Research Center for Medical Sciences
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General Summary

The aim of our researches is to develop and establish novel cancer therapies. Concepts of new anti-cancer therapy generated from unique idea of the researchers would be verified by basic and clinical studies in order to apply such concepts to the clinical cancer treatment. Most of our researches have been based on antitumor immunity.

Research Activities

Identification and functional validation of the neo-antigens of glioblastoma mutiforme (GM) recognized by specific cytotoxic T lymphocytes (CTLs) activated by the dendritic cell (DC) vaccine

Treatment with the DC vaccine generated by cell fusion of autologous DCs and GM cells demonstrated significant clinical benefit for the prolongation of progression free survival and overall survival of the post-operative GM patients. Recent progression of cancer immunotherapy indicated that tumor antigens recognized by the antitumor CTLs should be neo-antigens generated by the gene mutation of tumor cells. Because central nervous system is lack in lymphoid organs, the neo-antigens expressed in GM cells may be hardly recognized by systemic immune system. Consequently, secondary immune-suppression to neo-antigens of GM cells could not be induced, allowing the DC vaccine effective for GM treatment. Analysis using the next generation sequencer identified various neo-antigen peptides in GM cells. It has been under investigation whether CD8+CTLs from the DC vaccine-treated GB patients could acquire the responsiveness to these neo-antigen peptides. Peripheral mononuclear cells from the patients were stimulated with the candidate neo-antigen peptides *in vitro*, and T cell response will be examined by the analysis on T cell proliferation and cytokine production. The neo-antigen(s) responsible for the anti-tumor T cell immunity induced by the DC vaccine would be identified.

Soluble programmed cell death ligand-1 (sPD-L1) as a novel biomarker for nivolumab therapy for non-small cell lung cancer

Biomarkers for predicting the effect of anti-programmed cell death-1 (PD-1) mAb against non-small cell lung cancer (NSCLC) are urgently required. Although it is known that blood levels of sPD-L1 are elevated in various malignancies, nature of sPD-L1 has not been thoroughly elucidated. We investigated the significance of plasma sPD-L1 levels as a biomarker for anti-PD-1 mAb, nivolumab therapy. Thirty-nine NSCLC patients were prospectively studied. The patients were treated with nivolumab at the dose of 3 mg/kg every 2 weeks, and the effects of nivolumab on NSCLC were assessed according to

change of tumor size, time to treatment failure (TTF) and overall survival (OS). Baseline plasma sPD-L1 concentration was determined by enzyme-linked immunosorbent assay. The area under the curve of the receiver operating characteristic curve was 0.761. The calculated optimal cut-off point for sPD-L1 level of the plasma samples was 3.357 ng/ml. Fifty-nine percent of the patients with low plasma sPD-L1 levels achieved CR/PR, while 25% of those with high plasma sPD-L1 levels did. In contrast, 22% of the patients with low plasma sPD-L1 levels underwent PD, but 75% of those with high plasma sPD-L1 levels did. TTF and OS were significantly longer in patients with low plasma sPD-L1 levels than in those with high plasma sPD-L1 levels. The clinical benefit by nivolumab therapy was significantly associated with baseline plasma sPD-L1 levels. Plasma sPD-L1 levels could represent a novel biomarker for the prediction of the efficacy of nivolumab therapy against NSCLC.

Encryption of Agonistic Motifs for TLR4 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 two categories: "physical adjuvants" increase the efficacy of antigen presentation by antigen-presenting cells (APC) and "signal adjuvants" induce the maturation of APC. Our previous study has demonstrated that a physical adjuvant can be encrypted into proteinous antigens by creating artificial proteins from combinatorial assemblages of epitope peptides and those peptide sequences having propensities to form certain protein structures (motif programming). However, the artificial antigens still require a signal adjuvant to maturate the APC; for example, co-administration of the Toll-like receptor 4 (TLR4) agonist monophosphoryl lipid A (MPLA) was required to induce an in vivo immunoreaction. In this study, we further modified the previous artificial antigens by appending the peptide motifs, which have been reported to have agonistic activity for TLR4, to create "adjuvant-free" antigens. The created antigens with triple TLR4 agonistic motifs in their C-terminus have activated NF-κB signaling pathways through TLR4. These proteins also induced the production of the inflammatory cytokine TNF-α, and the expression of the co-stimulatory molecule CD40 in APC, supporting the maturation of APC in vitro. Unexpectedly, these signal adjuvant-encrypted proteins have lost their ability to be physical adjuvants because they did not induce cytotoxic T lymphocytes (CTL) in vivo, while the parental proteins induced CTL. These results confirmed that the manifestation of a motif's function is context-dependent and simple addition does not always work for motif-programing. Further optimization of the molecular context of the TLR4 agonistic motifs in antigens should be required to create adjuvant-free antigens.

Basic studies on a novel dendritic cell (DC) vaccine against multiple myeloma (MM) using an anti-CD38 monoclonal antibody (Daratumumab)

CD38 is highly expressed on MM cells. Daratumumab is a newly established monoclonal antibody to CD38, and treatment of MM patients with daratumumab showed significant clinical benefits. MM cells treated with daratumumab might be efficiently engulfed into DCs mediated by Fc-receptor mediated ingestion. DCs engulfing daratumumab-treated

autologous MM cells could become a preventive or therapeutic vaccine against MM. It was demonstrated that daratumumab-treated Daudi cells were ingested by DCs more efficiently than untreated Daudi cells in vitro.

Publications

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