Cutaneous Papilloma with Viral Replication in an Old Dog
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Cutaneous papilloma is a benign neoplastic disease commonly affects many species of domestic animals [7] and man. Most of papillomas have been clarified to be caused by infection of papillomavirus and show characteristic site preference, age susceptibility and clinical course in each host.

In the dog, two types of papillomas have been well recognized; i.e., oral papilloma in young dogs of average age of 1 year and cutaneous papilloma in older dogs [3, 6]. In contrast to the well-established pathogenetic relation of viral infection to oral papilloma, etiologic agents have not been fully confirmed in cutaneous papilloma because of lack of viral inclusion bodies in the proliferating epidermal cells except for several cases [2, 8]. We encountered a case of cutaneous papilloma in the old dog which showed typical papillomatous proliferation and frequent viral inclusion bodies. This paper describes histological feature of this papilloma and fine structure of viral inclusion bodies.

A twelve-year-old male Maltese showed a projection sized about 1.5 cm in diameter from the skin near the foot pads of left hind leg with cauliflower-like appearance. Surgical biopsy specimens were fixed in 10% neutral buffered formalin, automatically processed and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin (HE) and Feulgen's reaction for DNA. Tissue blocks from formalin fixed tumor materials were further fixed in 2% glutaraldehyde solution for 2 hours and postfixed in 1% solution of osmium tetroxide for 1 hr, then dehydrated in the ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (JEM-1200EX).

Light microscopically, the lesions showed typical characteristics of squamous papilloma. Irregularly branched fine stalks of connective tissue covered with thick epidermal cell layer and the top of each papillary projection was incompletely keratinized (Fig. 1). Epidermal cells near the stalk were hyperplastic and resembled the basal cells in normal skin except for the more frequent mitotic figures. Over the three to several layers of these epidermal cells of basal cell type, large polygonal epithelial cells with hypertrophic nuclei proliferated without normal epidermal maturation sequence (Fig. 2). These large epithelial cells never formed distinct intercellular bridges or cytoplasmic spines. The cytoplasm of these cells was filled with basophilic filaments and stained pale. Some epithelial cells also contained keratohyaline granules sparsely scattered among the filaments. The nuclei of these cells near the superficial layer frequently contained basophilic inclusion bodies which were positive for DNA by Feulgen's reaction (Figs. 3 & 4). Vacuolated or pale nuclear inclusions with cytoplasmic invaginations were also observed in some cells.

Electron microscopically numerous virus particles were aggregated in the nuclei of the epidermal cells, corresponding to the basophilic intranuclear inclusion bodies in light microscopy (Fig. 5). Nuclear inclusions contained numerous particles of various electron density sized from 41 to 49 nm (average 45 nm). Tubular structures partially connected to the particle were also scattered occasionally among the particles. Branching tubular structures containing small irregular-sized particle of about half size of mature virus particles were also observed infrequently in the nuclei (Fig. 6). Sparse array of tonofilbrils and relatively small keratohyaline granules filled the whole cytoplasm. Neither crystalline structures in the nuclear inclusion bodies nor the cytoplasmic viral particles was found in these cells.

From the histological and electron microscopic findings the present case was diagnosed as a cutaneous papilloma caused by papillomavirus in an old dog. Contrary to many etiologic evidences of papillomavirus infection in the oral papillomatosis, only several cases with virus replication have been reported in the cutaneous papilloma in dogs [2, 8]. Among the canine cutaneous papillomas frequent viral infection has been reported in some special type, inverted papilloma located on the ventral part of the skin, showing morphological characteristics similar to intracutaneous cornifying epithelioma [2]. Therefore, close etiological participation to the site preference might be suggested even in an cutaneous type of the papilloma in the dog. In addition to the previous two cases [8], our case presents evidence for possible pathogenetic association of ordinal cutaneous papilloma with the papillomavirus.

Canine oral papilloma and cutaneous inverted papilloma with evident viral infection commonly affect the young dogs under 2 years, and two cases of the cutaneous papilloma in the previous report were nine-month-old and two-year-old. Therefore the present dog is the first case of canine cutaneous papilloma in old dog with viral replication. As the reasons for the occurrence of viral cutaneous papilloma in old dog, low immunity for papillomavirus in the dog population in these areas where the present dog was kept might be considered. Because, in spite of rare vaccination, the epidemics of viral papilloma were absent for long time in addition to the low opportunity of house-keeping dog to contact with other dogs.
Fig. 1. Low power view of the cutaneous papilloma. Typical appearance of squamous papillomatous growth. HE stain. × 8.

Fig. 2. Increased number of enlarged keratinocytes in the granulosa layer. Loss of intercellular bridge or keratohyaline granules suggesting loss of normal keratinizing process. HE stain. × 160.

Fig. 3. Enlarged pale keratinocytes (koilocytic cells) in the superficial layer, filled with basophilic filaments and small number of keratohyaline granules in the cytoplasm. Some nuclei contained basophilic inclusion bodies with frequent nuclear invagination. HE stain. × 530.

Fig. 4. Positive reaction for DNA of nuclear inclusion bodies in the keratinocytes. Feulgen's reaction. × 530.
Recently, the relationship between the type of papillomavirus and clinical course or site of the lesions was extensively studied in the proliferative lesions of human genital tract [4, 5]. In the canine papilloma, difference of the type of papillomavirus is also postulated from the unsuccessful establishment of cutaneous papilloma by transmission from oral papillomatosis [1] and unsuccessful hybridization with oral papillomavirus DNA in paraffin sections of inverted papillomas [2]. However, there has been little information for the typing of canine papillomavirus and type specific DNA probe is unavailable also in the present case. Therefore, identification of the type of papillomavirus involving the present case needs further investigation.

REFERENCES