Biochemical and Biophysical Research Communications 402 (2010) 88–93

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The inhibition of the T-cell immunoglobulin and mucin domain 3 (Tim3) pathway enhances the efficacy of tumor vaccine

Mi Jin Lee, Min-Yeong Woo, Yoo Mi Heo, Jung Sik Kim, Myung-Hee Kwon, Kyongmin Kim, Sun Park *

Department of Microbiology, Ajou University School of Medicine, Youngtongku Wonchondong San 5, Suwon 442-749, Republic of Korea

ARTICLE INFO

Article history:
Received 21 September 2010
Available online 25 October 2010

Keywords:
Tim3
Tim3-inhibition
Tregs
Tumor vaccine
Immunotherapy

ABSTRACT

T-cell immunoglobulin and mucin domain 3 (Tim3) plays an important role in the Th1-mediated immune response; however, its effect on the efficacy of tumor vaccines has not been fully evaluated. Here, we demonstrate the effect of Tim3 pathway inhibition on tumor growth in mice. Lewis lung carcinoma (3LL) cells expressing a Tim3 pathway inhibitor, when injected into mice, showed suppressed tumor growth and a reduced frequency of $CD4^+CD25^+Foxp3^+$ T-cells. Furthermore, Tim3 pathway inhibition significantly enhanced the efficacy of a prophylactic tumor vaccine and marginally enhanced the efficacy of a therapeutic tumor vaccine. However, when given in combination with the chemotherapeutic agent, 5-fluorouracil, the therapeutic tumor vaccine capable of Tim3 pathway inhibition had no additional anti-tumor effect. Our results show that Tim3 pathway inhibition can enhance tumor vaccine efficacy.

1. Introduction

Cancer immunotherapy relies on promoting the patient's immune response against the malignant tumor. Immune adjuvants such as BCG and imiquimod have been approved for the treatment of superficial bladder cancer and basal cell carcinoma, respectively [1]. Prophylactic cancer vaccines against oncogenic viruses have also proved to be effective. However, because of their tendency to promote tumor tolerance, therapeutic cancer vaccines such as antigen-specific vaccines and cytokine-based whole tumor-cell vaccines remain in development [1]. Tumor tolerance is established through immunoregulatory molecules, including CTLA-4, and cells, including $CD4^+CD25^+Foxp3^+$ regulatory T-cells (Tregs), both of which have served as immunotherapeutic targets [2–6]. For example, treatment with anti-CTLA-4 monoclonal antibody is associated with augmented anti-tumor immunity in a subset of patients previously vaccinated with autologous tumor cells [2,3].

Tim3 is also regarded as a potential target molecule for immunotherapy. Activation of Tim3 inhibits the activity of Th1 cells and production of IFN-γ, both of which play important roles in anti-tumor immunity [7,8]. Tim3, a transmembrane protein that contains an immunoglobulin- and a mucin-like domain, is expressed on many cells including Th1 cells, CD8 T-cells, monocytes, and dendritic cells [9–12]. Interaction of Tim3 with its ligands, phosphatidylserine and galectin-9, modulates the function of these cells [13,14]. Tim3 expressed by monocytes and dendritic cells facilitates phagocytosis of apoptotic cells and up-regulates cross-presentation of apoptotic cell-associated antigens through interaction with phosphatidylserine [12,14]. Galectin-9 induces the death of Th1 cells, which results in the suppression of both Th1 autoimmunity and the generation of Th17 cells [13,15]. Stimulation of Tim3 with an agonistic antibody promotes inflammation through the activation of innate immune cells [16]. However, the role of Tim3 in tumor biology has been the subject of a small number of studies. To evaluate Tim3 as a target for tumor immunotherapy, we examined the tumor-suppressive efficacy of cells expressing a Tim3 pathway inhibitor, a protein consisting of the Tim3 extracellular domain fused to the human IgG1 Fc region (Tim3-hIg). We found that the administration of cells expressing Tim3-hIg before, during, or after tumor development in mice suppressed tumor growth but did not enhance the tumor-suppressive effect of the chemotherapeutic agent 5-fluorouracil (5-FU).

2. Materials and methods

2.1. Plasmids and cell clones

The Tim3-hIg expression vector pIRES-EGFP-Tim3-hIg was constructed as previously described [17]. Lewis Lung Carcinoma cell line (3LL) was cultured in DMEM with 10% fetal bovine serum, 100 μg/mL streptomycin sulfate, and 100 IU/mL penicillin sulfate (Gibco–BRL, Invitrogen, Carlsbad, CA, USA), and transfected with pIRES-EGFP-Tim3-hIg, pIRES-EGFP-hIg, or pIRES-EGFP using Lipofectamine reagent (Invitrogen). Transformed cells were selected in medium containing 2 mg/mL neomycin, sorted twice for the expression of green fluorescence protein (GFP) using a FACS Vantage (Becton Dickinson, San Jose, CA, USA), and then cloned

* Corresponding author. Fax: +82 31 219 5079.
E-mail address: sinsun@ajou.ac.kr (S. Park).

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doi:10.1016/j.bbrc.2010.09.121

twice by limiting dilution. Clones secreting Tim3-hIg were named T1 and T2; control clones secreting human IgG1 Fc region were named H1, H2, and H3; and clones containing the empty vector pIRES-EGFP were named G1 and G2. All clones express GFP.

2.2. Reverse transcription (RT)-PCR

RNA was isolated from stable cell clones using RNAzol Bee (Tel-Test Inc., Friendswood, TX, USA). cDNA was synthesized from total RNA using RNase H^- reverse transcriptase (Invitrogen) and oligo$(dT)_{12-18}$ (Invitrogen). Amplification of cDNA for Tim3-hIg was performed using primers TIM-3F, 5′-GCT AGC ATG TTT TCA GGT CTT ACC CTC AAC TGT G-3′, and HIgCR, 5′-CGG GAT CCT CAT TTA CCC TGC GAC AG-3′. Amplification of cDNA for hIg was performed using primers HIgCF, 5′-GAA GAT CTG CAC CTG AAC TCC TGG GG-3′, and HIgCR.

2.3. MTT assay

Cell growth was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. Cells (5×10^2) were seeded in 96-well culture plates and incubated for the indicated days. Then, MTT solution (5 mg/mL in PBS) (Sigma–Aldrich) was added to each well and, after 3 h of incubation, the medium was replaced with DMSO. The optical density was measured at 540 nm. Cell numbers were calculated using a standard curve, which was devised using cells with various known cell numbers.

2.4. Evaluation of tumor growth

C57BL/6 mice (8-week old males) were injected subcutaneously with 3×10^5 3LL-derived tumor cells on the right flank. Tumor growth was monitored every other day by measuring the length (a) and width (b) of the tumor with a Vernier caliper. Tumor volume was calculated using the formula: $(a \times b^2) \times 0.523$.

2.5. Tumor vaccination

For tumor vaccination with transiently transfected cells, 3LL cells were transfected with plasmid 36 h prior to injection, treated with mitomycin (50 μg/mL) for 1 h, washed three times, and then injected subcutaneously on the back of the mouse. Mice then were challenged subcutaneously with G1 cells on the right flank. For 5-FU treatment, mice were injected peritoneally with 70 mg/kg 5-FU (Choongwae Pharm. Co., Seoul, Korea) on day 9 and day 11 after injection of G1. Animal care and experimental procedures were carried out under approval from the Animal Care Committee of Ajou University School of Medicine (AMC35).

2.6. Flow cytometry

Lymphocytes were isolated from tumor-draining, right axillary lymph nodes (dLNs) and from non-draining, left internal jugular lymph nodes (ndLNs). Cells were stained with FITC-conjugated anti-FoxP3 antibody (Ab) (eBioscience, San Diego, CA, USA), PE-conjugated anti-CD25 Ab (BD Pharmingen), and APC-conjugated anti-CD4 Ab (BD Pharmingen). Stained cells were analyzed on a FACS Calibur flow cytometer (Becton–Dickinson).

2.7. Immunoprecipitation and Western blotting (IP–Western blotting)

Anti-human IgG Ab and protein G agarose were used to immunoprecipitate Tim3-hIg from the culture supernatant of cells transiently transfected with pIRES-EGFP-Tim3-hIg. Immunoprecipitates were washed, applied to SDS–PAGE, and then subjected to Western blotting with horseradish peroxidase-conjugated anti-human IgG Ab and ECL Plus Western blot detection system (Pierce, Rockford, IL, USA).

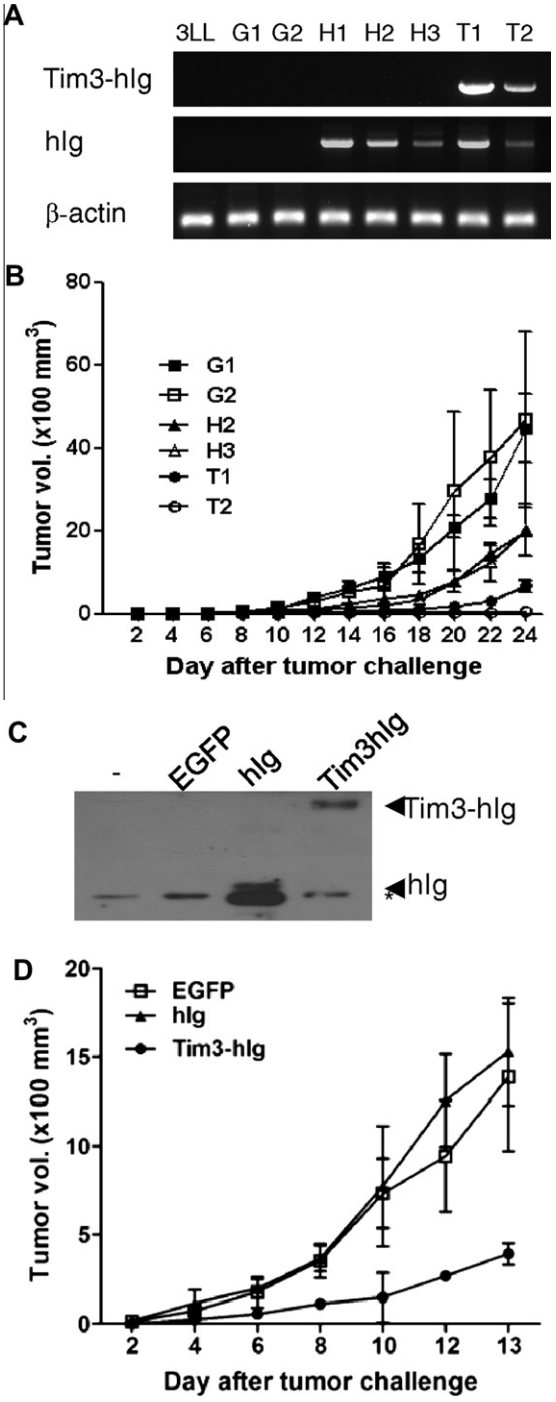

Fig. 1. The effect of Tim3-hIg expression by tumor cells on tumor development. (A) RT-PCR for Tim3-hIg and hIg expression in stable clones. Specific primers for Tim3-hIg and hIg, respectively, were used. (B) Tumor growth of stable clones. Cells were injected subcutaneously into C57BL/6 mice, and tumor volume was measured. (C) IP-Western blot for Tim3-hIg expression. –, untransfected 3LL cells; EGFP, 3LL cells transiently transfected with pIRES-EGFP; hIg, 3LL cells transiently transfected with pIRES-EGFP-hIg; Tim3-hIg, 3LL cells transiently transfected with pIRES-EGFP-Tim3-hIg; *, light chain of Ab specific to human IgG. (D) Tumor growth of cells transiently transfected with pIRES-EGFP-Tim3-hIg. Mice were injected subcutaneously with 3LL cells that had been transiently transfected with expression vector and cultured for 36 h before injection. (B,D) More than 5 mice per group were used. Representative data from two independent experiments are shown.

3. Results

3.1. Tim3-hIg expression in tumor cells suppresses tumor growth in mice

To examine the effect of Tim3 pathway inhibition on tumor growth, we established a selection of 3LL-derived stable, test and control clones. We verified these clones by flow cytometric analysis for GFP (data not shown) and by RT-PCR for Tim3-hIg and hIg mRNA expression (Fig. 1A). We then injected these cell clones into mice subcutaneously and analyzed tumor growth every other day for 3 weeks (Fig. 1B). The tumor growth rate was highest in those injected with G1 or G2 and lowest in mice injected with T1 or T2. However, *in vitro,* the growth rate of H2 and H3 was higher than that of G1, G2, T1, or T2 (Supplementary Fig.). The differences in growth rate of the clones *in vitro* and *in vivo* could possibly be due to the expression of foreign antigen, GFP and hIg; which could serve as tumor Ags, however, tumor growth was not correlated with GFP or hIg expression level. These results suggest, therefore, that the reduced tumor growth rate of clones T1 and T2 could be due to the expression of Tim3 pathway inhibitor, Tim3-hIg. To investigate further, we examined tumor growth in mice injected with cells transiently transfected with the Tim3-hIg expression vector. The transfection efficiency of the Tim3-hIg expression vector and control vectors was similar (36.6% for EGFP, 57.2% for hIg, and 39.7% for Tim3-hIg) (data not shown). We first confirmed, by IP-Western blotting (Fig. 1C), that Tim3-hIg was being expressed appropriately and then observed tumor growth in mice injected with these cells. Tumor growth was significantly slower in mice injected with cells transiently expressing Tim3-hIg compared to controls (transiently expressing hIg or EGFP) (Fig. 1D). Concordantly, the survival rate was significantly higher in mice injected with cells transiently expressing Tim3-hIg rather than control cells (data not shown). These results imply that Tim3 pathway inhibition, even if it is transient in the early stages of tumor development, may suppress tumor growth.

3.2. Tim3-hIg expression enhances the efficacy of prophylactic tumor vaccine

Our observation of tumor suppression by Tim3-hIg prompted us to investigate the effect of Tim3-hIg on anti-tumor immunity. We reasoned that if Tim3-hIg enhanced tumor immunity, prophylactic tumor vaccination with cells expressing Tim3-hIg would suppress growth when mice were subsequently challenged with tumor cells. We therefore immunized mice with 3LL cells expressing Tim3-hIg or hIg, the proliferative capacity of which had been eliminated by mitomycin-treatment. Ten days later, mice were challenged with

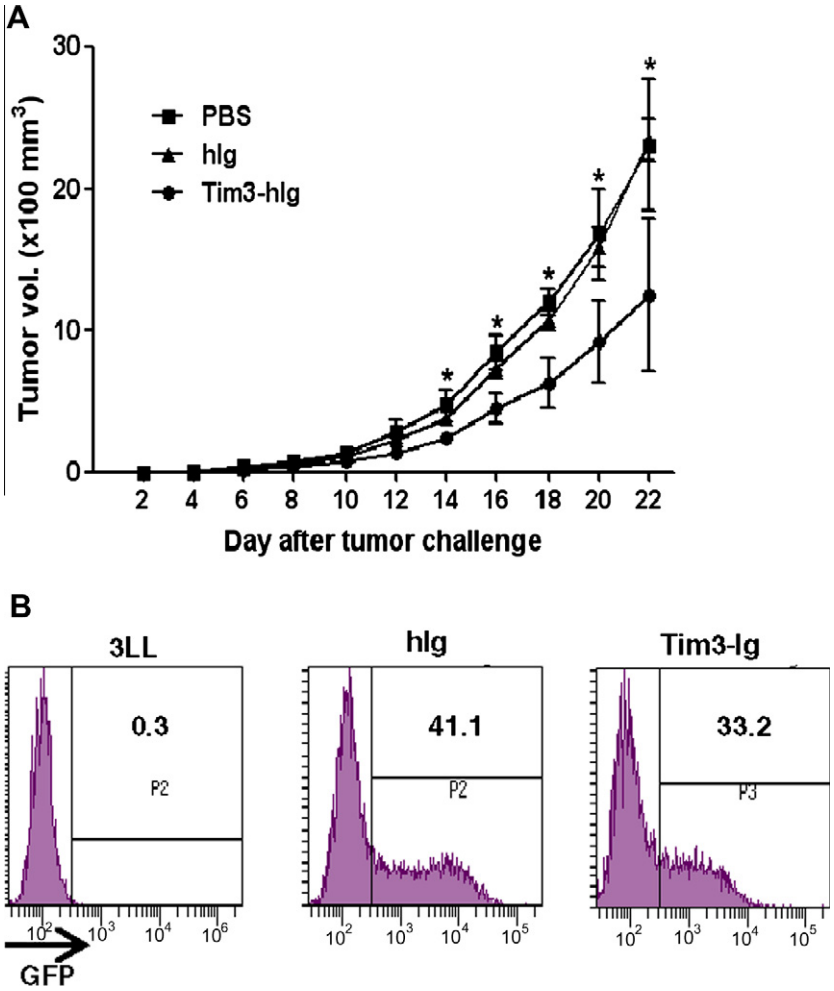

Fig. 2. The tumor-suppressive effect of prophylactic vaccine with whole tumor cells expressing Tim3-hIg. (A) The mice were vaccinated subcutaneously with 3LL cells that had been transiently transfected with pIRES-EGFP-hIg (hIg) or pIRES-EGFP-Tim3-hIg (Tim3-hIg) and treated with mitomycin to prevent proliferation. Mice injected with PBS were used as a control. Ten days later, the mice were challenged with G1 cells and tumor growth was measured. Five mice per group were used. Representative data from two independent experiments are shown. Data represent means ± SD. $^{*}p < 0.005$ between hIg and Tim3-hIg. (B) Transfection efficiencies of cells used for vaccination determined by detection of GFP using flow cytometry.

G1 cells. Tumor growth was similar among mice that were unvaccinated and those that had been vaccinated with hIg-expressing cells. However, tumor growth was significantly suppressed in mice vaccinated with Tim3-hIg-expressing cells compared to that in mice vaccinated with hIg-expressing cells ($p < 0.005$, Fig. 2). These results imply that Tim3-hIg may enhance the efficacy of prophylactic tumor vaccine.

3.3. The frequencies of $CD4^+CD25^+Foxp3^+$ regulatory T-cells in mice given Tim3-hIg expressing tumor cells

As tumors develop, the frequency of $CD4^+CD25^+Foxp3^+$ regulatory T-cells (Tregs) increases, and these cells can suppress the anti-tumor immune response [18,19]. Furthermore, Tim3 pathway inhibition suppresses the function of Tregs in auto- and alloimmune responses, as well as in transplantation models [9,20]. However, the relevance of the relationship between Tim3 pathway inhibition and Tregs in tumor models has not been well studied. We wanted to know, therefore, whether Tim3-hIg expression affected the frequency of Tregs. Using 3-color flow cytometry, we analyzed the frequencies of Tregs in mice at 21 days after the injection of tumor cells stably expressing Tim3-hIg (Fig. 3A). The frequencies of Tregs in non-draining LNs were similar among all mice; however, the frequencies of Tregs in draining LNs were significantly decreased in mice with T1 or T2 Tim3-hIg expressing tumors, compared to mice given G1 or G2 clones. To determine whether the decreased frequency of Tregs was the result of direct modulation by Tim3-hIg or a reflection of the reduced tumor mass, we analyzed the frequency of Tregs in LNs from mice vaccinated with tumor cells expressing either Tim3-hIg or hIg, and then challenged with G1 tumor cells (Fig. 3B). Ten days after challenge, the tumor volume was similar in all mice and the frequency of Tregs was slightly, but significantly, increased in mice with tumor cells compared to the tumor-free control mice ($p < 0.05$). However, the frequency of Tregs in tumor-bearing mice was similar in all groups regardless of vaccination. These results suggest that Tim3-hIg may reduce the frequency of Tregs indirectly through the inhibition of tumor growth.

3.4. Tim3-hIg expression marginally enhances the efficacy of the therapeutic tumor vaccine but not of the chemotherapy

We evaluated whether Tim3 pathway inhibition might enhance the efficacy of the therapeutic tumor vaccine (Fig. 4A). Mice with a tumor between 45 and 80 mm^3 on day 6 after tumor challenge were vaccinated on day 7 and on day 16 with mitomycin-treated cells expressing either Tim3-hIg or hIg. Although there was no significant difference between unvaccinated control mice and mice vaccinated with cells expressing hIg, tumor growth was significantly reduced in mice vaccinated with cells expressing Tim3-hIg compared to unvaccinated control mice ($p < 0.05$ on day 8, 14, 20, and 21 after tumor challenge). However, there was no significant difference between mice vaccinated with cells expressing hIg and with cells expressing Tim3-hIg.

Next, we examined whether a therapeutic tumor vaccine expressing Tim3-hIg might enhance the efficacy of chemotherapy (Fig. 4B). The tumor-bearing mice were administered with 5-FU on day 9 and day 11 after tumor challenge, and then with mitomycin-treated cells expressing Tim3-hIg on day 11 and on day 13. Tumor growth was markedly reduced in mice administered with 5-FU; however, there was no significant difference between mice administered with 5-FU alone and administered subsequently with cells expressing Tim3-hIg in addition to 5-FU. Using this protocol, there was a tendency for decreased tumor growth in mice vaccinated with cells expressing Tim3-hIg compared to control mice; however, the difference in growth was not statistically significant.

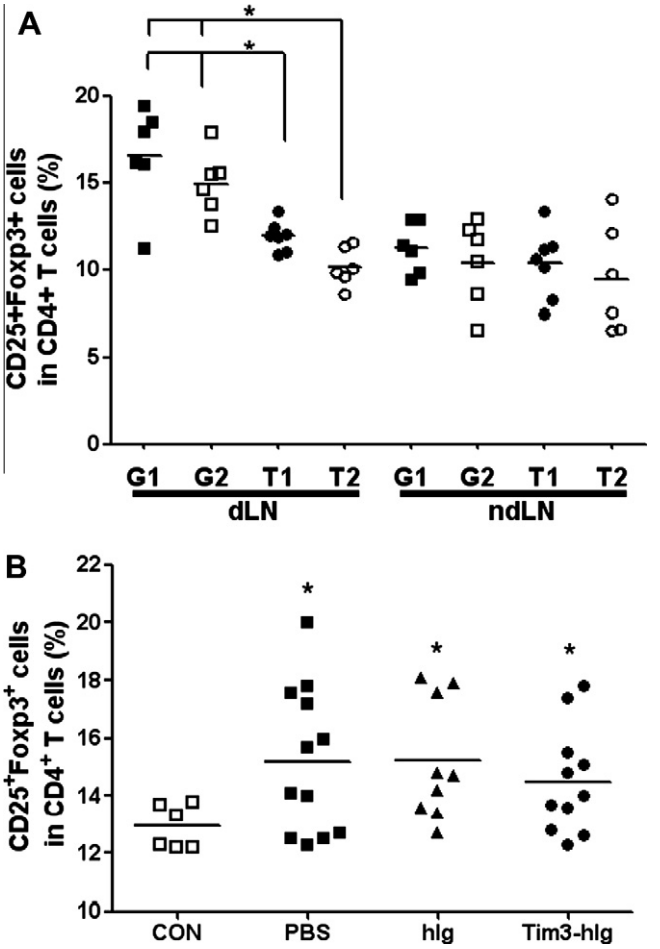

Fig. 3. The effect of Tim3-hIg expression by tumor cells on the frequency of $CD4^+CD25^+Foxp3^+$ Treg cells. (A) The frequency of Treg cells in dLN and ndLN of mice injected subcutaneously with G1, G2, T1, or T2, 23 days before isolation of lymph nodes. (B) The frequency of Treg cells was determined in dLN of mice vaccinated with cells expressing hIg or Tim3-hIg, and then challenged with G1 cells 10 days after the vaccination. Ten days after G1 cell challenge, dLN cells were labeled with APC-conjugated anti-CD4, PE-conjugated anti-CD25, and FITC-conjugated anti-Foxp3 and then analyzed by flow cytometry. CON, tumor-free, normal mice; PBS, tumor-bearing mice without tumor vaccine; hIg, tumor-bearing mice vaccinated with 3LL cells transiently expressing hIg; Tim3-hIg, tumor-bearing mice vaccinated with 3LL cells transiently expressing Tim3-hIg. Each symbol represents each mouse (6–12 mice per group). The bar represents the mean ($*p < 0.001$).

These results suggest that Tim3 pathway inhibition can enhance the efficacy of therapeutic tumor vaccine depending on the administration protocol.

4. Discussion

Recent findings regarding the role of Tim3 in tumor development suggest that Tim3 is a promising target for anti-tumor immunotherapy. The interaction of Tim3 with galectin-9 enhances anti-tumor immunity and prolongs the survival of tumor-bearing mice [21]. Nasopharyngeal tumors secrete exosomes containing galectin-9, which have been implicated in immune escape by the tumor through the induction of tumor-specific $Tim3^+$ T-cell death [22]. Furthermore, Tim3 expressed in lymphoma-associated endothelium leads to tumor progression through the inhibition of $CD4^+$ T-cell activation and Th1 polarization [23]. In addition, the interaction of Tim3 expressed by endothelial cells with melanoma cells facilitates metastasis through the activation of NF-kB in the melanoma cells [24]. In this study, we demonstrate the tumor-suppressive effect of Tim3 pathway inhibition by subcutaneous

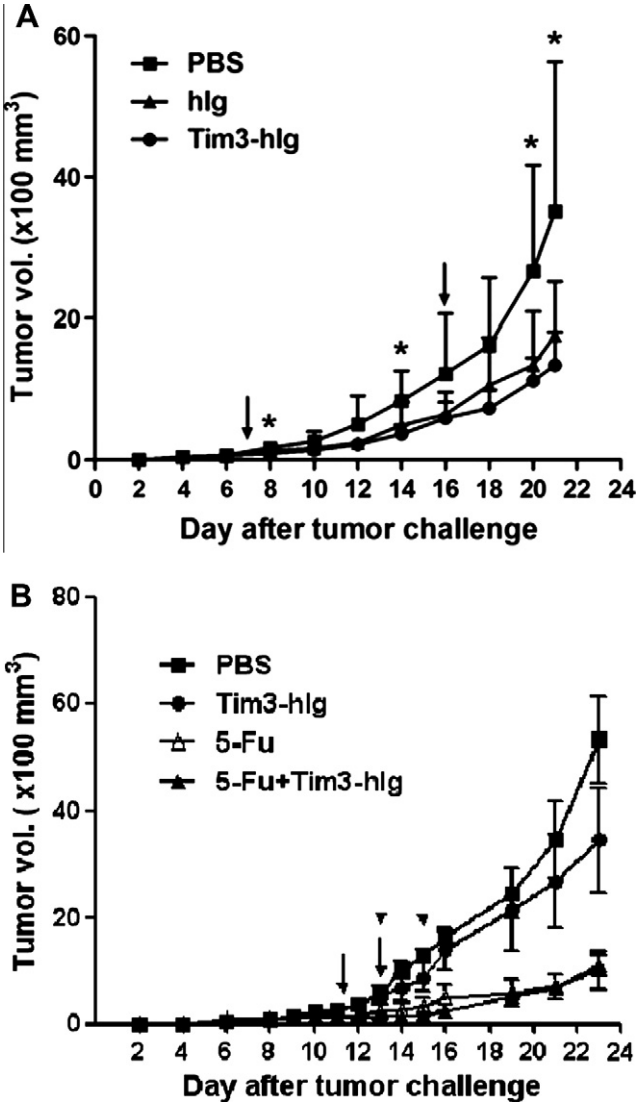

Fig. 4. The effect on tumor progression of therapeutic vaccine with whole tumor cells expressing Tim3-hIg. (A) Mice were injected subcutaneously with 3×10^5 G1 cells and then vaccinated on day 7 and day 16 with mitomycin-treated 3LL cells transiently transfected with pIRES-EGFP-hIg or pIRES-EGFP-Tim3-hIg. Mice injected with PBS were used as a control. *PBS vs Tim3-hIg $p < 0.05$. (B) Mice injected subcutaneously with G1 cells were given intraperitoneal 5-FU on day 9 and day 11 after G1 inoculation (5-Fu). One group of mice was subcutaneously vaccinated with mitomycin-treated 3LL cells (6×10^5 cells) transiently expressing Tim3-hIg on day 11 and day 13 after G1 inoculation (5-Fu+ Tim3-hIg). PBS, control group not treated with 5-FU nor vaccinated; Tim3-hIg, vaccinated without 5-FU treatment. (A,B) More than five mice per group were used. Representative data from two independent experiments are shown.

administration of tumor cells transiently expressing Tim3-hIg. The expression of Tim3-hIg by tumor cells delayed tumor growth in mice even when only around 30% of tumor cells were transiently expressing Tim3-hIg. ELISA analysis shows that this level of expression corresponds to a dose of approximately 35 ng of Tim3-hIg protein (data not shown). Therapeutic vaccination with tumor cells expressing Tim3-hIg also showed a transient inhibitory effect on tumor growth and, compared to the report of Dardalhon and colleagues [25], in which 100 μg of inhibitory anti-Tim3 Ab was given intraperitoneally, the quantity of Tim3-hIg administered in our study was decreased by 1000-fold. An increased amount and frequency of Tim3-hIg expressing tumor vaccine may further enhance the tumor-suppressive effect.

Tim3 modulates innate and adaptive immune cell function. It up-regulates cross-presentation of antigen by dendritic cells through increased phagocytosis of apoptotic cells, whereas it down-regulates Th1, CD8 T-, and NK-cell activation [9,12,14,25,26]. The Tim3 pathway also regulates the generation and function of immunosuppressive cells such as $CD11b^+Ly\text{-}6G^+$ myeloid cells (MDSC) and $CD4^+CD25^+Foxp3^+$ Tregs in a Tim3 transgenic mouse and an autoimmune murine model, respectively [9,27]. An elevated proportion of Treg cells in the total $CD4^+$ T-cell population have been detected in several different human cancers, including lymphoma, lung, and ovarian tumors [5,19,28]. We also observed an increased frequency of Tregs in tumor-draining LNs and a lower frequency of Tregs in mice bearing Tim3-hIg expressing tumors than in control mice; however, we could not conclude that Tim3-hIg expression directly regulated Treg generation since decreased frequency could be due to lower tumor burden rather than Tim3 pathway inhibition. Our findings show that Tim3-hIg expressing prophylactic tumor vaccine suppressed tumor growth through the inhibition of the Tim3 pathway, independent of the Treg cell frequency.

Although we could not dissect the underlying mechanisms of the tumor-suppressive effect of Tim3 pathway inhibition, two possibilities are conceivable. First, Tim3 pathway inhibition may promote anti-tumor immunity by suppressing the generation of MDSCs. The frequency of MDSCs is increased in various tumors, the depletion of which leads to anti-tumor immunity and tumor regression [29–32]. In a Tim3 transgenic mouse model and a galectin-9 (Tim3 ligand) transgenic mouse model, an increase in MDSCs and inhibition of immune responses are observed [27]. Interestingly, we found that Tim3-hIg expressing therapeutic tumor vaccine did not exhibit an additive inhibitory effect on tumor growth when given in combination with 5-FU, which has recently been reported to selectively kill MDSCs and thus enhance anti-tumor immunity [33]. Second, Tim3 pathway inhibition may enhance anti-tumor immunity by antagonizing phagocytosis of apoptotic cells and thereby circumventing the immunosuppressive effect of apoptosis. Tim3 binds phosphatidylserine on the apoptotic cell surface and facilitates phagocytosis of apoptotic cells. Tim3 pathway inhibition reduces phagocytosis of apoptotic cells and induces autoantibody production, indicating the break of peripheral tolerance [12]. In concordance with this, the efficacy of a therapeutic vaccine for melanoma is significantly augmented when the phagocytosis of apoptotic cells is inhibited by the binding of milk fat globule epidermal growth factor 8 to phosphatidylserine [34].

Conclusively, our results suggest that Tim3 pathway inhibition can enhance the efficacy of tumor vaccination and anti-tumor immunity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health Welfare, Republic of Korea (A080228).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.09.121.

References

[1] M. Dougan, G. Dranoff, Immune therapy for cancer, Annu. Rev. Immunol. 27 (2009) 83–117.
[2] F.S. Hodi, M. Butler, D.A. Oble, et al., Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients, Proc. Natl. Acad. Sci. USA 105 (2008) 3005–3010.

[3] F.S. Hodi, M.C. Mihm, R.J. Soiffer, et al., Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients, Proc. Natl. Acad. Sci. USA 100 (2003) 4712–4717.
[4] T.J. Curiel, Regulatory T cells and treatment of cancer, Curr. Opin. Immunol. 20 (2008) 241–246.
[5] Z.Z. Yang, A.J. Novak, M.J. Stenson, et al., Intratumoral $CD4^+CD25^+$ regulatory T-cell-mediated suppression of infiltrating $CD4^+$ T cells in B-cell non-Hodgkin lymphoma, Blood 107 (2006) 3639–3646.
[6] J. Dannull, Z. Su, D. Rizzieri, et al., Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells, J. Clin. Invest. 115 (2005) 3623–3633.
[7] J.B. Swann, M.J. Smyth, Immune surveillance of tumors, J. Clin. Invest. 117 (2007) 1137–1146.
[8] C.A. Sabatos, S. Chakravarti, E. Cha, et al., Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance, Nat. Immunol. 4 (2003) 1102–1110.
[9] A. Sanchez-Fueyo, J. Tian, D. Picarella, et al., Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance, Nat. Immunol. 4 (2003) 1093–1101.
[10] L. Monney, C.A. Sabatos, J.L. Gaglia, et al., Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease, Nature 415 (2002) 536–541.
[11] T. Oikawa, Y. Kamimura, H. Akiba, et al., Preferential involvement of Tim-3 in the regulation of hepatic $CD8^+$ T cells in murine acute graft-versus-host disease, J. Immunol. 177 (2006) 4281–4287.
[12] M. Nakayama, H. Akiba, K. Takeda, et al., Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation, Blood 113 (2009) 3821–3830.
[13] C. Zhu, A.C. Anderson, A. Schubart, et al., The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nat. Immunol. 6 (2005) 1245–1252.
[14] R.H. DeKruyff, X. Bu, A. Ballesteros, et al., and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells, J. Immunol. 184 (2010) 1918–1930.
[15] M. Seki, S. Oomizu, K.M. Sakata, et al., Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis, Clin. Immunol. 127 (2008) 78–88.
[16] A.C. Anderson, D.E. Anderson, L. Bregoli, et al., Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells, Science 318 (2007) 1141–1143.
[17] M.J. Lee, Y.M. Heo, S.H. Hong, et al., The binding properties of glycosylated and non-glycosylated Tim-3 molecules on CD4CD25 T Cells, Immune Netw. 9 (2009) 58–63.
[18] M.L. Chen, M.J. Pittet, L. Gorelik, et al., Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo, Proc. Natl. Acad. Sci. USA 102 (2005) 419–424.
[19] E.Y. Woo, C.S. Chu, T.J. Goletz, et al., Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer, Cancer Res. 61 (2001) 4766–4772.
[20] F. Wang, L. Wan, C. Zhang, et al., Tim-3-Galectin-9 pathway involves the suppression induced by $CD4^+CD25^+$ regulatory T cells, Immunobiology 214 (2009) 342–349.
[21] K. Nagahara, T. Arikawa, S. Oomizu, et al., Galectin-9 increases $Tim\text{-}3^+$ dendritic cells and $CD8^+$ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions, J. Immunol. 181 (2008) 7660–7669.
[22] J. Klibi, T. Niki, A. Riedel, et al., Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein–Barr virus-infected nasopharyngeal carcinoma cells, Blood 113 (2009) 1957–1966.
[23] X. Huang, X. Bai, Y. Cao, et al., Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion, J. Exp. Med. 207 (2010) 505–520.
[24] F.H. Wu, Y. Yuan, D. Li, et al., Endothelial cell-expressed Tim-3 facilitates metastasis of melanoma cells by activating the NF-kappaB pathway, Oncol. Rep. 24 (2010) 693–699.
[25] Y. Ju, N. Hou, J. Meng, et al., T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B, J. Hepatol. 52 (2010) 322–329.
[26] L. Golden-Mason, B.E. Palmer, N. Kassam, et al., Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional $CD4^+$ and $CD8^+$ T cells, J. Virol. 83 (2009) 9122–9130.
[27] V. Dardalhon, A.C. Anderson, J. Karman, et al., Tim-3/galectin-9 pathway: regulation of Th1 immunity through promotion of $CD11b^+$ $Ly\text{-}6G^+$ myeloid cells, J. Immunol. 185 (2010) 1383–1392.
[28] Z.Z. Yang, A.J. Novak, S.C. Ziesmer, et al., Attenuation of CD8(+) T-cell function by CD4(+)CD25(+) regulatory T cells in B-cell non-Hodgkin's lymphoma, Cancer Res. 66 (2006) 10145–10152.
[29] B. Almand, J.I. Clark, E. Nikitina, et al., Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer, J. Immunol. 166 (2001) 678–689.
[30] H.H. Yan, M. Pickup, Y. Pang, et al., $Gr\text{-}1^+$ $CD11b^+$ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung, Cancer Res. 70 (2010) 6139–6149.
[31] E. Suzuki, V. Kapoor, A.S. Jassar, et al., Gemcitabine selectively eliminates splenic $Gr\text{-}1^+/CD11b^+$ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity, Clin. Cancer Res. 11 (2005) 6713–6721.
[32] D.I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system, Nat. Rev. Immunol. 9 (2009) 162–174.
[33] J. Vincent, G. Mignot, F. Chalmin, et al., 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity, Cancer Res. 70 (2010) 3052–3061.
[34] M. Jinushi, Y. Nakazaki, M. Dougan, et al., MFG-E8-mediated uptake of apoptotic cells by APCs links the pro- and antiinflammatory activities of GM-CSF, J. Clin. Invest. 117 (2007) 1902–1913.