

Expression, Purification and Activity Identification of Extracellular Domain of EGFR

Chuan Liu ^a, Ju Wang ^b

Institute of Biomedicine, National Engineering Research Center of Genetic Medicine, Jinan University, Guangzhou 510632, China

^a2449935390@qq.com, ^bwangju168899@qq.com.

Abstract

The extracellular region of the EGFR was expressed *in vitro* using the prokaryotic expression system, purified and identified. The synthesized EGFR extracellular segment cDNA was inserted into plasmid pCreat-SII and transfected into *E. coli* BL21 (DE3), and IPTG induced protein expression. Proteins were purified by Affinity chromatography. CCK-8 method was used to determine the protein activity. Double digestion results show target gene was successfully inserted into the vector. Proteins are mainly expressed as inclusion bodies. CCK-8 results showed that the EGFR extracellular segment had good biological activity. This experiment successfully used the prokaryotic expression system to express the extracellular protein of EGFR with good biological activity, which is more convenient and economical than the traditional insect cell expression system.

Keywords

EGFR extracellular segment, prokaryotic expression system, CCK-8.

1. Introduction

Epidermal growth factor receptor (EGFR) is one of ErbB protein kinase family members. The EGFR gene encodes a transmembrane protein consisting of 1186 amino acids, of which 1-621 residues constitute the extracellular domain, and the extracellular domain can be divided into four subdomains (L1, CR1, L2, CR2)^[1]. EGFR signaling pathway is involved in the growth, proliferation, damage repair, invasion and metastasis, and the formation of blood vessels in many types of tumor cells^[2]. In many types of cancers, most of the EGFR family members exist in overexpressed forms, including lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, breast cancer, kidney cancer, pancreatic cancer, prostate cancer, brain cancer, and bladder cancer^[3]. Therefore, the design and development of drugs that use the extracellular domain of EGFR as a drug target have increasingly become research hot spots. Compared with the costly eukaryotic expression system, the prokaryotic expression system was used to express, purify and identify the EGFR extracellular domain protein *in vitro*^[4;5].

2. Experimental detail

2.1 Materials

1640 medium, fetal bovine serum was purchased from HyClone. BamH-I, Xho-I and T4 ligase are all derived from NEB. Cell Counting Kit-8 was purchased from DOJINDO.

2.2 Reagent formulation

LB Medium

Chemicals	Amount
Peptone	1g
Yeast	0.5g
NaCl	1g
Ultra-pure water volume to	100ml

Denaturation fluid

Chemicals	Amount
Tris-Hcl	1.21g
EDTA	0.074g
Guandine-Hcl	57.32g
DTT	0.154g
Ultra-pure water volume to	100ml pH=8.0

Elution buffer

Chemicals	Amount
NaCl	500mM
PB	20mM
Imidazole	250mM
Ultra-pure water volume to	1L pH=8.0

2.3 Experimental Procedure

A: Plasmid Transformation

The plasmid was transformed into BL21 (DE3) competent cells, plated and cultured overnight at 37°C.

B: Expression Identification and Soluble Analysis

a, Select a single clone in 1 tubes of LB medium, cultivate at 37°C , when OD600 = 0.6-0.8 add IPTG = 0.5mM, cultured 4h, centrifuged, collected, and analyzed by SDS-PAGE.

b, Inoculated BL21 in LB medium, when OD600 =0.6-0.8, 0.5mM IPTG was added. After 4h, the bacteria were collected, sonicated, centrifuged, and the supernatant and precipitate were prepared separately. Analyze by SDS-PAGE.

C: Amplification and affinity purification

a, We choose the best clonal strain for expansion culture, cultured to OD600=0.6-0.8, 37°C, and induced with 0.5mM IPTG for 4 h.

b, Centrifugal collection of bacteria, ultrasonic breaking;

c, Nickel column affinity purification

The supernatant and precipitate were separated by centrifugation. The precipitate was dissolved in a denaturation buffer. Then the centrifuged supernatant was added to the refolding solution for renaturation. After renaturation, the sample was purified by affinity chromatography on a Ni column. The equilibration buffer was used to equilibrate the nickel column. Perform the sample, after loading, equilibration buffer was used again for elute the non-specific binding protein or the less-binding protein. Finally, the elution buffer was used to obtain the target protein.

D: CCK8 detects the biological activity of EGFR extracellular

The well-grown cells were seeded in 96-well plates at a density of 4000-5000 cells/well and cultured in a cell culture incubator for 24 hours. 0.4% serum medium was starved for 12 h, and different concentrations of EGFR extracellular protein were dissolved in 0.4% FBS 1640 medium, induce 48 hours. Configure the CCK-8 working fluid according to the instructions of CCK-8. After adding the working fluid, incubate the incubator at 37 °C for about 1 hour. Read the OD value at a wavelength of 450 nm on the plate reader

2.4 Statistical analysis

All data were presented as mean \pm SD and statistically significant was determined by one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results and discussion

The plasmid was double digestion and analyzed by agarose gel electrophoresis, the result as shown in Fig 1, the target gene was successfully inserted into the plasmid vector.

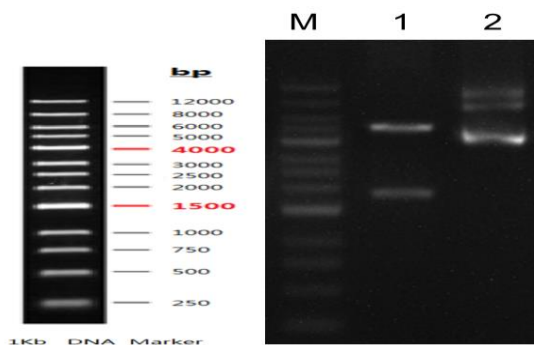


Fig.1 Electrophoresis detection of double digestion products

M: DNA Marker, 1: Plasmid digested with BamH-I and Xho-I, 2: Plasmid

We sequenced the monoclonal strain and selected the correct strain for subsequent experiments, inoculate strains in LB medium what containing kanamycin, cultured in a 37°C shaker, when OD=0.6-0.8, induced by adding 0.5mM IPTG, induction for 4h, SDS-PAGE analysis the sample of before and after the induction, as shown in Fig.2. The protein of interest was expressed successfully.

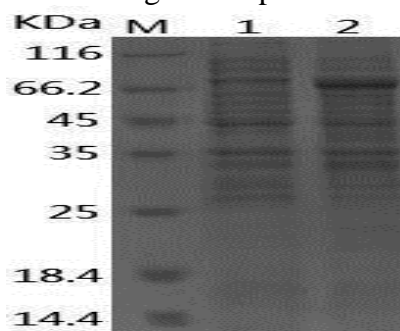


Fig.2 Expression Identification SDS-PAGE Results

M: protein marker, 1: pre-induction; 2: post-induction

After 4 h induction, the bacterial cells were collected by centrifugation and disrupted. The supernatant and precipitated were prepared separately. SDS-PAGE plus dyeing analysis, as show in Fig.3, proteins are mainly expressed as inclusion bodies.

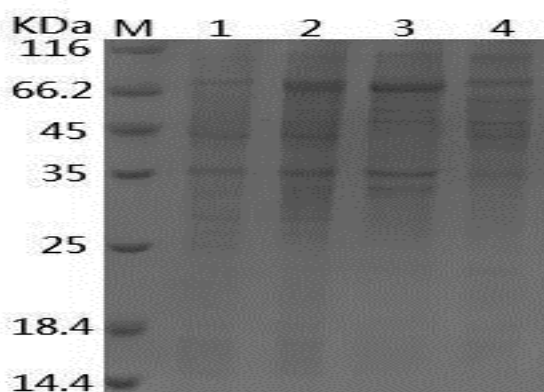


Fig.3 Soluble Analysis SDS-PAGE Results

M: protein marker, 1: pre-induction, 2: post-induction, 3: precipitation after induction, 4: supernatant after induction

The purified EGFR extracellular segment protein solution was prepared and subjected to SDS-PAGE gel electrophoresis plus dyeing analysis. As show in Fig 4, it can be seen that the target protein with higher purity was obtained after affinity purification on the Ni column.

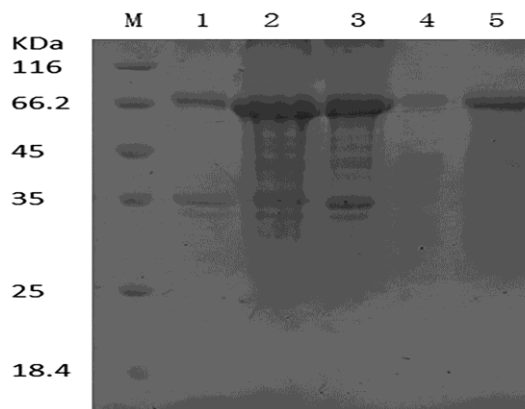


Fig.4 Test results of components in the purification process

M: protein marker, 1: sedimentation after renaturation, 2: Supernatant after renaturation, 3: penetrating fluid, 4: Wash, 5: Elution.

CCK8 assay detect the biological activity of EGFR extracellular domain proteins purified in our laboratory, as show in Fig 5, with the concentration increases, its inhibitory effect on EGF-induced proliferation on H1648 cell was increasing until an optimal inhibitory concentration was reached at 1280 ng/ml.

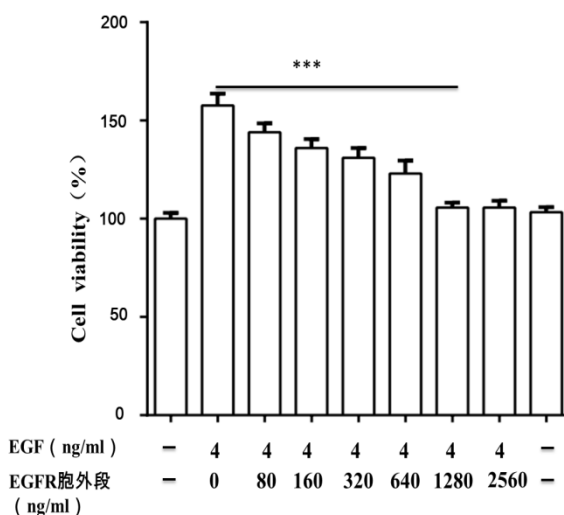


Fig.5 CCK8 in H1299 cells was used to detect the biological activity of EGFR extracellular segment.

*** indicates that the difference between the induction group and the blank group is extremely significant

4. Conclusion

In this study, we successfully constructed an EGFR extracellular domain protein that with good biological activity. Can be further used for EGFR immunology, tumor related immunotherapy and functional studies.

Acknowledgements

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References

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