Combination therapy with dendritic cell-based vaccine and anti-CD69 antibody enhances antitumor efficacy in renal cell carcinoma-bearing mice

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Background/aim: Dendritic cell-based vaccine therapy for renal cell carcinoma is effective but requires improvement. Here we explored whether combination therapy with dendritic cell-based vaccine and anti-CD69 antibody can enhance antitumor efficacy in renal cell carcinoma-bearing mice.

Materials and methods: Balb/c mice were challenged subcutaneously with murine renal cell carcinoma (Renca) cells. On day 3 after tumor cell inoculation, tumor-bearing mice either were left untreated or were treated with Renca tumor lysate-pulsed dendritic cells (i.e. dendritic cell-based vaccine), anti-CD69 antibody, or a combination of Renca tumor lysate-pulsed dendritic cells with anti-CD69 antibody. The mice were sacrificed on day 28. Tumor volume was measured for analysis of antitumor efficacy. Spleens were excised to evaluate antitumor immunological responses by measuring the proliferation and activation of T cells, which have the capacity to recognize and destroy tumor cells.

Results: Combination treatment with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody resulted in significant decreases in tumor volume and significant increases in T-cell proliferation and activity, compared with no treatment or either treatment alone.

Conclusion: These findings indicate that anti-CD69 antibody can potentiate antitumor efficacy of dendritic cell-based vaccine. The augmented therapeutic efficacy conferred by the combination therapy may be associated with increased T-cell proliferation and activity.

Key words: Dendritic cell, cancer vaccine, CD69, monoclonal antibody, renal cell carcinoma model

1. Introduction
Renal cell carcinoma accounts for 4% of all malignant tumors (1) and has an incidence of about 8–9 persons per 100,000 each year, which is increasing (2). There is no obvious symptom in the early stage of this cancer. As a result, approximately 25%–30% of patients exhibit tumor metastasis at first diagnosis and lose the opportunity for surgery (3). Although patients with primarily localized renal cell carcinoma can be successfully treated by partial or radical nephrectomy, one third of cases will subsequently develop metastases (4,5). Therapy of metastatic renal cell carcinoma is particularly difficult because of its poor response to radiotherapy or chemotherapy. Therefore, the prognosis for patients with metastatic renal cell carcinoma is poor, and the survival rate for 5 years is less than 10% (6). These lead to a rising demand for other treatment modalities.

Prospective studies note spontaneous regression rates of up to 7% in patients with metastatic renal cell carcinoma (7,8). In addition, immunohistochemical studies of renal cell carcinoma often reveal tumor-infiltrating dendritic cells and T lymphocytes able to recognize and kill tumor cells (9). Therefore, renal cell carcinoma is considered an immunogenic tumor. These findings also lead to attempts to develop immunotherapeutic strategies using immunostimulatory cytokines such as interleukin-2 and interferon-α (10). It has been reported that treatment with interleukin-2 and interferon-α can induce antitumor activity in less than 20% of patients with metastatic renal cell carcinoma, but is associated with a high incidence
of toxic side effects such as lung edema, hypertension, and hepatic and renal injury (10). Consequently, novel therapeutic agents with more effectiveness and less toxicity are needed.

Targeted therapy has revolutionized treatment for metastatic renal cell carcinoma in the past decade. Nowadays, there are seven approved targeted agents regarded as standard therapy for metastatic renal cell carcinoma: the monoclonal antibody to vascular endothelial growth factor (VEGF) bevacizumab; the VEGF receptor tyrosine kinase inhibitors (VEGFr-TKIs) pazopanib, sunitinib, axitinib, and sorafenib; and the mammalian target of rapamycin (mTOR) inhibitors temsirolimus and everolimus (11,12). These targeted agents have markedly improved treatment outcome (13). However, they rarely achieve durable complete responses, and patients with metastatic renal cell carcinoma eventually develop resistance to targeted therapy (13). In addition, treatment with targeted agents is frequently accompanied by various side effects such as hypertension, hand-foot syndrome, nausea, diarrhea, and fatigue (13). These observations prompt consideration of new therapeutic approaches for metastatic renal cell carcinoma.

More recently, emphasis has shifted to the use of dendritic cell-based vaccine to treat patients with metastatic renal cell carcinoma. Dendritic cells are the most powerful antigen-presenting cells and play a critical role in the induction of T-cell immune responses (14). Dendritic cells have the unique ability to process and present antigens derived from tumor cells to both naive CD4+ and CD8+ T cells. The interaction between T cells and dendritic cells results in activation and proliferation of T cells. Activated CD8+ T cells become cytotoxic T lymphocytes with the ability to recognize and kill tumor cells expressing the same antigen on the cell surface (15). Activated CD4+ T cells can provide help for CD8+ T cells. The interaction between T cells and dendritic cells results in activation and proliferation of T cells. Activated CD8+ T cells become cytotoxic T lymphocytes with the ability to recognize and kill tumor cells expressing the same antigen on the cell surface (15). Activated CD4+ T cells can provide help for CD8+ T cell activation by secreting interleukin-2 (16). A total of 166 patients with metastatic renal cell carcinoma were treated with dendritic cell-based vaccine in 11 clinical trials (17). Clinical responses to vaccine were evaluated according to the World Health Organization criteria (18): complete response was defined as complete disappearance of tumor, partial response was defined as ≥50% reduction in the product of two longest perpendicular diameters of tumor without the appearance of new metastases, stable disease was defined as <50% decrease to <25% increase in tumor size, and progressive disease was defined as ≥25% increase in tumor size or the appearance of new metastases. Among the 166 patients who finished therapy, 77 (46%) had clinical response with 6 complete responses, 13 partial responses, and 58 disease stabilizations (17). Treatment with dendritic cell-based vaccine was only accompanied by mild adverse effects such as local reactions at the injection site, flu-like symptoms, and fever. Although dendritic cell vaccination was proven to be safe and effective in clinical trials, the clinical efficacy remained limited. Approaches to improve these immune responses induced by dendritic cell-based vaccine will strengthen the clinical efficacy of this vaccine.

CD69 is a type II transmembrane protein and belongs to the C-type lectin superfamily (19). Activation of T-cells leads to upregulated expression of CD69 on the cell surface (20). CD69 acts as a co-stimulatory molecule for T-cell activation and proliferation (21). Cross-linking of CD69 by anti-CD69 monoclonal antibody can induce activation and proliferation of T cells, thereby promoting T-cell effector functions (22). We postulated that dendritic cell-based vaccine and anti-CD69 antibody may act as therapeutically synergistic partners against renal cell carcinoma. This hypothesis is based on the following reasons. Dendritic cell-based vaccine presents tumor antigen to T cells and stimulates T cell activation. CD69 expression on T cells is upregulated after T cell activation. Subsequent addition of anti-CD69 antibody further induces T-cell activation and proliferation via cross-linking of CD69 on T cells, thereby increasing anticancer efficacy. To test this hypothesis, we evaluated the combined efficacy of dendritic cell-based vaccine with anti-CD69 antibody in the treatment of murine renal cell carcinoma.

2. Materials and methods

2.1. Mice

Balb/c mice were purchased from Shanghai Laboratory Animal Center (Shanghai City, China) and housed under specific pathogen-free conditions. They had free access to standard rodent chow and water. Mice were used for experiments at the age of 8–10 weeks. The Animal Use and Care Committee at our college approved the protocol for all animal experiments, which conformed to the Principles of Laboratory Animal Care published by the US National Institutes of Health.

2.2. Generation of dendritic cell-based vaccine

Dendritic cell-based vaccine was generated from bone marrow progenitors as described by Lim et al (23). In brief, erythrocyte-depleted bone marrow cells of Balb/c mice were cultured in RPMI-1640 medium supplemented with 2 mmol/L fresh L-glutamine, 10% heated-inactivated fetal bovine serum (FBS), 100 µg/mL streptomycin, 100 units/mL penicillin (all from Life Technologies, Grand Island, NY, USA), 10 ng/mL recombinant mouse interleukin-4 (rm IL-4)(R&D Systems, Minneapolis, MN, USA), and 10 ng/mL recombinant mouse granulocyte-macrophage colony-stimulating factor (rm GM-CSF) (R&D Systems). On day 2, nonadherent cells were removed by washing. The adherent cells were cultured in fresh RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 10 ng/mL rm GM-CSF, and 10 ng/mL rm IL-4. The cultures
were fed every other day by removing half of the medium and replacing it with fresh RPMI-1640 medium containing 10% heat-inactivated FBS, 10 ng/mL rm GM-CSF, and 10 ng/mL rm IL-4. On day 7, suspended and loosely attached cells (i.e. immature dendritic cells) were collected. The murine renal cell carcinoma (Renca) cell line, syngeneic to Balb/c mice, was purchased from the American Type Culture Collection (Manassas, VA, USA). For preparation of tumor lysate, Renca tumor cells were suspended in phosphate-buffered saline and subjected to four successive cycles of rapid freezing in liquid nitrogen and thawing in a 37 °C water bath. In order to induce dendritic cell maturation, immature dendritic cells were incubated with Renca tumor cell lysate at a tumor cell equivalent to dendritic cell ratio of 3:1 in RPMI-1640 medium. After 8 h of culture, dendritic cell further maturation could be achieved by adding 10 ng/mL recombinant mouse tumor necrosis factor-α (R&D Systems) and 10 ng/mL recombinant mouse interferon-γ (R&D Systems). After culture for 16 h, Renca tumor lysate-pulsed dendritic cells (i.e. mature dendritic cells) were harvested and used as dendritic cell-based vaccine in our study. Dendritic cells were verified by phenotypic analysis.

2.3. Immunophenotypic analysis of dendritic cells by flow cytometry
Immature and mature dendritic cells were collected, immunostained with fluorescein isothiocyanate-conjugated anti-CD11c (dendritic cell marker) monoclonal antibody (BD PharMingen, San Diego, CA, USA), and phycoerythrin-conjugated anti-CD83 (maturation marker of dendritic cells) monoclonal antibody (BD PharMingen), and then analyzed by flow cytometry for expressions of CD11c and CD83 on the cell surface.

2.4. Analysis of CD69 expression on T cells
Balb/c mice either were left untreated or were immunized subcutaneously with Renca tumor lysate-pulsed dendritic cells (1 × 10⁶). Some of the mice were killed at 24-h intervals and their spleens were excised. Cell suspensions were prepared from the spleens. T cells were purified by the Magnetic Activated Cell Sorting device (Miltenyi Biotec, Auburn, CA, USA), using the T-cell isolation kit according to the manufacturer’s protocol (Miltenyi Biotec). Purified T cells were immunostained with fluorescein isothiocyanate-conjugated anti-CD3 (T-cell marker) monoclonal antibody (BD PharMingen) and phycoerythrin-conjugated anti-CD69 monoclonal antibody (BD PharMingen), and then analyzed by flow cytometry for CD69 expression.

2.5. Renal cell carcinoma model and therapeutic protocol
On day 0, Balb/c mice were challenged subcutaneously with 5 × 10⁵ Renca viable tumor cells in the right flank. On day 3, tumor-bearing mice were divided into 4 groups with 10 mice in each group: control, dendritic cell-treated, antibody-treated, and combination-treated groups. Mice in the control group received no treatment. The dendritic cell-treated group underwent subcutaneous injection of Renca tumor lysate-pulsed dendritic cells (1 × 10⁶) in the left flank on days 3 and 10. In the antibody-treated group, anti-CD69 monoclonal antibody (100 μg; R&D Systems) was injected intraperitoneally on days 3 and 10. Mice in the combination therapy group received both subcutaneous administration of Renca tumor lysate-pulsed dendritic cells (1 × 10⁶) on day 3 and intraperitoneal injection of anti-CD69 monoclonal antibody (100 μg) on day 7. The tumors were measured twice a week using mechanical calipers in two dimensions. Tumor volume was calculated by the formula A × B²/2, where A is the longest diameter and B is the next longest diameter perpendicular to A. The mice were sacrificed on day 28.

2.6. Determination of T cell proliferation
Spleens were collected from control (untreated) or variously treated mice 28 days after tumor implantation. T cells were purified from splenocytes by the use of a T cell isolation kit (Miltenyi Biotec). Purified T cells were seeded into 96-well round-bottom plates (1 × 10⁵ per well) together with 1 × 10⁴ Renca tumor lysate-pulsed dendritic cells. After incubation for 4 days, cultures were pulsed for 16 h with 1 μCi [³H]thymidine (PerkinElmer, Waltham, MA, USA). The cells were harvested on glass microfiber filters with a cell harvester. T cell proliferative response was determined by measuring [³H]thymidine incorporation using a liquid scintillation counter. The results were expressed as counts per minute.

2.7. Cytotoxicity assessment
Cell-mediated cytotoxicity was determined using a standard 4-h ⁵¹Cr release assay. In brief, Renca tumor cells were used as target cells and incubated with Na₂⁵¹CrO₄ (PerkinElmer) for 1 h at 37 °C. Control (untreated) or variously treated mice were killed 28 days after tumor challenge and their spleens were harvested. CD8+ T cells were purified from splenocytes by the use of a CD8+ T cell isolation kit (Miltenyi Biotec) and were co-cultured with Renca tumor lysate-pulsed dendritic cells at a ratio of 10:1. After 5 days, the activated CD8+ T cells (effector cells) were collected, washed twice, and co-incubated in round-bottom 96-well plates for 4 h with ⁵¹Cr-labeled Renca tumor cells (target cells) at a 100:1 (effector:target cell ratio). The ⁵¹Cr release was measured in culture supernatants with a gamma counter. The percentage of target cell-specific lysis was calculated as follows: [(experimental ⁵¹Cr release – spontaneous ⁵¹Cr release)/(maximal ⁵¹Cr release – spontaneous ⁵¹Cr release)] × 100. The amount of maximal ⁵¹Cr release was determined by incubating target cells in 1% Triton X-100 (Sigma-Aldrich, St. Louis, MI, USA). The amount of spontaneous ⁵¹Cr release was assessed by incubation of target cells in medium alone.
2.8. Detection of interleukin-2 by enzyme-linked immunosorbent assay
Twenty-eight days after tumor challenge, spleens were collected from control (untreated) or variously treated mice. CD4+ T cells were purified from splenocytes by the use of a CD4+ T cell isolation kit (Miltenyi Biotec), and were stimulated in vitro with Renca tumor lysate-pulsed dendritic cells at an effector/stimulator ratio of 10:1 for 24 h at 37 °C. Cell-free supernatants were harvested and analyzed for secreted amount of interleukin-2 using an enzyme-linked immunosorbent assay kit (BD PharMingen).

2.9. Statistical analysis
All data were presented as the mean ± standard deviation. One-way analysis of variance with Student–Newman–Keuls test was used for the data comparisons among the groups. Statistical significance was defined as P < 0.05. The data were analyzed by GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA).

3. Results
3.1. Surface phenotype of dendritic cell
As shown in Figure 1, cultured cells expressed high levels of CD11c, which is a typical dendritic cell marker. These results provide evidence that cultured cells are dendritic cells. To determine the maturation status of dendritic cells, we explored the expression of the maturation marker CD83. Unpulsed dendritic cells expressed low levels of CD83. These results indicate that unpulsed dendritic cells are immature dendritic cells. In contrast, Renca tumor lysate-pulsed dendritic cells showed high expression of CD83, suggesting that these cells are mature dendritic cells.

3.2. Renca tumor lysate-pulsed dendritic cell immunization modulates CD69 expression on T cells
CD69 expression on T cells was assessed in the spleen before and after immunization of mice with Renca tumor lysate-pulsed dendritic cells. As depicted in Figure 2, CD69 expression on T cells was negligible before immunization. However, Renca tumor lysate-pulsed dendritic cell immunization induced upregulation of CD69 expression on T cells.

3.3. Renca tumor lysate-pulsed dendritic cells in combination with anti-CD69 antibody highly increases the therapeutic efficacy
Tumor size is presented in Figure 3. Tumor volume in Renca tumor lysate-pulsed dendritic cells or anti-CD69 antibody-only-treated mice was significantly reduced in comparison with the control (untreated) mice (P < 0.05). The combination treatment with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody caused a much greater reduction in tumor volume than either treatment alone (P < 0.05), showing a synergistic antitumor effect of the combination therapy.

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**Figure 1.** Dendritic cell characterization. Dendritic cells were cultured as described in the Materials and methods section. Immature and mature dendritic cells were collected, immunostained with fluorescein isothiocyanate-conjugated anti-CD11c (dendritic cell marker) monoclonal antibody and phycoerythrin-conjugated anti-CD83 (maturation marker of dendritic cells) monoclonal antibody, and then analyzed by flow cytometry. Expression rates of CD11c and CD83 in unpulsed dendritic cells (i.e. immature dendritic cells) and Renca tumor lysate-pulsed dendritic cells (i.e. mature dendritic cells) were 84.7% (i.e. 3.1% + 81.6%) and 3.1%, and 85.3% (i.e. 68.4% + 16.9%) and 68.4%, respectively.
Figure 2. CD69 expression is upregulated on T cells induced by Renca tumor lysate-pulsed dendritic cells. Balb/c mice either were left untreated or were immunized subcutaneously with Renca tumor lysate-pulsed dendritic cells. Some of mice were sacrificed at 24-h intervals and their spleens were collected. T cells were purified from splenocytes and double immunostained with fluorescein isothiocyanate-conjugated anti-CD3 (T-cell marker) monoclonal antibody and phycoerythrin-conjugated anti-CD69 monoclonal antibody. CD69 expression on T cells was analyzed by flow cytometry. (A) CD69 expression on T cells 4 days after immunization or no treatment. Approximately 2.1% of T cells expressed CD69 in untreated mice. However, 50.8% of T cells expressed CD69 4 days after immunization of mice with Renca tumor lysate-pulsed dendritic cells. These results were representative of three independent experiments. (B) Dynamic change in CD69 expression on T cells. The peak level of CD69 expression on T cells was observed 4 days after immunization of mice with Renca tumor lysate-pulsed dendritic cells.
3.4. Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody combined treatment improves T-cell proliferation

Renca tumor lysate-pulsed dendritic cells or anti-CD69 antibody treatment significantly increased T-cell proliferation compared with the control (untreated) group (P < 0.05) (Figure 4). However, the combination therapy with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody led to the greatest proliferation of T cells (P < 0.05) (Figure 4).

3.5. Anti-CD69 antibody potentiates cytotoxic T-lymphocyte activity induced by Renca tumor lysate-pulsed dendritic cells

As depicted in Figure 5, therapy with Renca tumor lysate-pulsed dendritic cells or anti-CD69 antibody significantly increased CD8+ T-cell activity (P < 0.05), with the highest increase in the combination treatment (P < 0.05).

3.6. Co-treatment with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody results in enhanced production of interleukin-2

As shown in Figure 6, mice treated with Renca tumor lysate-pulsed dendritic cells or anti-CD69 antibody alone showed a significant increase in interleukin-2 level in comparison with the control (untreated) mice (P < 0.05). However, co-treatment with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody resulted in higher interleukin-2 level than any other groups (P < 0.05).

4. Discussion

Renal cell carcinoma cells are known to secrete both interleukin-10 and transforming growth factor-β (24). These cytokines inhibit the differentiation and maturation of dendritic cells in vivo, as well as their function to present tumor antigen, and to stimulate T lymphocytes, thereby reducing antitumor immunity (24). Therefore, the approach to culture dendritic cells in vitro has been developed recently. Immature dendritic cells can be obtained in vitro by culturing bone marrow cells in the presence of interleukin-4 and granulocyte-macrophage colony-stimulating factor (23). Further maturation can be achieved by adding tumor antigen and cytokines such as interferon-γ, tumor necrosis factor-α, and interleukin-1β (23). Our data showed that cultured cells highly expressed CD11c (dendritic cell marker) (Figure 1), suggesting that...
Figure 4. T-cell proliferation in mice treated with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody. Renca tumor-bearing mice (10 per group) either were left untreated or were treated with Renca tumor lysate-pulsed dendritic cells, anti-CD69 antibody, or combination of Renca tumor lysate-pulsed dendritic cells with anti-CD69 antibody. T cells were purified from splenocytes of control (untreated) or variously treated mice and were cocultured with Renca tumor lysate-pulsed dendritic cells in 96-well plates. After 4 days, 1 µCi [3H]thymidine was added to each well for an additional 16 h. T-cell proliferative response was determined by measuring [3H]thymidine incorporation. Results were expressed as counts per minute.*: Value significantly different from control group (P < 0.05). #: Value significantly different from other groups (P < 0.05).

Figure 5. Effect of Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody on cytotoxic T-lymphocyte activity. CD8+ T cells purified from splenocytes of Renca tumor-bearing mice (10 per group), which either were left untreated or were treated with Renca tumor lysate-pulsed dendritic cells, anti-CD69 antibody, or Renca tumor lysate-pulsed dendritic cells plus anti-CD69 antibody, were seeded into 96-well plates together with Renca tumor lysate-pulsed dendritic cells. After 5 days, the activated CD8+ T cells (effector cells) were collected and coincubated with 51Cr-labeled Renca tumor cells (target cells) at effector/target cell ratio of 100:1 for 4 h. Cytotoxic T-lymphocyte activity against Renca tumor cells was determined by the 51Cr release assay. Results are shown as the percentage of target cell lysis.*: Value significantly different from control group (P < 0.05). #: Value significantly different from other groups (P < 0.05).
cultured cells are dendritic cells. The maturation status of dendritic cells in tumor immunotherapy affects not only immune response but also clinical outcome. Several pilot clinical trials indicate that vaccination of mature dendritic cells is associated with stronger immune response and better clinical outcome than vaccination with immature dendritic cells (25). These findings provide a scientific rationale for the use of mature dendritic cells (i.e. Renca tumor lysate-pulsed dendritic cells) in the present study.

Dendritic cell-based vaccine is an important immunotherapy for patients with metastatic renal cell carcinoma. Many clinical trials have proven it to be effective, but only low clinical efficacy has been observed to date (17). Based on the reasons as described in the Introduction section, we hypothesized that combination of dendritic cell-based vaccine and anti-CD69 antibody may enhance clinical efficacy. In the present study, we found that CD69 expression on T cells reached a peak level on day 4 after immunization of mice with Renca tumor lysate-pulsed dendritic cells and then gradually declined (Figure 2B). Therefore, mice in the combined therapy group received an intraperitoneal injection of anti-CD69 antibody 4 days after immunization with Renca tumor lysate-pulsed dendritic cells. The combination treatment resulted in a significant decrease in tumor volume in comparison with either treatment alone (Figure 3). These results confirm our hypothesis that combination therapy can achieve a synergistic effect and increase therapeutic efficacy.

The primary objective of immune monitoring after dendritic cell-based vaccination is to find the association between vaccine-induced immune response and clinical outcome. Numerous assays for analysis of immune response are available. Frequently used immunoassays include enzyme-linked immunosorbent assay for detection of interleukin-2, cytotoxicity test, T-cell proliferation assay, delayed-type hypersensitivity skin test, and so on. In the current study, the combination treatment with Renca tumor lysate-pulsed dendritic cells and anti-CD69 antibody induced a much higher T-cell proliferation than either monotherapy (Figure 4), implying that combined therapy increases T-cell proliferation. In addition,
significantly stronger cytotoxic T-lymphocyte activity was observed in mice that underwent combination therapy than those treated with either agent alone (Figure 5). These data demonstrate that combination treatment enhances CD8+ T-cell activity to recognize and destroy tumor cells. Mice immunized with Renca tumor lysate-pulsed dendritic cells plus anti-CD69 antibody showed a higher interleukin-2 level in comparison with mice immunized with either agent singly (Figure 6). These results suggest that combined treatment improves CD4+ T-cell activity to release cytokine interleukin-2. It has been shown that interleukin-2 production can increase CD8+ T-cell activity (16). Taken together, these findings indicate that combined treatment induces enhanced T-cell proliferation and activity. T cells have the ability to recognize and kill tumor cells (15,16). Moreover, combination therapy significantly inhibits tumor growth in comparison with either treatment alone, and thus potentiates antitumor efficacy as discussed above. When all these findings are taken together, we think that the augmented antitumor efficacy conferred by combination therapy may be associated with increased T-cell proliferation and activity.

Many studies have demonstrated the effectiveness of dendritic cells pulsed with tumor antigens for cancer immunotherapy (17). Tumor antigens have various forms, such as tumor lysate, apoptotic tumor bodies, tumor-derived exosomes, tumor RNA or DNA, or peptide antigens (17). The choice of antigenic material is one of the most crucial aspects of designing an effective dendritic cell-based immunotherapeutic protocol. In our study, tumor lysate was selected as antigenic material. The selection of tumor lysate as antigenic material has two important advantages. Tumor lysate generally provides the whole set of tumor antigens. Consequently, it can induce a full immune response against multiple targets on the tumor cells, which diminishes the likelihood for tumor escape from immunological surveillance. Moreover, the selection of tumor lysate as antigenic material eliminates the requirement to identify and characterize the tumor antigens. For some tumors in which there is a lack of well-characterized tumor antigens, the use of tumor lysate may be the most practical method.

In conclusion, we have demonstrated for the first time that anti-CD69 antibody can potentiate antitumor efficacy of dendritic cell-based vaccine in renal cell carcinoma-bearing mice. The augmented therapeutic efficacy conferred by the combined therapy with dendritic cell-based vaccine and anti-CD69 antibody may be associated with increased T-cell proliferation and activity. The combined therapy may represent a new immunotherapeutic approach for patients with renal cell carcinoma. This new approach should be further evaluated in clinical trials.

Acknowledgments
This research was supported by grants from the Medical Science and Technology Planning Project of Zhejiang Province (Grant No. 2015KYB099) and the National Natural Science Youth Foundation of China (Grant No. 81201408).

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