学位論文の要約

博士の専攻分野の名称：博士（獣医学）　氏名：竹内 寛人

学位論文題名

Research for elucidation of immune evasion mechanisms and development of a novel immunotherapy for cancer in dogs

(イヌ腫瘍における免疫回避機構の解明と新規治療法の開発に向けた臨床研究)

While deaths from infectious diseases, which used to be a major cause of death, have declined dramatically, cancer has become a major cause of death in dogs due to the increasing life expectancy by improvements in the rearing environment and veterinary medicine. Studies in spontaneous cancers in dogs have attracted attention as a good model for cancers in humans because they share many features in their etiology and in the response to therapy. Currently, surgery, chemotherapy, and radiotherapy serve as the standard therapeutic modalities for canine cancers. However, in some cases, these treatments cannot be applied for some reasons, such as limited access for surgical resection and irradiation due to its anatomical sites as well as adverse effects and complications of the treatment. Due to the success of antibody therapies targeting immune checkpoint molecules, including programmed cell death 1 (PD-1) and PD-ligand 1 (PD-L1), immunotherapy has become one of the standard therapies for cancer in humans. In the field of veterinary medicine, immunotherapy has also emerged as a breakthrough therapy. Previous studies showed the expression of PD-L1 in various types of canine cancers, suggesting the potential of using immunotherapy targeting the PD-1/PD-L1 axis in dogs. However, the clinical trial in canine oral malignant melanoma (OMM) using a chimeric anti-PD-L1 antibody (c4G12) revealed that the therapeutic effect is limited despite PD-L1 expression in most of the cancer tissues. Thus, it is necessary to investigate the resistance mechanisms to c4G12 treatment and to develop combinational therapeutic strategies to enhance the efficacy of antibody therapy in canine cancers. Additionally, to facilitate the development of antibody drugs in dogs, the establishment of a cost-effective purification process is warranted.

In chapter I, for a better understanding of the regulation mechanisms of PD-L1

expression in canine cancers, the nucleotide sequences of canine CKLF-like MARVEL transmembrane domain containing protein 6 (*CMTM6*) and *CMTM4* genes were identified. Interestingly, the deduced amino acid sequences of canine CMTM6 and CMTM4 were highly similar to their orthologs in other mammalian species, and the predicted functional domain, MAL and related proteins for vesicle trafficking and membrane link domain, was conserved in dogs. The gene expression of *CMTM6* and *CMTM4* was confirmed by quantitative reverse transcription polymerase chain reaction in canine immune cells and cancer cells. Immunohistochemical analysis in canine malignant melanoma and osteosarcoma (OSA) revealed that all tissue samples co-expressed CMTM6, CMTM4, and PD-L1. Gene knockdown of *CMTM6* and *CMTM4* in a PD-L1-expressing cell line reduced the cell surface PD-L1 expression, suggesting that both CMTM6 and CMTM4 maintain the cell surface expression of PD-L1.

In chapter II, as an additional therapeutic target for combination therapy with anti-PD-L1 antibody, expression and functions of transforming growth factor (TGF)-β1 in dogs with cancer was examined. Canine melanoma cell lines produced TGF-β1 in the culture supernatant, and the serum TGF-β1 levels in dogs with metastatic OMM were elevated compared to those in healthy dogs. The immunosuppressive effects of TGF-β1 on canine peripheral blood mononuclear cells support the potential of TGF-β1 as a therapeutic target for cancer treatment. To reverse immunosuppression exerted by TGF-β1, a decoy receptor for TGF-β, TGF-β receptor 2-Ig (TGF-βRII-Ig), was prepared as a fusion protein of the extracellular region of canine TGF-βRII and the Fc region of canine IgG-B. TGF-βRII-Ig activated canine immune cells in the presence of exogenous TGF-β1, highlighting that its potential as a candidate therapeutic strategy for enhancing anti-tumor immunity. The anti-tumor efficacy of inhibiting the TGF-β1 pathway should be further investigated in clinical studies involving dogs with cancers.

In chapter III, to establish a robust and efficient purification method for canine therapeutic antibodies, the molecular characterization of SpsQ from *Staphylococcus pseudintermedius* was performed. From *S. pseudintermedius* isolates, the nucleotide sequence of SpsQ, which contained five immunoglobulin binding domains (Ig-BDs), was determined. To compare the binding characteristic of SpsQ and SpA, recombinant proteins of Ig-BDs from SpsQ (r-SpsQ) or SpA (r-SpA) were prepared and subjected to the binding assay. The binding capacity of r-SpsQ to canine IgG-A and IgG-D was higher than that of r-SpA, supporting the potential of SpsQ as an affinity chromatography ligand for a highly efficient purification process of canine therapeutic antibodies whose subclass are IgG-A and IgG-D.

Taken together, this study contributes to (1) the elucidation of PD-L1

regulation mechanism and providing a clue to fully understand the resistance mechanism to anti-PD-L1 therapy in dogs, (2) the development of a novel biologic in the field of veterinary medicine with enhanced efficacy, and (3) the improvement of purification process for canine therapeutic antibody with acceptable cost-effectiveness. Further studies including the investigation of regulators of PD-L1 and TGF-β1, clinical studies of a combination therapy involving anti-PD-L1 antibody and an TGF-β1 inhibitor in dogs with cancer, and the optimization of purification processes using SpsQ affinity chromatography are required to accelerate the development of novel antibody-based immunotherapies for canine cancers. Given the similarity in antitumor immunity between dogs and humans, the accumulated data from clinical studies in dogs with cancer will also contribute to the advance of cancer research in humans.