

Cancer Immunotherapy Project

Introduction

The Section for Frontier Medicine comprises the Cancer Immunotherapy Project (Head: T. Nakatsura), Dr. M. Ito (Colorectal Surgery), and Dr. T. Mukohara (Medical Breast, Investigational Drug Development). The aim of this section is to go on developing Frontier Medicine for cancer.

The Cancer Immunotherapy Project aims to investigate evidenced-based cancer immunotherapy, repeating basic research and translational research. This project is focused on developing not only more effective immunotherapies but also immunological methods for suppression of recurrence or for cancer prevention.

Glypican-3 (GPC3)

Glypican-3 (GPC3) was overexpressed, specifically in hepatocellular carcinoma (HCC) and melanoma in humans, making it useful as a novel tumor marker. The preimmunization of BALB/c mice with dendritic cells pulsed with H-2K^d-restricted mouse GPC3₂₉₈₋₃₀₆ (EYILSLEEL) peptide prevented the growth of tumor-expressing mouse GPC3. Because of similarities in the peptide-binding motifs between H-2K^d and HLA-A24 (A*2402), the GPC3₂₉₈₋₃₀₆ peptide appeared to be useful for immunotherapy of HLA-A24⁺ patients with HCC and melanoma. The GPC3₁₄₄₋₁₅₂ (FVGEFFTDV) peptide may induce peptide-reactive CTLs in HLA-A2.1 (HHD) transgenic mice without inducing autoimmunity. In five of eight HLA-A2⁺ GPC3⁺ HCC patients, the GPC3₁₄₄₋₁₅₂ peptide-reactive CTLs were generated from PBMCs by *in vitro* stimulation with the peptide and the GPC3₂₉₈₋₃₀₆ peptide-reactive CTLs were also generated from PBMCs in four of six HLA-A24⁺ GPC3⁺ HCC patients. The inoculation of these

CTLs reduced the human HCC tumor mass implanted into nonobese diabetic/severe combined immunodeficiency mice (100).

To elicit protective immunity against GPC3-expressing mouse tumors, we examined the capacity of an ES-DC expressing mouse homologue of human GPC3, a recently identified oncofetal antigen expressed in human melanoma and hepatocellular carcinoma. CTLs specific to multiple GPC3 epitopes were primed by the *in vivo* transfer of GPC3-transfectant ES-DC (ES-DC-GPC3). The transfer of ES-DC-GPC3 protected the recipient mice from subsequent challenge with B16-F10 melanoma, naturally expressing GPC3, and with GPC3-transfectant MCA205 sarcoma. Treatment with ES-DC-GPC3 was also highly effective against intravenous B16-F10. No harmful side effects, such as autoimmunity, were observed as a result of these treatments. The depletion experiments and immunohistochemical analyses suggest that both CD8⁺ and CD4⁺ T cells contributed to the observed antitumor effect (101).

Heat Shock Protein 105 (HSP105)

Heat shock protein 105 (HSP105), identified by serological analysis using a recombinant cDNA expression library (SEREX) employing serum from a pancreatic cancer patient, was by immunohistochemical analysis to be overexpressed in various human tumors and in the testis of adult men. The expression of HSP105 has not been studied in skin cancers. HSP105 is overexpressed in squamous cell carcinoma and extramammary Paget disease but not in basal cell carcinoma (102).

HSP105 is a strongly immunogenic tumor antigen that elicits both cellular and humoral immunity. Vaccination with HSP105 DNA induced anti-

tumor immunity; mice vaccinated with recombinant HSP105 whole protein-pulsed BM-DCs were significantly protected from the growth of subcutaneous tumors, accompanied by a massive infiltration of both CD4⁺ T cells and CD8⁺ T cells into the tumors. In depletion experiments, we proved that both CD4⁺ T cells and CD8⁺ T cells play a crucial role in anti-tumor immunity. Both CD4⁺ T cells and CD8⁺T cells specific to HSP105 were induced by stimulation with HSP105-pulsed DCs. As a result, the vaccination of mice with BM-DCs pulsed with HSP105 could elicit a stronger tumor rejection than DNA vaccination (103).

NIH3T3 cells overexpressing murine HSP105 (NIH3T3-HSP105) acquired resistance to apoptosis induced by heat shock or doxorubicin. The small interfering RNA (siRNA)-mediated suppression of HSP105 protein expression induced apoptosis in human cancer cells but not in fibroblasts. Apoptosis was induced synergistically in a human colon cancer cell line, HCT116, by a combination of siRNA introduction and doxorubicin or heat shock treatment. *In vivo*, siRNA inoculation into the human gastric cancer cell line KATO-3 established in the flank of an NOD SCID mouse suppressed the tumor's growth. This siRNA-induced apoptosis was mediated through caspases, but not the p53 tumor suppressor protein, even though the HSP105 protein bound to wild-type p53 protein in HCT116 cells. These findings suggest that the constitutive overexpression of HSP105 in cancer cells is involved in malignant transformation by protecting tumor cells from apoptosis. HSP105 may thus be a novel target molecule for cancer therapy, and a treatment regimen using synthetic siRNA to suppress the expression of HSP105 protein may provide a new strategy for cancer therapy (104).

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