Animal Cells and Systems 12: 193-201, 2008

Animal Cells
and
Systems
http://www.AnimalCells.or.kr

Anti-tumor Immunity Induced by Tumor Cells Expressing a Membrane-bound Form of IL-2 and SDF-1

Jin Wha Choi, Ho Yong Lim, Mi-Ra Chang, Ji-Yeon Cheon, and Young Sang Kim*

Department of Biochemistry, College of Natural Sciences, Chungnam National University, Daejeon 305-764, Korea

Abstract: The eventual goal of tumor immunotherapy is to develop a vaccine inducing a specific anti-tumor immunity. Cytokine gene therapy is an effective way at least in animal models, but limited efficacy and various side effects obstruct clinical applications. In this study, we developed a tumor vaccine expressing a membrane-bound form of IL-2 (mbIL-2) and SDF-1 in B16F10 melanoma cells. The tumor clones expressing mbIL-2 showed reduced tumorigenicity, and additional expression of SDF-1 to mbIL-2 expressing tumor cells caused more severe reduction in tumorigenicity. However, expression of the SDF-1 alone did not affect on the tumorigenicity, probably because of limited production of SDF-1 in the SDF-1 transfected clones. When the mice once rejected mbIL-2/SDF-1 expressing tumor clone were re-challenged with wild type B16F10 tumor cells, all of the mice survived. This result suggests that mbIL-2/SDF-1 tumor clone is effective in inducing systemic anti-tumor immunity against wild type B16 melanoma. Furthermore, culture supernatant of tumor clones expressing SDF-1 induced lymphocyte migration *in vitro*. These results, all together, suggest that expression of mbIL-2 and SDF-1 in tumor cells enhances anti-tumor immune responses through different roles; the secreted SDF-1 may function as a chemoattractant to recruit immune cells to tumor vaccine injection site, and the mbIL-2 on tumor cells may provide costimulatory signal for CTL activation in physical contacts.

Key words: membrane-bound form of IL-2, SDF-1, tumorigenicity, systemic anti-tumor immunity

INTRODUCTION

Various strategies employing cytokines to enhance host immunity against cancer cells have been studied intensively for tumor immunotherapy. With the successful clinical results using recombinant cytokines (Atkins et al., 1999; Leonard et al., 1997), genetic modification of tumor cells with cytokine genes has been evaluated extensively, such as IFN-γ, IL-2, IL-4, IL-12, GM-CSF, IL-15 (Allione et al., 1994; Armstrong et al., 1996; Blankenstein et al., 1991; Dranoff et al., 1993; Kimura et al., 1999; Nanni et al., 1999; Tepper and Mule, 1994). As soon as the biological functions of chemokines were characterized, chemokine gene transfer into tumor cells was also tested for their efficacy in inducing anti-tumor immunity (Dilloo et al., 1996).

Compared to the professional antigen presenting cells, many tumor cells, particularly those of non-hematopoietic origin, do not express MHC class II and costimulatory molecules such as B7, thus fail to activate tumor-associated antigen (TAA)-specific CD4+ T cells by themselves. Moreover, tumor cells generally express reduced levels of MHC class I molecules and become low immunogenic. Gene transfer of MHC class II or costimulatory molecules into tumor cells enhanced immunogenicity and successful tumor therapy (Baskar et al., 1993; Huang et al., 1994; Lu et al., 2003; Luo et al., 2004; Townsend and Allison, 1993), supporting the notion that the modified tumor cells can prime directly T cells as non-professional antigen-presenting cells.

Antigenicity of tumors have been endowed with immunostimulatory properties by transfecting them with genes encoding cytokines or costimulatory B7.1 molecule (Fearon et al., 1990; Gansbacher et al., 1990; Tepper et al., 1989; Townsend and Allison, 1993). The IL-2 gene transduction into tumor cells compensated for the defected Th function in tumor bearers (Fearon et al., 1990; Mizoguchi et al., 1992; Salvadori et al., 1994; Zier et al., 1996). Malfunction of immune system in tumor bearers is also implicated with disabled dendritic cells (Gabrilovich et al., 1996), so that GM-CSF transduced tumor cells could induce differentiation and activation of dendritic cells with therapeutic effect (Dranoff et al., 1993).

*To whom correspondence should be addressed.
Tel: +82-42-821-5487; Fax:+82-42-822-7548
E-mail: young@cnu.ac.kr

In previous study, we modified B16F10 melanoma cells to express membrane-bound forms of IL-2 (mbIL-2) as a costimulatory molecule to test its ability to induce anti-tumor immunity by direct priming of cytotoxic T ylmphocytes (CTL). The mbIL-2 tumor clone showed substantially increased immunogenicity, but failed to stimulate immune system sufficiently to cure pre-formed tumors (Chang et al., 2005). To enhance anti-tumor immune responses by mbIL-2 expressing tumor vaccine, forced recruitment of CTL to tumor growing site may be helpful. To this end, we adopted SDF-1 (CXCL12) as a chemoattractant for CTL. SDF-1 is expressed by stromal cells including fibroblasts and endothelial cells, and interacts specifically with the CXC chemokine receptor 4 (CXCR4) (Peled et al., 1999). SDF-1 regulates transendothelial migration and homing of hemtopoietic stem cells and progenitor cells (Mohle et al., 2001), and functions as a costimulatory factor for CD4+ T cell activation (Kumar et al., 2006). The SDF-1 transduced tumor cells induced anti-tumor immunity by affecting T cell migration (Dunussi-Joannopoulos et al., 2002; Nomura et al., 2001; Shi et al., 2005); CTL migration was also mediated by CXCR4 expressed on CTL and SDF-1 secreted by the tumor cells (Zhang et al., 2006).

In the present study, to facilitate the interactions between the mbIL-2 expressing tumor vaccine and TAA-specific CTL *in situ*, SDF-1 gene was additionally introduced into the B16F10 cells with the mbIL-2. The SDF-1 and the mbIL-2 expressed in tumor cells were expected to recruit CTL to tumor vaccine injection site and provide co-stimulatory signal selectively to TAA-specific CTL, respectively. We report here that the mbIL-2/SDF-1 tumor clone induces more potent systemic anti-tumor immunity than mbIL-2 alone expressing tumor vaccine.

MATERIAL AND METHODS

Tumor cell line and mice

The B16F10 melanoma tumor cell line was maintained in RPMI-1640 medium (Gibco-BRL, Rockville, MD) supplemented with 10% heat-inactivated FBS (Gibco-BRL, Rockville, MD). The tumor cells were periodically selected *in vivo* to maintain tumorigenicity. Female C57BL/6 mice were obtained from Daehan Biolink (Eumseong-gun, Korea) and used at 6 to 8 weeks of age.

Antibodies and reagents

Monoclonal antibody to murine IL-2 (S4.B6, rat IgG1) was kindly provided by Dr. Charles Janeway, Jr. (Yale University). PE- or FITC-conjugated goat anti-rat IgG antibody were purchased from BD Biosciences (San Jose, CA) and recombinant murine SDF-1 was purchased from R&D systems (Minneapolis, MN).

Construction of expression vectors encoding murine IL-2 and SDF-1

The mbIL-2 expression on cell surface of B16F10 cells was previously reported (Chang et al., 2005). To clone murine SDF-1 cDNA, RT-PCR was performed with isolated SDF-1 cDNA using following primer set and total RNA from spleen cells of C57BL/6 mouse: Forward primer 5'-TGGTGGTACCATGGACGCCAAGGTCGTCGCCGTG-3', reverse primer 5'-CTCCCGGGAATTACTTGTTTAAA GCTTTCTCC-3'. The *Kpn I/Afl II* SDF-1 cDNA was subcloned into pcDNA3.1(-)/hygro vector (Invitrogen, Carlsbad, CA).

Transfection and selection of transfected cells

To transfect SDF-1 and mbIL-2 expression vectors, B16F10 cells (5×10^6 cells) were mixed with 20 μg of linearized vector DNAs (after *Sal I* and *Bgl II digestions*, respectively). The transfected cells were selected G418 and/or hygromycin-containing media, and colonies were visible after 2 weeks.

ELISA and flow cytometry

The amount of SDF-1 in the supernatants was quantified by a sandwich ELISA using a purified anti-hmSDF-1 monoclonal antibody as a capturing antibody and a biotinylated anti-hmSDF-1 polyclonal antibody as a detection antibody (R&D systems, Minneapolis, MN). The whole procedure of ELISA was performed by the manufacturer's instructions. For analysis of mbIL-2 expression on tumor clones, cells were stained with anti-IL-2 monoclonal antibody (S4.B6.34, rat IgG1) at 4°C for 30 min, and followed by FITC-conjugated goat anti-rat IgG antibody (Sigma-Aldrich, St. Louis, MO). The stained cells were analyzed on a FACSCalibur flow cytometer (Beckton Dickinson, San Jose, CA).

Tumorigenicity and systemic immunity analysis

Cells (1×10^6 cells/mouse) of exponentially growing tumor clones were harvested and injected into mice (5 mice/group) subcutaneously. The tumor growth was monitored daily basis, and once tumor growth was visible, the longest diameter of growing tumors was measured with calipers. The survival of mice also monitored daily basis, but severely moribund individuals were sacrificed and recorded as a dead mouse. The mice survived from transfected tumor clone were re-challenged with wild type B16F10 cells (1×10^4 cells/mouse) subcutaneously. As a control, age matched new group of mice (5 mice) were also injected with the B16F10 cells at the same time, then tumor growth and survival were monitored as above.

In vitro migration assay

Migration of lymphocytes was measured by migration through 5 μm pore polycarbonate filters in 24-well trans-well culture chambers (Corning Costar, Cambridge, MA). Culture supernatants of transfected B16F10 tumor clones (1×10^6 cells, 48 hr, in 2 mL medium) or culture medium containing 100 ng/mL recombinant SDF-1 were transfer to low-chamber of trans-well plate, and lymphocytes (5×10^4 cells) from normal mice were then added to upper-chamber. After 3 hr of incubation at 37°C, the cells migrated to the low-chamber were collected and counted by FACSCalibur flow cytometry. Acquiring events was recorded for a fixed time period of 60 sec using the CellQuest software (Becton Dickinson).

RESULTS

In previous study, we produced genetically engineered tumor clones that express membrane-bound forms of IL-2 (mbIL-2) on surface of B16F10 mouse melanoma tumor cells. The mbIL-2 expressing tumor clone (mbIL-2 tumor clone) was expected to stimulate selectively tumor specific CD8+ T cells, because tumor-specific CD8+ T cells receive two stimulatory signals; one from TAA peptide/MHC class I, and the other from mbIL-2 acting as a costimulatory molecule. With the mbIL-2 tumor clone, we could induce anti-tumor immunity to parental B16F10 cells, but therapeutic effect to pre-formed tumor was marginal; showed 90% lethality in 3 months post-injection with mbIL-2 tumor clone (Chang et al., 2005). This led us to incorporate SDF-1 into the mbIL-2 tumor clone additionally as a mean to increase infiltration of CTL to injection site of mbIL-2 tumor clone.

Expression of mbIL-2 and SDF-1 in transfected B16F10 tumor cells

B16F10 cells were transfected with the pNeoSRαII mock vector, pNeoSRαII-mbIL-2 and/or pcDNA3.1(-)/Hygro-SDF-1 plasmid DNAs (Fig. 1), and selected in G418- and/ or hygromycin-containing media. The drug-resistant clones of each group were then analyzed for expression of the mbIL-2 and the SDF-1 by RT-PCR, ELISA, or FACS analysis. As expected, both parental tumor cells and mock vector-transfected clones did not express mRNAs of IL-2 and SDF-1, whereas the mbIL-2, the SDF-1, or the mbIL-2/ SDF-1 transfectant clones expressed corresponding mRNAs (Fig. 2A). Additionally, both the SDF-1 and the mbIL-2/ SDF-1 clones were found to secrete SDF-1 about 5 ng/mL of SDF-1 into culture supernatants in a culture of 1×10^6 cells in 2 mL volume for 48 hr (Fig. 2B). It may be equivalent to 2.5 ng/mL of SDF-1 in the traditional 24 hr culture assay for SDF-1 quantification. In a flow cytometry analysis for mbIL-2 expression, both the mbIL-2 and the mbIL-2/SDF-1 transfectant clones were found to express similar levels of mbIL-2 on cell surface (Fig. 2C). The expression of mbIL-2 and SDF-1 did not change proliferation rate of the tumor clones *in vitro* (data not shown).

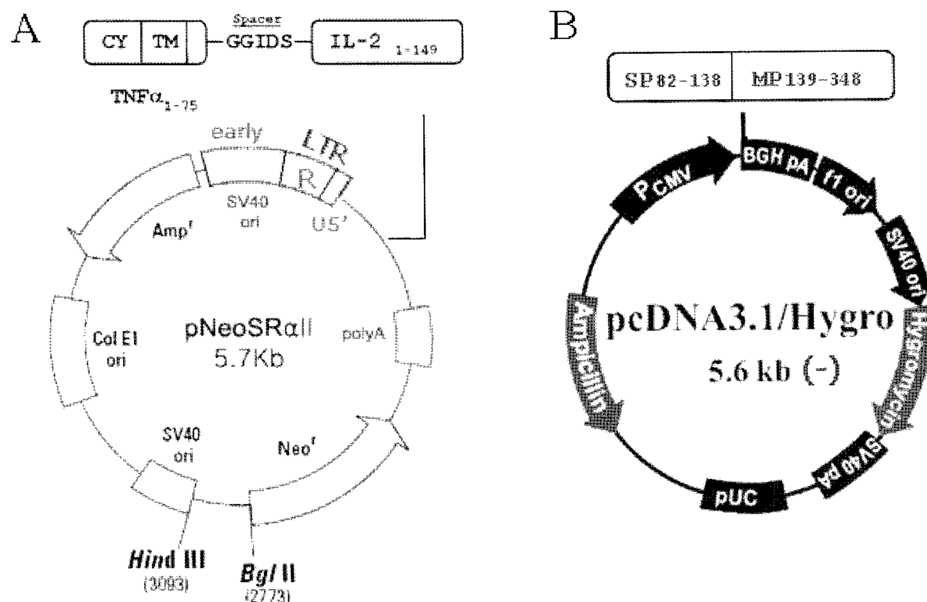

Fig. 1. Construction of expression vectors for mbIL-2 and SDF-1 cDNAs. The mbIL-2 chimeric gene encodes cytoplasmic domain (from -75 to -45), transmembrane domain (from -44 to -24), and 19 extracellular amino acids (from -23 to -5) of TNF, and genetic information of five amino acids spacer (GGIDS), and whole amino acids of IL-2 (from 1 to 149) without signal peptide. The SDF-1 cDNA encodes signal peptide (from 82 to 138) and mature peptide (from 139 to 348).

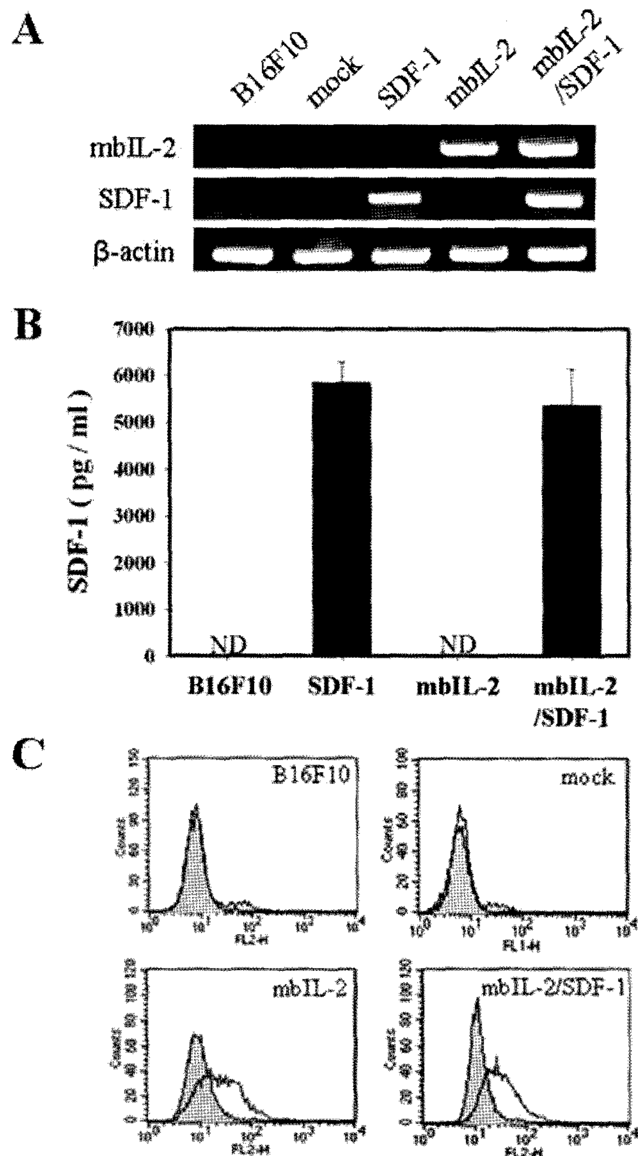

Fig. 2. Expression of SDF-1 and mbIL-2 on transfected clones. (A) Total RNA was isolated from each clone, and mRNAs of IL-2 and SDF-1 were amplified by RT-PCR using corresponding primer sets. (B) Cell cultures were started with 1×10^6 cells in 2 mL volume, and supernatants were collected after 48 hr incubation to determine SDF-1 using a sandwich ELISA. (C) For analysis of expression of mbIL-2 on tumor clones, cells were incubated with 100 μL of S4.B6.34 (rat anti-mouse IL-2 mAb, IgG) hybridoma culture supernatant at 4°C, and followed by PE or FITC-conjugated anti-rat IgG mAb. As control, an isotype matching antibody was used as a primary antibody (closed histograms). Open histograms indicate cells stained with anti-IL-2 antibody.

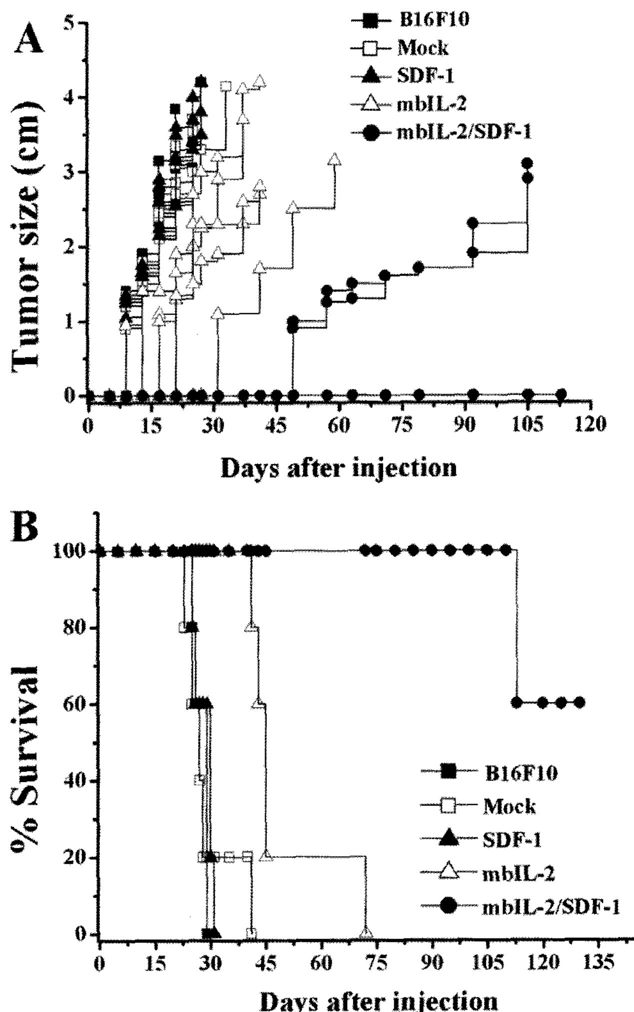

Fig. 3. Tumorigenicity of transfected clones expressing mbIL-2 and/or SDF-1. C57BL/6 mice (5 mice per group) were injected subcutaneously with live cells of B16F10, mock vector transfected, SDF-1, mbIL-2, and mbIL-2/SDF-1 clones (1×10^6 cells/mouse), respectively. Tumor growth was measured using calipers, and survival of mice was monitored.

Tumorigenicity of mbIL-2 and/or SDF-1 transfected B16F10 clones

To investigate the effect of mbIL-2 and/or SDF-1 expression on tumorigenicity of tumor cells, B16F10 parental cells, a mock vector-transfected clone, mbIL-2 clone, SDF-1 clone, and mbIL-2/SDF-1 clones were injected subcutaneously into syngeneic C57BL/6 mice, and tumor growth and survival were monitored regularly. There was a delay in tumor progression by the mbIL-2 and the mbIL-2/SDF-1 expressing clones compared with those by wild type B16F10, mock vector-transfectant, or the SDF-1 clone (Fig. 3A). Especially, tumor formation by the mbIL-2/SDF-1 clone was most delayed. Among five mice injected with the mbIL-2/SDF-1 clone, tumor was formed only in two mice, and the tumor growth was slower in the two mice than those by any other clones and parental tumor cells. When survival was examined, the group of mice injected with mbIL-2 expressing clones lived longer than the mice injected with wild type of B16F10 cells, mock transfectant or SDF-1 clone (Fig. 3B). Also mice injected with the mbIL-2/SDF-1 cells survived much longer than those injected with the mbIL-2 clone. Tumor was never formed in three out of five mice injected with mbIL-2/SDF-1 cells.

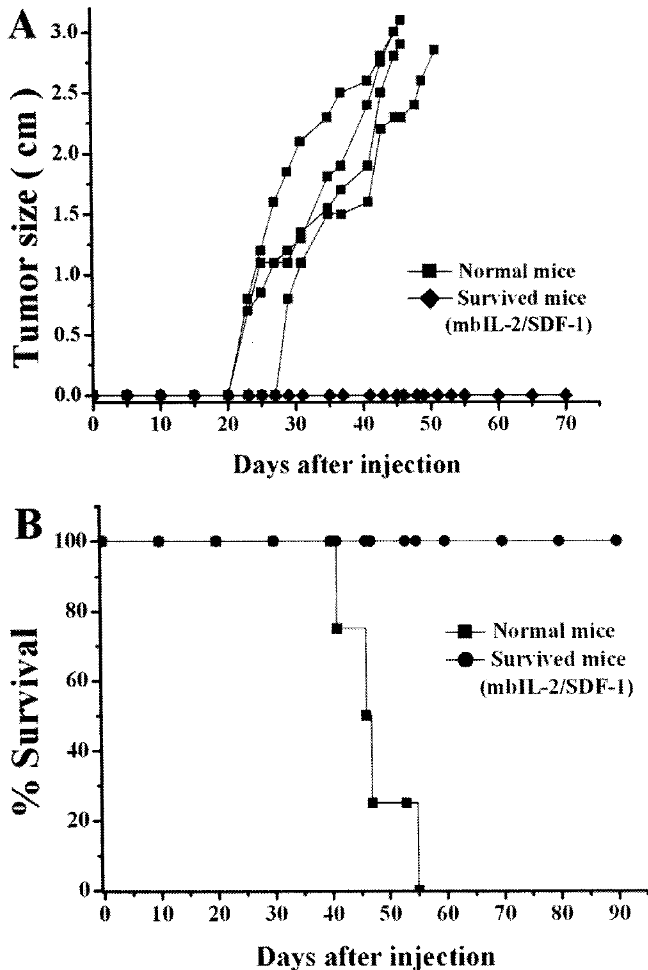

Fig. 4. Induction of systemic anti-tumor immunity. The survived mice (3 mice) from mbIL-2/SDF-1 clone were re-challenged 3 months later with 1×10^4 wild type B16F10 cells. As control, age matched five normal mice were challenged with 1×10^4 wild type B16F10 cells at the same time. Tumor growth and survival of mice were monitored periodically.

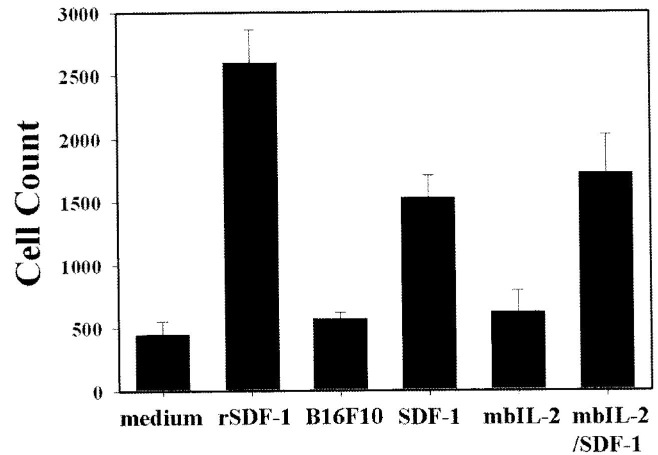

Fig. 5. Migration of lymphocytes by SDF-1. Culture supernatants from transfected tumor clones or wild type B16F10 cells (1×10^6 cells in 2 mL for 48 hr) were transferred to lower chambers of a trans-well plate, and lymphocytes (5×10^4 cells) from normal mice were applied upper chamber of the plate. As a control, recombinant SDF-1 (100 ng/mL) was placed in lower chamber. After 3 hr of incubation at 37°C, lymphocytes migrated to the lower chamber were counted by a flow cytometry. The cell count indicates the number of cells counted for 1 min through the flow cytometry.

This result indicates that expression of mbIL-2 on tumor cells reduces tumorigenicity of tumor cells, and additional expression of SDF-1 stimulates anti-tumor immunity further. The expression of SDF-1 alone was not beneficial in reducing tumorigenicity.

Systemic anti-tumor immunity by mbIL-2/SDF-1 clone

To determine whether the survived mice from the injection of the mbIL-2/SDF-1 clone acquired systemic anti-tumor immunity to B16F10 cells, the mice were re-challenged with wild type B16F10 cells subcutaneously after 3 months. As a control, a group of normal mice were injected with the wild type of B16F10 cells at the same time, and tumor growth was monitored. Tumor growth was found in all mice of new group of mice, whereas the wild type B16F10 tumor cells failed to form tumor in the mice once rejected the mbIL-2/SDF-1 clone (Fig. 4). These results indicate that the mbIL-2/SDF-1 clone effectively induces systemic anti-tumor immunity to B16F10 melanoma.

Effect of SDF-1 expression on lymphocyte migration

To analyze the effect of SDF-1 on lymphocyte migration, a migration assay was set up. Culture supernatants of wild type or various tumor clones were transferred to low-chamber and lymphocytes of normal mice were then added to the upper-chamber. After 3 hr incubation, the migrated lymphocytes into lower chamber were counted. As shown in Fig. 5, SDF-1 expressing clones (the SDF-1 and the mbIL-2/SDF-1 clones) induced lymphocyte migration effectively. When we placed tumor clones directly to lower chamber instead of culture supernatants from the clones, similar pattern of lymphocyte migration were found (data not shown). These results indicate that the SDF-1 secreted by tumor clones functions as a chemoattractant for lymphocytes.

DISCUSSION

In this study, we analyzed the effect of mbIL-2 and SDF-1 expression in B16F10 mouse melanoma cells on tumorigenicity. The SDF-1 and the mbIL-2 expressed in tumor cells were expected to recruit CTL to tumor vaccine injection site and provide costimulatory signal selectively to tumor-associated antigen (TAA)-specific CTL, respectively. The expression of mbIL-2 in B16F10 caused a reduction in tumorigenicity, and additional expression of SDF-1 reduced the tumorigencity more severely. SDF-1 expression alone

did not affect on tumorigenicity, but tumor clones expressing SDF-1 was effective in inducing lymphocyte migration. Furthermore, mice once rejected mbIL-2/SDF-1 clone were resistant to wild type B16F10 tumor cells, indicating acquisition of systemic anti-tumor immunity.

The transfer of IL-2 gene into tumor cells has advantage that IL-2 secreted by tumor cell itself can induce local immune responses at tumor growing site by IL-2 transduced tumor cells (Fearon et al., 1990; Gansbacher et al., 1990; Hillman et al., 2004; Jackaman et al., 2003). However, tumor cells engineered to produce cytokines have unexpected side effects (Dranoff et al., 1993; Lollini and Forni, 2003; Tjuvajev et al., 1995) that may be caused by activation of bulk bystander cells, or differentiation and expansion of T cells without evident specificity for TAA antigens (Colombo et al., 1992; Rivoltini et al., 1990). The multifunctional redundancy of cytokine itself and multicellular expression of cytokine receptors may cause these. To avoid such side effects of tumor vaccine engineered to secrete cytokines, a strategy for oriented selective targeting of immune responses is indispensable. To this end, we expressed IL-2 on tumor cells as a membrane-bound form rather than a secretory form; the membrane-bound form of IL-2 may give costimulatory signal selectively to CTL precursors specific to processed TAA/MHC class I molecule. In previous studies, the mbIL-2 tumor clones were found to be effective in inducing CTL activity and systemic anti-tumor immunity in MethA fibrosarcoma and B16F10 melanoma models (Chang et al., 2005; Sonn, 2006). The additional expression of SDF-1 was expected to stimulate anti-tumor immunity further, because the SDF-1 was known to function as a chemoattractant for various immune cells, especially CTL.

Among cytokines, IL-1 (Kurt-Jones et al., 1987), TNFα (Kriegler et al., 1988), LTα (Browning et al., 1993), and IFN-γ (Assenmacher et al., 1996) are expressed as membrane-bound form initially, and then cleaved by specific proteases to be released from cells. Genetically engineered expression of membrane-bound form of TNFα or LTα on tumor cells induced anti-tumor immunity with lower toxic side effect (Browning et al., 1993; Marr et al., 1997). Such approach using membrane-bound form of cytokines has been expanded to GM-CSF (el-Shami et al., 1999; Soo Hoo et al., 1999; Yei et al., 2002), Flt3 ligand (Chen et al., 1997), IL-4 (Chang et al., 2005; Kim et al., 2000), IL-12 (Cimino et al., 2004) and IL-2 (Chang et al., 2005; Sonn, 2006). Furthermore, anchoring of IL-2 via diphtheria toxin T domain on tumor cells induced successful anticancer immunity (Nizard et al., 2003). Although the intended target cells are different depending on the applied cyokines, these approaches share the common rationale that tumor cells expressing membrane-bound form of cytokine affect immune target cells selectively, that is, that are in direct physical contacts through interaction of cytokine with corresponding receptors. The direct priming of CTL by non-professional antigen-presenting cells was functional with fibroblasts (Kundig et al., 1995), so that the mbIL-2 molecule on tumor cells could function as costimulatory molecule selectively for CTL specific for TAA.

SDF-1 is a chemokine that regulates development, growth, survival, adherence, and migration of multiple cell types (Campbell and Butcher, 2000). It also functions as a chemotactic factor and has an effect on T cell rolling and tight adhesion to activated endothelial cells. Moreover, SDF-1 is known to provide costimulatory signal for T cell activation through CXCR4 receptor (Kumar et al., 2006). The local secretion of SDF-1 by SDF-1 transduced tumor cells reduced tumorigenicity of these cells, possibly owing to the development of anti-tumor immune responses involving CD4+ T cell and CD8+ T cells (Dunussi-Joannopoulos et al., 2002; Nomura et al., 2001; Shi et al., 2005). In the study by Xiang and colleagues, the number of tumor infiltrating T cells into SDF-1 transduced tumor cells was much higher than that of wild type tumor cells (Shi et al., 2005). Furthermore, the tumor-infiltrating T cells in SDF-1 expressing tumor were mainly CD8+ T cells, but the tumor infiltrating T cells in wild type tumor was CD4+ T cells, suggesting that SDF-1 is effective to recruit CD8+ T cells. However, the effect of the SDF-1 expression in tumor cells on the T cell migration appeared not to be consistent, or even opposite; the high expressor of SDF-1 (58 ng/mL) was chemorepulsive, but lower expressor (7.6 ng/mL) was chemoattractive for T cells (Vianello et al., 2006). The dose-dependent effect of SDF-1 was also reported in other SDF-1 transduced tumors. Low expressor of SDF-1 transduced B16F10 melanoma cells (2 ng/mL) resulted in reduced tumorigenicity and induced long-lived tumor-specific CTL responses, whereas therapeutic immunity against tumors was not observed in the mice immunized with high number (more than 0.2 million cells) of irradiated tumor cells expressing SDF-1. Moreover, high expressor of SDF-1 transduced MethA fibrosarcoma and HM-1 ovarian carcinoma (90 and 55 ng/mL, respectively) could induce immune responses only when tumors were engineered to co-express IL-2 or GM-CSF (Nomura et al., 2001). In our study, the SDF-1 and the mbIL-2/SDF-1 clones secreted about 5 ng/mL of SDF-1 in the conditioned media of 1×10^6 cells cultured in 2 mL volume for 48 hr that is equivalent to about 2.5 ng/mL of SDF-1 in 24 hr culture, indicating that the clones are in the range of low expressors of SDF-1. The low expression of SDF-1 may explain that the little change in tumorigenicity of SDF-1 alone expressing tumor clone (Fig. 3). However, SDF-1 expressing clones secreted enough amount of SDF-1 to show chemoattractive property for lymphocytes (Fig. 5). Taken together, all the results

suggest that the concentration of SDF-1 for tumor therapy should be controlled carefully.

We adopted SDF-1 originally as a chemoattractant to induce CTL migration into tumor vaccine injection sites, but pleiotropic effects of SDF-1 may contribute to induce anti-tumor immunity beyond the chemoattractive function. SDF-1 regulates expression of adhesion molecules; induction of ICAM-1 aids lymphocyte adhesion to endothelial cells (Emoto et al., 1999). SDF-1 also increases T cell proliferation and cytokine secretion (Nanki and Lipsky, 2000), supports T cells activation (Kumar et al., 2006), and inhibit activation-induced cell death of T cells (Nanki et al., 2000). These various function of SDF-1 may cooperate with mbIL-2 on tumor cells to induce CTL activation in a TAA-specific manner. For developing effective cancer therapeutic vaccines, strategies for manipulating immune system to activate selectively TAA-specific immune cells should be developed in parallel with the goals to enhance immunogenicity of tumor cells.

ACKNOWLEDGMENTS

This study was financially supported by research fund of Chungnam National University in 2006.

REFERENCES

Allione A, Consalvo M, Nanni P, Lollini PL, Cavallo F. Giovarelli M, Forni M, Gulino A, Colombo MP, Dellabona P *et al.* (1994) Immunizing and curative potential of replicating and nonreplicating murine mammary adenocarcinoma cells engineered with interleukin (IL)-2, IL-4, IL-6, IL-7, IL-10, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, and gamma-interferon gene or admixed with conventional adjuvants. *Cancer Res* 54: 6022-6026.

Armstrong CA, Botella R, Galloway TH, Murray N, Kramp JM, Song IS, and Ansel JC (1996) Antitumor effects of granulocyte-macrophage colony-stimulating factor production by melanoma cells. *Cancer Res* 56: 2191-2198.

Assenmacher M, Scheffold A, Schmitz J, Segura Checa JA, Miltenyi S, and Radbruch A (1996) Specific expression of surface interferon-gamma on interferon-gamma producing T cells from mouse and man. *Eur J Immunol* 26: 263-267.

Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M *et al.* (1999) High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol* 17: 2105-2116.

Baskar S, Nabavi N, Glimcher LH, and Ostrand-Rosenberg S (1993) Tumor cells expressing major histocompatibility complex class II and B7 activation molecules stimulate potent tumor-specific immunity. *J Immunother* 14: 209-215.

Blankenstein T, Rowley DA, and Schreiber H (1991) Cytokines and cancer: experimental systems. *Curr Opin Immunol* 3: 694-698.

Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP, Hession C, O'Brine-Greco B, Foley SF, and Ware CF (1993) Lymphotoxin beta, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. *Cell* 72: 847-856.

Campbell JJ and Butcher EC (2000) Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr Opin Immunol* 12: 336-341.

Chang MR, Lee WH, Choi JW, Park SO, Paik SG, and Kim YS (2005) Antitumor immunity induced by tumor cells engineered to express a membrane-bound form of IL-2. *Exp Mol Med* 37: 240-249.

Chen K, Braun S, Lyman S, Fan Y, Traycoff CM, Wiebke EA, Gaddy J, Sledge G, Broxmeyer HE, and Cornetta K (1997) Antitumor activity and immunotherapeutic properties of Flt3-ligand in a murine breast cancer model. *Cancer Res* 57: 3511-3516.

Cimino AM, Palaniswami P, Kim AC, and Selvaraj P (2004) Cancer vaccine development: protein transfer of membrane-anchored cytokines and immunostimulatory molecules. *Immunol Res* 29: 231-240.

Colombo MP, Modesti A, Parmiani G, and Forni G (1992) Local cytokine availability elicits tumor rejection and systemic immunity through granulocyte-T-lymphocyte cross-talk. *Cancer Res* 52: 4853-4857.

Dilloo D, Bacon K, Holden W, Zhong W, Burdach S, Zlotnik A, and Brenner M (1996) Combined chemokine and cytokine gene transfer enhances antitumor immunity. *Nat Med* 2: 1090-1095.

Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, and Mulligan RC (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* 90: 3539-3543.

Dunussi-Joannopoulos K, Zuberek K, Runyon K, Hawley RG, Wong A, Erickson J, Herrmann S, and Leonard JP (2002) Efficacious immunomodulatory activity of the chemokine stromal cell-derived factor 1 (SDF-1): local secretion of SDF-1 at the tumor site serves as T-cell chemoattractant and mediates T-cell-dependent antitumor responses. *Blood* 100: 1551-1558.

el-Shami KM, Tzehoval E, Vadai E, Feldman M, and Eisenbach L (1999) Induction of antitumor immunity with modified autologous cells expressing membrane-bound murine cytokines. *J Interferon Cytokine Res* 19: 1391-1401.

Emoto M, Mittrucker HW, Schmits R, Mak TW, and Kaufmann SH (1999) Critical role of leukocyte function-associated antigen-1 in liver accumulation of CD4+NKT cells. *J Immunol* 162: 5094-5098.

Fearon ER, Pardoll DM, Itaya T, Golumbek P, Levitsky HI, Simons JW, Karasuyama H, Vogelstein B, and Frost P (1990) Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* 60: 397-403.

Gabrilovich DI, Ciernik IF, and Carbone DP (1996) Dendritic cells in antitumor immune responses. I. Defective antigen presentation in tumor-bearing hosts. *Cell Immunol* 170: 101-110.

Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, and Gilboa E (1990) Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. *J Exp Med* 172: 1217-1224.

Hillman GG, Slos P, Wang Y, Wright JL, Layer A, De Meyer M,

Yudelev M, Che M, and Forman JD (2004) Tumor irradiation followed by intratumoral cytokine gene therapy for murine renal adenocarcinoma. *Cancer Gene Ther* 11: 61-72.

Huang AY, Golumbek P, Ahmadzadeh M, Jaffee E, Pardoll D, and Levitsky H (1994) Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* 264: 961-965.

Jackaman C, Bundell CS, Kinnear BF, Smith AM, Filion P, van Hagen D, Robinson BW, and Nelson DJ (2003) IL-2 intratumoral immunotherapy enhances CD8+ T cells that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2. *J Immunol* 171: 5051-5063.

Kim YS, Sonn CH, Paik SG, and Bothwell AL (2000) Tumor cells expressing membrane-bound form of IL-4 induce antitumor immunity. *Gene Ther* 7: 837-843.

Kimura K, Nishimura H, Hirose K, Matsuguchi T, Nimura Y, and Yoshikai Y (1999) Immunogene therapy of murine fibrosarcoma using IL-15 gene with high translation efficiency. *Eur J Immunol* 29: 1532-1542.

Kriegler M, Perez C, DeFay K, Albert I, and Lu SD (1988) A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 53: 45-53.

Kumar A, Humphreys TD, Kremer KN, Bramati PS, Bradfield L, Edgar CE, and Hedin KE (2006) CXCR4 physically associates with the T cell receptor to signal in T cells. *Immunity* 25: 213-224.

Kundig TM, Bachmann MF, DiPaolo C, Simard JJ, Battegay M, Lother H, Gessner A, Kuhlcke K, Ohashi PS, Hengartner H *et al.* (1995) Fibroblasts as efficient antigen-presenting cells in lymphoid organs. *Science* 268: 1343-1347.

Kurt-Jones EA, Fiers W, and Pober JS (1987) Membrane interleukin 1 induction on human endothelial cells and dermal fibroblasts. *J Immunol* 139: 2317-2324.

Leonard JP, Sherman ML, Fisher GL, Buchanan LJ, Larsen G, Atkins MB, Sosman JA, Dutcher JP, Vogelzang NJ, and Ryan JL (1997) Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* 90: 2541-2548.

Lollini PL and Forni G (2003) Cancer immunoprevention: tracking down persistent tumor antigens. *Trends Immunol* 24: 62-66.

Lu X, Kallinteris NL, Li J, Wu S, Li Y, Jiang Z, Hillman GG, Gulfo JV, Humphreys RE, and Xu M (2003) Tumor immunotherapy by converting tumor cells to MHC class II-positive, Ii protein-negative phenotype. *Cancer Immunol Immunother* 52: 592-598.

Luo L, Chapoval AI, Flies DB, Zhu G, Hirano F, Wang S, Lau JS, Dong H, Tamada K, Flies AS *et al.* (2004) B7-H3 enhances tumor immunity in vivo by costimulating rapid clonal expansion of antigen-specific CD8+ cytolytic T cells. *J Immunol* 173: 5445-5450.

Marr RA, Addison CL, Snider D, Muller WJ, Gauldie J, and Graham FL (1997) Tumour immunotherapy using an adenoviral vector expressing a membrane-bound mutant of murine TNF alpha. *Gene Ther* 4: 1181-1188.

Mizoguchi H, O'Shea JJ, Longo DL, Loeffler CM, McVicar DW, and Ochoa AC (1992) Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* 258: 1795-1798.

Mohle R, Bautz F, Denzlinger C, and Kanz L (2001) Transendothelial migration of hematopoietic progenitor cells. Role of chemotactic factors. *Ann N Y Acad Sci* 938: 26-34; discussion 34-25.

Nanki T, Hayashida K, El-Gabalawy HS, Suson S, Shi K, Girschick HJ, Yavuz S, and Lipsky PE (2000) Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J Immunol* 165: 6590-6598.

Nanki T and Lipsky PE (2000) Cutting edge: stromal cell-derived factor-1 is a costimulator for CD4+ T cell activation. *J Immunol* 164: 5010-5014.

Nanni P, Forni G, and Lollini PL (1999) Cytokine gene therapy: hopes and pitfalls. *Ann Oncol* 10: 261-266.

Nizard P, Gross DA, Babon A, Chenal A, Beaumelle B, Kosmatopoulos K, and Gillet D (2003) Anchoring cytokines to tumor cells for the preparation of anticancer vaccines without gene transfection in mice. *J Immunother* 26: 63-71.

Nomura T, Hasegawa H, Kohno M, Sasaki M, and Fujita S (2001) Enhancement of anti-tumor immunity by tumor cells transfected with the secondary lymphoid tissue chemokine EBI-1-ligand chemokine and stromal cell-derived factor-1alpha chemokine genes. *Int J Cancer* 91: 597-606.

Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L *et al.* (1999) Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283: 845-848.

Rivoltini L, Gambacorti-Passerini C, Squadrelli-Saraceno M, Grosso MI, Cantu G, Molinari R, Orazi A, and Parmiani G (1990) In vivo interleukin 2-induced activation of lymphokine-activated killer cells and tumor cytotoxic T-cells in cervical lymph nodes of patients with head and neck tumors. *Cancer Res* 50: 5551-5557.

Salvadori S, Gansbacher B, Pizzimenti AM, and Zier KS (1994) Abnormal signal transduction by T cells of mice with parental tumors is not seen in mice bearing IL-2-secreting tumors. *J Immunol* 153: 5176-5182.

Shi M, Hao S, Su L, Zhang X, Yuan J, Guo X, Zheng C, and Xiang J (2005) Vaccine of engineered tumor cells secreting stromal cell-derived factor-1 induces T-cell dependent antitumor responses. *Cancer Biother Radiopharm* 20: 401-409.

Sonn CH, Hee Ryung Yoon, In Ock Seong, Mi-Ra Chang, Yong Chan Kim, Han-Chul Kang, Seok-Cheol Suh, and Young Sang Kim (2006) MethA Fibrosarcoma Cells Expressing Membrane-Bound Forms of IL-2 Enhance Antitumor Immunity. *J Microbiol Biotech* 16: 1919-1927.

Soo Hoo W, Lundeen KA, Kohrumel JR, Pham NL, Brostoff SW, Bartholomew RM, and Carlo DJ (1999) Tumor cell surface expression of granulocyte-macrophage colony-stimulating factor elicits antitumor immunity and protects from tumor challenge in the P815 mouse mastocytoma tumor model. *J Immunol* 162: 7343-7349.

Tepper RI and Mule JJ (1994) Experimental and clinical studies of cytokine gene-modified tumor cells. *Hum Gene Ther* 5: 153-164.

Tepper RI, Pattengale PK, and Leder P (1989) Murine interleukin-4 displays potent anti-tumor activity in vivo. *Cell* 57: 503-512.

Tjuvajev J, Gansbacher B, Desai R, Beattie B, Kaplitt M, Matei C, Koutcher J, Gilboa E, and Blasberg R (1995) RG-2 glioma

growth attenuation and severe brain edema caused by local production of interleukin-2 and interferon-gamma. *Cancer Res* 55: 1902-1910.

Townsend SE and Allison JP (1993) Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. *Science* 259: 368-370.

Vianello F, Papeta N, Chen T, Kraft P, White N, Hart WK, Kircher MF, Swart E, Rhee S, Palu G *et al.* (2006) Murine B16 melanomas expressing high levels of the chemokine stromal-derived factor-1/CXCL12 induce tumor-specific T cell chemorepulsion and escape from immune control. *J Immunol* 176: 2902-2914.

Yei S, Bartholomew RM, Pezzoli P, Gutierrez A, Gouveia E, Bassett D, Soo Hoo W, and Carlo DJ (2002) Novel membrane-bound GM-CSF vaccines for the treatment of cancer: generation and evaluation of mbGM-CSF mouse B16F10 melanoma cell vaccine. *Gene Ther* 9: 1302-1311.

Zhang T, Somasundaram R, Berencsi K, Caputo L, Gimotty P, Rani P, Guerry D, Swoboda R, and Herlyn D (2006) Migration of cytotoxic T lymphocytes toward melanoma cells in three-dimensional organotypic culture is dependent on CCL2 and CCR4. *Eur J Immunol* 36: 457-467.

Zier K, Gansbacher B, and Salvadori S (1996) Preventing abnormalities in signal transduction of T cells in cancer: the promise of cytokine gene therapy. *Immunol Today* 17: 39-45.

[Received November 13, 2008; accepted December 12, 2008]