Characterization and analysis of human papillomaviruses of skin warts

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Abstract. We analysed human papillomavirus (HPV) infections in 61 tissue specimens of skin warts of Taiwanese patients by DNA hybridization. The prevalence of HPV infection was 69% by Southern blot hybridization. The typing of HPVs was performed by dot blot hybridization under highly stringent conditions with each probe separately. The prevalence of HPV-1, 2, 3, 4, 5, 8, 11, 16 and 18 in skin warts was 13, 7, 16, 2, 0, 5, 2 and 8%, respectively. Chi-squared analysis revealed that there was a correlation between HPV type and copy number. Most HPV-4-induced warts were verruca vulgaris. HPV-1 DNA was detected in verruca plantaris and verruca vulgaris. No specific histopathological features were found to be indicative of the presence or absence of HPV, or of the various types of HPV infection.

Key words: HPV – Skin warts – Dot blot hybridization – Southern blot hybridization

A close clinical relationship between verruca plana, laryngeal papilloma and condylomata acuminata has been known for many years [24]. Copious evidence shows that these conditions are caused by human papillomaviruses (HPVs) [14, 16]. HPVs are naturally occurring DNA tumour viruses that induce epithelial cell proliferation during the course of a productive infection. There are now at least 60 known types of HPV [25]. Each type is distinct, but like all members of the papillomavirus group, they possess an 8-kb virus genome, which is double stranded but contains all of the genetic information on one strand, and is divided into early, late and long control regulatory regions [14].

Among the distinct types of HPVs associated with epithelial or mucosal lesions are types 1 and 4 associated with plantar warts, type 2 with common cutaneous warts, types 5 and 8 with the lesions of epidermodysplasia verruciformis, type 7 with hand warts of patients handling meat [13], type 41 with some cutaneous squamous cell carcinomas [9], types 13 and 32 with oral mucosa [2, 21], types 6 and 11 with laryngeal papilloma, condylomata acuminata and penile condyloma, and types 16 and 18 with carcinoma in situ and invasive malignant neoplasm of the cervix [5, 13, 14]. The oncogenic potential of HPVs is reflected by the association of certain mucosotropic HPVs with varying grades of premalignant squamous lesions and invasive carcinoma of the cervix. Thus the HPV type determines, in part, the site of infection, the pathological features, the clinical appearance, and the clinical course of the respective lesion. Therefore, HPV typing could be clinically important for determining the putative biological potential of some productively infected HPV-associated lesions, particularly benign and low-grade premalignant anogenital tract lesions.

HPVs have been grouped for convenience into those associated with lesions of mucosal origin and those mainly found in cutaneous lesions [25]. The study of non-genital lesions has been fragmentary, and there has been no epidemiological study of HPV skin infections in Taiwan. It is important to establish the role of the various types of HPV in the pathogenesis of skin warts including verruca plantaris, verruca vulgaris, and verruca plana identified clinically. In this study, the existence of the HPV genome was detected by Southern blot hybridization with mixed HPV-1, 2, 3, 4, 5, 8, 11, 16 and 18 as probes under less stringent conditions. The HPV types in skin warts were identified by dot blot and Southern blot hybridization under highly stringent conditions. Patient record were reviewed for HPV infection and lesion locations, clinical diagnosis, sex, initial/recurrent lesions and copy number.

Materials and methods

Tissue specimens

Biopsy specimens from skin warts which included 9 cases on the head/neck (including face, scalp, neck and buccal), 4 on the chest/back, 26 on the hand (including hand, thumb, arm, index, wrist, elbow, palm and finger etc), 12 on the leg (including leg, knee and toe) and 10 on the sole were collected from the Dermatology Clinic at the Tri-Service General Hospital, Taipei, Tai-
The DNA from the tissue specimens was extracted as described previously [18]. Briefly, the DNA was isolated from the tissue by detergent lysis and protease digestion, followed by phenol and then chloroform extraction. The RNA was removed by digestion with ribonuclease. The DNA was concentrated by ethanol precipitation and 3 μg was digested with restriction endonucleases (Bethesda Research Laboratories) under the conditions specified by the vendor. The digested DNA was fractionated by 0.8% agarose gel electrophoresis, stained with ethidium bromide and examined by UV fluorescence photography. The DNA fragments were transferred to nitrocellulose filters (Schleicher & Schuell; BA 85) [3] which were baked under a vacuum, treated with 10X Denhardt’s solution [6] in 6X NaCl/Cit (1X NaCl/Cit = 0.15 M sodium chloride/0.015 M sodium citrate) for 3 h at 55 °C (less stringent conditions) or 68 °C (highly stringent conditions) prior to hybridization, and incubated at 55 °C or 68 °C for 48 h in 0.6 M sodium chloride, 0.06 M sodium citrate, 50 mM sodium phosphate (pH 7.2), 0.1X Denhardt’s solution and 0.5% sodium dodecyl sulphate (SDS) with 2 × 10⁶ Cerenkov cpm of radioactively labelled HPV DNA (generous gift from Dr. zur Hausen).

Less stringent conditions allow hybridization of weakly homologous regions and so permit detection of multiple HPV types with any one HPV probe [15]. These conditions are employed for screening specimens. Stringent conditions are employed for detection of specific HPV types. For ³²P labelling by nick translation, the HPV sequences were separated from the vector by digestion with the appropriate enzyme and recovered after agarose gel electrophoresis. The filters were washed at 55 °C or 68 °C for 4 h in 0.25 M sodium chloride, 0.03 M sodium citrate, 0.5% SDS, and in 0.15 M sodium chloride and 0.015 M sodium citrate for 1 h and air dried. The patterns of hybridization were detected by autoradiography using Kodak X-omat film.

**Dot blot hybridization**

The DNA samples (0.5 μg per tissue specimen) were denatured with NaOH (final concentration 0.3 M) for 0.5–1 h and spotted on to nitrocellulose membranes [12]. The filters were then neutralized with 1.5 M NaCl, 0.5 M Tris, pH 7.4. The nine replica filters containing the DNA samples were hybridized with an HPV probe (HPV-1, 2, 3, 4, 5, 8, 11, 16, 18) under highly stringent condition. The hybridization, washing and exposing of the filters were carried out as described for Southern blot hybridization.

**Histological examination**

The specimens were fixed in 4% neutral buffered formalin, dehydrated in a graded series of alcohol, and embedded in paraffin. The histopathological changes of the lesions were routinely evaluated in 4-μm thick sections stained with haematoxylin and eosin (HE) [10, 23].

**Results**

**Detection and typing of HPV infections in skin warts by hybridization**

We investigated 61 specimens from patients with skin warts diagnosed clinically as verruca plantaris, verruca vulgaris and verruca planta. The HPV type was determined by Southern blot hybridization. DNA (3 μg per patient) was digested with BamHI, transferred to nitrocellulose filters and hybridized with the mixed HPV-1, 2, 3, 4, 5, 8, 11, 16 and 18 probes under the less stringent conditions as described in ‘Materials and methods’. The results show that only 42 of the 61 cases contained HPV DNA (Fig. 1).

The typing of the various HPVs (HPV-1, 2, 3, 4, 5, 8, 11, 16 or 18) was performed by dot blot hybridization under highly stringent conditions (68 °C) with individual HPV probes. Figure 2 shows the data with the HPV-1 probe, the six dots on the top line of the filter from dot 1 to dot 6 were the 10 ng DNA of HPV-1, 2, 3, 4, 5, and 8, respectively (panel A). The results show that a positive signal was present only in HPV-1 DNA dot (panel A) and there were eight positive dots from 61 tissue specimens (panel B). The HPV typing was also done by Southern hybridization from the same filter sequentially hybridized with the different probes under highly stringent conditions. The results of typing by dot and Southern hybridization were the same.

The presence and distribution of the various types of HPV DNA in the warts are shown in Table 1. There were eight cases with HPV-1 infection, four cases with HPV-2 and HPV-3, ten cases with HPV-4, one case with HPV-5, three cases with HPV-11 (including two mixed infections—HPV-11 and 16; HPV-11 and 18), one case with HPV-16 (one mixed infections—HPV-11 and 16) and