Induction of Antitumor Immunity with Combination of HER2/neu DNA Vaccine and Interleukin 2 Gene-modified Tumor Vaccine

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ABSTRACT

The therapeutic effects of both cytokine-secreting tumor vaccine and DNA vaccine were studied using mouse MBT-2 bladder cancer cells as a model. Cytokine-secreting MBT-2 cells were obtained by infecting cells with retroviral particles containing interleukin (IL)-2, IL-4, or granulocyte-macrophage colony-stimulating factor (GM-CSF)-expression vector. The MBT-2-IL-2 cells were not tumorigenic in syngeneic C3H mice at all. Tumor formation decreased significantly for the MBT-2-GM-CSF cells, MBT-2-IL-2, -IL-4, and -GM-CSF cells were killed by irradiation and tested as tumor vaccines. The irradiated MBT2-IL-2 cells could complete protect mice from the growth of the preexisting tumor cells, and the immune memory lasted for 8 months. On the other hand, irradiated MBT-2-IL-4 and MBT-2-GM-CSF cells were less effective. When the loading tumor mass increased, all tumor vaccines lost protective effects. DNA vaccine encoding the tumor antigen neu was additionally tested to improve the therapeutic efficacy. Co-injection of 60 µg pSV-neu DNA was effective in enhancing the antitumor effects of MBT2-IL-2; however, DNA vaccine alone cannot prevent the progression of the preexisting tumor. Immunohistochemical analysis of tumor infiltrate revealed massive increase of CD4^+ T lymphoid cells in the group of mice treated with both DNA vaccine and IL-2-secreted tumor vaccine. Western blotting demonstrated the presence of anti-neu antibody in the serum from immunized mice. In contrast, combination of DNA vaccine and MBT-2-GM-CSF has no additive effect. The results indicate the combination of DNA vaccine and IL-2-secreting tumor vaccine can additionally improve therapeutic efficacy, and the efficacy is correlated with the increase of CD4^+ T lymphocytes and anti-neu antibody.

INTRODUCTION

The residual tumor mass or metastatic tumor cells after surgery is usually responsible for the therapeutic failure in clinical oncology. Active immunization against the patient tumor may provide a therapeutic modality for cancer patients whose primary tumors have been removed. Initial experiments of tumor vaccines consisting of irradiated tumor cells usually showed little protective effect (1). Systemic administration of cytokines, such as IL-4, had profound inhibitory effects on tumor progression, but the side effects of these cytokines limited the therapeutic use (2). An approach to achieve highly localized secretion of cytokines at the site of the tumor is genetic insertion of cytokine gene into tumor cells (3, 4). Several studies have shown that genetically engineered tumor cells expressing IL-2 (5–9), IL-4 (10), or GM-CSF (11–13) could immunize mice against a subsequent challenge with parental tumor cells. The antitumor effects of endogenous secreted cytokines are more potent than the locally applied exogenous cytokines at the tumor sites (11, 14). The antitumor responses induced by different cytokines seemed to operate through different mechanisms. For example, cytotoxic CD8^+ T cells play a major role in the IL-2-induced immune response (15), whereas CD4^+ and CD8^+ T cells mediate the GM-CSF antitumor activity (16). Most of the studies indicate that tumor vaccine is very effective in preventing subsequent tumor challenge but is only partially effective against preexisting tumors (9, 17). Combination of tumor vaccine and other types of cancer therapy is necessary for achieving better therapeutic efficacy.

DNA vaccine represents a novel method with delivery of naked plasmid DNA expressing one or several antigens, which usually leads to strong and persistent cellular and humoral immune response (for review see Refs. 18 and 19). Inoculation of plasmid DNA has been found to be protective against many infectious diseases (20–25). The success of DNA vaccine against foreign antigen led to the trial of DNA vaccine for relatively specific tumor-associated antigen. Vaccination of mice with plasmid encoding human CEA elicited CEA-specific T-cell response and protected the mice from subsequent challenge with syngenic CEA-expressing cell lines (26). In the

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mouse model, injection of plasmid-encoding gp100 could protect mice from subsequent challenge of syngenic B16 melanoma-expressing human gp100 (27).

The HER2/erbB2/neu oncogene encodes a M, 185,000 protein that is a transmembrane tyrosine kinase (28, 29). Amplification or overexpression of neu is frequently observed in breast, ovary, bladder, and many other types of cancer, and its amplification often correlates with a poor prognosis (30, 31).

Neu-derived peptide epitopes were recognized by cancer-specific CTLs in several types of cancer (32–38). Vaccination of peptide or extracellular domain of p185neu can prevent subsequent tumor formation (39, 40). DNA vaccines encoding full-length or truncated neu induce protective immunity against neu-expressing tumor in a transgenic mice model (40–44). Although these results indicate the potential use of oncogene neu as a DNA vaccine, the effect of neu-DNA vaccine against the preexisting native tumor has never been tested. The combination of DNA vaccine and other types of immunological vaccine also require investigation.

In this study, mouse bladder MBT-2 cells and C3H syngenic mice was used as a model because MBT-2 cells show high similarity to human bladder cancer grossly and histologically (9). Overexpression of neu was also observed in the MBT-2 cells.5 We first compared the effects of cytokines (IL-2, IL-4, and GM-CSF) on the therapeutic efficacy of tumor vaccine and then tested the combinatory therapy of DNA vaccine encoding neu and tumor vaccine. Our results indicate that IL-2-secreted tumor vaccine is most effective and that DNA vaccine encoding oncogene neu can additionally enhance the IL-2-secreting tumor vaccine in bladder cancer animal model.

MATERIALS AND METHODS

Retroviral Vectors and Establishing MBT-2-IL-2, MBT-2-IL-4, and MBT-2-GM-CSF Transfectants. The construction of murine GM-CSF retroviral vector was previously described (17). The IL-2 and IL-4 retroviral vectors were constructed by replacing the GM-CSF gene with the human IL-2 or murine IL-4 gene. Retrovirus-cell line producing cells for expressing IL-2, IL-4, and GM-CSF were cultured in a 10-cm dish, and medium was harvested for retrovirus particles 24 h later. Two ml of supernatants were used to infect 105 MBT-2 cells, and stable transfectants were obtained by G418 selection. The stable transfectants were named MBT-2-IL-2, MBT-2-IL-4, and MBT-2-GM-CSF, respectively.

Determination of Titers of Cytokines. The titers of MBT-2-IL-4 and MBT-2-GM-CSF were determined by ELISA kit (Endogen Inc., Boston, MA). The amount of IL-4 or GM-CSF in 50 μl of culture medium of transfectants was assayed following manufacturer’s instruction. The titer of IL-2 was assayed by stimulation of thymidine incorporation on HT-2 cells (IL-2-dependent cell line). HT-2 cells were treated with IL-2 by twofold dilution for 20 h to establish the bioassay standard curve. Thymidine incorporation was measured after a 4-h incubation with 0.5 μCi [H]thymidine. Fifty μl of culture medium from each cell line were incubated with HT-2 cells, and the thymidine incorporation experiment was performed. The values of thymidine incorporation stimulated by sample culture medium were compared with the standard curve of IL-2.

Analysis of Tumor Growth in Vivo. One million parental MBT-2 cells were injected s.c. into the middle back of 6- to 8-week-old syngenic C3H female mice. Tumor size was measured using a caliper, and mice that developed no palpable tumor (usually <0.5 cm) 60 days after injection were defined as “tumor-free” mice.

Preparation of Tumor Vaccines (e.g., Irradiated MBT-2-IL-2). MBT-2-IL-2 cells were irradiated with 60 Gy γ-ray and used immediately to inoculate s.c. on the back of C3H mice (5 × 105 cells in 0.2 ml Dulbecco’s PBS). The site of vaccination was on the same side as the tumor implantation, close to the tail. The vaccination was performed after tumor implantation following the specific schedule described in “Results.”

DNA Vaccine Injection. HER2/neu cDNA was cloned under the control of the SV40 promoter. Plasmid DNA was affinity-purified by Qiagen Mega kit and resuspended in sterile saline at the concentration of 60 μg/0.1 ml. Mice were injected i.m. on the upper thigh with plasmid DNA at weekly intervals.

Immunohistochemistry. Mice were killed by perfusion with PBS via cardiac puncture. Tumor tissues were removed and embedded in OCT compound and then frozen in liquid nitrogen. Cryosections (5-μm) were made and fixed with 3.7% formaldehyde and acetone. Endogenous peroxidase was removed with 3% hydrogen peroxide. The cryosections were washed with PBS three times and incubated with primary antibody overnight at 4°C. After additional reaction with peroxidase-conjugated secondary antibody, aminothiol carbazole substrate kit (Zymed Laboratories, San Francisco, CA) was used for color developing.

Western Blotting. Total cell lysates were prepared in 2× SDS loading buffer. The protein concentration was determined by Bio-Rad protein assay. Twenty-five μg of total cell lysates was analyzed on 8% SDS polyacrylamide minigel and transferred to nitrocellulose membrane. The nitrocellulose filter was preblocked with 5% skim milk (Difco Laboratories, Inc., Detroit, MI) for 1 h and probed with primary antibody (anti-neu Ab-3, Oncogene Research). The affinity-purified antibody was used as suggested by the manufacturer.

RESULTS

Establishment of the MBT-2 Transfectant Secreting IL-2, IL-4, and GM-CSF and the Tumorigenicity of MBT-2-IL-2, MBT-2-IL-4, and MBT-2-GM-CSF Cells in Mice. The MBT-2 cells were infected with retrovirus containing IL-2-, IL-4-, or GM-CSF-expressing vectors. The stable transfectants selected with G418 were cultured in fresh medium for 24 h, and the incubation medium was assayed for secreted cytokines. The titers of the selected clones for tumor vaccines are as follows: MBT-2-IL-2, 50 units/106 cells; MBT-2-IL-4, 198 ng/106 cells; and MBT-2-GM-CSF, 207 ng/106 cells. To determine whether

5 Unpublished results.
the cytokines secreted from the genetically modified tumor cells affect the tumorigenicity in vivo, one million live MBT-2-IL-2, MBT-2-IL-4, or MBT-2-GM-CSF cells were injected s.c. into syngenic C3H mice. The tumor formation was monitored 1 month later. The tumor cells that secrete cytokines are less tumorigenic, especially those cells secreting IL-2 and GM-CSF (Table 1). The results indicated that the secreted cytokine inhibited tumor formation, probably through enhancing the immune system to reject tumor formation.

**MBT-2-IL-2 Cells Act as a Tumor Vaccine to Prevent Tumor Formation from Preexisting Tumor Cells and Can Establish a Long-lasting Immune Memory.** The protocol for gene therapy was shown in Fig. 1A. MBT-2-IL-2, -IL-4, -GM-CSF cells were killed by 60 Gy irradiation and immediately used as tumor vaccine. At day 1, the mice were injected with $2 \times 10^6$ live MBT-2 cells. Then at days 7, 14, and 21, the mice were inoculated with irradiated MBT-2-IL-2, -IL-4, and -GM-CSF tumor vaccine. Tumor formation was observed 60 days later. The irradiated MBT-2-IL-2 cells could successfully work as tumor vaccine against the preexisting tumor cells. On the other hand, irradiated MBT-2-IL-4 and MBT-2-GM-CSF was less effective (Table 2). We also asked whether the immune memory was established in the mice that have been inoculated with MBT-2-IL-2 tumor vaccine. Three and 8 months after the last inoculation of tumor vaccines, the mice were injected with $10^6$ MBT-2 tumor cells, and no tumor formation was observed (data not shown). The result indicated that the immune memory lasted for at least 8 months in the mouse animal model.

**Tumor Vaccine Was Not Effective When Tumor Mass Increased.** The initial experiment was effective at the dose of $2 \times 10^4$ tumor cells. Similar experiments with tumor burden up to $10^6$ tumor cells were then tested. At day 1, $10^6$ MBT-2 cells were injected into mice. At day 10, when the tumors were almost palpable in these mice, the tumor vaccines were inoculated at days 10, 17, and 24 (Fig. 1B). Tumor formation was $\sim100\%$ in all groups of mice (Fig. 1B). Tumor formation was observed. To test the efficacy of tumor vaccines on various tumor burden, MBT-2-IL-2 tumor vaccines were inoculated at 2, 9, and 16 or at 5, 12, and 19 days after implanting tumor cells. The tumor vaccine was only partially effective at day 2 after tumor implantation (Table 4) but had no effect after day 5. The results indicated that the tumor vaccine can only be functional against a small amount of tumor mass. The result was similar to a previous report on GM-CSF-modified tumor vaccine (17).

**DNA Vaccine Enhanced the Efficacy of IL-2-transduced Tumor Vaccine.** Because single tumor vaccine was not effective against large tumor mass, we also tested the efficacy of a combination of cytokine-modified tumor vaccine and DNA vaccine against neu. One day after the initial injection of $1 \times 10^6$ tumor cells, the mice were inoculated with different combinations of $5 \times 10^5$ MBT-2-IL-2-irradiated cells, 60 μg pSV-neu plasmid DNA, and 60 μg pSV-lacOZ plasmid DNA. The inoculation was repeated twice at 7-day intervals. The protocol is shown in Fig. 1C. DNA vaccines encoding full-length rat neu cDNA alone did not provide protection against tumor progression. However, DNA vaccine against neu was effective in enhancing antitumor effects of IL-2-secreting tumor vaccine (Table 5). The combination of IL-2-secreting tumor vaccine and DNA vaccine was statistically significantly better

### Table 1
Tumorigenicity of live MBT-2-IL-2, MBT-2-IL-4, and MBT-2-GM-CSF cells in C3H mice

<table>
<thead>
<tr>
<th>Cells (10^6)</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBT-2</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>MBT-2-IL-2</td>
<td>0/9 (0%)*</td>
</tr>
<tr>
<td>MBT-2-IL-4</td>
<td>5/7 (71.4%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF</td>
<td>1/8 (12.5%)*</td>
</tr>
</tbody>
</table>

* Statistically significant when compared with the MBT-2 group ($P < 0.01$) by $\chi^2$ analysis.

### Table 2
Efficacy of gene therapy with low tumor burden

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>MBT-2-IL-2</td>
<td>0/10 (0%)*</td>
</tr>
<tr>
<td>MBT-2-IL-4</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF</td>
<td>4/10 (40%)</td>
</tr>
</tbody>
</table>

* Statistically significant when compared with the control group ($P < 0.01$) by $\chi^2$ analysis.
DNA Vaccine Enhances IL-2-secreting Tumor Vaccine

Mice were injected with 1 × 10^6 MBT-2 cells and inoculated with vaccines at days 7, 14, and 21. The tumor formation was monitored 60 days later.

Table 3  Efficacy of gene therapy with high tumor burden

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9/10 (90%)</td>
</tr>
<tr>
<td>MBT-2-IL-2</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>MBT-2-IL-4</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF</td>
<td>10/10 (100%)</td>
</tr>
</tbody>
</table>

Table 4  Efficacy of gene therapy with different tumor loading

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>MBT-2-IL-2 (Day 2)</td>
<td>3/7 (43%)</td>
</tr>
<tr>
<td>MBT-2-IL-2 (Day 5)</td>
<td>7/7 (100%)</td>
</tr>
</tbody>
</table>

Histological Analysis of the Tumor Infiltrates. To investigate the cellular mechanisms of the tumor rejection induced by IL-2-secreting tumor vaccine and DNA vaccine, histological analyses on the tumor sites were performed. Immunostaining did not detect the presence of macrophages and natural killer cells in the tumor sites (data not shown). As for CD8^+ T cells, immunostaining in the control mice and DNA vaccine mice did not reveal significant differences (Fig. 2). In contrast, a significant increase of infiltration of CD4^+ helper T cells was observed in the group of mice that was treated with both IL-2-secreting tumor vaccine and DNA vaccine.

Anti-neu Antiserum Response in Mice Injected with MBT-2-IL-2 Cells and pSV-neu Plasmid DNA. To determine the anti-neu antibody response in the serum of mice injected with DNA vaccine, mice were killed, and the serum pooled from heart was used for Western blotting. Cell lysates of three cell lines with variable expression of p185^{neu} were analyzed on SDS-PAGE and probed with commercial anti-neu antibody, serum from control mouse, or serum from mice injected with DNA vaccine and MBT-2-IL-2 tumor vaccine. The mouse MBT-2 bladder cancer cell line contains high endogenous mouse neu; the TCC-SUP human bladder cancer cell line has very low expression of human neu protein; and TCC-SUP-N5 (derived by exogenous transfection of pSV-neu) expressed a high level of rat neu protein. The commercial anti-neu antibody detected all mammalian p185^{neu} protein specifically and served as a positive control. Both rat and mouse neu protein in TCC-SUP-N5 and MBT-2 cells were detected by the serum of mice immunized with DNA vaccine and MBT-2-IL-2 tumor vaccine, but the minute amount of human neu in TCC-SUP was not detected (Fig. 3). As a negative control, the serum from control mouse cannot detect p185^{neu} protein. The serum of the group of the mice injected with MBT-2-IL-2 tumor vaccine or DNA vaccine alone was unable to detect p185^{neu} protein (data not shown).

DNA Vaccine Did Not Enhance the Therapeutic Effect of the GM-CSF-transduced Tumor Vaccine. We also tested the effect of a combination of HER-2/neu DNA vaccine and MBT-2-GM-CSF tumor vaccine. In contrast to the MBT-2-IL-2 tumor vaccine, addition of MBT-2-GM-CSF tumor vaccine to HER-2/neu DNA vaccine had no therapeutic advantage compared with the tumor vaccine alone (Table 6). In addition, no increase of infiltration of macrophages, natural killer cells, CD4^+ lymphocytes, or CD8^+ lymphocytes was observed in the tumor sites of the mice treated with MBT-2-GM-CSF and/or DNA vaccine (data not shown).

Table 5  Efficacy of combination of IL-2-modified tumor vaccine and DNA vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32/38 (84%)</td>
</tr>
<tr>
<td>MBT-2-IL-2</td>
<td>13/22 (59%)</td>
</tr>
<tr>
<td>LacZ DNA vaccine</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Neu DNA vaccine</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>MBT-2-IL-2 + Neu DNA vaccine</td>
<td>4/18 (22%)</td>
</tr>
<tr>
<td>MBT-2-IL-2 + LacZ DNA vaccine</td>
<td>8/10 (80%)</td>
</tr>
</tbody>
</table>

* Statistically significant when compared with either the MBT-2-IL-2 group or the control group (P < 0.05) by χ² analysis.

DISCUSSION

Our results indicated that the irradiated cytokine-secreting tumor cells could work as a good vaccine against tumor progression of a small tumor burden. Among the three types of cytokines, IL-2 is the most effective one in the mouse bladder cancer model. Combination of HER2/neuDNA vaccine and MBT-2-IL-2 tumor vaccine can additionally enhance the immunological effects of tumor vaccine.

In several comparative studies, IL-12 and GM-CSF are most potent (45). However, IL-2 is more effective than the IL-4 and GM-CSF in our bladder cancer model. This result is in agreement with an earlier report that IL-2 is more potent than IFN-γ in stimulating immunity in a bladder cancer model (9). These results altogether suggest that IL-2-secreting tumor vaccine may be one of the best tumor vaccines in treating bladder cancer.

DNA vaccine encoding a specific antigen is effective in protection from infectious disease. One contributing factor in the efficacy of DNA vaccine is the CpG motif. DNA motifs consisting of an unmethylated CpG dinucleotide (46, 47) stimulate an immune response with the production of several cytokines, such as IL-6, IL-12, and IFN-γ (48). Combination of DNA vaccine and other types of immunological therapy may use CpG motifs in DNA as immune adjuvants (49). In our experiments, pSV-LacZ control plasmid cannot enhance the...
antitumor effect of MBT-2-IL-2, suggesting that the CpG motif does not work as an adjuvant in stimulating immunological response in this assay. Control DNA pSVLacZ may even enhance the tumor progression attributable to unidentified side effects (Tables 5 and 6).

The use of DNA vaccine in the challenge against tumor progression was in progress with the identification of tumor-associating antigen (34, 50). Injection of DNA vaccine encoding a specific tumor-associating antigen could reduce tumor formation in a subsequent challenge of tumor cells (27, 41, 51). Many of the experimental systems used to evaluate the efficacy of DNA vaccine against tumor progression suffer several drawbacks: (a) immunization of healthy animals against a subsequent challenge with tumor cells was assayed rather than treatment of a tumor-bearing animal with DNA vaccine; (b) a transgenic animal system, which might not mimic native tumor, was used; and (c) DNA vaccine encoding an exogenous transfected antigen rather than an endogenous tumor antigen was studied.

In our assay system, overexpression of endogenous p185
neu is present in the MBT-2 mouse bladder cell line. Our result indicated that DNA vaccine alone was not effective against the progression of preexisting tumor (Tables 5 and 6). This may be attributable to several causes: (a) SV40 promoter may be weaker than the commonly used cytomegalovirus promoter; (b) the dose of DNA vaccine (60 μg) may be too low; and (c) vaccination against a specific antigen on a native complex tumor is not sufficient to prevent tumor progression. Vaccination of 100 μg DNA was sufficient for dog (52), and pSV promoter appears to be a relatively strong promoter in fibroblasts and several epithelial types. In addition, we have recently expressed the extracellular domain of HER2/neu under cytomegalovirus promoter and injected i.m. 100 μg of this DNA into mice to prevent tumor progression. The immunized mice can develop anti-neu antibody, as demonstrated by Western blotting, but are provided no protection from tumor progression. Therefore, it is likely that DNA vaccine against a specific tumor-

Fig 2. Cellular infiltrates at the tumor site. Immunohistochemical analysis of CD4+ and CD8+ cells was performed with cryosections of tumor and detected with primary antibody specific for CD4+ and CD8+ cells. Dark spots, peroxidase-stained cells.
DNA Vaccine Enhances IL-2-secreting Tumor Vaccine

CD4+ T lymphocytes played an essential role in protection from tumor growth when IL-2 was combined with other therapy, such as DNA vaccine in this study. Our results indicated that a combination of DNA vaccine and IL-2-secreting tumor vaccine has additive therapeutic efficacy against tumor progression. The enhancing effect of DNA vaccine is probably attributable to both an increase of CD4+ helper T cells and the presence of anti-neu antibody.

Table 6  Efficacy of combination of GM-CSF-modified tumor vaccine and DNA vaccine

Mice were injected with 1 × 10^6 MBT2 cells and inoculated with vaccines at days 2, 9, and 16. The tumor formation was monitored 60 days later.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor formation</th>
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<tbody>
<tr>
<td>Control</td>
<td>5/6 (84%)</td>
</tr>
<tr>
<td>Neu DNA vaccine</td>
<td>5/6 (84%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF + Neu DNA vaccine</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>MBT-2-GM-CSF + LacZ DNA vaccine</td>
<td>4/6 (67%)</td>
</tr>
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</table>

Fig 3  The serum from the DNA vaccine group of mice detect 185 neu in Western blot analysis. Three cell lines containing different amounts of 185 neu were analyzed on SDS-PAGE and subjected to Western blot analysis with the serum from two groups of mice: control mice inoculated with MBT-2 cells only; and mice inoculated with both MBT-2-IL-2 tumor vaccine and DNA vaccine pSV-neu. Arrow, the position of 185 neu. Commercial anti-neu antibody (Oncogene Science) was used as a control. *M*, Mr in thousands.

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Induction of Antitumor Immunity with Combination of HER2/neu DNA Vaccine and Interleukin 2 Gene-modified Tumor Vaccine

Shi-An Chen, Ming-Huan Tsai Fu-Tien Wu, Ali Hsiang, et al.


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