the papillomavirus has been isolated from a wide variety of mammals, including human beings. The papillomavirus is a small DNA virus and is a member of papova viridae. Its virion particle has an icosahedral symmetry with 72 capsomeres, approximately 55 nm in diameter. The papillomavirus genome is a double-stranded, closed, circular molecule containing about 8,000 base pairs of genetic information. The ability of papillomavirus to cause benign epithelial papilloma or warts in man and other animals, and fibropapilloma in the sheep and deer dermis is well known.

Recent advances in molecular virology have demonstrated a remarkable pleurality of human papillomavirus (HPV). Based on the restriction endonuclease cleavage pattern of HPV and molecular hybridation techniques, more than 25 different HPVs have been found so far. Specific types of HPVs preferentially associate with specific types of warty lesions and epidermodyplasia verruciformis.

Recently, zur Hausen's group and others have found some HPV-related DNA sequences in precancerous and cancerous lesions of the human skin and cervix and stressed the possible relationship between HPV infection and neoplastic transformation. The presence of HPV-5, -6, -8, -11, -16, and -18-related genomes has already been shown in human cancer materials. In particular, HPV-16 and -18 are reported to be frequently present in premalignant and malignant lesions of the uterine cervix.

The aim of this study was to confirm papillomavirus infection in various lesions of Japanese patients by immunohistochemical methods and, furthermore, to evaluate the possible relationship between HPV infection and neoplastic transformation.

MATERIALS AND METHODS

Tissues Various cases, which were morphologically suspected of papillomavirus infection, were collected from the pathology files of the
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National Cancer Center Hospital. In the field of dermatology, hyperkeratotic papillomatous lesions and Bowen's disease were investigated. In the anogenital area, condyloma acuminatum and Bowenoid papulosis were included in this study. In the oropharynx and upper respiratory tract, the target of this study was squamous cell papilloma including laryngeal papilloma. In the field of gynecology, cervical dysplasias with koilocytotic change and cervical dysplasias which developed later into in situ or invasive carcinoma were studied. The cases are summarized in Table I.

Immunohistochemical Detection of pgs-Antigen Various human tissues for routine surgical pathology, fixed with 15% formalin and embedded in paraffin, were used in this study. After being dewaxed with xylene, 3 µm thick paraffin sections were immersed in absolute ethanol containing 0.3% H2O2 for 30 min at room temperature in order to eliminate endogenous peroxidase activity. The sections were rinsed with tris-buffered saline (TBS; 0.05M tris-HCl buffer containing 0.8% NaCl), then pretreated with 10% normal goat serum in TBS for 30 min at room temperature. The antibody for papillomavirus genus-specific common structural antigen (pgs-antigen) was purchased from DAKO Company (Denmark). This antibody was diluted 5,000 times with 5%, normal goat serum in TBS and used as primary antibody. The primary antibody was reacted with each section overnight at 4°. After being washed thoroughly with TBS, the section was reacted with biotinylated anti-rabbit IgG goat IgG for 30 min at room temperature, followed by avidin-biotin-peroxidase complex (ABC) solution for 1 hr at room temperature, as described in the Vectastain ABC (Vector Lab., U.S.A.) staining procedure.14) Subsequently, sections were colored with 0.05M ammonium acetate-citric acid buffer containing 20 mg/dl of 3',3-diaminobenzidine hydrochloride and 0.005% H2O2, lightly counterstained with hematoxylin, and mounted for light microscopic study.

For the controls, normal rabbit serum was substituted for primary antibody. For confirmation of the reactivity, a case of verruca vulgaris positive for pgs-antigen was examined by electron microscopy of paraffin-embedded material; a typical crystalline array of viral particles was seen in the nuclei of the keratinocytes (Fig. 1).

RESULTS

The results of all cases investigated immunohistochemically for pgs-antigen are summarized in Table I.

Among the 11 of 23 cases of verruca vulgaris positively stained for pgs-antigen, the majority showed typical morphological features of verruca vulgaris. However, almost all cases negatively stained for pgs-antigen differed somewhat from the typical morphology of verruca vulgaris. A positive immunoreaction product for pgs-antigen was

Fig. 1. In a case of verruca vulgaris, a crystalline array of virus particles, about 50 nm in diameter, was ultrastructurally observed in the nucleus of the keratinocyte. Bar, 0.5 µm; × 18,000.

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diffusely seen in the nucleus of cells located in the superficial layer of the epidermis. In about one-half of the pgs-antigen-positive verruca vulgaris cases, the nuclei with positive immunoreaction for pgs-antigen were irregular, sometimes shrunken and at times binucleated. In other cases they were round or oval and usually associated with kerato-hyaline granules. Only in one case did nuclei with prominent eosinophilic inclusions in the granular layer of the epidermis stain positively for pgs-antigen and in this case nuclei without nuclear inclusions were also positive for pgs-antigen (Fig. 2).

No other benign hyperkeratotic and papillomatous skin lesions, such as seborrheic keratosis, keratoacanthoma, squamous cell papilloma, etc., disclosed any positive immunoreaction for pgs-antigen. Exceptionally, several foci of pgs-antigen-positive cells were observed in a hyperkeratotic papillomatous lesion resembling verruca vulgaris (Fig. 3), seborrheic keratosis or condyloma acuminatum, which developed mainly in the anogenital region and the thighs of an old woman, but were also scattered widely

| Table I. The Immunohistochemical Detection of Papillomavirus Infection in Various Human Lesions |
|----------------------------------|------------------|
| Skin                            |                  |
| Verruca vulgaris                | 11/23            |
| Keratoacanthoma                 | 0/15             |
| Seborrheic keratosis            | 0/9              |
| Solar keratosis                 | 0/3              |
| Bowencoid papulosis             | 2/2              |
| Bowen's disease                 | 0/23             |
| Condyloma acuminatum            | 13/26            |
| Squamous cell carcinoma of the genital lesion | 0/2 |
| Other keratotic papillomatous lesion | 1/7 |
| Upper respiratory and digestive tracts |
| Inverted papilloma of nasal cavity | 0/5 |
| Squamous cell papilloma of the pharynx | 0/18 |
| Laryngeal papilloma (adult)     | 3/12             |
| Hyperkeratotic lesion of the esophagus | 0/24 |
| Verrucous papilloma and carcinoma | 0/7 |
| Cervix                          |                  |
| Cervical papilloma              | 1/1              |
| Cervical inverted condyloma     | 1/1              |
| Dysplasia with koilocytic change | 4/18             |
| Dysplasia which later developed into in situ or invasive carcinoma | 9/60 |
| Total 256 cases                |                  |

Fig. 2. In verruca vulgaris, the shape of cell nuclei positive for pgs-antigen varies from case to case. a) Round nuclei associated with cytoplasmic kerato-hyaline bodies (arrows). b) Tortuous or shrunken nuclei. Nuclear inclusion is unclear. c) Round nuclei with true inclusion bodies. Lightly counterstained with hematoxylin. × 330.
in the skin of the chest, arms and abdomen. This unique case will be further studied and reported separately.

As for Bowen's disease, all 23 cases investigated were immunohistochemically negative for pgs-antigen. Early invasive carcinomas progressing from Bowen's disease also failed to demonstrate pgs-antigen in tumor tissues.

Both cases of Bowenoid papulosis revealed many pgs-antigen-positive nuclei in the superficial layer of the epidermis (Fig. 4). Intraepithelial lesions compatible with Bowen's disease were composed of severely atypical cells with large hyperchromatic nuclei, often multinucleated, and contained atypical mitotic figures. These Bowenoid papulosis cases were associated with condylomatous lesions with characteristic koilo-

Fig. 3. Verruca vulgaris-like lesions developed mainly in the anogenital area of an old woman and histologically showing hyperkeratosis, acanthosis and papillomatosis. This lesion was associated with HPV infection. Hematoxylin and eosin stain. ×15.

Fig. 4. Bowenoid papulosis with histology quite similar to that of ordinary Bowen's disease and containing pgs-antigen-positive cells in the superficial layer of the epidermis. Arrows indicate atypical giant cells. Lightly counterstained with hematoxylin. ×75.
cytotic cells in the superficial layer of the epidermis, in which many pgs-antigen positive nuclei were present.

In the upper respiratory and digestive tracts, three cases out of 12 laryngeal papillomas were immunohistochemically positive for pgs-antigen. In all three cases, the laryngeal papillomas were multiple and developed in adults from age 36 to 62. The pgs-antigen was demonstrated in koilocytic cell nuclei in the superficial layer of the papilloma (Fig. 5). The keratinization was minimal and the histology was reminiscent of condyloma acuminatum.

Fig. 5. In some adult laryngeal papillomas, HPV was immunohistochemically demonstrable in the nuclei of koilocytic cells by staining for pgs-antigen. Lightly counterstained with hematoxylin. ×165.

Fig. 6. Cervical inverted condyloma contained numerous pgs-antigen-positive koilocytic cells in the superficial layer. Lightly counterstained with hematoxylin. ×75.
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No pgs-antigen was detected in inverted papilloma of the nasal cavity, various squamous cell papilloma of the pharynx or verrucous lesions including verrucous carcinoma. Hyperkeratotic lesions and squamous cell papilloma of the pharynx and esophagus were also negative for pgs-antigen.

In the genital region, the pgs-antigen was immunohistochemically demonstrated in 13 of 26 condyloma acuminata. The immunoreaction product was localized in the nuclei of koilocytotic cells in the superficial layer. The number of nuclei stained for pgs-antigen varied from case to case. The condyloma acuminatum with positive immunoreaction for pgs-antigen generally showed marked epithelial hyperplasia with koilocytosis as compared to that negative for pgs-antigen.

In the uterine cervix, the pgs-antigen-positive nuclei were numerous in both cervical papilloma and inverted condyloma (Fig. 6). An inverted condyloma was accompanied with a more atypical intraepithelial lesion, namely moderate to severe dysplasia, with a few pgs-antigen-positive cells in the superficial layer.

Biopsy specimens showing mild to moderate dysplasia associated with koilocytic change were about 20% positive (4 of 18 cases) for pgs-antigen. However, the number of pgs-antigen-positive nuclei was usually low.

Furthermore, the presence of HPV infection was immunohistochemically investigated in 60 cases of cervical dysplasia which later developed into in situ or invasive cervical cancer. Of 60 cases, nine cases were demonstrated to possess pgs-antigen in koilocytotic cell nuclei (Fig. 7). The pgs-antigen was found in mild to moderate dysplastic lesions but not in severe dysplasia or carcinoma in situ. The average age at which cancer developed following dysplasia was 47 years in all 60 cases, 43 years in the 9 pgs-antigen-positive cases and 48 years in the 51 pgs-antigen-negative cases.

DISCUSSION

HPV has been demonstrated by morphological and virological methods in various warts of human skin. Based on immunohistochemical identification of papillomavirus capsid antigen or pgs-antigen, the detection rate of HPV is reported to be about 50% in common warts, plantar warts, and anogenital warts. The results of this study are consistent with previous reports. Furthermore, HPV-related DNA has been demonstrated in almost all types of warts by molecular hybridation techniques.

Combined histological and virological examination has shown that HPV-3, -4 and -6 induced warts have a close correlation between histology and virus type, though the histologies of HPV-1 and -2 induced warts are heterogeneous. As shown in this study, a verruca vulgaris or common wart (mainly induced by HPV-2) showed various histological features, especially various cytopathological changes in the superficial layer of the wart. Further study is needed to conclude whether the morphological heterogeneity in common warts is caused by the
pleurality of HPV-2 DNA or host-virus interaction.

Bowenoid papulosis is a newly defined disease entity,\(^\text{23}\) which is histologically quite similar to Bowen's disease but shows quite different clinical features from ordinary Bowen's disease. Previous electron microscopic studies suggested a viral etiology for this disease.\(^\text{7, 18}\) Recently, not only papillomavirus pgs-antigen, but also HPV-16 related DNA have been demonstrated in Bowenoid papulosis.\(^\text{1, 12, 15}\) On the other hand, the correlation between ordinary Bowen's disease and HPV still remains unclear, except in the case of anogenital Bowen's disease and Bowenoid lesion in epidermodysplasia verruciformis.\(^\text{15, 25}\) No pgs-antigen was observed in any case of ordinary Bowen's disease in this study.

In other skin lesions such as seborrheic keratosis, keratoacanthoma and solar keratosis, previous reports indicate that papillomavirus is unlikely to play an important role.\(^\text{1, 16}\) However, there are several reports suggesting papillomavirus infection in solar keratosis and keratoacanthoma.\(^\text{34, 39}\) In this study, a peculiar skin lesion different from epidermodysplasia verruciformis and giant condyloma was found to be related to HPV infection. Further virological studies are necessary to clarify this peculiar disease.

In the upper respiratory and digestive tracts, HPV has been demonstrated immunohistochemically in various warts and condylomata of the oral cavity.\(^\text{16, 27}\) In our own experience, oral warts and condylomata are very rare among Japanese. In this study, no pgs-antigen was detected in oral squamous cell papilloma. This might be attributable, at least in part, to cultural differences from other countries, such as bowing instead of kissing as a greeting, which reduces the opportunity of acquiring HPV infection.

Juvenile laryngeal papillomas are well known to be caused by papillomavirus infection,\(^\text{3, 22}\) especially HPV-6 and -11,\(^\text{10, 25}\) from maternal genital condyloma during the birth.\(^\text{2}\) On the other hand, little work has been done on papillomavirus infection in adult laryngeal papilloma.\(^\text{29}\) This study revealed that papillomavirus pgs-antigen was present in about 25\% of adult laryngeal papillomas. This might be due to a different route of infection from that of juvenile laryngeal papillomas.

In the field of gynecology, HPV infection is noted in about 1–2\% of all women,\(^\text{29}\) and is well-known to be closely related to cervical intraepithelial neoplasia (CIN).\(^\text{28, 36, 37, 42}\) By the immunohistochemical method, papillomavirus capsid antigen or pgs-antigen has been demonstrated to varying degrees in cervical dysplasia.\(^\text{6, 20, 21, 30, 31}\) This study revealed that in Japanese women about 15\% of cervical dysplasia which later progressed to carcinoma, mostly carcinoma in situ (CIS), contained pgs-antigen-positive cells. This result suggests that the infection rate of HPV in Japanese is roughly similar to that in other countries. In general, the immunohistochemically demonstrable HPV infection rate gradually decreases with increase in the grade of dysplasia.\(^\text{20, 36}\) Subsequently, no HPV infection is immunohistochemically demonstrable in CIS and invasive cervical carcinoma.\(^\text{20, 31}\)

Recent advances in molecular hybridation techniques have shown that HPV-related DNA is frequently present in CIN, and even in invasive cervical cancer. In this respect, HPV infection has been strongly suspected to be associated with human cervical oncogenesis.\(^\text{4, 5, 8, 10, 28, 41}\) In particular, both HPV-16 and -18 related DNAs have been detected at a high rate in human uterine cervical premalignant and malignant lesions.\(^\text{4, 5, 8}\)

In conclusion, papillomavirus infection was frequently observed in human hyperplastic and atypical surface epithelial lesions of Japanese patients, and might have some association with human neoplastic transformation, especially in the cervix and skin.

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