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

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学位論文題名
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Study on the Pathogenesis of Canine Hemangiosarcoma Focusing on Epigenetics and Tumor Metabolism

（エピジェネティクスおよび腫瘍代謝に着目したイヌ血管肉腫の病態解明に向けた研究）

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Canine hemangiosarcoma (HSA) is a highly aggressive malignant tumor derived from vascular endothelial cells, characterized by rapid invasion, early metastasis, and frequent rupture. Although surgery and chemotherapy remain the standard of care, clinical outcomes have shown little improvement, highlighting the need for new therapeutic approaches. Besides genetic abnormalities, tumor cells acquire epigenetic and metabolic alterations that enhance their malignant behavior. These systems are closely connected, as metabolites such as acetyl-CoA and lactate support epigenetic modifications. Therefore, targeting this metabolic–epigenetic interplay may provide new opportunities for therapeutic intervention. This dissertation investigates the molecular pathogenesis of HSA focusing on the epigenetic regulation and tumor metabolism.

Chapter 1 investigated the role of histone acetylation in HSA using two histone deacetylase (HDAC) inhibitors (SAHA and VPA) and one BET inhibitor (JQ1), a small molecule that blocks acetylated histone recognition. HSA cell lines exhibited high histone acetylation levels, whereas clinical HSA tissues showed heterogeneous acetylation patterns. SAHA and JQ1 induced apoptosis in HSA cells. SAHA and VPA upregulated inflammatory-related genes and enhanced macrophage recruitment, suggesting that HDAC inhibition may modulate immune responses within the tumor microenvironment. JQ1 stimulated autophagy and induced cell-cycle arrest. *In vivo*, JQ1, but not SAHA or VPA, suppressed tumor growth. These findings suggest that dysregulated histone acetylation contributes to HSA biology and that BET inhibitors may represent a potential therapeutic option.

Chapter 2 analyzed the implication of lysine lactylation under glucose-limited conditions, in which tumor cells experience metabolic stress to survive. Although global histone lactylation decreased during glucose deprivation, lactylation peaks redistributed to the

transcription start sites of ATF4-regulated stress-response, asparagine-synthesis, and immune-related genes. The expression of these genes was upregulated, and ATF4 polyclonal knockout abolished this transcriptional activation of stress-response genes. Furthermore, [U-^{13}C]glutamine tracing demonstrated that HSA cells synthesized asparagine from glutamine under glucose starvation, and asparagine supplementation modestly supported cell proliferation. In clinical HSA tissues, H3K18la levels were heterogeneous, and M2-like macrophages preferentially infiltrated around tumor cells with low histone lactylation levels. These findings suggest that lysine lactylation is associated with regulating stress responses and establishment of pro-tumor microenvironment under glucose starvation.

Chapter 3 investigated the roles of lactate and LDH in HSA. Pharmacological inhibition of LDHA/LDHB with (R)-GNE-140 reduced cell proliferation and downregulated cholesterol-related genes. CRISPR/Cas9 knockout analysis using single-cell derived clones showed that the loss of LDHA, but not LDHB, reduced cholesterol-related gene expression and lipid droplet formation. Based on this link, I evaluated the therapeutic effects of fluvastatin, an inhibitor of cholesterol synthesis, with or without dipyridamole, which inhibits SREBP2 processing, thereby enhancing fluvastatin efficacy. While fluvastatin monotherapy had limited effects, the combination therapy significantly inhibited tumor growth in patient-derived xenograft models. These results suggest that LDH is involved in cholesterol metabolism of HSA and that targeting cholesterol synthesis may offer a promising therapeutic approach.

In summary, this study describes epigenetic and metabolic aspects of HSA, specifically histone acetylation, stress response-related lactylation, and LDH-linked cholesterol metabolism. These findings deepen the comprehensive understanding of HSA pathogenesis and provide a molecular basis for future treatment strategies.