Expression and Purification of a Foreign Gene Delivery Fusion Protein anti-LMP1scFv/tP in *Escherichia coli*

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Abstract Epstein-Barr virus (EBV)-encoded latent membrane protein-1 (LMP1) constitutively expresses on cell surface of various cancer cells. In this study, we aim to construct a fusion protein that contains a domain of the single chain of the human variable fragment (scFv) against LMP1 and a nucleotide-binding domain of truncated protamine (tP). The fusion protein anti-LMP1scFv/tP was designed with the tP coding sequence linked to the 3′-terminus of the scFv against LMP1, which will not only target LMP1 but also maintain nucleotide binding activity. The anti-LMP1scFv/tP gene was obtained by PCR-based gene assembly. The fusion protein was expressed in inclusion bodies in *Escherichia coli* Rosseta and was purified efficiently by immobilized metal (Ni^{2+}) affinity chromatography with His-tag under denaturation condition, and then the purified product was refolded by dialysis against urea concentration gradient. Indirect cellular immunofluorescence staining confirmed that refolded anti-LMP1scFv/tP maintained antigen binding activity, which could bind to CNE1-GL cells expressing LMP1 and couldn't bind to CNE1 cells not expressing LMP1. Gel shift assay demonstrated that refolded anti-LMP1scFv/tP had DNA binding activity. Thus, the fusion protein provides a basis for further application for targeting gene delivery to LMP1 expressing tumor cells.

Key words latent membrane protein1; single-chain antibody fragment; truncated protamine; fusion protein; gene delivery

Although there is some progress on therapeutic modalities, cancer remains a serious threat to human health [1]. Currently, surgical resection, radiotherapy and chemotherapy are the major therapeutic modalities for many malignancies. Radiotherapy and chemotherapy would damage normal cells in the same time of killing tumor cells [1]. With the increasing understand of molecule mechanism for tumorigenesis and development, cancer gene therapy has been a main research focus. But, the lack of an optimized, systemic gene delivery vehicle remains a key limiting blockade for developing effective treatment applications [2].

Recently, there are some reports on utilizing single-chain antibody fragment against specific antigen or receptor on cell surface for siRNA or gene delivery. To obtain the fusion protein which could maintain antigen binding activity and nucleotide binding ability, single-chain antibody fragment was fused with truncated protamine (tP), thus nucleotides could be delivered to the targeted cells and internalized to the cells accompanied with the antibody. This would be a most perspective strategy for targeted nucleotide delivery. Epstein-Barr virus (EBV) is a prototype gamma herpes virus that infects the majority of the population worldwide and has been implicated in the pathogenesis of several human malignancies including nasopharyngeal carcinoma [3], Burkitt's and Hodgkin's lymphomas [4], gastric carcinoma [5], colon carcinoma [6] and hepatoma [7]. EBV infection is mainly characterized by the expression of latent genes including EBNA1, LMP1, LMP2 and EBER. Latent membrane protein-1 (LMP1) is a transmembrane

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protein which was the first EBV latent gene found to be able to transform cell lines and alter the phenotype of cells due to its oncogenic potential. Protamine is a basic protein which was initially discovered in sperm. Protamine has strong nucleotide binding ability and could condense DNA at the head of sperm to ensure the transmission of genetic information [8,9]. Tp is a short-peptide containing twenty-two basic amino acids sequence which maintains nucleotide binding ability [10~13]. The fusion protein anti-LMP1scFv/tP contains a single-chain antibody against human LMP1 and a truncated protamine. The fusion protein maintains the binding activity to LMP1 and nucleotide binding ability.

Expression of recombinant proteins in *E.coli* is a common method to obtain large quantities of desired proteins [14,15]. However, it is often difficult to obtain soluble and active proteins, which is one of the major problems encountered when expressing proteins in *E. coli* [16]. Overexpression leads to the production of inclusion bodies [17]. Improper formation of disulfide bonds also results in aggregation of disulfide bond-rich protein in reducing cytoplasm of *E.coli* [18]. The expressed protein could be released from the inclusion bodies by treatming with strong denaturant solution. These proteins must be refolded in order to regain their activity [19,20].

In this study, a fusion protein anti-LMP1scFv/tP was constructed, expressed, purified and refolded which was produced as an inactive form as inclusion body in *E.coli*. In addition, we characterized the antigen binding activity and DNA binding ability of this fusion protein.

1 Materials and Methods

1.1 Strains and reagents

E.coli strains DH5α and Rosseta (DE3) were used as the hosts for plasmid manipulation and protein expression, respectively. The pMD18-T simple vector (TaKaRa) was used as the vector for cloning of the PCR product of anti-LMP1scFv/tP gene and pET32a (+) was used for protein expression vector. The Luria-Bertani (LB) medium contained 1% tryptone, 1% NaCl, and 0.5% yeast extract (plus 1.5% agar in plates). 100mg/L ampicillin was used for selection of transformants when supplemented into LB medium. Restriction endonucleases (*Eco*RI, *Hin*dIII), PrimeSTAR HS DNA polymerase, Taq DNA polymerase, dNTP, IPTG, X-gal, DNA marker and T4 DNA ligase were purchased from TaKaRa; BugBuster® protein extraction reagent, Benzonase nuclease, His tag monoclonal antibody and His bind kits were purchased from Novagen. All oligonucleotide primers were synthesized by the United States of America IDT Biotech. All other reagents used in this study were at least of analytical grade.

1.2 Construction of plasmids

The coding sequence of tP was ligated to the 3'-terminus of the anti-LMP1scFv (GenBank accession No. DQ201313), the anti-LMP1scFv/tP gene was designed as 22 oligonucleotides(Table 1) that overlapped each other according to the instruction of the DNAWORKS program [21]. The anti-LMP1scFv/tP gene was obtained by PCR-based gene assembly [22]. PrimeSTAR HS DNA polymerase was used for PCR. The PCR product was purified using a PCR purification kit, and then the purified PCR product was subcloned into pMD18-T simple vector. Then the anti-LMP1scFv/tP gene was cloned into *Eco*RI/*Hin*dIII sites of pET32a(+) to produce pET32a-LMP1scFv/tP and sequenced (Fig.1). The fusion protein anti-LMP1scFv/tP was expressed using the Rosseta.

1.3 Expression of the fusion protein anti-LMP1scFv/tP

Expression of the fusion protein anti-LMP1scFv/tP was performed according to the methods of Kou G, *et al* [23]. In brief, the overnight culture of bacteria harboring pET32a-LMP1scFv/tP was diluted 1∶100 into LB medium containing 100 mg/L ampicillin and 34 mg/L amphemycin. The culture was incubated at 37 °C with shaking until it reached A_{600} of 0.6. Isopropyl-1-thio-β-galactopyranoside (IPTG) was added to final concentrations of 0.05, 0.1, 0.5, 1, 2 mmol/L to determine the optimal time and the optimal concentration of IPTG for maximal induction, respectively. The culture was incubated for indicated time points at 37 °C. The bacteria were harvested by centrifugation at 5 000 r/min for 2 min. The bacterial pellets were resuspended in BugBuster Protein Extraction Reagent containing 5 mmol/L PMSF, 25 U/ml Benzonase nuclease, and 200 μg/ml lysozyme and incubated at RT for 20 min with gentle shaked. The soluble and insoluble fractions were separated by centrifugation at 10 000 r/min for 15 min at

Table 1 Oligonucleotides for the synthesis of anti-LMP1scFv/tP

Primers	Sequences
P1	5'-TTT GAA TTC ATG GCC CAG GTG CAG CTG GTG CAG TCT-3'
P2	5'-GAC CTT CAC TGA GGC CCC AGG CTT CTT CAC CTC AGC CCC AGA CTG CAC CAG CTG C-3'
P3	5'-GGG CCT CAG TGA AGG TCT CCT GCA AGG CTT CTG GAT ACA CCT TCA CCG GCT ACT A-3'
P4	5'-AAG CCC TTG TCC AGG GGC CTG TCG CAC CCA GTG CAT ATA GTA GCC GGT GAA GGT G-3'
P5	5'-CCC TGG ACA AGG GCT TGA GTG GAT GGG ATG GAT CAA CCC CAA CAG TGG TGG CAC A-3'
P6	5'-GGT CAT GGT GAC CCA GCC CTG AAA CTT CTG TGC ATA GTT TGT GCC ACC ACT GTT G-3'
P7	5'-CTG GGT CAC CAT GAC CAG GGA CAC GTC CAT CAG CAC AGC CTA CAT GGA GCT GAG C-3'
P8	5'-CAC AGT AAT ACA CGG CCG TGT CCT CAG ATC TCA GCC TGC TCA GCT CCA TGT AGG C-3'
P9	5'-CGG CCG TGT ATT ACT GTG CAA GAC CTT TTA GTG AGC CGG TGT GGG GCC AAG GTA C-3'
P10	5'-CCG CCT GAA CCG CCT CCA CCA CTC GAG ACG GTG ACC AGG GTA CCT TGG CCC CAC A-3'
P11	5'-GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT AGT GCA CTT CAG TCT GTG CTG ACT C-3'
P12	5'-CCC TCT GCC CGG GGG TCC CAG ACG CTG AGG GTG GCT GAG TCA GCA CAG ACT GAA G-3'
P13	5'-CCC CGG GCA GAG GGT CAC CAT CTC TTG TTC TGG AAG CAG CTC CAA CAT CGG AAG T-3'
P14	5'-CCG TTC CTG GGA GCT GCT GGT ACC AGT ATA CAT AAT TAC TTC CGA TGT TGG AGC T-3'
P15	5'-CAG CTC CCA GGA ACG GCC CCC AAA CTC CTC ATC TAT AGG AAT AAT CAG CGG CCC T-3'
P16	5'-CCA GAC TTG GAG CCA GAG AAT CGG TCA GGG ACC CCT GAG GGC CGC TGA TTA TTC C-3'
P17	5'-TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC AGT GGG CTC CGG TCC G-3'
P18	5'-GCT GTC ATC CCA TGC TGC ACA GTA ATA ATC AGC CTC ATC CTC GGA CCG GAG CCC A-3'
P19	5'-CAG CAT GGG ATG ACA GCC TGT CTG CGC TTG TAT TCG GCG GAG GGA CCA AGC TGA C-3'
P20	5'-TGC TCC GGC TCT GGC TGC GTG CGG CCG CAC CTA GGA CGG TCA GCT TGG TCC CTC C-3'
P21	5'-GCC AGA GCC GGA GCA GAT ATT ACC GCC AGA GAC AAA GAA GTC GCA GAC GAA GGA G-3'
P22	5'-CCC AAG CTT CTA GCT CCG CCT CCT TCG TCT GCG ACT-3'

4 °C. Soluble and insoluble protein fractions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

1.4 SDS-PAGE and Western blot

The total cell lysate before and after induction, soluble and insoluble protein fractions were analyzed by 10% SDS-PAGE, and then stainied with Coomassie brilliant blue R-250 to confirm the quality of fusion protein. For Western blot analysis, proteins were electro-transferred to PVDF membranes using Mini Trans-Blot cell (Bio-Rad) following manufacturer's instruction. The target protein was immunodetected by mouse anti-His monoclonal antibody and HRP-labeled goat anti-mouse IgG (Zymed, USA). This was followed by signal enhancement with the enhanced chemiluminescence detection system.

1.5 Preparation and purification of the fusion protein anti-LMP1scFv/tP

The fusion protein anti-LMP1scFv/tP was expressed as described above. One thousand two hundred milligrams of wet weight bacterial pellets were resuspended in BugBuster Protein Extraction Reagent containing 5 mmol/L PMSF, 25 U/ml Benzonase nuclease, and 200 μg/ml lysozyme and incubated at RT for 20 min with gentle shake. The lysate was separated by centrifugation at 10 000 r/min for 20 min at 4 °C when it was transparented. The pellet was washed three times with washing buffer (20 mmol/L Tris-HCl, pH 7.5, 10 mmol/L EDTA, 1% Triton X-100, and 2 mol/L urea) and then resuspended in inclusion body solubilization buffer (20 mmol/L Tris-HCl, pH 7.9, 8 mol/L urea, 500 mmol/L NaCl and 5 mmol/L imidazole) and gently shaked at RT for 1h to completely solubilize protein. The solubilized inclusion bodies were centrifuged at 10 000 r/min for 20 min. The supernatant was filtered through a 0.45 μm membrane to prevent clogging of resins prior to performing purification.

The prepared extract was loaded onto the Ni^{2+} chelating column packed with 2.5 mL of Ni^{2+}-chelating resin which had been previously changed with 50 mmol/L $NiSO_4$ in H_2O and equilibrated with binding buffer (20 mmol/L Tris-HCl, pH 7.9, 8 mol/L urea, 500 mmol/L NaCl and 5 mmol/L imidazole). The column was washed using 10 volumes of binding buffer and 6 volumes of washing buffer (20 mmol/L Tris-HCl, pH 7.9, 8 mol/L urea, 500 mmol/L NaCl and 60 mmol/L imidazole) after

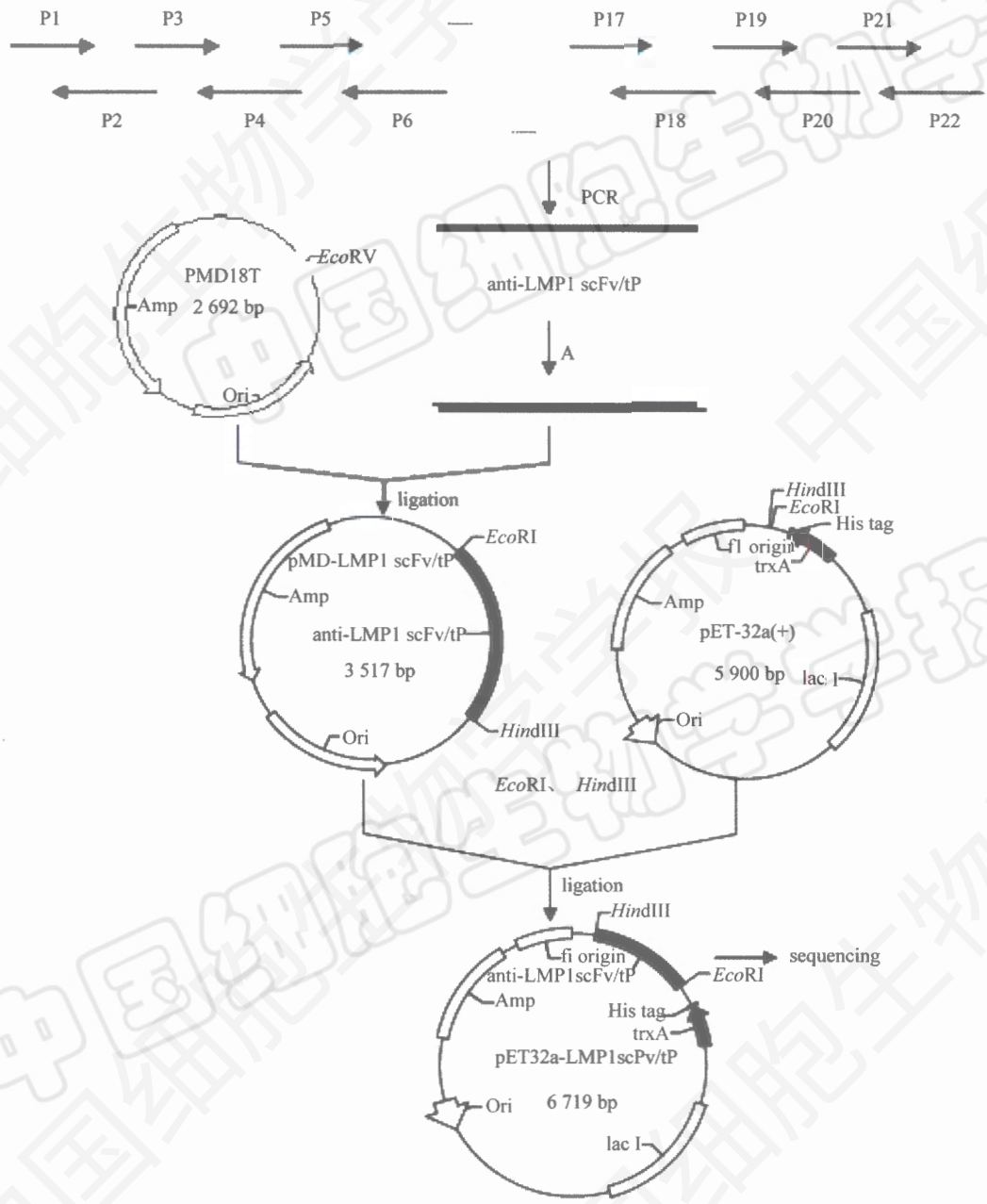

Fig. 1 Schematic representation of the cloning steps of anti-LMP1scFv/tP fragment into the expression vector pET32a(+)

the sample loading and penetration, and finally the bound protein was eluted with elute buffer (20 mmol/L Tris-HCl, pH 7.9, 1 mol/L imidazole, 500 mmol/L NaCl, and 8 mol/L urea), and the eluate was captured which containing the fusion protein anti-LMP1scFv/tp. The purified protein was analyzed by SDS-PAGE.

1.6 *In vitro* refolding of the fusion protein anti-LMP1scFv/tP

The eluate was loaded in the treated bag filter and refolded by dialysis against urea concentration gradient at 4 °C. Refolding was performed by dialysis against different buffers as described below [24-27]. Refolding was performed in 20 mmol/L Tris-HCl (pH8.5) containing 0.1 mmol/L dithiothreitol (DTT), 0.2 mmol/L PMSF, 0.4 mol/L L-Arginine and 6 mol/L urea, followed by dialysis against the same buffer with step-wise reductions in the urea concentration (4, 2, 1, 0.5 mol/L stages). Each dialysis was performed for 12 h. Finally, the refolding system was dialyzed against 10 mmol/L Tris-HCl (pH7.2) containing 0.2 mmol/L PMSF for 48 h

to remove the remaining denaturant. The dialyzed protein solution was centrifuged at 10 000 r/min for 10 min at 4 °C. The protein concentration of refolded product was determined by bicinchoninic acid (BCA) assay. The refolded protein was kept at 4 °C.

1.7 Indirect cellular immunofluorescence staining

The antigen binding activity of fusion protein anti-LMP1scFv/tP was detected and identified by indirect cellular immunofluorescence staining. CNE1 (LMP1 negative cell) and CNE1-GL (LMP1 positive cell) cells were suspended in growth medium at a concentration of 1×10^5 cells/ml/well in a 24-well flat-bottomed plate (Cell Wells, Corning, NY) and cultured at 37 °C in a humidified atmosphere with 5% CO_2 (70% confluence). The cells were incubated with refolded fusion protein anti-LMP1scFv/tP and PBS (as control) for 30 min at 37 °C after washed three times with PBS, respectively. Cells were fixated with 4% paraformaldehyde in PBS after being incubated in 0.5% Triton X-100 in PBS and washed, and cells were blocked with 10% bovine serun albumin in PBS after washed three times with PBS. Cells were then stained by anti-His antibody (1 ∶ 400) and FITC-labeled goat anti-mouse antibody (1 ∶ 50). Cell nucleus was counterstained with 5μg/ml propidium iodide (PI) and analyzed by fluorescence microscope.

1.8 Gel-Shift assay

One hundred and fifty nanograms whole pET32a (+)-LMP1scFv/tP plasmid DNA was incubated with increasing amounts of the fusion protein anti-LMP1scFv/tP in 0.2 mol/L NaCl solution at RT for 30 min. The protein-DNA complexes were then analysed by 0.8% agarose gel electrophoresis.

2 Results

2.1 Construction of the expression vector for anti-LMP1scFv/tP

The anti-LMP1scFv/tP gene was obtained by PCR-based gene assembly. An 825 bp DNA fragment was obtained after two rounds of PCR amplification (Fig. 2). The PCR product was subcloned into pMD18-T simple vector. Then the anti-LMP1scFv/tP gene was cloned into *Eco*RI/*Hin*dIII sites of pET32a(+) to produce expression vector pET32a-LMP1scFv/tP.

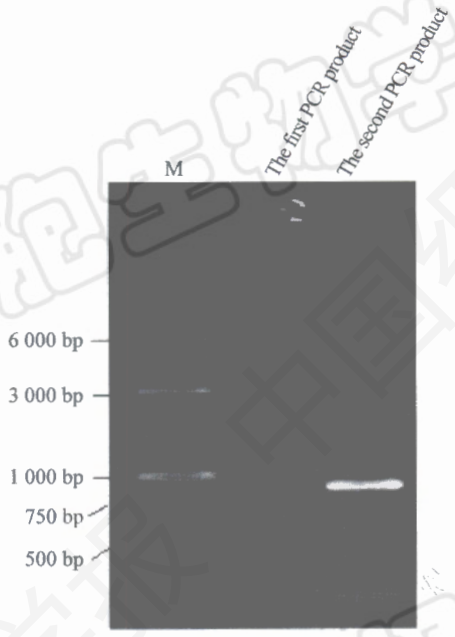

Fig.2 Electrophoresis analysis of products of PCR based gene assembly

The first PCR: 22 oligonucleotides(P1~P22) were added to PCR reaction in a 50 μL volume and included PrimeSTAR HS DNA polymerase (TaKaRa) and its corresponding buffer according to the manufacturer's instructions, oligonucleotides at a final concentration of 200 nmol/L each. The PCR program consisted of 35 cycles at 98 °C for 30 s, 70 °C for 30 s and 72 °C for 50 s. The secondry PCR: the previous reaction product was diluted 100-fold and then 1 μL was added to PCR reaction in a 50 μL volume and included PrimeSTAR HS DNA polymerase and its corresponding buffer according to the manufacturer's instructions, P1 and P22. P1 and P22 at a final concentration of 20 μmol/L. The PCR program consisted of 94 °C for 5 min, 35 cycles at 98 °C for 30 s, 70 °C for 30 s and 72 °C for 50 s.

2.2 Expression, purification and refolding of the anti-LMP1scFv/tP

The molecular weight of the fusion protein anti-LMP1scFv/tP should be approximately 48 kDa. The fusion protein was expressed in Rosseta after 2 h induction with 0.1 mmol/L IPTG, the expression level was the highest at 4 h, about 49.1% of the total proteins of *E.coli*. The fusion protein is the major band present at all time points of induction (Fig.3A). The expression level of the fusion protein with a final concentration of 0.1 mmol/L IPTG was the highest (Fig.3C) after incubation for 4 h at 37 °C, the relative percent of the fusion protein was approximately 52.6% as analysed by gray scale scaning. The inclusion body expression of the fu-

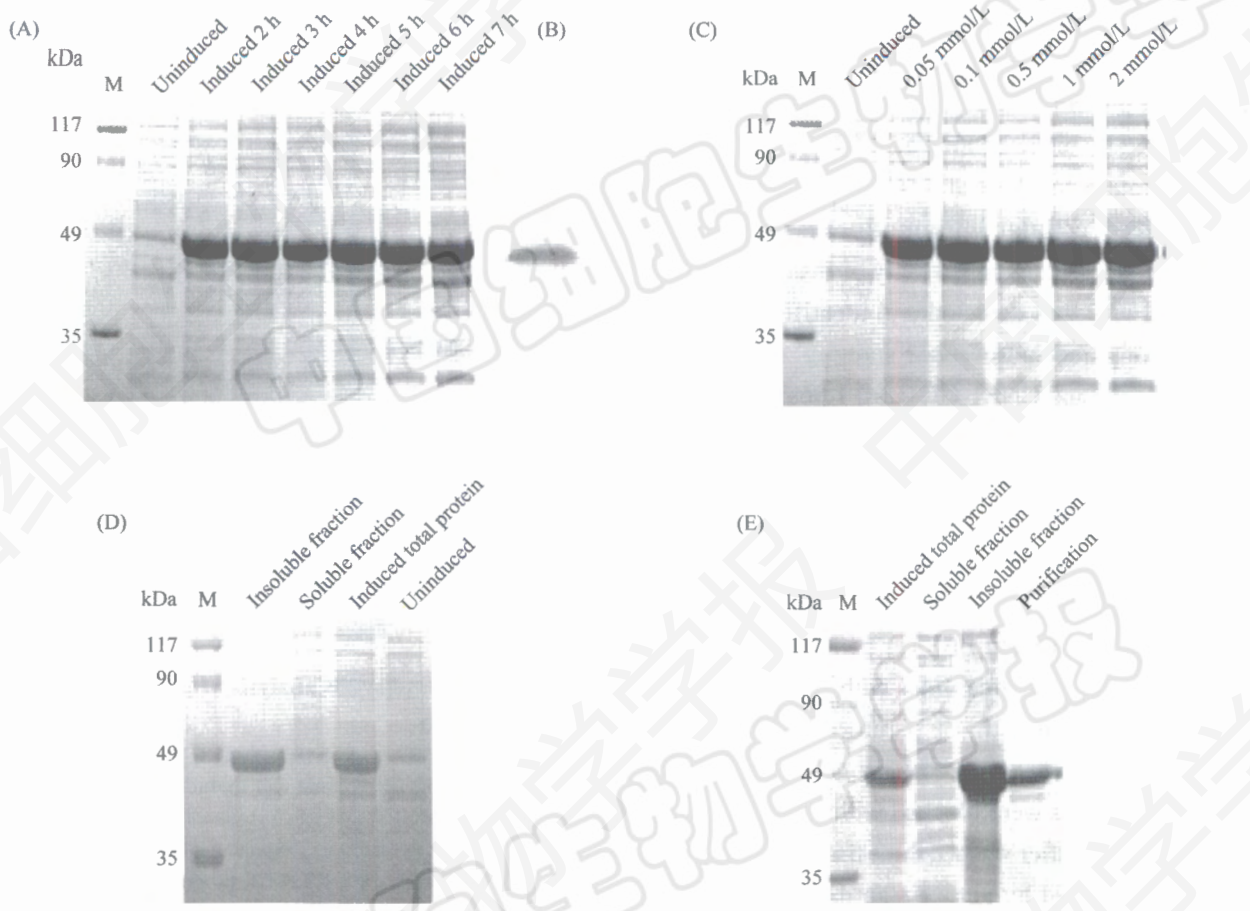

Fig.3 Expression and purification of anti-LMP1scFv/tP in Rosseta

A: 10%SDS-PAGE and Coomassie brilliant blue R250 staining of expression of anti-LMP1scFv/tP fusion protein induced by 0.1mmol/L IPTG; B: the expression of anti-LMP1scFv/tP was confirmed by Western blot using anti-His monoclonal antibody and a HRP-labeled goat anti-mouse IgG; C: SDS-PAGE and Coomassie brilliant blue R250 staining analysis of expression of anti-LMP1scFv/tP fusion protein induced by different concentration of IPTG at 4h in Rosseta; D: SDS-PAGE and Coomassie brilliant blue R250 staining analysis of expression and location of the fusion protein anti-LMP1scFv/tP in the cellular fractions of Rosseta; E: SDS-PAGE and Coomassie brilliant blue R250 staining analysis of the purified anti-LMP1scFv/tP fusion protein.

sion protein was confirmed using Western blot analysis (Fig.3B). Taken together, results showed that the fusion protein is His-immunoreactive and, has a molecular weight of approximately 48 kDa and was found only in the inclusion body fraction, not in the soluble fraction (Fig.3D).

The inclusion bodies were isolated and washed with buffers containing 2 mol/L urea to remove the contaminated proteins following the successful overproduction of fusion protein as inclusion body in *E.coli*, and then the inclusion bodies were dissolved in the presence of 8 mol/L urea solution. The fusion protein was purified on immobilized Ni^{2+}-chelating ion-affinity chromatography column using the internal His-tag. The purified protein was refolded by dialysis against urea concentration gradient (a linear gradient from 6 mol/L to 0 mol/L urea in the dialysate). No aggregation during refolding has been observed. Using this method, the refolded fusion protein could be obtained at a stable final concentration of 233 μg/ml. The resulting purity of the refolded anti-LMP1scFv/tP could reach 95% or more (Fig.3E).

2.3 Antigen binding activity of anti-LMP1scFv/tP towards LMP1

We assessed the antigen-binding ability and specificity of the refolded anti-LMP1scFv/tP for native LMP1 by indirect cellular immunofluorescence staining using CNE1 and CNE1-GL cells. The refolded anti-LMP1scFv/

tP maintained antigen binding activity and could probe LMP1 expressing CNE1-GL cells but could not probe CNE1 cells without LMP1 expression (Fig.4A). These results suggested that the fusion protein anti-LMP1scFv/tP renatured from the inclusion body fraction of *E.coli* was active and possessed antigen specific binding activity to LMP1.

2.4 Gel shift assay

The DNA binding activity of the refolded anti-LMP1scFv/tP was examined by gel shift assay (Fig. 4B). When plasmid DNA was mixed with increasing amounts of the fusion protein anti-LMP1scFv/tP, there was a reduction in the migration of whole plasmid DNA. This demonstrated that the refolded fusion protein possessed DNA binding ability.

3 Discussion

We aim to obtain a fusion protein not only targeting LMP1 but also possessing nucleotide binding ability which providing a delivery system for siRNA or DNA plasmid. In the present study, we constructed the fusion protein containing a single-chain antibody specifically against LMP1 and the tP for targeted delivering ectogenic nucleotide.

We obtained the anti-LMP1scFv/tP gene by PCR-based gene assembly from overlapping oligos *in vitro*.

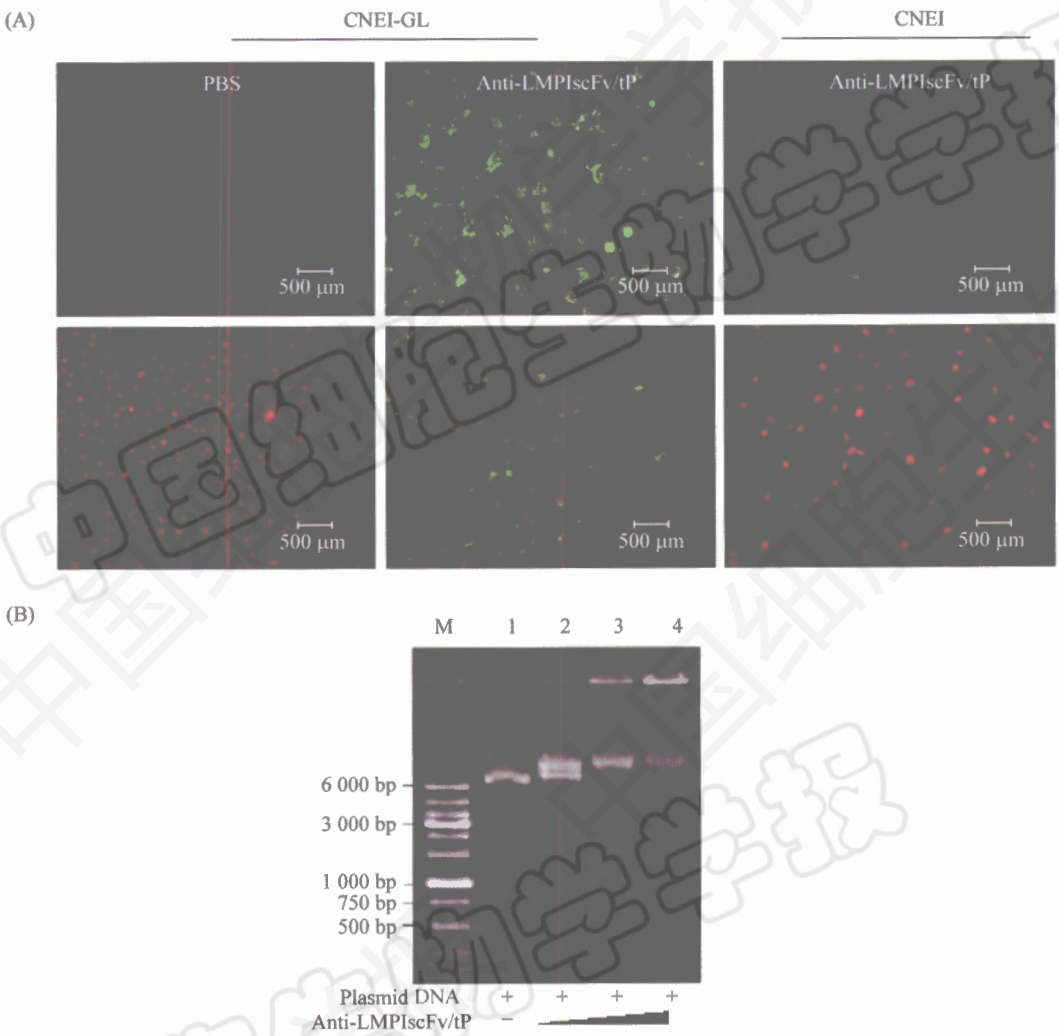

Fig.4 The identification of binding activity of anti-LMP1scFv/tP fusion protein with LMP1 antigen and DNA

A: indirect cellular immunofluorescence staining and PI staining analysis of antigen binding activity of the anti-LMP1scFv/tP fusion protein. Cells were processed as described in Materials and Methods by anti-His antibody and FITC-labeled goat anti-mouse antibody (green) (top panels), and cell nucleus was counterstained with propidium iodide (PI) (red) (bottom panels) and analyzed by fluorescence microscope. immunofluorescence; B: DNA binding ability of anti-LMP1scFv/tP fusion protein determined by Gel mobility-shift assay.

This gene assembly method as described in this paper did not require the DNA template, all the oligos can be designed efficiently by following the instruction of the DNAWORKS program and synthesized without the need for further amplification. It is an effective method to obtain a target gene as long as the gene product it codes for can be identified.

The fusion protein anti-LMP1scFv/tP was expressed in Rosseta. As the host of prokaryotic expression, different strain of *E.coli* has effect on expression of exogenous protein. Some codons of eukaryotic gene may be rare codons for prokaryotic cells. The expression of exogenous protein will be low or as premature termination if eukaryotic gene contains generous rare codons with continuous distribution. In this study, the expression vector pET32a-LMP1scFv/tP was transformed into the BL21, only a small amount of fusion protein produced at different inducing conditions. One of the possible reasons is four arginines which continuously distribute at the 3'-terminus of the anti-LMP1scFv/tP gene. Rosseta is a strain especially for expression of eukaryon protein, it contains *E.coli* rare codons tRNA, AUA, AGG, AGA, CUA, CCC and GGA. Therefore, we switched to Rosseta for producing the fusion protein and the expression level of the fusion protein significantly increased in Rosseta transformants.

In this study, huge amounts of fusion protein produced as an insoluble, inactive form in cytoplasmic inclusion bodies. During expression, we tried to increase the proportion of soluble fusion protein in total proteins expressed by optimizing inducing conditions, including initial culture time, IPTG concentration and inducing temperature. There is soluble fusion protein in the cytoplasm when inducing at 16 °C (data not shown), however, the native fusion protein anti-LMP1scFv/tP could not bind onto the Ni^{2+}-chelating column. Maybe the His-tag at the N-terminal of anti-LMP1scFv/tP is not exposed under native condition. Therefore, the fusion protein was induced with 0.1 mmol/L IPTG for 4 h at 37 °C that could produce the highest amount of inclusion bodies, about 52.6% of the total proteins of *E. coli*. The inclusion body protein anti-LMP1scFv/tP was purified under denaturing condition.

Inclusion bodies were solubilized at alkaline pH in the presence of 8 mol/L urea solution. The solubilized protein was purified. Refolding of inclusion body protein into bioactive form is cumbersome. Therefore, an efficient, stable and convenient refolding system would be needed. There are many methods for refolding, such as dilution, dialysis, and gel filtration [28,29]. In this study, we chose dialysis against urea concentration gradient at 4 °C for protein refolding. In general, the recovery of refolded protein actually makes decision competition between the correct folding process and aggregation. Protein concentration plays an important role in determining refolding recovery. High protein concentration generally shifts the kinetics of the refolding pathway to favor aggregation [30], and the protein concentration was controlled between 0.1~1 mg/ml commonly. Furthermore, the purity of inclusion body proteins is a key factor. In the present study, inclusion bodies were washed with the buffer containing 2 mol/L urea and 1% Triton X-100 which could effectively remove remaining cellular proteins and facilitate the following purification and refolding. In addition, we added deoxidize reagent DTT and micromolecule mass additive L-arginine in the dialysate. DTT would ensure to form correct and stable disulfide bond. L-arginine could enhance the solubility of folding intermediate and suppress aggregation of proteins [31]. The results indicated that this process for refolding of anti-LMP1scFv/tP was suitable and effective.

Finally, we confirmed that anti-LMP1scFv/tP was functionally active and was able to bind specifically to LMP1 expressed on the surface of CNE1-GL, while it did not react with CNE1. We further tested the DNA binding ability of anti-LMP1scFv/tP by gel shift assay and found that anti-LMP1scFv/tP could efficiently bind to DNA and slow its mobility. Our preliminary studies indicated that fusion protein anti-LMP1scFv/tP was successfully refolded and had the activities of binding antigens and DNA binding ability, which provides a foundation for the application of this scFv for targeted delivery of DNA or siRNA.

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Anti-LMP1scFv/tP 融合蛋白基因递送载体在大肠杆菌中的表达与纯化

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摘要 潜伏膜蛋白 1(LMP1) 是一种 EB 病毒编码的膜蛋白，组成型表达于各种肿瘤细胞表面。通过体外基因拼装技术，构建抗LMP1单链抗体/鱼精蛋白截短体融合蛋白基因(anti-LMP1scFv/tP)。在抗 LMP1 单链抗体基因的 3' 末端连接上编码鱼精蛋白截短体的基因序列，设计 anti-LMP1scFv/tP 融合蛋白基因。采用 PCR 为基础的基因拼接获得 anti-LMP1scFv/tP。在大肠杆菌 Rosseta 中诱导表达 anti-LMP1scFv/tP 融合蛋白并利用其 His 标签在变性条件下通过 Ni^{2+} 亲合层析介质进行纯化，纯化产物通过尿素浓度梯度透析的方法进行复性。间接免疫荧光染色检测 anti-LMP1scFv/tP 融合蛋白的抗原结合活性，DNA 凝胶迁移阻滞实验检测 anti-LMP1scFv/tP 的 DNA 结合活性，结果显示 anti-LMP1scFv/tP 融合蛋白能够与 LMP1 阳性表达的 CNE1-GL 细胞结合，而不能与 LMP1 阴性表达的 CNE1 细胞结合；同时复性 anti-LMP1scFv/tP 融合蛋白也具有与 DNA 结合的活性。本研究为该单链抗体用于靶向输送外源核苷酸的研究奠定了基础。

关键词 潜伏膜蛋白 1；单链抗体；鱼精蛋白截短体；融合蛋白；基因递送

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