Institute of DNA Medicine
Department of Oncology

Yoshikatsu Eto, *Professor and Director*
Sadamu Homma, *Associate Professor*
Tetsuro Kikuchi, *Associate Professor*
Mikio Zeniya, *Professor*
Junko Horiguchi-Yamada, *Associate Professor*

General Summary

We continue to study immunological therapies for cancer and a model of leukemia cell differentiation. To further develop immunotherapy, we investigated the mechanisms of immunological reactions against cancer and strategies to modify them to enhance clinical responses.

Research Activities

Mass spectrometric identification of a tumor-associated antigen expressed on mouse hepatocellular carcinoma cells that regulates host antitumor immunity

Tumor-associated antigens might stimulate or suppress host antitumor immunity depending on host tumor burden or immune environment. To identify a tumor antigen expressed on cancer cells, MHC class II binding peptide was isolated from I-A^k/peptide complexes obtained from bone marrow-derived dendritic cells (DCs) loaded with MIH-2 mouse hepatocellular carcinoma (HCC) cells, and peptide ion peaks were analyzed by means of mass spectrometry. One representative peptide, EMTK, was considered to be derived from amino acids 284-287 of the cytochrome P450 2j subfamily (CYP2js) expressed in MIH-2 cells. Amino acids adjacent to EMTK showed an I-A^k-binding structural motif. Accordingly, the peptide consisting of EMTK and the I-A^k-binding motif (DFIDAFLKEMTKYPE) was considered to be an antigenic peptide presented to $CD4^+$T cells in the context of MHC class II. Administration of several doses of the synthesized CYP2js peptide to naïve mice induced interferon (IFN)-gamma production by splenocytes. However, repeated immunization of mice with CYP2js peptide suppressed IFN-gamma production and enhanced MIH-2 tumor growth *in vivo*. Production of interleukin (IL)-4, IL-10, and transforming growth factor beta was not involved in this immune-suppression. Increased frequency of $CD4^+FoxP3^+$regulatory T cells and $CD11b^+Gr\text{-}1^+$immature myeloid cells was observed in splenocytes from mice repeatedly immunized with CYP2js peptide. These results indicate that CYP2js expressed on HCC cells could promote antitumor immunity at an early stage of tumor growth with low or moderate antigenic stimulation but would suppress antitumor immunity in an advanced stage of tumor growth with excess antigenic stimulation due to a large tumor burden.

Clinical immunotherapy for brain tumors

Several clinical trials of immunotherapy with DCs have been published. We investigat-

ed the safety and clinical response of immunotherapy with fusions of DCs and glioma cells and IL-12 for patients with malignant glioma. Fifteen patients with malignant glioma participated in this study. No severe adverse effects were observed. In 4 patients, magnetic resonance showed a reduction in tumor size of more than 50%. The results of the Phase I clinical trial of IL-12 and fusion cells indicated that this treatment safely induced immune responses, resulting in a clinically significant antitumor effect in some patients. We are now analyzing the antitumor effects of DC therapy in patients with both primary and recurrent malignant gliomas. Stat-3 is a potential negative regulator of inflammatory responses. Blocking Stat-3 in tumor cells increases expression of proinflammatory cytokines that activate innate immunity and DCs, leading to tumor-specific T-cell responses. We are investigating the antitumor effects of fusion cells transfected with Stat-3 small interfering RNA to block Stat-3 expression.

Differentiation of leukemia cells by adhesion

JASR is a novel leukemia cell line with megakaryoerythroid features. JASR cells adhere to extracellular matrices and acquire megakaryocytic features, including morphological changes. Among genes expressed in parallel to this alteration, FLI1, a transcription factor of the ets family, is a target of particular interest. FLI1 has been shown to be a key regulator for megakaryocytic progenitors. Furthermore, the adherent cells show greater resistance to chemotherapeutic agents. The precise mechanism of the cell-adhesion-mediated drug resistance is being investigated.

Publications

Homma S, Sagawa Y, Komita H, Koido S, Nagasaki E, Ryoma T, Okamoto M. Mechanism of antitumor effect on mouse hepatocellular carcinoma by intratumoral injection of OK-432, a streptococcal preparation. *Cancer Immunol Immunother* 2007; **56:** 1265–74.

Koido S, Hara E (Saitama Cancer Center), Homma S, Torii A, Mitsunaga M, Yanagisawa S, Toyama Y, Kawahara H, Watanabe M, Yoshida S, Kobayashi S, Yanaga K, Fujise K, Tajiri H. Streptococcal preparation OK-432 promotes fusion efficiency and enhances induction of antigen-specific CTL by fusions of dendritic cells and colorectal cancer cells. *J Immunol* 2007; **178:** 613–22.

Homma S, Sagawa Y, Ito M, Ohno T, Toda G. Cancer immunotherapy using dendritic/tumor-fusion vaccine induces elevation of serum antinuclear antibody with better clinical responses. *Clin Exp Immunol* 2006; **144:** 41–7.

Komita H, Homma S, Saotome H (Wyeth Res), Zeniya M, Ohno T, Toda G. Interferin-gamma produced by interleukin-12-activated tumor infiltrating CD8+T cells directly induces apoptosis of mouse heaptocellular carcinoma. *J Hepatol* 2006; **45:** 662–72.

Sekikawa T, Takahara S, Suzuki H, Takeda N, Yamada H, Horiguchi-Yamada J. Diffuse large B-cell lymphoma arising independently to lymphoplasmacytic lymphoma: a case of two lymphomas. *Eur J Haematol* 2007; **78:** 264–9.

Sekikawa T, Iwase S, Saito S, Arakawa Y, Agawa M, Horiguchi-Yamada J, Yamada H. JAS-R, a new megakaryo-erythroid leukemic cell line that secretes erythropoietin. *Anticancer Res* 2006; **26:** 843–50.

Yamada H, Arakawa Y, Saito S, Agawa M, Kano Y, Horiguchi-Yamada J. Depsipeptide-resistant KU812 cells show reversible P-glycoprotein expression, hyper-acetylated histones, and modulated gene expression profile. *Leuk Res* 2006; **30:** 723–34.

Homma S. Designs to enhance the antitumor effects of dendritic cell-based vaccine therapy (in Japanese). *BIO Clin* 2006; **21:** 59–64.

Reviews and Books

Homma S. Dendritic/tumor-fusion vaccine. *Mod Asp Immunobiol* 2006; **19:** 37.

Yamada H, Suzuki H, Arakawa Y, Horiguchi-Yamada J. Newly elucidated pathophysiology for acute myeloid leukemias (in Japanese). *Seijin-byou to Seikatu-Syukan-byou* 2007; **37:** 220–3.