生物化学与生物物理进展 *Progress in Biochemistry and Biophysics*, 2006, 33(8): 781~788
www.pibb.ac.cn

PIBB

Fusion Immunotoxin Anti-HER-2-scFv-SEC2 Expressed in *E. coli* With an Improved Expression Vector pASK75-EX: Its Construction and Functioning*

XU Ming-Kai[1], ZHANG Cheng-Gang[1]**, ZHANG Hui-Wen[1],
ZHOU Ya-Feng[2], ZHANG Xian-En[2], LIU Li[1]
([1]*Institute of Applied Ecology, The Chinese Academy of Sciences, Shenyang* 110016, China;
[2]*Wuhan Institute of Virology, The Chinese Academy of Sciences, Wuhan* 430071, China)

Abstract A tumor-targeting recombinant fusion immunotoxin B-L-SEC2 was constructed by fusing staphylococcal enterotoxin C2 (SEC2) and an anti-HER-2 single-chain Fv B1 through a peptide linker, and expressed in *E.coli* strain BL21(DE3) with an improved expression vector pASK75-EX as inclusion body. The denatured inclusion body was purified with Ni-NTA chelate agarose, and then re-natured by dialysis. FACS and MTT assays indicated that the re-natured fusion immunotoxin B-L-SEC2 could target the HER-2 over-expressing breast tumor cell SK-Br-3 *in vitro*, and inhibit the growth of SK-Br-3.

Key words staphylococcal enterotoxin C2 (SEC2), HER-2, fusion immunotoxin, tumor- targeting, pASK75

As a kind of superantigen, staphylococcal enterotoxin (SE) has the capability of interacting with the Vβ regions of T cell receptor (TCR) and major histocompatibility complex class Ⅱ molecules (MHC Ⅱ)[1], activating a large number of T cells to release massively cytokines including IL-2, IFN-γ and TNF, producing significant tumor inhibition *in vivo* and *in vitro*[2,3]. As a special member of SEs, SEC2 is a subtype of staphylococcal enterotoxin C (SEC) family, which has been used in China as an effective therapeutic agent for tumor treatment, and some encouraging results have been reported[4]. However, some normal cells express much more MHC Ⅱ than matured tumor cells do, which impairs SEC2 from recognizing tumor targets. Therefore, it is of significance to develop an appropriate vehicle to deliver SEs to tumor cells, especially for therapeutic purposes.

Immunotoxins with a targeting molecule linked to a cytotoxic agent have been widely used as therapeutic agents for cancers[5]. Recently, single chain Fv antibody fragment (scFv) is commonly used for targeting purpose, because its small size is suitable for drug development. Like many other tumor-associated antigens, Her2/neu, also known as c-erbB-2, is an oncogene product which is overexpressed on the cell surface of human breast and ovarian tumors by 25%～30% localized[6,7]. In the last decade, anti-HER-2 scFvs have been widely used as effective vehicles for targeting treatment of HER-2 over expressing tumors[8~11].

In this study, a novel fusion immunotoxin was constructed by fusing SEC2 and HER-2 specific scFv, and successfully expressed and purified. This immunotoxin retained anti-HER-2 scFv specificity and SEC2 bioactivity, and compared with native SEC2, had an enhanced antitumor activity against HER-2 overexpressing tumors *in vitro*.

1 Materials and methods

1.1 Materials

1.1.1 Cell line and bacteria strain. Human breast cancer cells SK-Br-3 and MCF-7 were provided by

*This work was supported by a grant from Sciece and Technology Project of Liaoning (2001305003)
**Corresponding author . Tel: 86-24-83970378, Fax: 86-24-83970300, E-mail: chgzhang0118@yahoo.com.cn
Received: March 2, 2006 Accepted: April 20, 2006

Wuhan Tongji Medical University, and cultured in RPMI 1640 supplemented with 10% FBS (Fetal Bovine Serum), 100 U/ml penicillin G and 100 mg/L streptomycin. *E.coli* BL21(DE3) in LB medium was purchased from Novagen Company (USA).

1.1.2 Vector. Expression vector pASK75 was purchased from Biometra Company (Germany). Plasmid pGEM-T-sec2 containing sec2 gene was from our laboratory[12]. Anti-HER-2 scFv plasmid pCynB1 was kindly provided by Dr. Jame Marks, University of California, San Francisco.

1.2 Methods

1.2.1 Construction of expression vector pASK75-EX. Two oligonucleotide fragments containing the recognition sites of normal restriction endonucleases were designed, based on the original expression vector plasmid pASK75. Fragment P-F contained a *Stu* Ⅰ end (sense chain) 5′ CC TGG CCC AGC CGG CCC ATG GCA TGC GAA TTC GCG GCC GCA GAT CTG CTC GAG CTG CAG CAT CAT CAT CAT CAT CAT A 3′, while fragment P-R contained a *Hin*d Ⅲ stick end (antisense chain) 5′ A GCT ATG ATG ATG ATG ATG ATG GTC CAG CTC GAG CAG ATC TGC GGC CGC GAA TTC GCA TGC CAT GGG CCG GCT GGG CCA GG 3′. These two fragments were annealed, and ligated to the pASK75 plasmid digested by *Stu* Ⅰ and *Hin*d Ⅲ to construct the expression vector pASK75-EX.

1.2.2 Production of scFv and sec2 DNA.

Two oligonucleotide polymerase chain reaction (PCR) primers for scFv-b were designed. Primer V-F contained a *Nco* Ⅰ restriction site (sense primer) 5′ TTA TCC ATG GCC CAG GTG CAG CTG GTG CAG TCT 3′, while primer V-R contained a *Not* Ⅰ restriction site (antisense primer) 5′ TTC TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT C A 3′. The plasmid DNA of pCynB1 was used as a template for PCR, and PCR amplification was performed in the procedure of 5 min at 94℃, followed by 30 cycles of 45 s at 94℃, 45 s at 55℃ and 2 min at 72℃, and 10 min at 72℃. The PCR amplification products were purified by electrophoresis on a 1% TAE-agarose gel then digested by endonucleases *Nco* Ⅰ and *Not* Ⅰ, and gel-purified again.

Two other oligonucleotide PCR primers for sec2 were designed. Primer S-F contained an *Eco*R Ⅰ restriction site (sense primer) 5′ TCT GAA TTC GAG AGT CAA CCA GAC CCT A 3′, while primer S-R contained an *Xho* Ⅰ restriction site (antisense primer) 5′ ATA CTC GAG TCC ATT CTT TGT TGT A 3′. The plasmid DNA of pGEM-T-sec2 was used as a template for PCR, and PCR amplification was performed in the procedure of 5 min at 94℃, followed by 30 cycles of 45 s at 94℃, 45 s at 58℃ and 1.5 min at 72℃, and 10 min at 72℃. The PCR amplification products were gel-purified then digested by endonucleases *Eco*R Ⅰ and *Xho* Ⅰ, and gel-purified again.

1.2.3 Construction of expression vector pASK75-EX-b-l-sec2

Two annealed oligonucleotide linkers were designed to ligate the genes of scFv and sec2 to fusion immunotoxin gene. Linker L1 contained a *Not* Ⅰ stick end (sense chain) 5′ G GCC GCA AGC GGC TCA GGA TCT GGA TCA GGA TCT GGC G 3′, while linker L2 contained an *Eco*R Ⅰ stick end (antisense chain) 5′ AA TTC GCC AGA TCC TGA TCC AGA TCC TGA GCC GCT TGC 3′.

The expression vector plasmid pASK75-EX DNA fragments digested by *Nco* Ⅰ and *Xho* Ⅰ, scFv-b DNA fragments, sec2 DNA fragments, and the annealed linker were mixed with a proportion of 1∶3∶3∶3, ligated overnight to construct fusion immunotoxin expression vector pASK75-EX-b-l-sec2, and transformed into *E.coli* BL21(DE3)[13]. The constructed fusion gene contained a fused His-tag for protein purification.

1.2.4 Expression and purification of fusion immunotoxin B-L-SEC2. Single colony *E. coli* strain BL21(DE3) with plasmid pASK75-EX-b-l-sec2 was grown overnight at 37℃ in LB medium containing 100 mg/L ampicillin. The cultures were diluted 50-fold in the same medium and grown at 37℃ to an A_{600} of 0.6～0.8. Fusion immunotoxin expression was induced with 0.2 mg/L anhydrotetracycline for 6 h at 37℃. Cells were harvested by 4 000 *g* centrifugation for 10 min at 4℃, re-suspended in ice-cold PBS (Phosphate-buffered Saline), and disrupted by sonication at 0℃. The inclusion bodies were isolated by 10 000 *g* centrifugation for 30 min at 4℃, washed in PBS containing 2 mol/L urea, and re-suspended in buffer A (20 mmol/L Tris-HCl, pH 7.5, 0.5 mol/L NaCl, 8 mol/L urea) for 30 min at room temperature. The suspension was clarified by 12 000 *g* centrifugation for 30 min at 4℃, and the supernatants were collected and loaded onto the Ni^{+}-saturated chelating sepharose column equilibrated with buffer A. After nonspecifically bound protein was washed off

with buffer A containing 40 mmol/L imidazole, the specifically bound protein B-L-SEC2 was eluted with buffer A containing 200 mmol/L imidazole, re-natured by rapid 1 ∶10 dilution into PBS containing 400 mmol/L L-arginine at 4℃, and subsequently dialyzed against PBS. The purity of the re-natured protein was determined by SDS-PAGE and Coomassie-brilliant-blue staining[14].

1.2.5 Western blotting analysis of B-L-SEC2. The re-natured immunotoxin B-L-SEC2 was analyzed with SDS-PAGE and then transferred into nitrocellulose membrane. The membrane was blocked with blocking solution (2% non-fat dried milk powder in PBS with 0.5% Tween 20) and then incubated with rabbit anti-SEC2 antibody diluted in blocking solution. Alkaline phosphatase-labeled goat anti-rabbit IgG was used as the secondary antibody and specific binding was detected with NBT/BCIP.

1.2.6 Cell-surface binding specificity of fusion immunotoxin B-L-SEC2. The cell-surface binding specificity of B-L-SEC2 was performed by fluorescence-activated cell sorting (FACS) analysis. Tumor cells MCF-7 and SK-Br-3 were washed two times and re-suspended to 0.5×10^6～1.0×10^6 cells/ml in PBA (PBS containing 2% BSA). The purified B-L-SEC2 was added to a final concentration of 100 mg/L, and incubated for 1h at 4℃. Tumor cells were washed twice and incubated subsequently with rabbit anti-His-tag antibody (primary antibody) and FITC-labeled goat anti-rabbit IgG (secondary antibody) diluted in PBA for 45 min at 4℃. In the negative control, the cells not exposed to B-L-SEC2 were incubated with primary and secondary Abs. Cells were washed and re-suspended in PBA, and detected by flow cytometry.

1.2.7 Stimulation of PBMC proliferation by fusion immunotoxin. Peripheral blood mononuclear cells (PBMCs) from healthy donor blood were isolated by ficoll density gradient centrifugation, and aliquoted to 2×10^5 cells/well in 96-well plate in RPMI 1640 supplemented with 10% FBS. A series of concentrations of purified fusion immunotoxin B-L-SEC2 and standard SEC2 were added to triplicate wells. BSA and phytohemagglutinin-P (PHA-P) were used as negative and positive controls, respectively. After 96 h incubation at 37℃ in 5% CO_2, 50 μl 5 g/L 3- (4,5-dimethylthiazole-2-yl)-2,5 diphenyltetrazolium bromide (MTT) in PBS was added into each well, and the incubation was continued for another 4 h. The crystals were collected by 500 *g* centrifugation for 10 min at room temperature and re-dissolved in 120 μl DMSO (Dimethyl Sulfoxide) for 20 min. The absorbance was measured at a wavelength of 570 nm in a microplate reader.

1.2.8 *In vitro* anti-tumor activity of fusion immunotoxin. Tumor cells SK-Br-3 or MCF-7 were seeded in 96-well plates at a density of 1×10^4 cells/well in RPMI 1640 supplemented with 10% FBS. Different concentrations of purified B-L-SEC2 and standard SEC2 were added separately to triplicate wells, and the cells were incubated for 4 h before 2×10^5 PBMC cells added. The blank wells (RPMI 1640 only), unsettled tumor cell wells (tumor cells only), and PBMC releasing wells (PBMC and toxin protein) were used as controls. The negative control was BSA.

After incubation at 37℃ for 72 h, the medium was removed from each well, and 150 μl fresh medium and 50 μl 5 g/L MTT in PBS were added into each well. Incubation was continued for another 4 h. The crystals were collected by 500 *g* centrifugation for 10 min at room temperature and re-dissolved in 120 μl DMSO for 20 min. The absorbance was measured at a wavelength of 570 nm in a microplate reader. The anti-tumor activity was calculated by the formula:

Tumor growth inhibition/% = 100 – ((A_{570} of protein treated cells well- A_{570} of PBMC releasing wells) / (A_{570} of unsettled tumor cells control wells - A_{570} of blank control wells)) ×100%

2 Results

2.1 Construction of expression vector pASK75-EX

Figure 1a outlines the construction of expression vector pASK75-EX. Strap-tag was replaced by His-tag to reduce the cost of purifying expressed foreign proteins. The constructed pASK75-EX was verified by digestion with restriction endonucleases whose recognition sites only existed in the new cloning/expression region (Figure 1b). DNA sequencing results verified this design. Compared to the original pASK75, there were more normal restriction endonuclease sites in the new cloning/expression region of pASK75-EX, which was convenient for the cloning and expression of foreign genes.

2.2 Construction of expression vector plasmid pASK75-EX-b-l-sec2

Anti-HER-2 scFv gene scFv-b (～770 bp) and SEC2 gene sec2 (～720 bp) were amplified by PCR,

and analyzed by electrophoresis in a 1%TAE-agarose gel (Figure 2a). These two genes were linked with DNA linker, and ligated into pASK75-EX plasmid to construct expression vector pASK75-EX-b-l-sec2 for protein B-L-SEC2 (Figure 2c). The constructed pASK75-EX-b-l-sec2 was verified by double digestion (Figure 2b) and DNA sequencing.

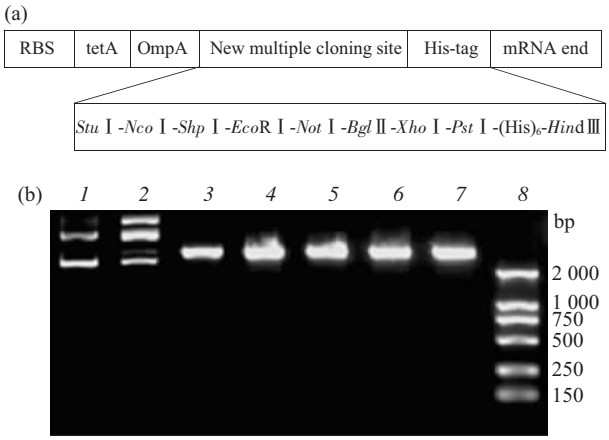

Fig. 1 Construction of expression vector pASK75-EX

(a) Cloning/expression region of constructed expression vector pASK75-EX. (b) Electrophoretogram of digested pASK75-EX (0.8% TAE-agarose gel). *1*: Original pASK75 without digestion, *2*: pASK75-EX without digestion, *3* ~*7*: Single RE digestion of pASK75-EX by *Sph* Ⅰ, *Eco*R Ⅰ, *Bgl* Ⅱ, *Xho* Ⅰ, *Pst* Ⅰ, respectively, *8*: DL 2000 DNA marker.

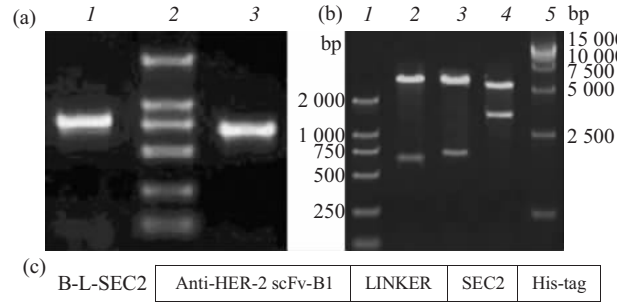

Fig. 2 Construction of expression vectors pASK75-EX-b-l-sec2

(a) PCR products of scFv-b gene and SEC2 gene. *1*: scFv-b1, *2*: DL2000 DNA marker, *3*: sec2. (b) Verification of expression vector pASK75-EX-b-l-sec2 by double RE digestion. *1*: DL 2000 DNA marker, *2*: pASK75-EX-b-l-sec2 digested by *Eco*R Ⅰ and *Xho* Ⅰ, *3*: pASK75-EX-b-l-sec2 digested by *Nco* Ⅰ and *Not* Ⅰ, *4*: pASK75-EX-b-l-sec2 digested by *Nco* Ⅰ and *Xho* Ⅰ, *5*: DL 15 000 DNA Marker. (c) Structure of fusion immunotoxion B-L-SEC2.

2.3 Expression and purification of fusion immunotoxin B-L-SEC2

The expression of B-L-SEC2 was induced with anhydrotetracycline. After sonication and centrifugation, the bacterial supernatants were collected, and the pellets were washed with PBS containing 2 mol/L urea and re-suspended in buffer A, 1/5 of the supernatant volume. SDS-PAGE data showed the expected protein of about 60 ku in the inclusion bodies of the bacteria (Figure 3a). B-L-SEC2

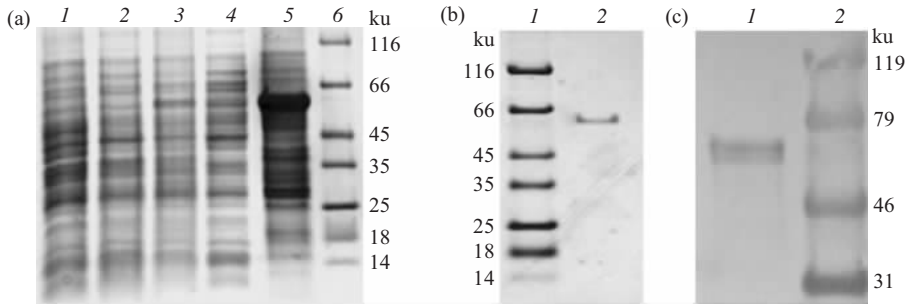

Fig. 3 Expression, purification and Western blotting of fusion immunotoxion B-L-SEC2

(a) Expression. *1*: Induced BL21(DE3)/pASK75-EX, *2*: Un-induced BL21(DE3)/ pASK75-EX-b-l-sec2, *3*: Total protein expression of BL21 (DE3)/pASK75-EX-b-l-sec2, *4*: Soluble expression of BL21 (DE3)/pASK75-EX-b-l-sec2, *5*: Inclusion expression of BL21(DE3)/pASK75-EX-b-l-sec2 (5 folds), *6*: Protein molecular mass marker. (b) Purification. *1*: Protein molecular mass marker, *2*: Re-natured B-L-SEC2. (c) Western blotting. *1*: B-L-SEC2, *2*: Prestained protein molecular mass marker.

was purified from solubilized inclusion bodies *via* a single round of Ni^{2+} affinity chromatography, and re-natured by rapid dilution and subsequent dialysis. The yield of re-natured fusion protein was about 30 mg/L of original bacterial culture, with a purity of more than 95% as determined by SDS-PAGE analysis (Figure 3b). Western blotting showed that the re-natured fusion protein could be recognized by rabbit anti-SEC2 antibody (Figure 3c).

2.4 Cell-surface binding specificity of fusion immunotoxin B-L-SEC2

FACS analysis showed that the cell-surface binding of B-L-SEC2 to HER-2 overexpressing SK-Br-3 tumor cells caused a peak shift in the fluorescence measurement, compared to MCF-7 cells without HER-2 overexpression (Figure 4), suggesting that B-L-SEC2 could target HER-2 overexpressing tumor cells specifically.

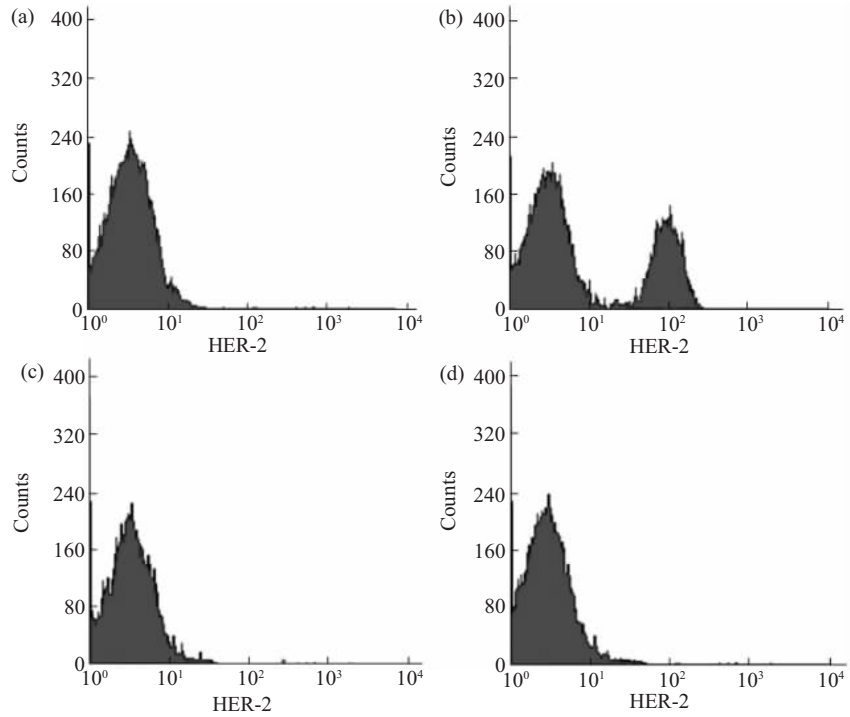

Fig. 4 Cell-surface binding ability of fusion immunotoxin B-L-SEC2 to HER-2 overexpressing tumor cells

(a) Negative control of HER-2 overexpressing cell line SK-Br-3. (b) Binding of B-L-SEC2 to HER-2 overexpressing cell line SK-Br-3. (c) Negative control of no HER-2 overexpressing cell line MCF-7. (d) Binding of B-L-SEC2 to no HER-2 overexpressing cell line MCF-7.

2.5 Stimulation of PBMC proliferation by fusion immunotoxin

The re-natured fusion protein B-L-SEC2 was used to stimulate PBMC proliferation, with BSA and PHA-P as negative and positive controls, respectively. Figure 5 showed that similar to the standard SEC2, B-L-SEC2 was very efficient ($P > 0.05$) in stimulating PBMC proliferation even at a concentration of 50 μg/L, indicating that the fused scFv fragment did not inhibit the PBMC proliferation stimulation activity of SEC2 in the constructed fusion immunotoxin.

2.6 *In vitro* anti-tumor activity of fusion immunotoxin

In comparing with standard SEC2, the re-natured fusion immunotoxin B-L-SEC2 demonstrated an enhanced inhibition activity against the growth of HER-2 overexpressing SK-Br-3 tumor cells, but had a less effect on the growth of MCF-7 cells (Figure 6), which indicates that the constructed immunotoxin had the anti-tumor activity specially targeting HER-2 overexpressing tumor cells.

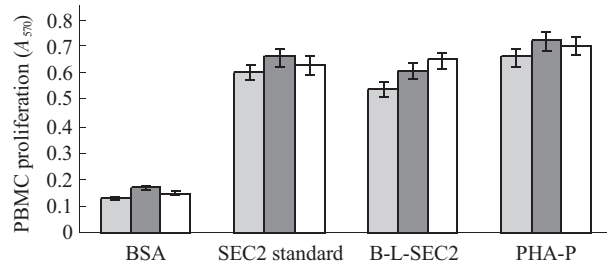

Fig. 5 Stimulation of PBMC proliferation by fusion immunotoxin B-L-SEC2

□: 20 ng; ■: 50 ng; □: 200 ng.

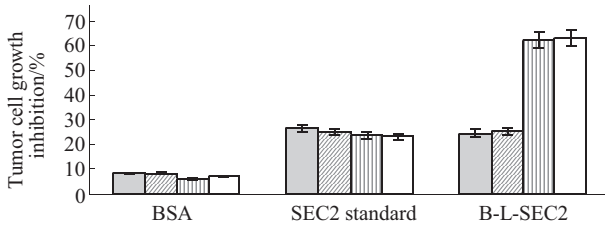

Fig. 6 *In vitro* anti-tumor activity by fusion immunotoxin B-L-SEC2

□: 100 ng to MCF-7; ▨: 200 ng to MCF-7; ▥: 100 ng to SK-Br-3; □: 200 ng to SK-Br-3.

3 Discussion

As a superantigen, SEs can direct T cell cytotoxicity against the MHC Ⅱ-positive cells they are bound to [15]. Feasible clinical application of superantigen-based antitumor strategies requires a compromising MHC Ⅱ binding capability. However, for human, the expressed MHC Ⅱ are finally localized on the surface of normal but not tumor cells, which causes the lack of tumor targeting in tumor therapy. The ability of high dosage superantigen to cause T cell anergy or deletion *in vivo* has also been documented [16,17]. Therefore, designing appropriate tumor-targeting vehicles to deliver SEs to tumor cells specifically could improve the efficiency of tumor therapy.

The HER-2 receptor has been shown to play an important role in tumor development and progression. In particular, overexpression of HER-2 receptor has been correlated with poor clinical outcomes in sub-sets of human malignancies[18]. Considering that expression of the HER-2 receptor, is higher on tumor cell surfaces and lower on normal cell surfaces, anti-tumor therapeutical effects could be increased by specifically targeting to HER-2. Antibodies specific to the extracellular domains of such receptors could be used to deliver highly cytotoxic moieties such as radionuclides, toxins or chemotherapeutic agents to HER-2 overexpressing tumors. Under such conditions, it would be desirable to efficiently minimize non-targeted toxicity to normal organs lacking HER-2 during the tumor targeting process. Because of their small size, scFvs are the most promising candidates for this purpose. Therefore, linking anti-HER-2 scFvs to cytotoxic effectors has great potential for growth suppression of HER-2 overexpressing tumor cells.

The production of heterologous proteins is often accompanied by an impaired growth of *E. coli* cells. Consequently, a regulation of heterologous biosynthesis is generally required. In the case that foreign protein is cytotoxic, a small quantity can damage or even kill *E. coli* cells harboring the expression plasmid. In such cases, a strong repression of the promoter is needed. Expression vector pASK75 carries the promoter/operator region from tetA resistance gene, and can be considered as a better candidate for such an inducible expression system [19]. The strength of the tetA promoter is comparable with that of lac-UV5 promoter. It can be fully induced by adding 0.2 mg/L of anhydrotetracycline, a concentration that is not antibiotically effective. The constitutive expression of the tet repressor gene, which is also encoded on the pASK75, guarantees the repression of the promoter in the absence of the inducer. In addition, the tetA promoter/operator is not coupled to any cellular regulation mechanism. Therefore, when using pASK75, there are no restrictions in the choice of culture medium or *E. coli* expression strain. Unfortunately, pASK75 lacks normal restriction endonuclease sites in its cloning/expression region, which limits its application. Also, the purification procedure using Strep-tag in pASK75 is costly, which is not fit for large scale foreign protein preparation. In this study, an improved expression vector pASK75-EX was constructed based on the original pASK75. Compared to the original one, the improved vector had more normal restriction endonuclease sites in the cloning/expression region, and the Strap-tag was replaced by His-tag to reduce the cost of purification of the expressed foreign proteins. Our results showed the validity of His-tag in pASK75-EX, and B-L-SEC2 expressed by pASK75-EX could be purified with Ni^{2+}-affinity chromatography. The OmpA signal peptide remained in pASK75-EX, but the expressed B-L-SEC2 could not be detected in the *E. coli* periplasmic space. Most of the fusion immunotoxin B-L-SEC2 was expressed as insoluble inclusion body, and the same phenomena have also been observed for other inducing conditions. A possible interpretation is that the large proportion of hydrophobic amino acids in scFv fragment inhibits transport into the periplasmic space as well as correct folding of the expressed B-L-SEC2.

In this study, the expression, purification and characterization of a novel fusion immunotoxin consisting of SEC2 and anti-HER-2 scFv pCynB1 were described. Compared with standard SEC2, the

purified and re-natured fusion immunotoxin B-L-SEC2 displayed antigen binding specificity and an enhanced growth-inhibitory effect on HER-2 overexpressing tumor cells *in vitro*. This anti-tumor targeting activity of B-L-SEC2 could be of great therapeutical value and should be further evaluated *in vivo*.

Acknowledgements We thank Dr. J. D. Marks, University of California, San Francisco for providing the pCynB1 plasmid and Prof. C. E. McKenna of University of Southern California for critically reading this manuscript.

References

1 Balaban N, Rasooly A. Staphylococcal enterotoxins. Int J Food Microbio, 2000, **61**: 1～10

2 Dohlsten M, Sundstedt A, Bjorklund M, *et al*. Superantigen-induced cytokines suppress growth of human colo-carcinoma cells. Int. Cancer, 1993, **54**: 482～488

3 Pardoll D M. Parakrine cytokine adjuvants in cancer immunotherapy. Annu Rev Immunol, 1995, **13**: 399～415

4 陈廷柞. 高聚生研发历程述评及其用于癌症治疗的理论基础. 微生物学免疫学进展, 2001, **29** (2): 63～69
Chen T Z. Prog Microbiol Immunol, 2001, **29** (2): 63～69

5 Kreitman R J. Recombinant toxins for the treatment of cancer. Curr Opin Mol Ther, 2003, **5** (1): 44～51

6 Slamon D J, Goldophin W, Jones L A, *et al*. Studies of the Her-2/neu proto-oncogene in human breast and ovarian cancer. Science, 1989, **244**: 707～712

7 Berchuck A, Kamel A, Whitaker R, *et al*. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res, 1990, **50**: 4087～4091

8 Marshall K W, Marks J D. Engineering and characterization of a novel fusion protein incorporating B7.2 and an anti-ErbB-2 single-chain antibody fragment for the activation of jurkat T cells. J Immunother, 2001, **24** (1): 27～36

9 Schmidt M, McWatters A, White R A, *et al*. Synergistic interaction between an anti-p185^{HER-2} pseudomonas exotoxin fusion protein [scFv(FRP5)-ETA] and ionizing radiation for inhibiting growth of ovarian cancer cells that overexpress HER-2. Gynecol Oncol, 2001, **80** (2): 145～155

10 Peng L S, Penichet M L, Morrison S L. A single-chain IL-12 IgG3 antibody fusion protein retains antibody specificity and IL-12 bioactivity and demonstrates antibody activity. J Immunol, 1999, **163** (1): 250～258

11 Altenschmidt U, Schmidt M, Groner B, *et al*. Targeted therapy of schwannoma cells in immunocompetent rats with an erbB2-specific antibody-toxin. Int J Cancer, 1997, **73** (1): 117～124

12 徐明恺, 张成刚, 周亚凤, 等. 金黄色葡萄球菌肠毒素 C2 的基因克隆、表达及其生物学活性. 生物化学与生物物理进展, 2005, **32** (3): 275～281
Xu M K, Zhang C G, Zhou Y F, *et al*. Prog Biochem Biophys, 2005, **32** (3): 275～281

13 Sambrook J, Fritsch E F, Maniatis T. Molecular Cloning. 3rd. New York: Cold Spring Harbor Laboratory Press, 2002. 96～99

14 Ausubel F M, Brent R, Kingston R E, *et al*. Short Protocols In Molecular Biology. 3rd. New York: John Wiley & Sons, Inc, 1995. 338～340

15 Kalland T, Hedlund G, Dohlsten M, *et al*. Staphylococcal enterotoxin-dependent cell-mediated cytotoxicity. Curr Top Microbiol Immunol, 1991, **174**: 81～92

16 Wahl C, Miethke T, Heeg K, *et al*. Clonal deletion as direct consequence of an *in vivo* T cell response to bacterial superantigen. Eur J Immunol, 1993, **23** (5): 1197～1200

17 MacDonald H R, Baschieri S, Lees R K. Clonal expansion precedes anergy and death of V beta 8^+ peripheral T cells responding to staphylococcal enterotoxin B *in vivo*. Eur J Immunol, 1991, **21** (8): 1963～1966

18 Hynes N E, Stern D F. The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta, 1994, **1198**: 165～184

19 Skerra A. Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in *Escherichia coli*. Gene, 1994, **151** (1～2): 131～135

抗 Her-2-scFv-SEC2 融合免疫毒素的构建和功能研究 *

徐明恺[1] 张成刚[1]** 张惠文[1] 周亚凤[2] 张先恩[2] 刘 丽[1]

([1]中国科学院沈阳应用生态研究所，沈阳 110016；[2]中国科学院武汉病毒研究所，武汉 430071)

摘要 用基因工程方法，将金黄色葡萄球菌肠毒素 C2 与抗人表皮生长因子受体 HER-2 单链抗体 scFv-B1，以一连接短肽连接，构建融合免疫毒素 B-L-SEC2，并用改进的新型表达载体 pASK75-EX，在大肠杆菌 BL21(ED3)中表达. 以不溶性包涵体形式表达的目的蛋白经变性后以镍离子螯和层析纯化，并以透析法进行复性. 流式细胞术和 MTT 实验结果表明，纯化复性的融合免疫毒素 B-L-SEC2，在体外具有与 HER-2 过表达的靶细胞 SK-Br-3 特异性结合的活性，并对该细胞产生显著的特异性生长抑制作用.

关键词 金黄色葡萄球菌肠毒素 C2，抗人表皮生长因子受体 HER-2，融合免疫毒素，肿瘤靶向， pASK75 表达载体

学科分类号 Q78

---

*辽宁省科技计划项目(2001305003).

** 通讯联系人. Tel: 024-83970378, Fax: 024-83970300, E-mail: chgzhang0118@yahoo.com.cn

收稿日期：2006-03-02，接受日期：2006-04-20