Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):15-18

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Expression of cdc42 protein in *Escherichia coli*

Lin Weiping

College of Biological Sciences and Technology, Weifang Medical University, WeiFang, China

---

ABSTRACT

phellinus igniarius is a filamentous fungi with good anti-cancer effect world-recognized now, which has broad prospects in the field of medicine because it contains a large number of active substances. cdc42 proteins are a class of key proteins in transporting cancer cells, this study clone and express cdc42 protein using molecular biology tools, successfully obtained gene sequence information and protein sequences and then detected their expression in E. coli, it provides some theoretical basis in further to research cdc42 proteins and phellinus igniarius exploitation.

Keywords: Cdc42, cancer, *phellinus igniarius*

INTRODUCTION

The advantages of medicinal fungi are safer than other similar drugs and have no side effects[1], many scholars are now working on their antitumor activity, anti-mutagenic activity and other activity. *phellinus igniarius* is now recognized as the best anti-cancer effect of filamentous fungi, and *phellinus igniarius* have many other biologically activity, so it has high research value, and become a hot spot in recent years.

As we all know, the spread of cancer cell is one of the problems in the treatment of cancer. Most cancer deaths are caused by the spread of cancer cells, there is no good treatment method to prevented the spread. In November 2012, researchers published research article said that they found an effective method to prevent cancers, A key protein that cancer cell metastasis required --cdc42 protein [2].

Cdc42 protein present in the interior of cancer cells can help cancer cells attached to the vessel wall, so that they can spread to other position through blood [3]. Cdc42 protein can also affect the number of β1 integrin (another cancer cell surface protein). To reduce the chance of cancer spread, it is necessary to prevent the cancer cells attach to the vessel wall, and now Cdc42 protein gives us that hope, if we can inhibit these proteins, it may be able to reduce the proliferation opportunities of cancer. Cdc42 protein allows scientists have a new understanding about metastasis of cancer cells, and then design a new future therapy [4].

EXPERIMENTAL SECTION

Material: *phellinus igniarius*, *E.coli*, pET22b plasmids are preserved in our laboratory.

Obtain the target fragments: Total RNA were extracted from *phellinus igniarius*, and reversed transcription to obtain cDNA, according to the report in the literature and sequence result designed primers:

P1 CGCCATATGCAAACCATCAAGTGTGTCGT *Nde* Ⅰ
P2 CTCAAGCTTCAGCACAACACACTTCGTGCCT *Hind*ⅢⅠ

Amplified to obtain gene sequence of cdc42 protein, massively fragment was recovered by agarose gel electrophoresis

confirmed.

Construction and Transformation of recombinant plasmid: Cdc42 protein PCR product and prokaryotic expression vector pET22b were double digested respectively with restriction enzymes *Nde* Ⅰ and *Hin*dⅢ, and then purified the restriction fragment, the products were ligated at 16 ℃ overnight and transformed into *E. coli* DH5α.

Verification of recombinant plasmid and sequencing: Monoclonal picked from LB solid plate after transformation, extracted plasmid and verification result by PCR, Successfully verified and sent to sangon biotech for sequencing.

Prokaryotic expression recombinant plasmid: Successfully constructed recombinant plasmids were transformed into *Escherichia coli* BL21. After cultured overnight and 2% transferred into new LB medium, when OD600 reached about 0.5, added IPTG and induced for 4h, after induction harvested by centrifugation, supernatants and precipitation were collected after sonication respectively, SDS-PAGE detected the expression of total bacterial, supernatant and precipitation.

RESULTS AND DISCUSSION

After the cultivation for 9d, *phellinus igniarius* were harvested by centrifugation, extracted total RNA using Triol method, detected RNA extraction quality by agarose gel electrophoresis, shown in Figure 1

Figure 1 Agarose electrophoresis of total RNA of *Phellinus igniarius*

Used TaKaRa PrimeScript ™ RT-PCR Kit reverse transcription to obtain cDNA, as a template, and designed specific primers of cdc42 protein to amplify fragments, fragment detected by agarose gel electrophoresis, result shown in Figure 2:

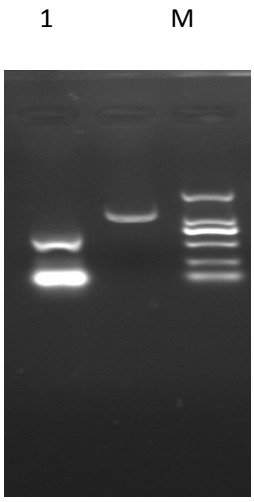

Figure 2 Agarose electrophoresis of products of PCR

1 : PCR product

M : Marker

Fragment and pET22b plasmid were digested, ligated and transformed into *E. coli* DH5α, and then detected recombinant plasmids with control, as shown in figure3

1 2 3 M

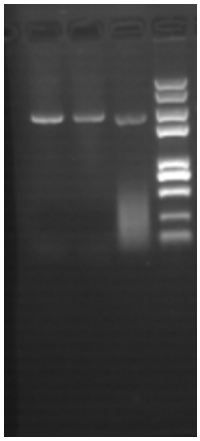

Figure 3 Agarose electrophoresis of the constructed plasmid
1/2:pET22b-cdc42
3:pET22b
M: marker

The recombinant plasmid that constructed successfully was sequenced, sequence result as follows, according to the gene sequences we obtained amino acid sequence.

Gene sequence:
ATGCAAACCATCAAGTGTGTCGTTGTGGGTGATGGTGCGGTTGGGAAGACATGTCTGCTGATATCGTAC
ACAACAAATAAATTCCCGAGCGAATATGTTCCGACTGTGTTTGACAACTACGCGGTGACTGTCATGATTG
GCGATGAGCCTTATACTTTGGGTCTGTTCGATACGGCTGGTCAAGAGGATTATGACCGCCTCAGACCACT
CTCATACCCTCAGACCGACGTTTTCCTCGTATGCTTTAGTGTGACATCGCCCGCTTCCTTTGAAAATGTAC
GCGAGAAATGGTTCCCTGAGGTATTCCACCACTGTCCTGGTGTCCCGTGCCTCATCGTTGGCACGCAAA
TCGATCTGCGGGACGACCCGAGTGTACGCGAAAAGCTCGCTCGCCAGAAACAGGCGCCGATCCAAGAG
GAAGACGGTAAAAGACTCGCCCATGAGCTCGGCGCAGTCAAATATGTCGAATGCTCTGCTCTTACCCAG
AAGGGACTGAAGAATGTCTTTGACGAGGCTATTGTTGCTGCGTTGGAGCCGCCTGTGGTTAAGAAGAA
AGGCACGAAGTGTGTTGTGCTGTAA

Amino Acid sequence:
MQTIKCVVVGDGAVGKTCLLISYTTNKFPSEYVPTVFDNYAVTVMIGDEPYTLGLFDTAGQEDYDRLRPLS
YPQTDVFLVCFSVTSPASFENVREKWFPEVFHHCPGVPCLIVGTQIDLRDDPSVREKLARQKQAPIQEEDGK
RLAHELGAVKYVECSALTQKGLKNVFDEAIVAALEPPVVKKKGTKCVVL

The recombinant plasmid was transformed into *E.coli* BL21 and detected expression after induced by SDS-PAGE, Figure 4 shows that the protein was not expressed obviously, it is necessary to further optimize the conditions to achieve prokaryotic expression of cdc42 protein.

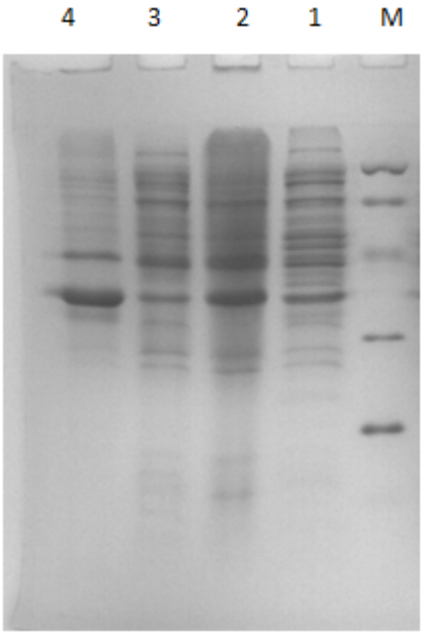

Figure 4 SDS-PAGE analysis of recombinant pET22b-cdc42 proteins in *E.coli*

M :standard protein molecular weight; 1: crude enzyme from BL21 at 30 ℃ for 5h; 2: crude enzyme from BL21 (DE3)/ pET22b-cdc42 induced by IPTG (0.5mM) at 30 ℃ for 5 h; 3: Supernatant of crude enzyme ;4: Precipitation of crude enzyme

DISCUSSION

Invasion and metastasis of malignant tumors is the key reason of treatment failure. Wherein the invasion and metastasis of tumor cell depends on the initiative migration, this view was confirmed gradually [5]. Studies have confirmed, Rho family proteins highly expressed in a variety of malignant tumors, and closely related invasion and metastasis. cdc42 is another subfamily in Rho family protein --Cdc42 subfamily (Cdc42, Tc10, Wrch1, chp / Wrch2) [6]. DNA sequences encode the product with the length of 191 amino acid [7] [8] [9]. Cdc42 protein present in the interior of cancer cells. This protein helps cancer cells attached to the vessel wall, so that they spread to other parts through the blood.

Cdc42 proteins, which help scientists to have a new understanding and awareness on cancer metastasis, and help to design a new therapy in future. Theoretically, an inhibitor through direct injection into the bloodstream can prevent the cancer cells to attach to the vessel wall or make the vessel wall endothelium denudation of cancer cell, thus reduce the chance of cancer metastasis, which is extremely possible in future.

Cdc42 protein rare report domestic , this study cloned and expressed cdc42 from *phellinus igniarius*, because the application potential of cdc42 protein, this experiment can provide data and theoretical basis for the application of cdc42 protein through realizing prokaryotic expression

REFERENCES

[1] Zhou Cunshan, Ma Haile. *edible fungi*, **2005**,27 (2): 50-51
[2] Dong Zhiwei, Qiao Youlin, Li liandi, etc. *Chinese Cancer*. **2002** (05)
[3] Rohatgi R, Ho H H, Kirschner M W. *The Joumal of cell biology*, **2000**,150(2):1299-1310.
[4] Maeks P W, Kwiatkowski D J. *Genomics*, **1996**,38(1):13-18.
[5] Cheng Daye, Liang Lin, Li Feng, *Chinese Journal of Cell Biology*. **2007** (03).
[6] Pan Yanglin, Bi Feng, Liu Na, etc. *Med J Chin PLA* .**2003** (06)
[7] Xuan Zhang, Ma Chunqi, Ann L.Miller,Hadia Arabi Katbi, *Developmental Cell*. **2008**(3)
[8] Amanda JKay,Craig P.Hunter. *cell*.**2003**(1)
[9] Huang Juan, Lang Nan, Liu Ming, etc . *J Sichuan Univ*(Med Scl Edi).**2009** (04)