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

Analysis of tumor evasion mechanisms contributing to tumor radio-resistance and the radio-sensitizing effects of Janus kinase inhibitor oclacitinib in canine tumor cell lines

（イヌ腫瘍細胞における放射線治療抵抗性に関わる

免疫回避機構の解析および Janus kinase 阻害薬オクラシチニブの

放射線増感効果に関する基礎的研究）

Radiation therapy is an effective treatment modality for tumors in both humans and animals, but later tumor recurrence or regrowth becomes a concern after radiation therapy. This is associated with the tumor radio-resistance. Therefore, analyzing the mechanisms of tumor radio-resistance is crucial for enhancing the treatment outcomes of radiation therapy. The aim of this study is to elucidate several mechanisms contributing to tumor radio-resistance in canine tumors focusing on programmed death ligand 1 (PD-L1) and Janus kinase (JAK) - signal transducer and activator of transcription (STAT) signaling pathway, and to investigate the radio-sensitizing effects of oclacitinib, an orally available JAK inhibitor in dogs, for the establishment of novel therapeutic strategies targeting these mechanisms to improve patient outcomes.

In Chapter I, to assess whether inflammatory signaling and DNA damage signaling is involved in PD-L1 regulation in canine tumors and to elucidate the underlying mechanisms, the effects of interferon (IFN)-γ, tumor necrosis factor (TNF)-α treatment and X-irradiation exposure were evaluated in two canine malignant melanoma cell lines (CMeC and LMeC) and an osteosarcoma cell line (HMPOS). The protein level of PD-L1 expression was increased on all tumor cells treated with IFN-γ and TNF-α, but not with

X-irradiation. Upon IFN-γ stimulation, higher gene expression of PD-L1, STAT1, STAT3, and genes regulated by STAT activation was demonstrated in all cell lines. Upregulation of these genes was inhibited by the addition of a JAK inhibitor, oclacitinib. In contrast, upon TNF-α stimulation, all cell lines indicated an increase in expression of the nuclear factor kappa B (NF-κB) gene (RELA) and genes regulated by NF-κB activation, whereas PD-L1 expression was upregulated in LMeC only. The addition of an NF-κB inhibitor, BAY 11-7082 suppressed the upregulated expression of these genes. The upregulation of PD-L1 expression induced by IFN-γ and TNF-α treatment was reduced by oclacitinib and BAY 11-7082, respectively, indicating that PD-L1 upregulation by IFN-γ and TNF-α stimulation is regulated via the JAK-STAT and NF-κB signaling pathways, respectively.

In Chapter II, the radio-sensitizing effect of oclacitinib in canine tumors and the underlying mechanisms were assessed in HMPOS and CMeC cell lines. Oclacitinib significantly enhanced the radio-sensitivity of tumor cells both in vitro and in vivo. Oclacitinib significantly enhanced radiation-induced apoptosis by inhibiting the expression of anti-apoptosis genes in HMPOS. Additionally, oclacitinib significantly inhibited the transcription of cell-cycle regulating genes and arrested cell cycle progression from the G1 phase to subsequent phases. These results indicated that oclacitinib facilitated radio-sensitivity by triggering apoptosis and impeding cell cycle progression via STAT3 inhibition in canine tumor cell lines. This study suggested the clinical therapeutic potential of oclacitinib in combination with radiation therapy, aiming to enhance treatment efficacy and outcomes in dogs with tumors.

In conclusion, the regulatory mechanisms of PD-L1 expression on canine tumors were elucidated. The JAK-STAT signaling pathway could play a pivotal role in the regulation of not only PD-L1 upregulation induced by IFN-γ but also the radio-sensitivity of canine tumors. In addition, oclacitinib targeting STAT3 was shown to be effective as a radio-sensitizer in canine tumors.