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学 位 論 文 要 旨

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題　　目：Analysis for Differential expression profiles of microRNA in Canine Mammary Gland Tumor （犬の乳腺腫瘍におけるマイクロ RNA 発現差異の解析）

論文要旨：

Canine mammary gland tumors (MGTs) are a common diagnosis in companion animal medicine and a potential model of human breast cancer. The genetics behind these tumors could thus be pivotal in enhancing diagnoses and therapies for canine patients and advancing translational medicine. The miRNAs are small noncoding-RNAs with a gene-regulating role. They have known roles as tumor suppressors and oncogenes, and have the potential as biomarkers for diagnosis and predicting response to therapy. Interestingly, miRNAs reportedly show differential expression between metastatic and non-metastatic tumors in dogs, which suggests they have potential utility as biomarkers of metastasis. However, to the best of my knowledge, research on miRNA expression in canine MGTs has largely been small-scale and focused on a few targets. Next generation sequencing (NGS) allows us to take further investigations on miRNAs in canine MGTs by screening for molecules of interest. Accordingly, in my PhD studies, I aimed to elucidate miRNAs differentially expressed in canine MGTs with a relatively large-scale cohort compared to normal tissue, and further investigated to target multiple histological types and subtypes of a tumor.

In the first chapter, I aimed to identify miRNAs differentially expressed in canine MGTs using next generation sequencing (NGS), with subsequent confirmatory qPCR and target gene analyses. Mammary gland tissue was collected from healthy dogs (n=7) and dogs with suspected tumors (n=80). A subset of samples was analyzed with NGS to identify differentially expressed miRNAs with CLC Genome Workbench. Normal (n=10), tumor-adjacent (n=6), and tumor-bearing (n=76) mammary gland tissue samples were analyzed for the identified miRNAs using qPCR. An *in silico* analysis (TargetScan) was performed to predict the miRNAs' target genes and investigated the bio-functional aspect by using gene ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (DAVID).

I identified four miRNAs (cfa-miR-1-3p, cfa-miR-133a-3p, cfa-miR-133b-3p, and cfa-miR-133c-3p) as down regulated in canine MGTs relative to normal and tumor adjacent tissues. KEGG analysis revealed the potential target genes of cfa-miR-1-3p were related to the Rap1 signaling pathway, adherens junction, and Ras signaling pathway, and those of the miR-133 family were related to the TGF-beta signaling pathway, synaptic vesicle cycle, and sphingolipid signaling pathway. In combination, these target genes were related to the regulation of transcription and DNA binding

transcription (GO analysis), and the Hippo signaling pathway, adherens junction, and endocytosis (KEGG analysis). Accordingly, I suggest these four miRNAs are promising potential biomarker candidates for canine mammary gland tumors warranting further investigation.

In the second chapter, I investigated the association between miRNA expression patterns and histological classification. Mammary gland tissue was collected from healthy dogs (n=7) and dog patients (n=80). Further samples (n=5) were obtained from established canine MGT cell lines. I targeted miRNAs differentially expressed in metastatic tumor tissue versus non-metastatic and normal tissue. A subset of samples was analyzed using small RNA-Seq with subsequent qPCR. Six differentially expressed miRNAs (cfa-miR-187-3p, cfa-miR-202-5p, cfa-miR-424-5p, cfa-miR-450a-5p, cfa-miR-450b-5p, and cfa-miR-542-3p) were selected from the NGS analysis and submitted for large-scale qPCR analysis. The large-scale qPCR analysis revealed greater alternations in miRNA expression. Large-scale analysis, based on 79 samples, revealed five clusters in a hierarchical clustering based on selected miRNAs. Cluster A contained samples from all tumor subtypes, and resembled Cluster D in its tumor subtype composition. In contrast, Cluster C contained mostly non-tumor samples. The composition of Cluster B was similar to that of Cluster C, although it contained a smaller number than Cluster C. The most markedly distinct cluster was Cluster E, because it contained only one sample of metastasized adenocarcinoma.

The qPCR-based heatmap hierarchical clustering yielded a markedly greater scattering of samples than that based on NGS, thus indicating the classification of the hierarchical clustering did not strikingly match the histopathological subtype classification. I successfully investigated the large-scale miRNA expression pattern in canine MGT and provided the signatures of whole miRNA expression. The selected miRNA demonstrated that there is no straightforward mapping between molecular signatures and histological classification of canine MGTs at the miRNA level.

In conclusion, I developed differential miRNA expression profiles pattern for canine MGTs, demonstrating downregulation of cfa-miR-1-3p and cfa-miR-133 family miRNAs in these tumors, and reported the whole miRNA expression pattern in metastatic and non-metastatic MGT. Then, I regard the miRNA expression diversity across histological canine MGT subtypes as the principal point of interest for this study. I believe these findings add to the state of existing knowledge of canine MGTs, and suggest future avenues of research that may lead improved diagnoses and therapies in canine and potentially human medicine.

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