学位論文の要約

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学位論文題名

Studies on immune checkpoint molecules in canine cancers and development of a novel immunotherapy targeting these molecules

**(イヌ腫瘍における免疫チェックポイント分子の解析と
それらを標的とした新規免疫療法の検討)**

Cancer treatment in dogs has become more important along with their extended lifespan. Spontaneous cancers are common cause of death and the current treatment for them includes surgery, radiation, and chemotherapy. However, novel treatment options, such as immunotherapy, are needed to improve prognosis of some malignant cancers, especially for those effective systemic therapy is not available. For example, malignant melanoma that develops in oral cavity represents intractable cancers in dogs with its local invasiveness, metastatic propensity, and resistance to chemotherapy. In this study, the development of a novel immunotherapy was aimed against canine malignant cancers, including oral malignant melanoma (OMM), by targeting immune checkpoint molecules programmed cell death 1 (PD-1) and PD-ligand 1 (PD-L1).

PD-1 is an immunoinhibitory receptor that attenuates T-cell effector functions when it binds to its ligand, PD-L1. Aberrant expression of PD-L1 is reported in various types of malignant cancers in humans, and the PD-1/PD-L1 axis is considered an immune evasion mechanism. On the other hand, the blockade of the PD-1/PD-L1 pathway using

monoclonal antibodies (mAbs) can induce antitumor activity in various types of malignant cancers in humans. For the success of PD-1/PD-L1 inhibiting-mAbs in clinical studies, immunotherapy has gained attention and now is considered the fourth therapeutic modality for human cancers. However, in dogs, no study had been performed on the PD-1/PD-L1 pathway and its association with cancers was unclear.

In chapter I, the sequences of canine *PD-1* and *PD-L1* genes were identified as the first step. The PD-1/PD-L1 pathway appeared to be immunosuppressive in dogs because functional motifs were conserved in deduced amino acid sequence of canine PD-1, and the PD-L1 blockade with an anti-PD-L1 mAb enhanced the immune cell function *in vitro*. The PD-1 and PD-L1 expressions were found in several canine cancers, confirming that the PD-1/PD-L1 axis could be an immune evasion mechanism in canine cancers. Most importantly, the treatment with anti-PD-L1 mAb enhanced the IFN-γ production from tumor-infiltrating mononuclear cells, encouraging the development of a therapeutic antibody that can be used for a future clinical study.

In chapter II, to clarify the cancer types that could respond to this therapeutic strategy, immunohistochemical analysis of the PD-L1 expression was further performed in various types of malignant cancers in dogs. Most tissue samples of OMM, osteosarcoma, hemangiosarcoma, mast cell tumor, and several other cancers expressed PD-L1, suggesting that these cancers could be candidates for immunotherapy using anti-PD-L1 antibody.

In chapter III, to prepare a therapeutic antibody that can be repeatedly administrated to dogs, blocking anti-PD-L1 mAbs were canine-chimerized using genetic engineering techniques and produced in a mammalian cell-based expression system. Three anti-PD-L1 mAbs were compared, and 4G12 was selected as the source of variable regions with its sufficient blocking ability. A stable high-producer cell line for canine-chimerized 4G12, named c4G12, was established using dihydrofolate reductase (dhfr)-deficient Chinese hamster ovary-DG44 cells and the dhfr/methotrexate method. Because c4G12 showed immunostimulatory effects on canine peripheral blood mononuclear cells *in vitro*, the use of c4G12 in a future clinical study was warranted.

In chapter IV, a pilot clinical study was performed in the Veterinary Teaching Hospital of Hokkaido University to evaluate the safety and antitumor activity of c4G12 in dogs. Dogs with OMM or undifferentiated sarcoma were treated with c4G12 at 2 or 5 mg/kg, every 2 weeks. Evident cancer regression was found in a dog with OMM and another with undifferentiated sarcoma, with objective response rates of 14.3% (1/7) in OMM and 50.0% (1/2) in undifferentiated sarcoma. Despite the repeated administration, no systemic toxicity or autoimmune disease was noticed during the observation period, demonstrating the safety of c4G12 in dogs.

Taken together, the blockade of the PD-1/PD-L1 pathway is a promising therapeutic strategy against various types of canine malignant cancers, and c4G12 is a candidate biological drug that deserves further investigation. Because the blockade of immune checkpoint molecules takes off the brake of antitumor immune responses, its combination with radiation, molecular-targeted drugs, cancer vaccines, or other immunotherapy may exert synergetic effect in cancer treatment. Therefore, further clinical studies are needed to fully elucidate the therapeutic potential of c4G12, and to find the way to elicit the maximum clinical benefit from the blockade of immune checkpoint molecules for canine cancers.