

Construction of eukaryotic expression vector of TSLC1 gene

Qi-Lian Liang¹, Bi-Rong Wang², Zhou-Yu Li¹, Guo-Qiang Chen¹, Yuan Zhou¹

¹Department of Oncology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China

²Department of Chemotherapy, Affiliated Tumor Hospital of Guangzhou Medical College, Guangzhou, China

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Corresponding author:

Prof. Qi-Lian Liang
Department of Oncology
Affiliated Hospital
of Guangdong Medical College
57 People Avenue
Zhanjiang 524001
Guangdong, China
Phone: 86-759-2387455
Fax: 86-759-2231754
E-mail: lianqilian@gdmc.edu.cn

Abstract

Introduction: To construct a eukaryotic expression vector of the tumour suppressor in lung cancer 1 (TSLC1) gene, so as to explore the mechanisms of tumour suppression of the gene theoretically.

Material and methods: The open reading frame (ORF) of TSLC1 gene was amplified with RT-PCR from normal human foreskin acrobystia, and cloned to pMD19-T simple vector (TA Clone method). The resultant plasmid was transformed into *Escherichia coli* JM109 for amplification. The TA Clone recombinant was digested by double restriction enzyme (Bgl II/EcoR I) and analysed with agarose gel electrophoresis. The positive one was sequenced. The inserted DNA fragment was recovered, and then it was mounted into the eukaryotic expression vector pIRES2-EGFP, transformed into *E. coli* JM109 for amplification. A positive recombinant plasmid named pIRES2-EGFP-TSLC1 was confirmed by Bgl II/EcoR I double-enzyme digestion analysis.

Results: RT-PCR amplified the ORF of the TSLC1 gene. It was approximately 1400 base pairs. The obtained DNA was confirmed a high degree of homology with the sequence of TSLC1 cDNA sequence (AY358334) stored at GenBank.

Conclusions: Construction of a TSLC1 eukaryotic expression vector was successful, and it has established a solid foundation for further study.

Key words: TSLC1 gene, construction, eukaryotic expression vector.

Introduction

Cancer is still an inextirpable disease. However, human beings have evolved an array of host anti-tumour surveillance, to combat the arising malignant cell clones [1]. Tumour suppressor in lung cancer 1 (TSLC1) is a cloned tumour suppressor gene [2-5]. It is expressed in a variety of tissues and organs in the human body, particularly in the normal lung, brain, liver and skin tissues [6], but silenced in many cancers such as larynx cancer [7], cervical cancer [8], breast [9] and nasopharyngeal cancer [10]. It is widely considered to play a negative role in tumour genesis and development. Mao *et al.* [11] transfected recombinant adenovirus encoding TSLC1 cDNA into non-small cell lung cancer (NSCLC) A549 cell line, and found that 60% of cells underwent apoptosis as TSLC1 accumulated, and induced the activation of caspase 3 at the same. The suppressive role of TSLC1 in genesis and progression of tumours is not fully understood, but

available data suggest that it regulates cell apoptosis and the cell cycle. So far, little is known about the effect of the TSLC1 gene on malignant cells; therefore, we constructed the TSLC1 gene eukaryotic expression vector, so as to establish a solid foundation for further study.

Material and methods

Normal foreskin acrobystia was obtained from patients admitted to Guangdong Medical College Hospital emergency operating room with written consent; TRIzol was purchased from Invitrogen (Carlsbad, CA, USA); *Escherichia coli* JM109 and plasmid pIRES2-EGFP were kind gifts from the biochemistry laboratory of Guangdong Medical College; High Fidelity PrimeScript™ RT-PCR Kit, DL2,000 DNA Marker, λ-Hind III DNA Marker, pMD19-T Simple vector, DNA A-Tailing Kit, DNA Ligation Kit, Agarose Gel DNA Purification Kit, restriction endonucleases EcoR I and Bgl II were purchased from TaKaRa Biotechnology Co, Ltd (Dalian, China). Mini Plasmid Purification Kit was obtained from Beyotime Institute of Biotechnology (Haimen, China).

Obtaining and identification of TSLC1

Design and synthesis of TSLC1 primer

According to GenBank, accessible human TSLC1 gene mRNA sequence data (serial accession: AY358334), using the primer design software Premier 5.0 to design primers containing Bgl II/EcoR I restriction site fragment for amplification of the ORF length of TSLC1. The primer was synthesized by TaKaRa Biotechnology Co, Ltd (Dalian, China) Primer: OUTER F 5'-GCGTGGGGTGCCCGACATGG-3' 20 mers
OUTER R 5'-AATAGGGCCAGTTGGACAC-3' 20 mers.

Total RNA extraction

Up to 100 mg of the normal foreskin (fat removed) specimen plus liquid nitrogen were ground to powder in a grinder followed by adding 1 ml Trizol, and extraction of total RNA (according to the instructions of the Trizol kit).

RT-PCR assay

With the High Fidelity Prime Script™ RT-PCR Kit for (according to instructions of the kit) PCR amplification, the PCR conditions were: 94°C, 3 min, 1 cycle; 98°C, 10 s, 55°C, 15 s, 72°C, 1 min, 30 cycles; 72°C, 10 min, 1 cycle.

Recovery and purification of PCR product

Take PCR products were loaded in 1 × TAE buffer agarose gel electrophoresis, 120 V, 30 min; agarose

gel including the purpose of DNA was excised under the UV lamp, purified using TaKaRa Agarose Gel DNA Purification Kit according to the instructions, and TSLC1 fragments were recovered.

TA Cloning

One µg of the recovered TSLC1 fragments was added "A" tail with A-Tailing Kit; 5 µl of the reaction solution was analysed with agarose gel electrophoresis; the new TSLC1 fragments were ligated to pMD19-T Simple vector with the proportion of 9 : 1 for 16°C, 30 min; 10 µl of pMD19-T Simple-TSLC1 solution was transformed into competent *E. coli* JM109 for amplification.

Extraction and identification of pMD19-T simple-TSLC1 recombinant plasmid

pMD19-T Simple-TSLC1 recombinant plasmid was extracted from *E. coli* JM109 with Mini Plasmid Purification Kit according to the instructions; 1 µg of recombinant plasmid was digested by restriction enzyme Bgl II and EcoR I for 37°C, 8 h; 5 µl of digestion product was analysed with agarose gel electrophoresis; the positive plasmid was sequenced by TaKaRa Biotechnology Co, Ltd (Dalian, China).

Subclone of pIRES2-EGFP-TSLC1 recombinant plasmid

Recovery of TSLC1

Double restriction enzymes (Bgl II and EcoR I) digested pMD19-T Simple-TSLC1 recombinant plasmid, and the remaining steps were the same as "Recovery and purification of PCR product".

Ligation of pIRES2-EGFP-TSLC1

The recovered TSLC1 fragment was inserted to pIRES2-EGFP vector with the concentration proportion of 9 : 1 for 16°C, 8 h (according to DNA Ligation Kit instructions), and then transformed into competent *E. coli* JM 109 for amplification.

Extraction and identification of pIRES2-EGFP-TSLC1 recombinant plasmid

This step is the same as "Extraction and identification of pMD19-T simple-TSLC1 recombinant" except for DNA sequencing.

Results

Evaluation of RT-PCR product

The total RNA extracted from normal foreskin tissue was reversely transcribed to cDNA as a template for PCR, and the full length of 1400 bp of TSLC1 was obtained. Figure 1 depicts the result

of purification of the TSLC1 gene fragment after adding the "A" tail. Figures 2 and 3 show the basic structure and restriction sites of plasmid pMD19-T Simple and pIRES2-EGFP respectively.

Evaluation of TA Clone recombinant plasmid

pMD19-T Simple-TSLC1 recombinant plasmid was digested by restriction enzymes Bgl II and EcoR I and analysed with agarose gel electrophoresis. The positive plasmid was sequenced. The result is shown in Figure 4. Obtained data were aligned with the sequences deposited in GenBank (AY 358334).

Evaluation of pIRES2-EGFP-TSLC1 recombinant plasmid

pIRES2-EGFP-TSLC1 recombinant plasmid was digested by restriction enzymes Bgl II and EcoR I, and then analysed together with pIRES2-EGFP vector (after being digested by restriction enzymes Bgl II and EcoR I) and TSLC1 (from "Recovery of TSLC1") recovered with agarose gel electrophoresis (Figure 5). The result confirmed that the pIRES2-EGFP-TSLC1 eukaryotic expression vector was successfully constructed.

Discussion

Since identification of TSLC1, many scholars have found that it was low or missing expression in a variety of human cancer tissues, while widely present in normal human tissues, especially in the skin, lungs and liver tissues. Therefore, TSLC1 is widely considered as a tumour suppressor gene. The gene has been a hot spot in cancer research, especially the mechanisms of potential to induce apoptosis in malignant cells. In this study, extracts from skin and constructs TSLC1 gene eukaryotic

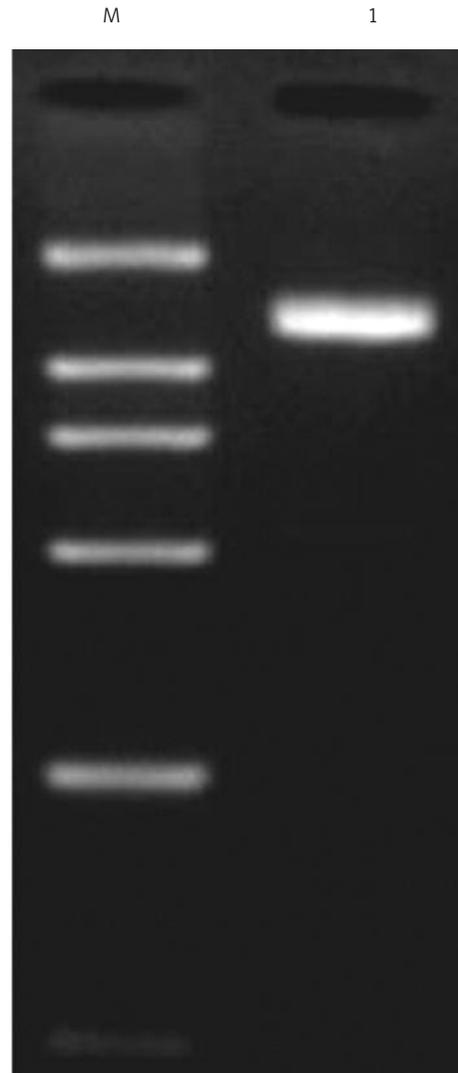


Figure 1. Purification of the TSLC1 gene fragment
M – DL2.000 DNA marker, 1 – TSLC1-PCR product (added "A" tail)

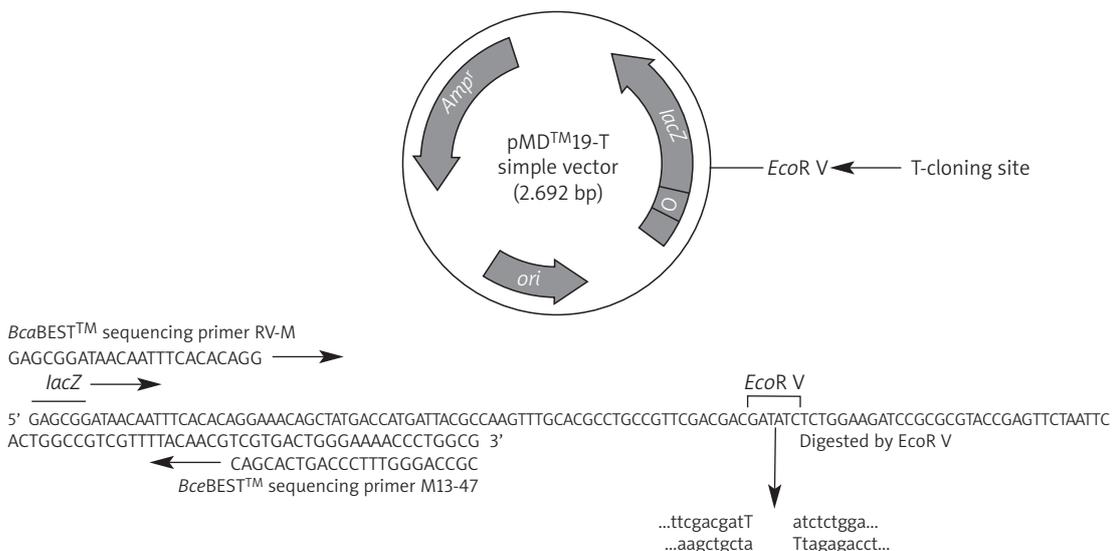


Figure 2. The structure and important restriction sites of pMD19-T simple vector

pIRES2-EGFP vector information

PT3267-5
Catalog #6029-1

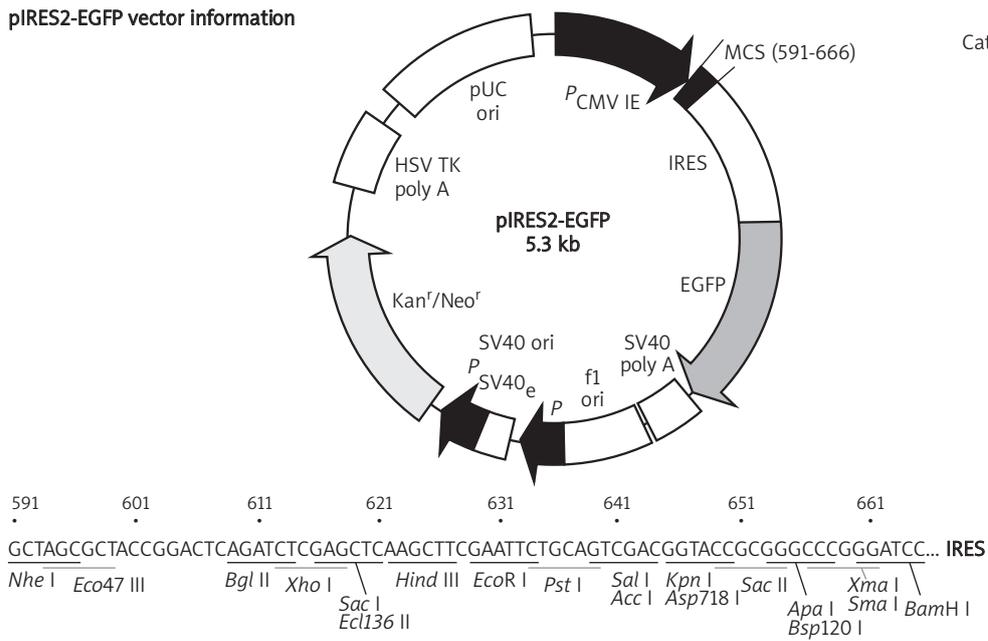


Figure 3. The structure and digestion sites of pIRES2-EGFP plasmid

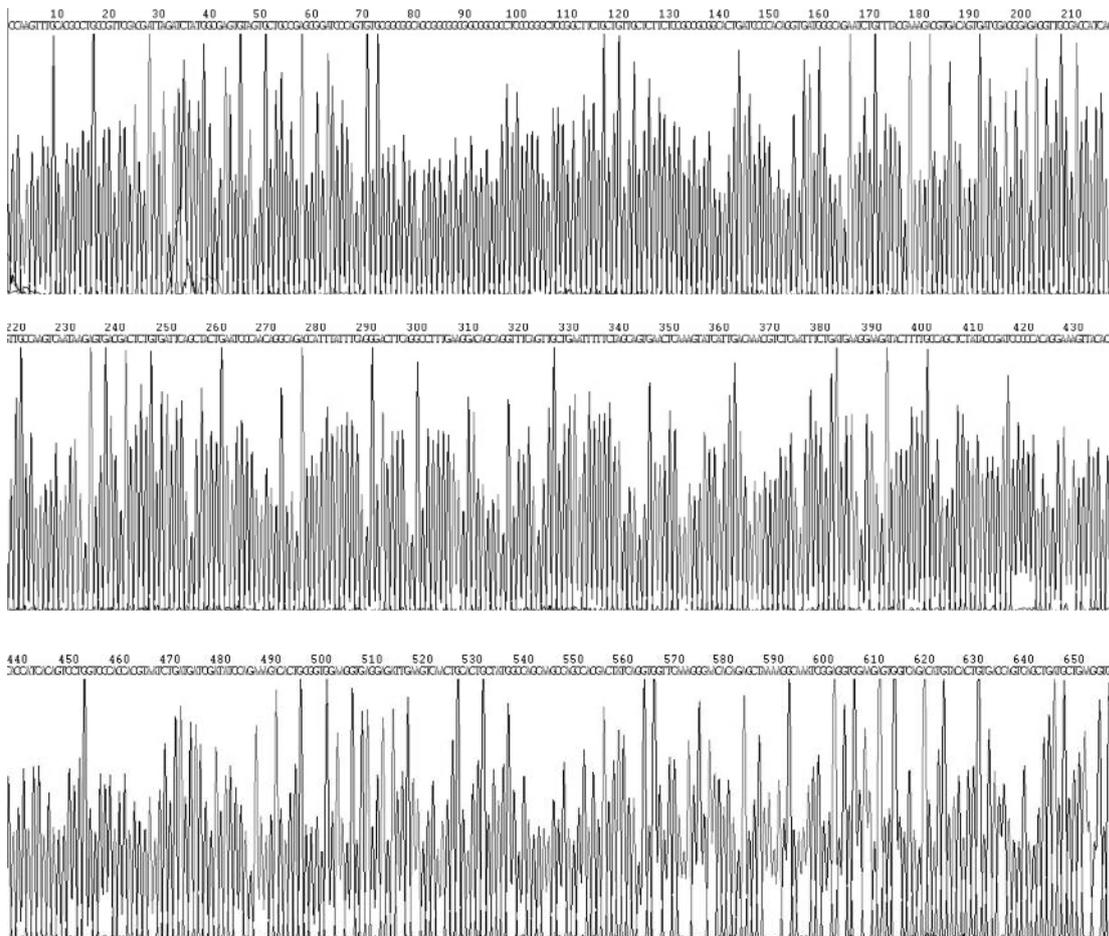


Figure 4. Full-length 1393 bp of the TSLC1 gene sequence diagram

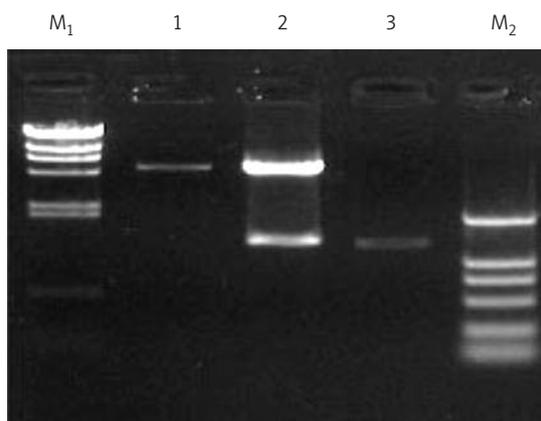


Figure 5. Bgl II/EcoR I double-enzyme digestion analysis of pIRES2-EGFP-TSLC1 recombinant plasmid *M*₁ – λ -Hind III DNA marker *1* – pIRES2-EGFP vector, *2* – pIRES2-EGFP-TSLC1 double-enzyme digestion, *3* – TSLC1 INSERT, *M*₂ – DL2,000 DNA marker

expression vector for further study of the mechanism of tumour suppressor genes.

The key of the TSLC1 gene clone is the vector; it determines whether the exogenous gene can express steadily after being transfected into cells. The present study shows that the pIRES2-EGFP eukaryotic expression vector has the following advantages: 1) It contains internal ribosome entry site 2 (IRES2) and enhanced green fluorescent protein (EGFP) clone regions, enable transiently transfected into mammalian cells to express EGFP with high selectivity; furthermore, it is able not only to express EGFP alone but also to obtain a stable transfected cell line. 2) The EGFP is a kind of wild-type GFP, with characteristics of highly selective fluorescence and highly expressed in mammalian cells. Two amino acids (Phe-64 and Ser-65) in GFP mut1 variant6 of EGFP can be replaced by Leu and Thr, which makes its green fluorescence intensity increase greatly, thus screening exogenous gene expression in mammalian cells easily. 3) It contains an immediate early strong promoter from cytomegalovirus, which is conducive to a high level of transcription; furthermore, the EGFP gene downstream contains SV40 polyA signal, which can improve the stability of RNA. 4) It contains the neomycin and kanamycin resistance gene and the HSV TK gene poly A signal, which are favourable to screening positive cells after cell transformation or transfection [12-14].

Designing a pair of primers with Bgl II/EcoR I restriction enzyme sites to amplify the ORF of TSLC1, and mounted to pMD19-T Simple vector, *E. coli* transformation, sequencing, etc., have confirmed that the obtained cDNA extracted from the skin is submitted to GenBank release (accession number: AY 358334). In the current study, this DNA Ligation Kit is different from the ordinary Taq ligase enzyme, which can ligate the TSLC1 fragment into

the pIRES2-EGFP eukaryotic expression vector exactly and efficiently after double restriction enzyme digestion, without needing to consider the pros and cons ends of ligating fragments. The recombinant plasmid was excised out by Bgl II/EcoR I double-restriction enzyme digestion and analysed by agarose gel electrophoresis. The result shows that the ligated fragments were about 1.4 kb and 5.3 kb, which reveals that the TSLC1 gene and pIRES2-EGFP vector size was corrected, and the TSLC1 eukaryotic expression vector has been constructed successfully.

Acknowledgments

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