Identification of Tumor Rejection Antigens Using Mouse Tumor Rejection Models

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Introduction

Mice and Tumor Cell Line

Neu-tg mice (strain name: FVB-N-Tgf(MMTV-neu)-20Ma) were obtained from Charles River Laboratory (Bar Harbor, ME) and bred under SPF conditions at the University of Washington. The mice harbor nonmutated, nonactivated rat neu under control of the mouse mammary tumor virus (MMTV) promoter. Animal care and use were in accordance with institutional guidelines. Mouse mammary carcinoma (MMC) cells were derived from a spontaneous tumor in a neu-mouse.

Tumor Rejection Experiment

FVB-N or parental neu-mice were inoculated with 1 x 10^6 MMC cells subcutaneously on the mid-dorsum with a 23-gauge needle. Tumors were measured every other day with vernier calipers and tumor volume was calculated as the product of length x width x height x 0.5236. As vivo data are presented as mean ± s.e.m. For IL-2 immunostimulatory (ONTAK) treatment, mice were treated every 2-3 days with tail vein dosing of either 100 ul PBS as control or 100 ul of PBS containing varying concentration of the immunostimulant. For SEREX screening, serum samples were collected from each mouse before tumor challenge and at 1 month post tumor implantation.

Material and Methods

SEREX to Screen for Tumor Rejection Antigens

RNA was extracted from MMC (mouse mammary carcinoma) cells using RNA Isolation kit from Ambion (Austin, TX). Poly(A)^+ RNA was extracted with Poly(A) Pur Kit (Ambion). A cDNA library using the ZAP Express vector (Stratagene, La Jolla, CA) was constructed following the manufacturer’s instructions. After overnight plaque lifting, the introduction of antibody was first incubated with the neo-tumor, developed with NBT/BCIP kit and positive spots were marked. Then the membrane was washed and reprobed with post-rejection serum, new spots are followed by secondary screening. Positive clones that react to post-rejection sera were purified to mononucularity and adjusted to be two vectors using SLOX cells and Eschacharoid Helper pluge. Plasmid DNA was prepared using FastPlasmid kit (Eppendorf). DNA sequencing was performed using Big Dye reaction. What was used to search for sequence homology. The human homologues of the mouse tumor antigens were identified through NCBI database and the immunoepitogen of their human homologues was searched using the Human Cancer Immunome Database (http://ludwig-sun5.unil.ch/CancerImmunomeDB).

Vaccination and Tumor Protection Experiment

Six to 8 week old female nude mice were immunized with plasmid DNA (pcDNA3 or pcDNA3-HER2) expressing the mouse tumor antigens. Mice were immunized by intramuscular injection of 5ug DNA in combination with Complete Freund Adjuvant (CFA, for the 1st vaccine) or Incomplete Freund Adjuvant (IFA, for the 2nd and 3rd vaccines). The vaccine was given three times, two weeks apart. The plasmid that encodes intracellular domain of human HER2 (HER2) was used as positive control. Vaccination with the empty pCMV plasmid DNA together with CFA/IFA was included as a vaccine backbone control. One million tumor cells were injected to each mouse subcutaneously on the mid-dorsum 2 weeks post the third vaccine. Tumors were measured every other day with vernier calipers and tumor volume was calculated as the product of length x width x height x 0.5236. As vivo data are presented as mean ± s.e.m.

Results

1. Two tumor rejection models were established for screening of tumor rejection antigens. (A) Parental tumor rejection model. Shown are tumor growth in neu-mice (black columns), and parental mouse (white columns) with and without immunoactivation (white columns) or tumor rejection mice that received IL-2 immunostimulatory (ONTAK) to stimulate T regulatory cells (gray columns). There is significant increase in serum anti-neu Ab levels in parental FVB/N mice that rejected tumors and in neu-tg mice with immunostimulation (ONTAK). In neu-tg mice without immunostimulation, the serum anti-neu Ab level remains unchanged after tumor development.

2. Anti-neu neo antibody immunity was augmented in mice rejecting tumor. Shown are mean fluorescent intensity of MMC cells stained with serum from pre-tumor challenge mice (gray columns) and post-tumor development mice (black columns). There was significant increase in serum anti-neu Ab levels in parental FVB/N mice that rejected tumors and in neu-tg mice with immunostimulation (ONTAK) to elicit Tregs. In neu-tg mice without immunostimulation, the serum anti-neu Ab level remains unchanged after tumor development.

3. Novel Tumor rejection associated immunity can be identified via SEREX. (A) representative SEREX blot showing that Mvi1, one of the newly-identified antigens, is only reactive to post-rejection sera. (B) Western blot verifying the antibody response identified in SEREX. ONTAK is post-tumor serum from the same mouse. Lanes 1 and 4: ladder; Lanes 2 and 5: immune from bacteria transfected to express Mvi1; Lanes 3 and 6: immune from the control transfected bacteria.

Conclusions

1. The tumor antigen repertoire identified in tumor-rejecting mice is totally different from the antigen repertoire identified in tumor-bearing mice.

2. Vaccination targeting antigens identified from tumor-rejecting mice, but not tumor antigens identified from tumor-bearing mice, had tumor protective effect. (A) Vaccination using plasmid DNA encoding Mvi1 had the same level of protection as vaccination using pcDNA3 plasmid DNA and a whole tumor cell vaccine using stratified MMC cells. Other TRAs including Cyp50, Tum1, Tum2, or ONTAK were also shown to have tumor protective effect (data not shown). (B) Vaccination targeting antigens identified from tumor-bearing mice (Swap70 and Gsn) had no tumor protective effect.

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References


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