論文の内容の要旨

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論文題目：Pathological studies on canine papillomavirus-associated cutaneous lesions

（イヌパピローマウイルス関連皮膚病変の病理学的研究）

Papillomavirus infects the squamous epithelium of many mammalian, some avian and reptilian species. Canine papillomavirus (CPV) includes 19 genotypes and is classified into three genera of Lambda, Tau and Chi. Infection of CPV causes canine oral papillomatosis (CPV1 and 6), cutaneous papilloma (CPV1, 2, 6, 7, 13, 17, 18 and 19) and pigmented viral plaque (CPV3, 4, 5, 8, 9, 10, 11, 12, 14, 15 and 16). Some previous studies suggested that CPV-associated precancerous lesions may result in malignant tumors, though the mechanism toward malignancy was not been well elucidated. Immunohistochemistry (IHC) and polymerase chain reaction (PCR) assay have been used to detect viral protein and gene, respectively, in the lesions. However, the detection of viral L1 protein and the presence of virions in the lesions were nothing more than the positive results, which cannot necessarily illustrate the virus as a causative agent in the precancerous lesions. In human, oncogenic human papillomavirus (HPV) can result in the overexpression of p16 protein in both nuclei and cytoplasm with inhibiting pRb expression in HPV-associated tumors. However, to the present, whether CPV can also lead to this similar immunohistochemical characteristics is still unknown within the precancerous lesions in dogs.

The aim of this study is to identify CPV association in canine precancerous lesions. The results may provide more information about the localization of CPV nucleic acid of different genotypes and giving more details about the pathogenesis in CPV-related precancerous cutaneous lesions.

In Chapter 1, to clarify CPV association in cutaneous precancerous lesions, CPV was detected by IHC, PCR and nucleic acid sequence determination in totally 23 cases including 16 cases of papilloma, 1 case of papillary hyperplasia and 6 cases of pigmented viral plaque. Papillomavirus antigen was detected in 2 cases of papilloma (2/16, 12.5%), and 5 cases of pigmented viral plaque (5/6, 83.3%). CPV gene was detected by PCR in 2 cases of papilloma (2/16, 12.5%), and 6 cases of pigmented viral plaque (6/6, 100%). These results illustrated that PCR is a more sensitive method to detect papillomavirus infection in dogs. Moreover, the results of nucleic acid sequence analysis showed that 2 cases of papilloma were infected with CPV2; 5 cases of pigmented viral plaque were infected with CPV4, and 1 case with CPV18. In the case of pigmented viral plaque, CPV18 gene was also detected in the lesion of cytokeratin-14- and P63-positive basal cell tumor that had developed from pigmented viral plaque. This is the first finding of basal cell tumor associated with CPV18-infection in the dog. In addition, the number of Ki-67-positive cells was increased in the CPV-infected papilloma and basal cell tumor compared to that in the CPV-uninfected papilloma and pigmented viral plaque lesions. The result indicates a potential risk of malignant transformation in the lesions associated with CPV infection.

Information on the localization of CPV nucleic acid of each CPV types in the precancerous lesions have never been available. Therefore, in Chapter 2, an *in situ* hybridization (ISH) assays were performed to detect canine papillomaviral DNA on all CPV-positive tissue sections. The cases include 2 papilloma and 6 pigmented viral plaque lesions. The hybridization probes were produced by labeling PCR products (primer pair canPVf/FAP64; L1 gene) from cutaneous papilloma (CPV2) and pigmented viral plaque (CPV4 and CPV18), which did not show any cross reactivity each other, therefore they were used for detecting CPV2, CPV4 and CPV18, respectively. The ISH results showed that 2 cases of papilloma were positive for CPV2, 5 cases of pigmented viral plaque were for CPV4 and 1 case of pigmented viral plaque was for CPV18. The results of the *in situ* hybridization for CPV2, CPV4 and CPV18 were 100% concordant with those of the PCR. Moreover, CPV2 signals were observed in the nuclei of neoplastic cells within the perilesional thickened epidermis of 2 cases of papilloma. CPV4 signals were observed in the nuclei of epithelial cells in the spinous layer and granular layer of 5 cases of pigmented viral plaque. In one case of basal cell tumor which had developed adjacent to pigmented viral plaque lesion, nuclei of tumor cells were positive for the CPV18 gene. The results of this chapter implied that CPV2, CPV4 and CPV18 infection may cause cutaneous papilloma, pigmented vial plaque and basal cell tumor in dogs.

In Chapter 3, the IHC detection for p16 protein and pRb in CPV-infected skin tumors in dogs

was performed. In human, oncogenic HPV-infection can cause the overexpression of p16 protein in both the nucleus and cytoplasm of HPV-associated neoplastic cells through inhibiting the pRb expression. However, it is still unknown whether CPV can also represent the similar immunohistochemical characteristics within the precancerous lesions in dogs. In this chapter, Totally, 23 cases were applied to IHC for the detection of p16 and pRb. Among the cases, 16 were papilloma including, 2 positive for CPV2 and 14 negative for CPV. One was papillary hyperplasia negative for CPV. Other 6 cases were pigmented viral plaque including 5 positive for CPV4, and 1 positive for CPV18 (Chapter 1). Diffuse cytoplasmic expression of p16 protein with faint/no expression of pRb was detected in 1 case of CPV2-associated papilloma, 5 cases of CPV4-associated pigmented viral plaque and 1 case of CPV18-associated pigmented viral plaque. In lesions of the head and neck in a case of CPV2-associated papilloma, tumor cells were negative for p16 protein. Nuclear expression of pRb was observed in 1 case of CPV-negative papillary hyperplasia and 14 cases of CPV-negative papilloma, nonetheless no nuclear and cytoplasmic expression of p16 protein was observed in all the cases. The diffuse cytoplasmic expression of p16 protein in the neoplastic cells of CPV-positive cases was not consistent with the results of a previous report on papillomavirus pathogenesis in malignant skin tumors of human and dogs, which accompanied with the nuclear and cytoplasmic expression of p16 protein. Moreover, oncogenic CPV can also inhibit the expression of pRb in CPV-associated

precancerous lesions, like HPV-associated tumors in human.

Based on the results in this study, there is a high CPV infection rate in the precancerous lesions such as pigmented viral plaques, and neoplasias in dogs. This study also documents the detection and localization of infected cells in canine precancerous skin lesions, and may confirm a pathogenic role of CPV2, CPV4 and CPV18 during the development of the lesions. Moreover, the cytoplasmic expression of p16 protein and loss of pRb in CPV-associated lesions indicates the oncogenic potential of the molecules in the precancerous lesions. Therefore, the presence of CPV DNAs within canine precancerous cutaneous lesions was associated with the expression of p16 and the loss of pRb like oncogenic HPV. The findings in the present study may increase the awareness of the oncogenic potential of CPV2, CPV4 and CPV18 in the precancerous skin lesions in dogs.