学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Studies on DNA methylation changes in canine malignant melanoma
(犬の悪性黒色腫における DNA メチル化の変化に関する研究)

Canine malignant melanoma is a common cancer with a high mortality rate. Although previous studies have investigated various etiological factors including genetic mutations, exact mechanism of tumorigenesis remains unknown.

Epigenetic mechanisms, such as DNA methylation, have recently gained attention as aetiological factors for neoplasia. Although there are several reports for DNA methylation in canine malignant melanoma, DNA methylation status in canine malignant melanoma is still unclear. The aim of following studies is to reveal DNA methylation change in canine malignant melanoma from three different perspectives.

In chapter 1, genome-wide DNA methylation analysis in malignant melanoma cell lines and malignant melanoma clinical samples was performed by using NGS. Widespread DNA methylation alterations including hypermethylation in CGIs and hypomethylation in NCGIs are detected. These results were consistent with the findings in human cancers suggesting that changes in DNA methylation patterns are based on tumorigenic transformation. In addition, a large number of CpG sites in the CGI promoter region of genes were hypermethylated. Although the effects of aberrant DNA methylation on gene expression changes and their relevance to tumorigenesis were not assessed in this study, these signatures of aberrant DNA methylation could be used as diagnostic or prognostic markers in canine malignant melanoma.

In chapter 2, DNA methylation status of LINE-1 in canine mucosal malignant melanoma was investigated using bisulfite-pyrosequencing which is a highly sensitive and

high-throughput method to quantify CpG site methylation. LINE-1 repetitive elements are the most well recognized repetitive elements and LINE-1 DNA methylation status is considered to represent global methylation status. In this study, malignant melanoma showed hypomethylation of LINE-1 compared to normal tissue. Furthermore, LINE-1-low patients showed worse overall survival compared with LINE-1-high patients. These results indicated that LINE-1 DNA methylation level could be used as a surrogate marker of global DNA methylation level and prognosis in canine oral malignant melanoma.

In chapter 3, difference in DNA methylation status between epithelial and mesenchymal phenotype in canine malignant mucosal melanoma were assessed using genome-wide DNA methylation data analyzed by NGS. No remarkable difference was observed in global DNA methylation status between epithelial and mesenchymal phenotypes. However, the epithelial phenotype exhibited more hypermethylated CpG sites in CGI promoters than the mesenchymal phenotype. In addition, two genes associated with cell adhesion were significantly hypermethylated in the epithelial phenotype through de novo hypermethylation. The results from this study indicate the influence of DNA methylation on EMT in canine malignant mucosal melanoma and suggest the therapeutic potential of demethylating agents in the future.

In these studies, drastic DNA methylation changes in canine malignant melanoma were revealed. Although continuous studies with a greater number of clinical cases or investigation of functional effects of the observed DNA methylation changes are required, these results should contribute to elucidation of epigenetic mechanisms in canine malignant melanoma.