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• BASIC RESEARCH •

Expression and bioactivity identification of soluble MG7 scFv

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Abstract

AIM: **To examine the molecular mass and identify the bioactivity of MG_7 scFv for its application as a targeting mediator in gene therapy of gastric cancer.**

METHODS: **Two strongly positive recombinant phage clones screened from MG_7 recombinant phage antibody library were separately transfected into *E.coli* TG1. Plasmid was isolated from the transfected *E.coli* TG1 and digested by EcoR I and Hind Ⅲ to examine the length of exogenous scFv gene. Then, the positive recombinant phage clones were individually transfected into *E.coli* HB2151.The transfectant was cultured and induced by IPTG. Perplasmic extracts was prepared from the induced transfectant by osmotic shock. ELISA was used to examine the antigen-binding affinity of the soluble MG_7 scFv. Immunodoting assay was adopted to evaluate the yield of soluble MG_7 scFv produced by transfected *E.coli* HB2151. Western blot was used to examine the molecular mass of MG_7 scFv. Finally, the nucleotide sequence of MG_7 scFv was examined by DNA sequencing.**

RESULTS: **two positive recombinant phage clones were found to contain the exogenous scFv gene. ELISA showed that MG_7 scFv had strong antigen-binding affinity. Immuodoting assay showed that transfected *E.coli* HB2151 could successfully produce the soluble MG_7 scFv with high yield via induction by IPTG. The molecular mass of MG_7 scFv was 30 kDa by western blot. DNA sequencing demonstrated that the V_H and V_L genes of MG_7 scFv were 363bp and 321bp, respectively.**

CONCLUSION: **We have successfully developed the soluble MG_7 scFv which possessed strong antigen-binding affinity.**

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INTRODUCTION

Gastric cancer takes the leading place in the incidence of various tumors in china[1]. Many conventional approaches, including surgical, chemical and physical treatments, appear palliative in most advanced cases. Because these conventional approaches cannot selectively target at the tumor cells and completely eradicate them, and recurrence and metastasis of tumor may develop due to the existence of residual tumor cells. Therefore, targeting therapy for tumor is badly needed to achieve a greater curative effect and overcome the obstacle existing in the conventional approaches[2-13]. Recent studies showed that the targeting therapy had a promise in the treatment of gastric cancer[14-29]. In the present study, we produced the soluble MG_7 scFv and evidenced that MG_7 scFv is a favorable mediator for targeting therapy of gastric cancer.

MATERIALS AND METHODS

Restriction analysis of the strong recombinant phage clones

The strong positive recombinant phage clones (3 μL containing 2×10^9 pfu) were separately added into 5mL log-phase *E.coli* TG1 and incubated for 1 h at 37℃ with shaking at 250 $r\cdot min^{-1}$. Plasmid was isolated from the culture product and digested by *Eco*R I and *Hind* Ⅲ. Electrophoresis was performed to check the digested product.

Production and antigen-binding affinity test of the soluble MG_7 scFv

The strongly positive recombinant phage clones (3 μL containing 2×10^9 pfu) were separately added into 5mL log-phase *E.coli* HB2151 and incubated for 1 h at 37℃ with shaking at 250 $r\cdot min^{-1}$. 10 μL IPTG (isopropyl β-D-thiogalactopyanoside) were added and incubated overnight at 37℃ with shaking at 250 $r\cdot min^{-1}$. Cells were precipitated by centrifugation and supernatant was also collected. The precipitated cells were subjected to osmotic shock for preparation of soluble MG_7 scFvs. KATOⅢ cells in log phase were transferred into a 96 wells-plate and immobilized on the wall by centrifiguation at 1000g for 10min, and finally fixed in 0.25$mL\cdot L^{-1}$ glutaraldehyde. 0.2 mL perplasmic extracts and supernatant were applied to each well and incubated at 4℃ overnight. 0.2 mL anti-E tag antibody were applied to each well and incubated at 37℃ for 2 h. 0.1 ml HRP-labeled goat anti-mouse (HRP-GAM) Ig solution was added into each well. The absorbance value (A) at 450nm of reactant in each well was measured after incubation for 1 h at 37℃ and staining with TMB.

Immunodoting test of the yield of soluble MG_7 scFv

40 μL of perplasmic extracts and supernatant were separately dotted on the Hybond-C super membrane and dried at 60℃ for 30 min. After being blocked by 50 $mL\cdot L^{-1}$ nonfat milk for 2 h, the Hybond-C super membrane was incubated in 5mL diluted anti-E tag antibody solution at 37℃ for 2 h. 5 mL HRP- labeled goat anti-mouse (HRP-GAM) Ig solution were added for another incubation at 37℃ for 1 h and eventually stained by DAB.

Western blot test of the molecular mass of soluble MG_7 scFv

40 μL of perplasmic extracts and supernatant were firstly analysized by SDS-PAGE and then transferred onto the Hybond-C super membrane. After being blocked by 50 $mL\cdot L^{-1}$nonfat milk, the Hybond-C super membrane was incubated in 5mL diluted anti-E tag antibody solution at 37℃ for 2 h. 5 mL of HRP- labeled goat anti-mouse (HRP-GAM) Ig solution were added for another incubation at 37℃ for 1 h and

eventually stained by DAB.

DNA sequencing of MG_7 scFv

DNA sequencing was performed by ABI PRISM™ 377 DNA sequencer.

RESULTS

Restriction analysis of the strong positive recombinant phage clones

Two strongly positive clones were found to be recombinant clones which contained the exogenous gene. One of the two strongly positive clones contained a 450bp fragment of exogenous gene (Lane 1)and the other one contained a 750bp fragment of exogenous gene(Lane 2, Figure 1).

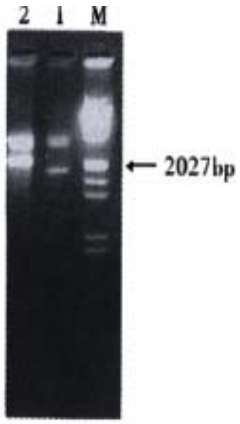

Figure 1 Restriction analysis of the strong positive recombinant phage clones. M: λ/EcoR I and Hind II; 1,2: Recombinant clones

Antigen-binding affinity of the soluble MG_7 scFv by ELISA

One of the strong positive clones was shown to produce soluble form of MG_7 scFv with Antigen-binding activity (Table 1). This clone was confirmed to contain a 750bp fragment of exogenous gene by restriction analysis above and chosen for later use.

Table 1 ELISA of the soluble MG_7 ScFv for binding with KATOIII cells (*A* value)

ELISA	Number of strong positive clones		Neg. ctrl
	1	2	
First round	0.287	0.776	0.201
Second round	0.346	0.802	0.223

The yield of soluble MG_7 scFv

The positive signal displayed on the dotting site with perplasmic extracts from induced *E.coli* HB2151 was significantly stronger than that from non-induced *E.coli* HB2151 (Figure 2). It implied that *E. coli* HB2151 had successfully produced the soluble MG_7 scFv via induction by IPTG.

Figure 2 Immunodoting of soluble MG_7 scFv.1: Perplasmic extracts from induced *E.coli* HB2151; 2: Supernatant from induced *E.coli* HB2151; 3: Perplasmic extracts from non-induced *E.coli* HB2151

The molecular mass of MG_7 scFv

A protein band with positive signal was found at *Mr* 30 indicating that the soluble MG_7 scFv was a protein of *Mr* 30 (Figure 3).

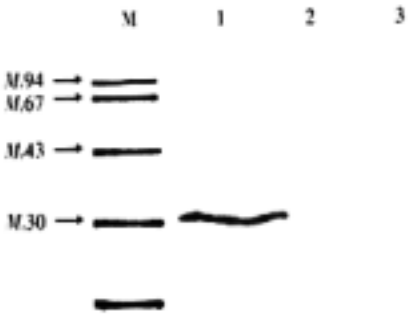

Figure 3 Western blot of the molecular mass of soluble MG_7 scFv. M: Protein marker; 1: Perplasmic extracts from induced *E.coli* HB2151; 2: Supernatant from induced *E.coli* HB2151; 3: Perplasmic extracts from non-induced *E.coli* HB2151

DNA sequence of MG_7 scFv

The V_H and V_L genes of MG_7 scFv were respectively 363 bp and 321 bp in length. The V_H gene has two conserved codon for cysteine at 67-69bp and 289-291bp.The V_L gene has two conserved codon for cysteine at 472-474bp and 664-666bp. Both of the V_H and V_L genes are highly homologous with the variable fragment of some known antibodies (Figure 4).

ATG GCC CAG GTG AAG CTG CAG CAG TCT GGG CCT GAA GTG GTA AAG CCT GGG GCT TCA GTG AAG TTG TCC TGC AAG GCT TCT GGC TAC ATC TTC ACA AGT TAT GAT ATA GAC TGG GTG AGG CAG ACG CCT GAA CAG GGA CTT GAG TGG ATT GGA TGG ATT TTT CCT GGA GAG GGG AGT ACT GAA TAC AAT GAG AAG TTC AAG GGC AGG GCC ACA CTG

VH

AGT GTA GAC AAG TCC TCC AGC ACA GCC TAT ATG GAG CTC ACT AGG CTG ACA TCT GAG GAC TCT GCT GTC TAT TTC TGT GCT AGA GGG GAC TAC TAT AGG CGC TAC TTT GAC TTG TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC GAG CTC ACT CAG

Linker

TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AGG GTC ACC ATG ACC TGC AGT GCC AGC TCA AGT ATA CGT TAC ATA TAT TGG TAC CAA CAG AAG CCT GGA TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC GTG GCT

VL

CCT GGA GTC CCT TTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAT TCT CTC ACA ATC AAC CGA ATG GAG GCT GAG GAT GCT GCC ACT TAT TAC TGC CAG GAG TGG AGT GGT TAT CCG TAC ACG TTC GGA GGG GCA CCA AGC TGG GAA ATC AAA CGG

Figure 4 Nucleotide sequence of MG_7 scFv

DISCUSSION

MG_7, a monoclonal antibody against human gastric cancer, was proved to possessa quite high specificity and sensitivity to gastric cancer associated antigen. It was successfully used in experimental targeting therapy in nude mice bearing transplanted human gastric cancer[30]. But owing to its murine origin, like many other similar antibodies, MG_7 antibody can elicit anti-mouse immunoreaction in man and thus its use in clinical practice is restricted[31,32]. One of the efficient strategies to this problem is to remove the constant region of antibody which makes main contribution to the immunogenicity of the murine antibody to human being[33-38]. On the other hand, antibody without constant region, termed scFv, is less antigenic and induces weaker repulsive reaction. In addition, it is a smaller molecule and comprises 1/6 of its original antibody in molecular mass which ensure that scFv can more readily penetrate into the tumor tissue *in vivo* and be easily cleared up from the normal tissue[39,40]. Besides, the scFv is more available than its original antibody by gene engineering technology which can provide an economical means for diagnosis of gastric cancer[41-47].

As mentioned above, the scFv is advantageous to its original antibody in clinical practice. Therefore, developing the MG_7 scFv is of great significance in both early diagnosis and therapy of gastric cancer. For example, MG_7 scFv fused with avidin can be used as a reagent in immuno-PCR for early diagnosis of gastric cancer. Additionally, a new immunotoxin for treatment of gastric cancer can be developed by fusing the MG_7 ScFv and A subunit of ricin together. MG_7 ScFv can direct the A subunit of ricin to MG_7 positive gastric cancer cells[48-50]. In our previous study, the MG_7 recombinant phage antibody derived from MG_7 hybridoma was constructed and subsequently two strong positive phage antibody clones were screened out from this library[51].

Targeting therapy for tumors in the last decade has become a highlight in the field of tumor therapy[52-57]. In past, the discoveries of many tumor specific antigen (TSA) and tumor associated antigen (TAA) assured the practicability of antibody as a tool in tumor targeting therapy[58-69]. Ascribed to its high specificity and sensitivity in recognizing associated antigen, antibody is the optimal candidate for targeting mediator. Therefore, targeting therapy mediated by antibody still remains as a promising curative modality among the ways of tumor therapy and attracts worldwide attention[70].

This study was conducted with the purpose to produce the soluble MG_7 scFv, identify its antigen-binding affinity, determine its molecular mass and get an understanding of its nucleotide sequence. We first examined the length of exogenous MG_7 scFv gene harbored in the two strong positive phage antibody clones by restriction analysis and found that one of the phage antibody clones contained a 750bp fragment of exogenous gene which was identical to many discovered scFvs in length. Secondly, we prepared the perplasmic extracts (containing majority of the soluble scFv) from IPTG-induced *E.coli* HB2151 and detected the antigen-binding affinity of MG_7 scFv by ELISA. One of the soluble MG_7 scFvs was shown to possess apparent antigen-binding activity. Subsequently, immunodoting test exhibited that *E.coli* HB2151 had successfully produced the soluble MG_7 scFv with high yields via induction by IPTG. Meanwhile, western blot suggested that the molecular mass of soluble MG_7 scFv was *Mr* 30. Finally, DNA sequencing unraveled that the MG_7 scFv had the common characteristics shared by other known scFvs in nucleotide sequence. Collectively, we have successfully developed the soluble MG_7 scFv and created an opportunity to study its application in targeting therapy of gastric cancer.

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