Research Center for Medical Sciences
Division of Oncology

Sadamu Homma, *Professor and Director*
Masaki Ito, *Assistant Professor*
Shigeo Koido, *Associate Professor*
Yasuharu Akasaki, *Assistant Professor*

General Summary

The aim of our research is to develop and establish novel cancer therapies. Concepts for new anticancer therapies, generated from the unique ideas of our researches, would be verified by basic and clinical studies so that they could be applied clinically. Most of our research has been on antitumor immunity.

Research Activities

Investigation of immunogenic mutated antigens in glioblastoma multiforme
Although the overall survival and progression-free survival of patients with glioma have significantly improved with dendritic/tumor cell vaccine therapy, a predictive marker of efficacy is unknown. Whole-exome and whole-transcriptome analyses were performed of patients treated with vaccine therapy to clarify the relationships of mutation-derived antigens and the gene expression profile to the efficacy of vaccine therapy. Mutated protein sequences obtained from detected variants by whole-exome analysis were predicted for binding affinity to human leukocyte antigen (HLA). Mutated peptides predicted as strong binders of HLA were selected as candidates for mutation-derived antigens. Approximately 40 candidates of mutation-derived antigens were detected in all cases. Some candidates were common in all cases. However, no candidates of mutation-derived peptides were found to be common in patients in whom vaccine therapy was effective. Expression of major histocompatibility complex (MHC) class II molecules in patients was higher when vaccine therapy was effective than when it was ineffective. These results suggest that the effectiveness of dendritic/tumor cell vaccine therapy in patients with glioma is related to MHC class II molecule expression rather than to the number of mutation-derived antigens or specific mutated-derived antigens.

Nafamostat mesilate suppresses interferon γ–inducible programmed cell death ligand-1 up-regulation in cancer cells
The binding of programmed cell death ligand 1 (PD-L1) on tumor cells to programmed cell death 1 (PD-1) on activated T lymphocytes leads to inactivation of T lymphocytes, resulting in the immune escape of tumor cells. Because PD-L1 up-regulation is induced by interferon (IFN) γ secreted from activated cytotoxic T lymphocytes (CTLs), suppression of IFNγ-inducible PD-L1 up-regulation is important for CTL-mediated antitumor activity. We have found that nafamostat mesilate (NM), a serine protease inhibitor, potently suppresses IFNγ-inducible PD-L1 up-regulation in cancer cells. HLA class I is an essential molecule for T cell response and up-regulated by IFN-gamma just like

PD-L1. Interestingly, NM did not suppress IFNγ-inducible HLA class I up-regulation, and activation of signal transducer and activator of transcription 1/IFN regulatory factor 1 pathway was not affected by NM treatment. These results suggest that NM has a unique mechanism for PD-L1 suppression different from that of inhibition of signal transducer and activator of transcription 1 signaling.

Encryption of agonistic motifs for Toll-like receptor 4 into artificial antigens augmented the maturation of antigen-presenting cells

Adjuvants are indispensable for achieving a sufficient immune response from vaccinations. From a functional viewpoint, adjuvants are classified into 2 categories: "physical adjuvants," which increase the efficacy of antigen presentation by antigen-presenting cells (APC), and "signal adjuvants," which induce the maturation of APCs. We developed artificial antigens by appending the peptide motifs, which have been reported to have agonistic activity for Toll-like receptor 4 (TLR4), to create "adjuvant-free" antigens. The created antigens with triple TLR4 agonistic motifs in their C termini have an activated nuclear factor kappa B signaling pathways through TLR4. These proteins also induced the production of the inflammatory cytokine tumor necrosis factor α and the expression of the co-stimulatory molecule CD40 in APCs, supporting the maturation of APCs *in vitro*.

Association of soluble PD-L1 in blood and PD-L1 expressed on peripheral immune cells in patients with advanced pancreatic cancer

We have found that plasma levels of soluble (s) PD-L1 in patients with advanced pancreatic cancer are significantly higher than those in healthy subjects. We have also found that PD-L1 expression in peripheral immune cells is higher in patients with cancer than in healthy subjects and that PD-L1 expression is highest in $CD4^+$ T cells. The sPD-L1 was detected with enzyme-linked immunosorbent assay (ELISA) in the culture supernatants of $CD4^+$ T cells from patients with pancreatic cancer, suggesting that sPD-L1 in blood is derived from $CD4^+$ T cells. The immune-suppressive activity of sPD-L1 is now being investigated.

sPD-L1 may become a prognostic marker for human lung cancer

Plasma levels of sPD-L1 in 96 patients with lung cancer, including 73 with adenocarcinomas, 7 with squamous cell cancer, 1 with large cell cancer, and 15 with small-cell cancer, were examined with ELISA. Our results showed that plasma levels of sPD-L1 were higher in patients with lung cancer than in healthy subjects and that overall survival and progression-free survival of patients with high plasma levels of sPD-L1 were significantly shorter than those of patients with low plasma levels of sPD-L1. These results suggest that the plasma level of sPD-L1 may become a prognostic marker for patients with lung cancer.

Publications

Takakura K, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Homma S, Ohkusa T,

Tajiri H. Prognostic markers for patient outcome following vaccination with multiple MHC class I/II-restricted WT1 peptide-pulsed dendritic cells plus chemotherapy for pancreatic cancer. *Anticancer Res.* 2015; **35:** 555-62.

Komita H, Koido S, Hayashi K, Kan S, Ito M, Kamata Y, Suzuki M, Homma S. Expression of immune checkpoint molecules of T cell immunoglobulin and mucin protein 3/galectin-9 for NK cell suppression in human gastrointestinal stromal tumors. *Oncol Rep.* 2015; **34:** 2099-105.

Kan S, Koido S, Okamoto M, Hayashi K, Ito M, Kamata Y, Komita H, Ishidao T, Nagasaki E, Homma S. Gemcitabine treatment enhances HER2 expression in low HER2-expressing breast cancer cells and enhances the antitumor effects of trastuzumab emtansine. *Oncol Rep.* 2015; **34:** 504-10.

Takakura K, Shibazaki Y, Yoneyama H, Fujii M, Hashiguchi T, Ito Z, Kajihara M, Misawa T, Homma S, Ohkusa T, Koido S. Inhibition of cell proliferation and growth of pancreatic cancer by silencing of carbohydrate sulfotransferase 15 in vitro and in a xenograft model. *PLoS One.* 2015; **10:** e0142981.

Kan S, Koido S, Okamoto M, Hayashi K, Ito M, Kamata Y, Komita H, Nagasaki E, Homma S. Up-regulation of HER2 by gemcitabine enhances the antitumor effect of combined gemcitabine and trastuzumab emtansine treatment on pancreatic ductal adenocarcinoma cells. *BMC Cancer.* 2015; **15:** 726.