Molecular Characteristics of Cutaneous Papillomavirus from the Canine Pigmented Epidermal Nevus

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ABSTRACT. To investigate the relation between the canine pigmented epidermal nevus (PEN) and cutaneous papillomavirus, we cloned and sequenced the L1 gene of papillomavirus from the canine pigmented epidermal nevus (PEN). Amplification of DNA sample with the L1 consensus primers yielded an expected fragment of approximately 450-bp. The nucleotide sequences of the fragment showed about 64% of sequence similarity to the L1 region of human papillomavirus isolate CP6108 and less than 57% sequence similarity to those of canine oral papillomavirus (COPV). In situ hybridization determined the presence of papillomavirus DNA mainly in the upper stratum granulosum of skin in this case. The results indicated that the canine cutaneous papillomavirus from the PEN lesion was genetically close to human papillomavirus rather than COPV.

KEY WORDS: canine, papillomavirus, pigmented epidermal nevus.

Papillomaviruses cause cutaneous and mucosal neoplasms in a wide range of hosts, including man, cattle, horses, dogs, rabbits and wild animals. The viruses are known to be host specific and tissue specific. Canine oral papillomavirus (COPV) induces benign papillomas in the mucous membrane of the oral cavity of dogs [6, 11]. It has recently been reported that canine oral papillomas may progress to squamous cell carcinomas in dogs [2]. The COPV was cloned from a canine oral papilloma which is common in dogs and analyzed for nucleotide sequence of the L1 gene, which encodes a major capsid protein [4]. Moreover, nucleotide sequence analysis of L1 gene has been utilized for the identification of human and animal papillomaviruses [1].

On the other hand, immunohistochemical analyses have been recently applied to canine cutaneous papillomavirus infection [12, 13]. However, molecular analysis of this papillomavirus has been infrequently reported. Nagata et al. (1995) reported clinical and histopathological study of the canine pigmented epidermal nevus (PEN) [9]. In their report, the presence of numerous papillomavirus particles were shown within lesions, suggesting that the nature of canine PEN was equivalent to that of epidermodysplasia verruciformis (EV) in humans [9]. EV is considered to be associated with unusual susceptibility to the infection by EV-specific human papillomaviruses in relation to the suppression of immunosurveillance exclusively against these viruses [2, 9].

In the present study, we cloned and sequenced the L1 gene of papillomavirus from the canine PEN lesion to investigate its relation with canine cutaneous papillomavirus.

The histologic examination, immunohistochemical analysis and electron microscopic examination of skin biopsy specimens from the PEN in intact male Pug were described by Nagata et al. [9]. These results suggested that papillomavirus was the etiologic agent of the canine PEN. The extraction method of DNA from paraffin-embedded tissue of the skin biopsy was reported in Leonardi et al. [5]. The DNA sample dissolved in TE buffer (10 mM Tris-hydrochloride, pH 8.0 and 1 mM EDTA) were used for polymerase chain reaction (PCR) amplification.

The DNA sample (200 ng) was amplified by PCR in a reaction mixture [30 µl] containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 200 µM each deoxynucleoside triphosphate, 1.0 unit of Taq polymerase (Takara, Kyoto, Japan) and 0.5 µg of a pair of primer. The sequences of the primers for L1 gene of human papilloma virus were based on the sequences reported in Leonardi et al. [5]: The PCR products were electrophoresed through 2% agarose gel and then stained with ethidium bromide.

The PCR products from each species were respectively gelpurified and cloned into a pCR4 vector (Invitrogen, CA, U.S.A.). The plasmid DNAs from more than three clones of each species were extracted with the QIAGEN plasmid kit (QIAGEN, CA, U.S.A.) and sequenced by dye-deoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, CA, U.S.A.).

In situ hybridization was performed using Micro-Probe staining with modifications of previously described methods [7, 8]. Briefly, 5 µm sections of ProbeOn Plus slides were deparaffinized, cleared, and dehydrated as described above. The tissue sections were then treated with 1.25 mg/ml of pepsin for 3 min at 105°C. Following protease pretreatment, biotinylated MY11 or MY09 probes were applied to the tissue sections, and the tissue were heated at 105°C for 5 min. Hybridization was then carried out for 2 hr at 37°C. The tis-
sues were then washed with standard saline citrate (SSC) × 2,
then SSC × 1 for 1 min at room temperature. The biotinylated
hybrids were detected with streptavidin-horseradish peroxi-
dase with signal generation using diaminobenzidine, as
described above.

Amplification of DNA sample from the canine PEN with
the L1 consensus primers yielded an expected fragment of
approximately 450-bp (Fig. 1). The nucleotide sequences of
the fragment showed about 64% of sequence similarity to the
L1 region of human papillomavirus isolate CP6108 of which
was the most sequence similar in the GenBank search
(GeneBank accession no. U12478). However, the nucleotide
sequences of the fragment showed less than 57% sequence
similarity to those of a COPV [3, 4, 7].
Fig. 2. *In situ* hybridization determined the presence of papillomavirus DNA (→), mainly in the upper stratum granulosum of skin in this case. Biotinylated MY11 probe was applied to the tissue sections, and the tissue were heated at 105°C for 5 min. Hybridization was then carried out for 2 hr at 37°C. The biotinylated hybrids were detected with streptavidin-horseradish peroxidase with signal generation using diaminobenzidine.

On the other hand, the primers did not amplify 450-bp of the fragment from genomic DNA of five normal skin samples from dogs.

*In situ* hybridization using MY11 probe determined the presence of papillomavirus DNA mainly in the upper stratum granulosum of skin in this case (Fig. 2). The MY11 probe did not determine the presence of papillomavirus DNA in five normal skin samples from dogs.

The papillomavirus are naturally oncogenic to induce species- and tissue-specific papillomas [2]. The COPV has been cloned and characterized. On the other hand, Nagata *et al.* suspected that the canine PEN could be associated with canine papillomavirus other than COPV, since the clinical features of the present dermatosis were completely different from those observed in previous canine papillomavirus infections [9].

Human papillomavirus (HPV) genomes usually isolated directly from clinical specimens and the types were identified by the analysis of L1 gene sequence [1]. The sequence similarity of more than 90% in the L1 genes with an established HPV type has been suggested as criterion type differentiation of HPV [1, 10].

The nucleotide sequences of L1 gene of the canine cutaneous papillomavirus from the canine PEN showed about 64% of sequence similarity to those of human papillomavirus isolate CP6108 and less than 57% sequence similarity to those of canine oral papillomavirus.

In the present study, to investigate the molecular characteristics of canine cutaneous papillomavirus from the canine PEN, we cloned and sequenced the L1 gene of the papilloma-virus. The *in situ* hybridization analysis also suggested that the presence of canine papillomavirus DNA in the skin lesion of canine PEN was determined. The CP6108 was isolated from cervical specimens of normal women and its pathogenicity is unknown. Although the papillomavirus in canine PEN genetically close to human one might not be directly transmissible to people, the prudent care would be paid whenever handling an infected dogs. Further investigations of this papillomavirus will be needed.

REFERENCES