Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2010, Article ID 121094, 8 pages
doi:10.1155/2010/121094

Research Article

Construction and Characterization of a Novel Fusion Protein MG7-scFv/SEB against Gastric Cancer

Qiang Tong, Ke Liu, Xiao-Ming Lu, Xiao-Gang Shu, and Guo-Bin Wang

Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan 430022, China

Correspondence should be addressed to Xiao-Gang Shu, shuxiaogang222@yahoo.com.cn

Received 20 September 2009; Revised 4 January 2010; Accepted 13 January 2010

Academic Editor: Xin-yuan Guan

Copyright © 2010 Qiang Tong et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Antibody-targeted superantigen has been developed into a new strategy to treat many malignant tumors. In this study, for specific targeting to gastric cancer cell, superantigen SEB (Staphylococcal Enterotoxin B) was genetically fused to the single-chain variable fragment of gastric carcinoma-associated antibody MG7(MG7-scFv) that recognizes the MG7 antigen frequently expressed in gastric cancer cell. The recombinant MG7-scFv/SEB fusion proteins are expressed in E. coli as inclusion bodies, and the purified MG7-scFv/SEB retains high binding affinity with gastric cancer cell SGC-7901 (positive MG7 antigen expression). When incubated with effector cell—peripheral blood mononuclear cells (PBMCs), MG7-scFv/SEB could effectively inhibit the proliferation and induce apoptosis of SGC-7901. After being treated with MG7-scFv/SEB, PBMCs remarkably increased the production of Th1 cytokines (IFN-gamma, IL-2), and slightly increased the production of Th2 cytokines (IL-4, IL-10) in vitro. It was observed that gastric-tumor-bearing rats administrated with MG7-scFv/SEB showed more inflammatory cell infiltration, more significant tumor inhibition, and longer survival time than those of rats treated with SEB or NS (Normal Saline). The data indicated that MG7-scFv/SEB fusion protein could specifically target gastric cancer cell, enhance the activity of T cells and induce tumor cell apoptosis to exert the antitumor effect on gastric cancer.

1. Introduction

In recent decades, oncologists have made strenuous attempts to improve the prognosis of gastric cancer patient; however, due to unresponsiveness to majority of the available therapies, gastric cancer still ranks as one of the most frequent and lethal cancers [1–3].

Therefore, alternative treatment approaches have been made to treat malignant tumor, such as monoclonal antibody that has become a new potential immunotoxin for anticancer therapy [4–7]. Nevertheless, monoclonal antibody has some deficiencies, such as high molecular weight, low penetration ability, slow clearance in the blood [5, 8–10], and so forth. These deficiencies result in the low tumor-to-blood biodistribution ratio [11–15]. In addition, patients may produce human anti-mouse antibody (HAMA) against most antibodies used for clinical therapy as they are from murine hybridoma [15, 16]. How can we circumvent these problems and enhance the antitumor effect of the immunotoxin? Single-chain variable fragment (scFv), denoted as the fusion of the immunoglobulin heavy and light chain variable regions, can partially get rid of the shortcomings of the whole antibody [17]. The scFv that consists of variable heavy-chain antibody connected to the variable light chain by a flexible linker is the smallest antibody fragment that retains the entire antigen-binding region for a particular antibody [18, 19], and the molecular weight of scFv is only one-sixth of the whole antibody [20]. Recently, scFv has had possible clinical utility [18, 21, 22].

The other part of this immunotoxin is the superantigen. Superantigen is a family of bacterial or viral protein, named from its ability to polyclonally activate large fractions (2%–20%) of the T-cell population cells at picomolar concentrations [23, 24]. Its unique feature is that it bypasses antigen-processing mechanism, specifically binds to T-cell receptor variable region beta-chain (TCR-V_β) segment, and forms a trimolecular complex along with major histocompatibility complex class II (MHC-II) [23, 25–27]. This trimolecular

complex can trigger T cells proliferation and affect cytokines production (e.g., IFN-γ, TNF-alpha, IL-2, and IL-12) and strong cytolytic activity [28–30]. This discovery confirms the effective application of superantigen in tumor immunotherapy. SEB, a well-characterized 28-kDa exotoxin produced by some strains of staphylococcus aureus, is one of the most commonly used exogenous superantigens for tumor therapy [30–32].

Here, we constructed a fusion protein consisting with chemically synthesized MG7-scFv against human gastric-cancer-associated antigen MG7 and superantigen SEB. The fusion protein was expressed in E. coli [33]. Then the antigen-binding activity of the fusion protein was determined by cell-ELISA and the antitumor effect was examined both in vitro and in vivo. So far as we know, it is the first time to report on a preliminary investigation into the target antitumor effect of MG7-scFv/SEB fusion protein on gastric cancer.

2. Materials and Methods

2.1. Reagents. The expression vector pET-32a (+) was purchased from Novagen (Germany). All enzymes were purchased from TaKaRa (Japan). Anti-SEB rabbit polyclonal antibody and HRP-labeled goat anti-rabbit were purchased from Sigma (US). Apoptotic DNA-Ladder kit was purchased from Roche (Switzerland). Lymphocyte-separating solution was produced by Shanghai No. 2 Chemical Reagent Factory (China).

2.2. Cell Lines and Animals. Human gastric adenocarcinoma SGC-7901 cell line and human normal gastric mucosal cell line GES-1 were preserved by our Laboratory. PBMCs were separated from healthy volunteers. Cells were maintained in RPMI-1640 and supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin G, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The 4–6 week old female SD rats were provided by experimental animal center of Tongji Medical College of Huazhong University of Science and Technology.

2.3. Construction, Expression, Purification, and Identification of Recombinant MG7-scFv/SEB. The plasmid pMD-18T/MG7-scFv-linker, encoding MG7-scFv (against human gastric-cancer-associated antigen MG7) with 15-residue C-terminal linker, was chemically synthesized by Sangon (Shanghai, China). The Plasmid pMD-18T/SEB, encoding SEB(S407030), was a kind gift from Dr. Gao Shi-tong (Shenzhen Center for Disease Control and Prevention, Shenzhen, China) [34]. The pMD-18T/MG7-scFv-linker was digested with Nco I and Bgl II; the pMD-18T/SEB was digested with BamH I and Sal I; the pET-32a (+) was digested with Nco I and Sal I. These three digested DNA fragments were ligated and transformed into E. coli BL21 (DE3). The transformed DE3 was grown in SB (super broth) culture, containing 2% glucose, 0.05% $MgSO_4$, and 100 μg/ml ampicillin. When the culture reached $A_{600} \approx 0.8$, induction was initiated by addition of 1 mM IPTG (isopropyl-L-D-thiogal-actoside). After incubation at 37°C for 2–6 hours, the cells were harvested at 8,000 g for 5 minutes at 4°C. The pellet was resuspended in 50 mM Tris-HCl, 20 mM EDTA, 0.5 M NaCl, 1% Triton X-100, pH 8.0, and then vortexed, sonicated for 5 minutes, and centrifugated at 10,000 g for 10 minutes at 4°C. The steps for sonication and centrifugation were repeated five times. The inclusion bodies were dissolved in freshly prepared denaturation buffer (8 M guanidine hydrochloride, 0.1 M Tris-HCl, 2 mM EDTA, 10 mg/mL DTE, pH 8.0), incubated at room temperature for 4 hours, and then centrifugated at 18,000 g for 20 minutes at 4°C. The supernatant was purified by Ni^{2+}-His Bind Resin affinity chromatography, and the purified fusion protein was eluted with 300 mM of imidazole. The solution was slowly dialyzed against renaturation solution I (2 M urea, 50 mM Tris-HCl, 2 mM GSH, and 1 mM GSSG) for 24 hours at 4°C, then continued dialyzed against renaturation solution II (50 mM Tris–HCl, 5 mM EDTA, pH 8.0) for 10 hours at 4°C. Then, the upstream 158-residue N-terminal fusion sequence was cleaved from enterokinase recognition sequence DDDDK by enterokinase. Digested proteins were dialyzed against 10 mM Tris-HCl pH 7.5 and then proteins were applied to a Superdex-75 column (2.6 × 100 cm) and eluted in 10 mM PBS (pH 7.6) containing 0.15 M NaCl. Fractions containing proteins of interest were collected and dialyzed against 10 mM Tris-HCl pH 7.5. The fusion protein was confirmed by Western blot, and stored at −70°C for further analysis.

2.4. Antigen-Binding Activity. Briefly, SGC-7901 or GES-1 cells were suspended at a concentration of 2×10^5/mL. 100 μL of the cell suspension was added to 96-well microplate, and incubated at 37°C for 48 hours. After discarding the medium, the plate was washed three times by filling the wells with 200 μL PBS, and then fixed with cold methanol/acetone solution (1 : 1) for 30 minutes, blocked with 0.3% H_2O_2/methanol at 37°C for 30 minutes. Serial diluted concentrations of MG7-scFv/SEB and SEB were added to the wells, respectively, (NS was added as negative control) and incubated at 37°C for 60 minutes. Washed twice with PBS, the well was then added with rabbit anti-SEB antibodies, incubated at 37°C for another 60 minutes. Washed twice again, the well was added with sheep anti-rabbit secondary antibodies, incubated at 37°C for 30 minutes. Each well was added with TMB (3,3′, 5,5′-tetramethylbenzidine) solution, incubated for 30 minutes, and then added with equal volume of 2 M H_2SO_4, and the optical density was read at 450 nm.

2.5. Effect of MG7-scFv/SEB on Gastric Cancer Cell Proliferation In Vitro. MG7 antigen positive-expression SGC-7901 cells were used as target cells. PBMCs were used as effector cells. SGC-7901 cells were suspended at a concentration of 5×10^4/mL, and 100 μL of the cell suspensions were added to 96-well microplate. After an overnight incubation, the cells were co-cultured with PBMCs at effector: target (E: T) ratios of 10: 1, and treated with various concentrations (0.01 μg/mL, 0.1 μg/mL, 1.0 μg /mL, and 10 μg/mL) of MG7-scFv/SEB or SEB for 48 hours, respectively. Moreover,

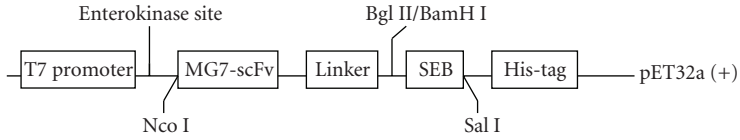

Figure 1: The schematic diagram of plasmid pET32a(+)/MG7-scFv/SEB. Enterokinase site: DDDDK; the linker: $(Gly_4Ser)_3$; His-Tag: six-histidine amino acid tag.

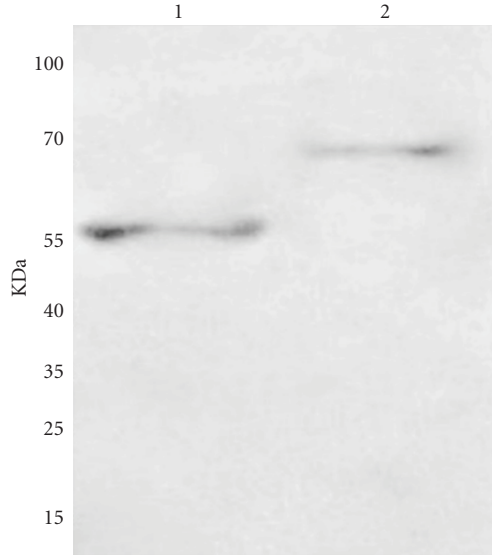

Figure 2: Western blot analysis using primary antibody anti-SEB and HRP-goat anti-rabbit IgG. lane 1: MG7-scFv/SEB protein after digestion with enterokinase; lane 2: MG7-scFv/SEB before digestion with enterokinase.

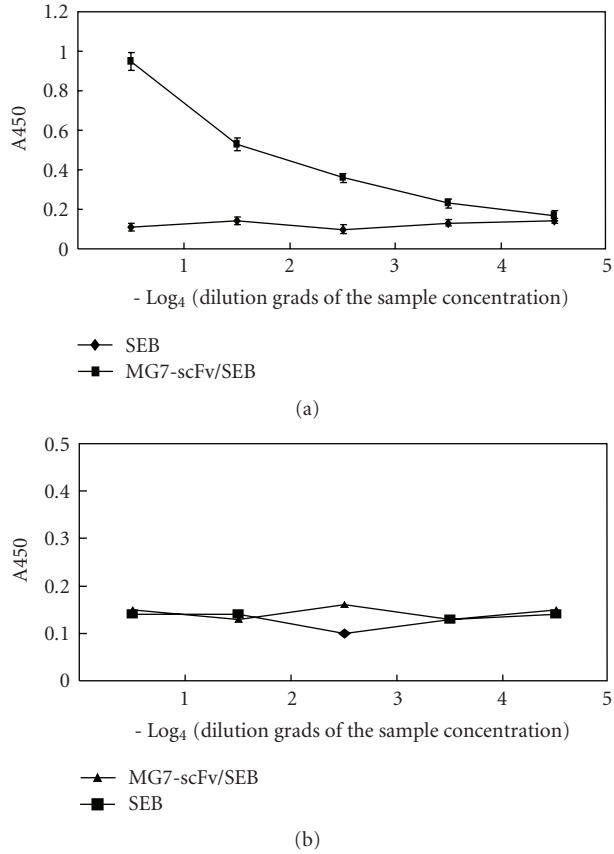

Figure 3: Binding affinity assay of MG7-scFv/SEB to MG7 positive or negative expression cells by cell-ELISA under different concentrations, same starting concentration 1.0 μg, 4 $\times$ serial dilution. (a) Binding assay of MG7-scFv/SEB to MG7 positive cell (gastric cancer cell SGC-7901) and (b) binding assay of MG7-scFv/SEB to MG7 negative cell (normal human gastric mucosal cell line GES-1).

cells were incubated with 1.0 μg/mL of MG7-scFv/SEB for different periods of time (0, 6, 12, 24, 48, and 72 hours). The cell viability after treating with MG7–scFv/SEB or SEB was measured by cell counting kit-8 (CCK-8) assay. In brief, CCK-8 (10 μL) was added to each well of a 96-well microplate containing 100 μL of culture medium and incubated at 37$\circ$C for 2 hours. The absorbance was then measured at 470cnm using a Bio-Rad 550 spectropho-tometric microplate reader. Six wells were repeated per group and all experiments were performed in triplicate.

2.6. Effect of MG7-scFv/SEB on Gastric Cancer Cell Apoptosis. SGC-7901 cells were incubated in 60 mm culture dish with PBMCs, allowed to adhere overnight, and then treated with different concentrations (0.1 μg/mL, 1.0 μg/mL, and 10 μg/mL) of MG7-scFv/SEB or NS (normal sodium). Each group of cells was harvested after 24 hours. The cell suspension was pelleted by centrifugation at 800 g for 5 minutes and resuspended in 400 μL lysis buffer, mixed by gentle vortex. The sample was incubated with proteinase K (10 μg/mL) at 65°C for 120 minutes, and centrifugated for 10 minutes to precipitate DNA. After supernatant removal, DNA pellet was washed with 750 μL 70% ethanol, centrifuged again to remove trace ethanol, and air dried for 10 minutes at room temperature. Each test sample was resuspended in the gel-loading buffer, and then 15 μL of each sample solution was loaded to a 1.5% agarose gel and electrophoresed at 100 V for 45 minutes. Ethidium bromide-stained DNA could be visualized by transillumination with UV light and photographed.

2.7. Cytokines Release In Vitro. PBMCs were adjusted to 1 $\times$ 10^6/mL. 100 μL of the cell suspension was seeded in 96-well microplate, and incubated at 37°C for 1 hour to adhere. The cells were cocultured with 1.0 μg/mL concentrations of SEB and MG7-scFv/SEB, respectively, (NS used as negative control). The supernatant was harvested at 12, 24, 36, 48,

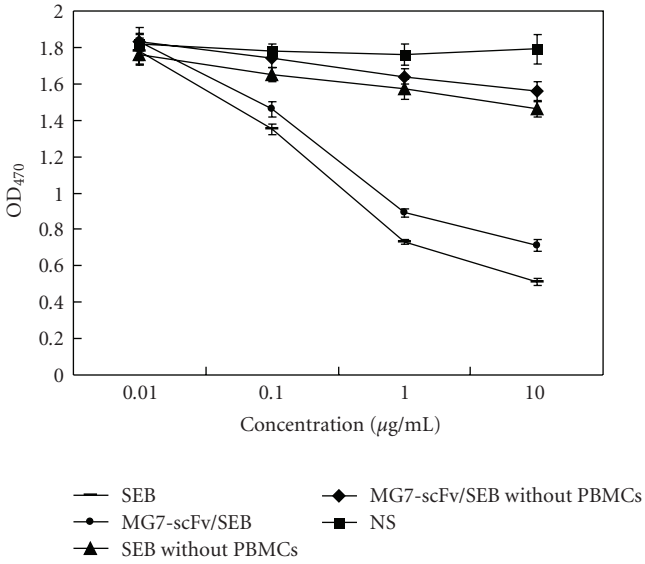

Figure 4: The effect of MG7-scFv/SEB fusion protein on SGC-7901 cell proliferation was detected by CCK-8 assay. When co-cultured with effector cell PBMCs, both SEB and MG7-scFv/SEB could effectively inhibit the proliferation of SGC-7901 cell ($P < .05$).

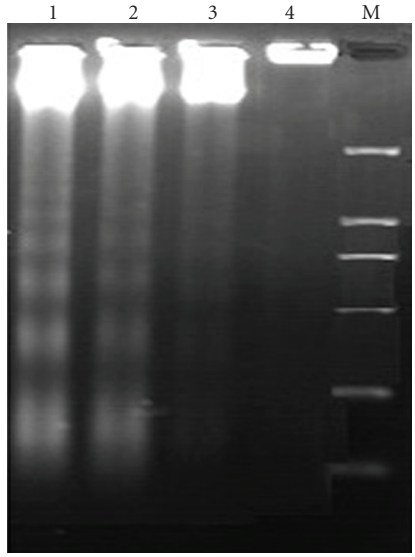

Figure 5: DNA ladder after treating with MG7-scFv/SEB. Lane 1: 10 μg/mL MG7-scFv/SEB group; lane 2: 1.0 μg/mL MG7-scFv/SEB group; lane 3: 0.1 μg/mL MG7-scFv/SEB group; lane 4: normal sodium group.

and 72 hours after incubation. Then, the cytokines (IL-2, IFN-gamma, IL-4, and IL-10) secreted by PBMCs were measured using a standard sandwich ELISA technique with corresponding kits.

2.8. Inhibition Effect of MG7-scFv/SEB on Gastric Cancer In Vivo. Rats models of gastric cancer were established by inoculating 1×10^7 SGC-7901 cells into left hind limbs of rats. After 12 days, the mean diameter of tumor nodule was nearly 1.0 cm. Sixty tumor-bearing rats were randomly divided into 3 groups: NS control group, SEB-treated group, and MG7-scFv/SEB-treated group. Then, rats in MG7-scFv/SEB group were administrated with 100 μg/mL MG7-scFv/SEB via the vena caudalis, once every other day for 6 times. The equivalent quality of SEB or NS was administrated in SEB group or control group, respectively. One-half rats in each group were sacrificed on the 15th day, and the subcutaneous tumors were isolated and weighed. The other half of rats in each group were observed for their survival period.

Table 1: The infiltration of inflammatory cell and necrosis of tumor nodule in gastric-cancer-bearing rats after combined therapy with MG7-scFv/SEB, SEB, or NS.

Groups	Tumor necrosis[1]	Infiltration of inflammatory cells inside the tumors[2]	Infiltration of inflammatory cells outside the tumors[2]
NS	−	−	−
SEB	+	+	++
MG7-scFv/SEB	++	++	+

[1]Tumor necrosis: −, no necrosis; +, less than 1/3 of the tumor size; ++, 1/3-2/3 of the tumor size; +++, more than 2/3 of the tumor size.
[2]Infiltration of inflammatory cells: −, no infiltration; +, minimal infiltration; ++, medium infiltration; +++, intense infiltration.

2.9. Inflammatory Cells Infiltration and Necrosis of Tumor Nodule. The tumor nodules were taken from tumor-bearing rats on the 15th day after the first injection. The subcutaneous tumor nodules were fixed in 10% formalin, and embedded in paraffin. Sections were observed by hematoxylin and eosin (H&E) dyeing. Grading evaluation criteria were referred to Q Wang (35). Briefly, the evaluation of tumor necrosis was −, no necrosis; +, less than 1/3 of the tumor size; ++, 1/3-2/3 of the tumor size; +++, more than 2/3 of the tumor size. The evaluation of the infiltration of inflammatory cell both in and around the tumor was −, no inflammatory cell infiltration; +, minimal inflammatory cell infiltration; ++, medium inflammatory cell infiltration; +++, intense inflammatory cell infiltration.

2.10. Statistical Analysis. All the results are expressed as mean ± standard error of the mean (SEM). Student's t-test or ANOVA was used to analyze the results. $P < .05$ was considered statistically significant.

3. Results

3.1. Construction and Expression of MG7-scFv/SEB. The expression vector pET32a(+)/MG7-scFv/SEB was constructed for the production of fusion protein MG7-scFv/SEB, in which the superantigen SEB was linked to the C-terminal of the anti-MG7 scFv via a $(Gly_4Ser)_3$ flexible linker facilitating both correct folding of the MG7-scFv and SEB (Figure 1). The expression of the fusion protein MG7-scFv/SEB was confirmed by Western blot analysis

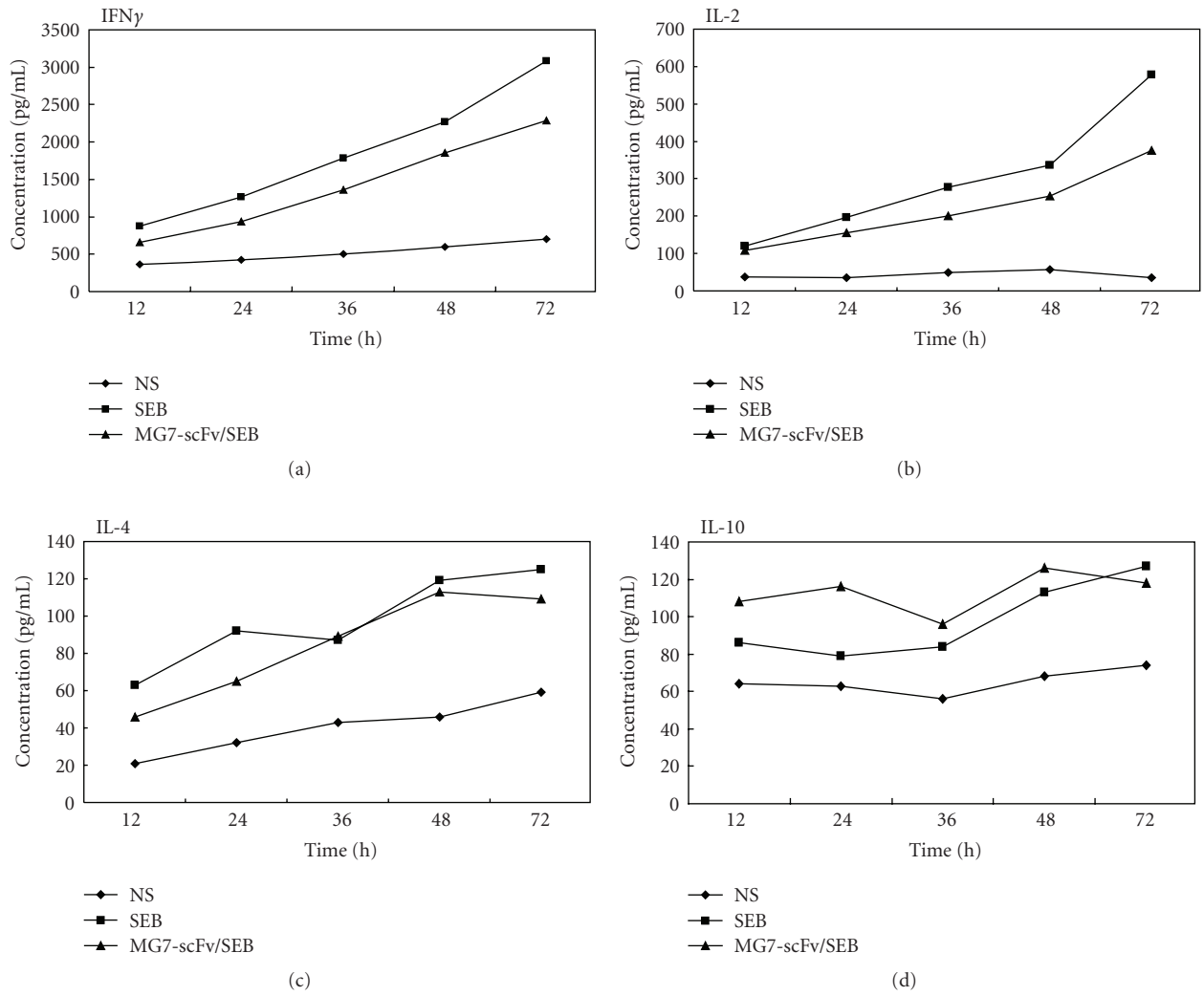

Figure 6: Production of cytokines by stimulated PBMCs: (a) IFN-γ production, (b) IL-2 production, (c) IL-4 production, (d) IL-10 production. The productions of IFN-γ and IL-2 are much more than those of IL-4 and IL-10 ($P < .05$). It indicated that SEB mainly activates Th1 cell to upregulate cellular immunity; meanwhile, it slightly activates Th2 cell.

using the anti-SEB antibody. As shown in Figure 2, Western blotting analyses revealed that a protein of about 76 kDa was strongly expressed before digestion with enterokinase, and a protein of about 56 kDa was expressed after digestion with enterokinase.

3.2. Binding Potential of MG7-scFv/SEB to SGC-7901. The binding potential of MG7-scFv/SEB to SGC-7901 gastric cancer cells was examined by using cell-ELISA. As shown in Figure 3(a), the MG7-scFv/SEB fusion protein demonstrated a high binding capacity to the SGC-7901 cells, whereas recombinant SEB exhibited very low binding affinity. As shown in Figure 3(b), both MG7-scFv/SEB fusion protein and SEB demonstrated a poor binding capacity with control cell GES-1 (human normal gastric mucosal cell line, negative expression of MG7 antigen). These findings indicated that MG7-scFv/SEB fusion protein can specifically target MG7-positive cell with high affinity.

3.3. Antitumor Effect of MG7-scFv/SEB on Gastric Cancer In Vitro. As shown in Figure 4, both SEB and MG7-scFv/SEB could effectively inhibit the proliferation of SGC-7901 cell which was cocultured with PBMCs, and the inhibitory effect exhibited a time- and dose-dependent manner (part of data not shown). In the absence of effective cell, the fusion protein MG7-scFv/SEB had no obvious inhibition effect of SGC-7901 cell. The data indicated that the antitumor effect of MG7-scFv/SEB fusion protein is dependent on the function of PBMC.

The characteristics of DNA ladders indicated internucleosomal cleavage of DNA during apoptosis. The results of agarose gel electrophoresis of DNA for the SGC7901 cells showed an overt ladder shaped band in 1.0 μg/mL and 10 μg/mL MG7-scFv/SEB-treates group; the ladder-shaped band became weaker in 0.1 μg/mL treated group, and no DNA ladders were detected in NS treating group (Figure 5).

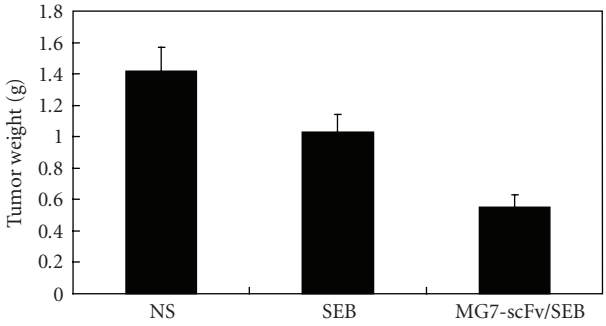

Figure 7: Ten gastric-cancer-bearing rats in each group were subcutaneously injected with same number tumor cells and observed for tumor nodules growth after being administrated with MG7-scFv/SEB, SEB, or NS. More potent inhibition of tumor growth was observed in MG7-scFv/SEB-treated group compared with the other two groups ($P < .05$).

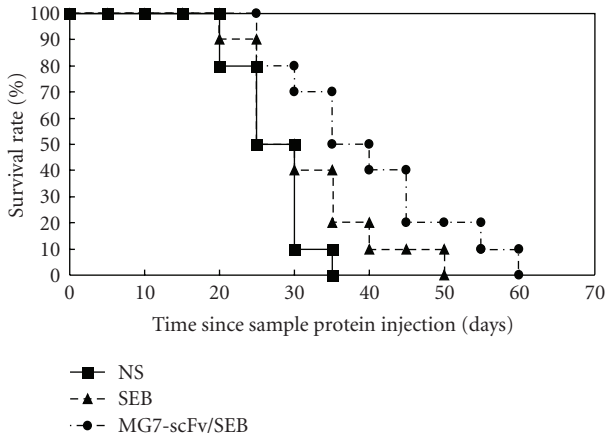

Figure 8: Ten gastric-cancer-bearing rats in each group were observed for their survival time after being administrated with MG7-scFv/SEB, SEB, or NS. The survival times of the rats in MG7-scFv/SEB and SEB group were longer than that of NS group within 60-day observation ($P < .05$). There were statistic differences among the three groups ($P < .05$).

As shown in Figure 6, both fusion protein MG7-scFv/SEB and SEB could dramatically induce the productions of cytokine IL-2 and IFN-gamma as compared with NS control group ($P < .01$). The production of cytokine IL-4 and IL-10 were also increased in MG7-scFv/SEB and SEB group ($P < .05$). The data indicated that MG7-scFv/SEB and SEB would mainly increase the production of Th1 cytokines (IL-2, IFN-gamma) and slightly upregulate the production of Th2 cytokines (IL-4, IL-10).

3.4. Antitumor Effect of MG7-scFv/SEB on Gastric Cancer In Vivo. The effect of MG7-scFv/SEB on SGC-7901 proliferation in vivo was detected via gastric-cancer-bearing rat. As shown in Figure 7, the average weights of tumor nodules in MG7-scFv/SEB- and SEB-treated groups were significantly lighter than that in NS group, suggesting that both SEB and MG7-scFv/SEB have obviously antitumor effect in vivo, especially the latter. The results in Figure 8 showed that the survival times of the rats in MG7-scFv/SEB and SEB groups were longer than that of NS group within 60-day observation ($P < .05$). These results indicated that MG7-scFv/SEB fusion protein effectively inhibited gastric cancer growth in vivo.

As shown in Table 1, extensive inflammatory cells were observed in tumor nodules of gastric-cancer-bearing rats treated with MG7-scFv/SEB; relatively less inflammatory cells were present in the tumors of SEB-treated rats; little inflammatory cells were found inside and around tumors of NS control group.

4. Discussion

SEB, an exotoxin produced by some strains of staphylococcus aureus, is a bacterial superantigen that has been deeply studied in tumor therapy [30, 31, 35]. As a superantigen, SEB can polyclonally activate CTL (cytotoxic T lymphocytes), and massively produce cytokines in vivo [31]. It is capable of killing tumor cells with similar injuring effect on normal cells, to some extent, limiting its clinical application. The application of hybridoma technology and genetic engineering technology to fuse superantigen with a tumor-specific vector to generate a fusion protein that can selectively target tumor cells can effectively limit these adverse effects. The antigen of MG7 had high specificity to gastric cancer, whose positive rate in gastric cancer was 82.8%–91.2% [36, 37]. For this reason, monoclonal antibody against MG7 has been recognized as an effective strategy for targeting therapy of gastric cancer.

In this study, we synthesized scFv against MG7, fused it with SEB to construct MG7-scFv/SEB fusion protein, and detected its biological characteristics. Cell-ELISA results showed that MG7-scFv/SEB fusion protein could specifically target MG7-positive gastric cancer cell SGC-7901 with high affinity, suggesting the correct folding of fusion protein during the process of renaturation. CCK-8 assay indicated that the fusion protein MG7-scFv/SEB had significant inhibitory effect on SGC7901; nevertheless, there was no obvious influence on the cell negative expression of MG7 antigen. Some further experiments showed that MG7-scFv/SEB could mainly increase the production of Th1 cytokines (IL-2, IFN-gamma) and activate cellular immunity to mediate tumor cell apoptosis and necrosis. These results indicated that MG7-scFv/SEB was not only endowed with the specificity target to the MG7 antigen, but also maintained the immunogenicity of superantigen SEB. Given the results we had, SEB was apparently more effective to produce cytokines and inhibit the proliferation of tumor cell than MG7-scFv/SEB at the same concentration. We considered that it was probably due to smaller molecular weight of SEB than that of MG7-scFv/SEB. But taking the antigen-specific antitumor effect into account, MG7-scFv/SEB is more potential for clinical application than SEB as the less cytotoxic effect on normal cells.

We used rat as the recipient in tumor xenograft model because rat has intact immune system which is necessary for specificity of SEB. Although the immune system of rat somehow can reject human tumor cells, the baseline of

rejection is same for all groups. So this model still can tell the difference between different groups even if there is xenograft rejection. Tumor-bearing rats model experiment showed that the average weight of tumor nodules in MG7-scFv/SEB group was significantly lighter than those in SEB or NS group ($P < .05$) at the same injection volume. The survival time of tumor-bearing rats in MG7-scFv/SEB group was prolonged compared with those in other two groups ($P < .05$). The immunohistochemistry results of tumor nodule showed that large number of inflammatory cells (neutrophils, lymphocytes, and monocytes) existed inside and around the tumor nodules in MG7-scFv/SEB-treated group ($P < .05$). The data suggested that MG7-scFv/SEB could effectively activate T cell to inhibit the growth of gastric cancer cell in vivo. Interestingly, the antitumor effect of MG7-scFv/SEB was significantly stronger than that of SEB in the same dosage. Maybe it was due to the specifically target ability to tumor cell of MG7-scFv/SEB.

Taken together, we finished the construction, expression, and purification of fusion protein MG7-scFv/SEB. The fusion protein retains high binding affinity to MG7-positive gastric cancer cell SGC-7901 and the immunogenicity of SEB. These characteristics ensure that MG7-scFv/SEB can specifically kill tumor cell without obvious side effect on normal cell. Our data provided a basis for further investigation of tumor immunotherapy.

Acknowledgments

The authors thank Dr. Gao Shi-tong for his kind gift of the plasmid pMD-18T/SEB. This work was supported by National Natural Science Foundation of China (no. 30901418) and the Natural Science Foundation of Hubei Province, P. R. China (no. NX200513). The first and the second authors contributed equally to this work.

References

[1] M. Garcia, A. Jemal, E. M. Ward, M. M. Center, Y. Hao, and R. L. Siegel, *Global Cancer Facts and Figures 2007*, American Cancer Society, Atlanta, Ga, USA, 2007.

[2] F. Kamangar, G. M. Dores, and W. F. Anderson, "Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world," *Journal of Clinical Oncology*, vol. 24, no. 14, pp. 2137–2150, 2006.

[3] C. Pinto, F. Di Fabio, S. Siena, et al., "Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study)," *Annals of Oncology*, vol. 18, no. 3, pp. 510–517, 2007.

[4] G. R. Thrush, L. R. Lark, B. C. Clinchy, and E. S. Vitetta, "Immunotoxins: an update," *Annual Review of Immunology*, vol. 14, pp. 49–71, 1996.

[5] T. A. Waldmann, "Monoclonal antibodies in diagnosis and therapy," *Science*, vol. 252, no. 5013, pp. 1657–1662, 1991.

[6] G. Hedlund, M. Dohlsten, C. Petersson, and T. Kalland, "Superantigen-based tumor therapy: in vivo activation of cytotoxic T cells," *Cancer Immunology, Immunotherapy*, vol. 36, no. 2, pp. 89–93, 1993.

[7] N. Intasai, K. Tragoolpua, P. Pingmuang, et al., "Potent inhibition of OKT3-induced T cell proliferation and suppression of CD147 cell surface expression in HeLa cells by scFv-M6-1B9," *Immunobiology*, vol. 214, no. 6, pp. 410–421, 2009.

[8] H. Zhang, S. Zhang, N.-K. V. Cheung, G. Ragupathi, and P. O. Livingston, "Antibodies against GD2 ganglioside can eradicate syngeneic cancer micrometastases," *Cancer Research*, vol. 58, no. 13, pp. 2844–2849, 1998.

[9] D. Colcher, A. Goel, G. Pavlinkova, G. Beresford, B. Booth, and S. K. Batra, "Effects of genetic engineering on the pharmacokinetics of antibodies," *Quarterly Journal of Nuclear Medicine*, vol. 43, no. 2, pp. 132–139, 1999.

[10] R. K. Jain, "Transport of molecules across tumor vasculature," *Cancer and Metastasis Review*, vol. 6, no. 4, pp. 559–593, 1987.

[11] D. E. Milenic, T. Yokota, D. R. Filpula, et al., "Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49," *Cancer Research*, vol. 51, no. 23, pp. 6363–6371, 1991.

[12] G. P. Adams, J. E. McCartney, M.-S. Tai, et al., "Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv," *Cancer Research*, vol. 53, no. 17, pp. 4026–4034, 1993.

[13] G. Pavlinkova, G. W. Beresford, B. J. M. Booth, S. K. Batra, and D. Colcher, "Pharmacokinetics and biodistribution of engineered single-chain antibody constructs of MAb CC49 in colon carcinoma xenografts," *Journal of Nuclear Medicine*, vol. 40, no. 9, pp. 1536–1546, 1999.

[14] U. A. Wittel, M. Jain, A. Goel, et al., "Engineering and characterization of a divalent single-chain Fv angiotensin II fusion construct of the monoclonal antibody CC49," *Biochemical and Biophysical Research Communications*, vol. 329, no. 1, pp. 168–176, 2005.

[15] A. Goel, D. Colcher, J. Baranowska-Kortylewiez, et al., "Genetically engineered tetravalent single-chain Fv of the pancarcinoma monoclonal antibody CC49: improved biodistribution and potential for therapeutic application," *Cancer Research*, vol. 60, no. 24, pp. 6964–6971, 2000.

[16] A. L. M. Oei, F. C. G. J. Sweep, L. F. A. G. Massuger, A. J. Olthaar, and C. M. G. Thomas, "Transient human anti-mouse antibodies (HAMA) interference in CA 125 measurements during monitoring of ovarian cancer patients treated with murine monoclonal antibody," *Gynecologic Oncology*, vol. 109, no. 2, pp. 199–202, 2008.

[17] T. Yokota, D. E. Milenic, M. Whitlow, and J. Schlom, "Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms," *Cancer Research*, vol. 52, no. 12, pp. 3402–3408, 1992.

[18] A. A. McCormick, S. Reddy, S. J. Reinl, et al., "Plant-produced idiotype vaccines for the treatment of non-Hodgkin's lymphoma: safety and immunogenicity in a phase I clinical study," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 29, pp. 10131–10136, 2008.

[19] R. E. Bird, K. D. Hardman, J. W. Jacobson, et al., "Single-chain antigen-binding proteins," *Science*, vol. 241, no. 4877, pp. 423–426, 1988.

[20] G. P. Adams and R. Schier, "Generating improved single-chain Fv molecules for tumor targeting," *Journal of Immunological Methods*, vol. 231, no. 1-2, pp. 249–260, 1999.

[21] P. J. Yazaki, T. Kassa, C. Cheung, et al., "Biodistribution and tumor imaging of an anti-CEA single-chain antibody-albumin fusion protein," *Nuclear Medicine and Biology*, vol. 35, no. 2, pp. 151–158, 2008.

[22] D. N. Krag, G. S. Shukla, G.-P. Shen, et al., "Selection of tumor-binding ligands in cancer patients with phage display libraries," *Cancer Research*, vol. 66, no. 15, pp. 7724–7733, 2006.

[23] G. Hedlund, M. Dohlsten, C. Petersson, and T. Kalland, "Superantigen-based tumor therapy: in vivo activation of cytotoxic T cells," *Cancer Immunology Immunotherapy*, vol. 36, no. 2, pp. 89–93, 1993.

[24] B. A. Torres, S. Kominsky, G. Q. Perrin, A. C. Hobeika, and H. M. Johnson, "Superantigens: the good, the bad, and the ugly," *Experimental Biology and Medicine*, vol. 226, no. 3, pp. 164–176, 2001.

[25] J. White, A. Herman, A. M. Pullen, R. Kubo, J. W. Kappler, and P. Marrack, "The Vβ-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice," *Cell*, vol. 56, no. 1, pp. 27–35, 1989.

[26] J. K. Russell, C. H. Pontzer, and H. M. Johnson, "The I-Aβ^b region (65–85) is a binding site for the superantigen, staphylococcal enterotoxin A," *Biochemical and Biophysical Research Communications*, vol. 168, no. 2, pp. 696–701, 1990.

[27] J. K. Russell, C. H. Pontzer, and H. M. Johnson, "Both α-helices along the major histocompatibility complex binding cleft are required for staphylococcal enterotoxin A function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 16, pp. 7228–7232, 1991.

[28] K. Mehindate, J. Thibodeau, M. Dohlsten, T. Kalland, R.-P. Sékaly, and W. Mourad, "Cross-linking of major histocompatibility complex class II molecules by staphylococcal enterotoxin A superantigen is a requirement for inflammatory cytokine gene expression," *Journal of Experimental Medicine*, vol. 182, no. 5, pp. 1573–1577, 1995.

[29] H. Fischer, M. Dohlsten, U. Andersson, et al., "Production of TNF-α and TNF-β by staphylococcal enterotoxin A activated human T cells," *Journal of Immunology*, vol. 144, no. 12, pp. 4663–4669, 1990.

[30] F. G. E. Perabo, P. L. Willert, A. Wirger, D. H. Schmidt, A. von Ruecker, and S. C. Mueller, "Superantigen-activated mononuclear cells induce apoptosis in transitional cell carcinoma," *Anticancer Research*, vol. 25, no. 5, pp. 3565–3573, 2005.

[31] L. Wang, H. Zhang, S. Zhang, M. Yu, and X. Yang, "Construction and characterization of a novel superantigen fusion protein: BFGF/SEB," *Cancer Investigation*, vol. 27, no. 4, pp. 376–383, 2009.

[32] F. G. E. Perabo, P. L. Willert, A. Wirger, et al., "Preclinical evaluation of superantigen (staphylococcal enterotoxin B) in the intravesical immunotherapy of superficial bladder cancer," *International Journal of Cancer*, vol. 115, no. 4, pp. 591–598, 2005.

[33] Z.-C. Yu, J. Ding, B.-R. Pan, D.-M. Fan, and X.-Y. Zhang, "Expression and bioactivity identification of soluble MG7 scFv," *World Journal of Gastroenterology*, vol. 8, no. 1, pp. 99–102, 2002.

[34] S. T. Gao, R. L. Zhang, H. J. Liu, et al., "Sub-cloning of the enterotoxin B gene from a wild *Staphylococcus aureus* strain and its expression in *Escherichia coli*," *Journal of Pathogen Biology*, vol. 2, no. 6, pp. 413–416, 2007.

[35] S. Swaminathan, W. Furey, J. Pletcher, and M. Sax, "Crystal structure of staphylococcal enterotoxin B, a superantigen," *Nature*, vol. 359, no. 6398, pp. 801–806, 1992.

[36] J. Ren, Z. Chen, S. J. Zhou, X. Y. Zhang, B. R. Pan, and D. M. Fan, "Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique," *Cancer*, vol. 88, no. 2, pp. 280–285, 2000.

[37] D.-L. Guo, M. Dong, L. Wang, L.-P. Sun, and Y. Yuan, "Expression of gastric cancer-associated MG7 antigen in gastric cancer, precancerous lesions and H. pylori-associated gastric diseases," *World Journal of Gastroenterology*, vol. 8, no. 6, pp. 1009–1013, 2002.