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Molecular cloning and characterization of a novel human papillomavirus, HPV 126, isolated from a flat wart-like lesion with intracytoplasmic inclusion bodies and a peculiar distribution of Ki-67 and p53

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ABSTRACT

Infection with certain human papillomavirus types induces warts with specific macroscopic and microscopic features. We observed multiple flat wart-like lesions on the chest, neck and extremities of an adult T-cell leukemia patient. Histologically, atypical intracytoplasmic inclusion bodies currently known to be pathognomonic for genus gamma or mu papillomaviruses were disclosed in some cells of the epidermis showing histological features compatible with flat warts. In the present study, a novel human papillomavirus was identified and its whole genome, 7326 bp in length, was cloned and characterized. Phylogenetic analysis showed the virus designated as HPV126 to be a novel type of genus gamma papillomavirus. Strikingly, Ki-67 and p53 expression was found to be increased in all layers of the epidermis except for horny layer, contrasting to expression restricted to the basal and lower spinous layers in ordinary flat warts.

Introduction

So far, more than one hundred twenty human papillomaviruses (HPVs) have been characterized based on nucleotide sequence diversity (Bernard et al., 2010). Infections of distinct types of HPVs are characterized by type-specific cytopathic/cytopathogenic effects (CPEs), i.e., macro- and microscopic features, pathological properties, and tissue tropisms. Hence, unusual CPEs which had not previously been described may suggest that lesions could be induced by a novel type of HPV (Egawa, 2005). We recently observed intracytoplasmic inclusion bodies (ICBs) resembling the HPV 4/60/65-associated homogenous ICB (Hg-ICB) (Egawa, 1994, 2005; Egawa et al., 1993) in flat wart-like lesions of a patient with adult T-cell leukemia (ATL). However, the clinical features of the lesions proved quite different from those of HPV 4/60/65-associated skin lesions, i.e., pigmented warts (Egawa, 1988; Egawa et al., 1993) or ridged warts (Honda et al., 1994), suggesting the presence of a previously unidentified papillomavirus. While the HPV type-specific CPEs are important in understanding the biological nature of the viruses, many of the novel HPV genotypes recently isolated lacked specific cell biological aspects.

The present report describes not only isolation and molecular biological characterization of a novel HPV genotype, HPV126, but also a clinical, histopathological and immunohistochemical characterization of HPV 126-associated skin lesions, revealing this novel human genus gamma papillomavirus to induce flat wart-like lesions with Hg-ICBs. Strikingly, Ki-67 and p53, well-known cell cycle proteins, were established to be expressed in all layers of the epidermis except for horny layer in the lesions, quite different from the expression pattern restricted to basal and lower spinous layers seen in ordinary flat warts.

Results

Histopathological features of wart lesions

Disseminated hypopigmented macules clinically resembling flat warts or epidermodysplasia verruciformis-related tinea versicolor-like lesions (Jablonska and Orth, 1985) were seen on the chest, neck, and extremities of a 56-year-old Japanese patient (Fig. 1A) (Kawai et al., 2009). A biopsy was taken from the disseminated fused lesion and adjacent normal-looking skin. Microscopically, at least two independent wart-

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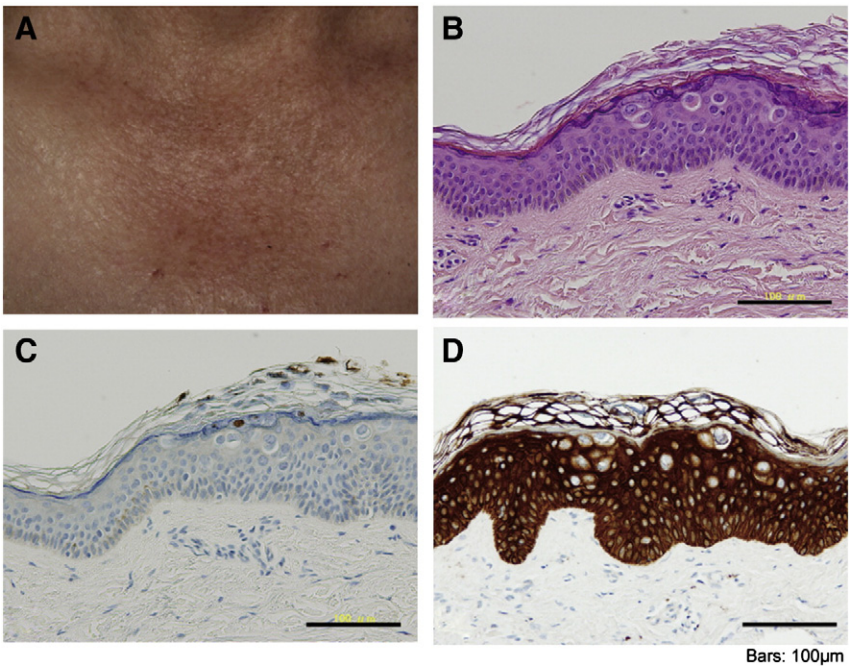

Fig. 1. Clinical (A), histopathological (B) and immunohistochemical (C and D) findings for the HPV 126-associated flat wart-like lesion. Macroscopic appearance of the flat wart-like lesions (A). In contrast to the normal skin adjacent to the flat wart-like lesion, Hg-ICBs are evident in balloon cells in the upper epidermal cell layers (B); strong positive signals for papillomavirus common antigen (L1 protein) in the nuclei of the cells in horny layer and cells with Hg-ICBs in granular layer (C); and positive signals for pan-cytokeratin are lacking in the cells with ICB (D). Scale bars; 100 μm.

like lesions separated by normal epidermis were included in the specimen. The epidermis showed mild acanthosis with basket-weave-like hyperkeratosis, partial hypergranulosis and mild papillomatosis, basic histological features compatible with those of flat warts (Fig. 1B) (Jablonska et al., 1985). However, additional unique histopathological features were also seen, i.e., keratinocytes with an enlarged nucleus, abundant blue-gray cytoplasm, occasional perinuclear haloes, and prominent keratohyalin granules observed in the granular and spinous layers, which are histopathological features consistent with EV (Jablonska and Orth, 1985). In addition, large clear cells contained homogeneous eosinophilic ICBs (Fig. 1B) resembling the homogeneous ICBs (Hg-ICBs) previously described in HPV 4/60/65-associated cutaneous warts (Egawa, 1994, 2005; Egawa et al., 1993) .

Cloning and characterization of the HPV 126 genome

Although highly sensitive PCR failed to detect the DNA of either genus beta or mu papillomaviruses from the frozen biopsy specimen, a segment of a putative novel type genus gamma papillomavirus was amplified with a gamma papillomavirus-specific degenerate primers (Kawai et al., 2009) (Supplementary Fig. 1). Based on the nucleotide sequence, the full genome was cloned as described in Materials and methods. Sequencing of two clones from independent PCR reactions revealed the full genome consists of 7326 bp in length with a GC content of 50.5%. With a cutaneotropic papillomavirus primer set FAP59/FAP64 (Forslund et al., 1999), only the corresponding region of the cloned genome was amplified, further indicating the HPV is a single type in the lesions of this patient. The cloned HPV was found to be closely related to genus gamma papillomavirus types with an L1 ORF nucleotide similarity ranging from 60.1% to 68.7% (Table 1). According to the established criteria for a new type of papillomavirus that a new type should have 10% divergence of the L1 ORF nucleotide sequence from that of any other papillomavirus type {de Villiers, 2004 #16}, the cloned HPV qualified as a new type of papillomavirus designated as HPV126. According to the proposed criteria for species that should share between 60% and 70% nucleotide identity within a genus, we propose that HPV126, which has less than 70% nucleotide identity with any other papillomaviruses, constitutes a new species of genus gamma papillomavirus. Generation of a phylogenetic tree based on complete L1 nucleotide sequences of representative HPV types indicated that HPV 126 is most closely related to HPV 129 (Fig. 3), with similarity of 68.7% (Table 1). HPV 126 has a typical genomic organization for a genus gamma papillomavirus, and it has seven ORFs, E6, E7, E1, E2, E4, L2 and L1, but no E5 (Supplementary Fig. 2).

Immunohistochemical features of the wart lesions

Strong signals of L1 capsid proteins were seen in the nuclei of the cells in horny layer and cells with the ICBs in granular layer (Fig. 1C), suggesting active production of virions. In the cells with ICBs, little cytokeratin staining was observed while strong staining was observed in all epidermal cell layers of the lesions as well as its adjacent normal skin (Fig. 1D).

Table 1
Nucleotide sequence pairwise comparison of HPV 126 ORFs with those of representative genus gamma papillomaviruses.

ORF / HPV type	E6	E7	E1	E2	E4	L1	L2
HPV 4	52.2	53.2	66.2	57.3	57.3	63.5	51.6
HPV 48	54.0	60.3	61.3	55.6	52.7	61.4	52.7
HPV 50	49.1	55.9	61.6	58.5	55.2	61.5	52.7
HPV 60	54.3	54.0	65.5	59.8	59.5	62.9	53.6
HPV 65	53.4	50.5	67.0	54.7	55.0	61.4	51.9
HPV 88	51.5	55.4	64.5	57.1	55.6	62.8	52.6
HPV 95	52.6	51.5	66.9	57.2	57.5	62.2	52.6
HPV 112	55.0	53.0	61.0	58.4	53.7	62.4	50.4
HPV 116	60.3	53.7	66.4	60.9	58.6	67.6	57.7
HPV 119	52.5	53.4	62.0	58.3	52.9	61.5	50.3
HPV 121	55.0	55.1	63.8	55.8	53.4	63.1	52.3
HPV 123	47.1	48.0	60.5	55.6	51.4	60.1	51.6
HPV 129	58.3	57.4	67.3	60.2	61.1	68.7	58.7

Similarities (%). Sequence for the genus gamma papillomaviruses were obtained from GenBank.