p53 암억제 유전자가 삽입된 재조합 pOPINEneo-3C-GFP 벡터의 효율 분석

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Analysis of Efficiency of Recombinant pOPINEneo-3C-GFP Vector with *p53* Tumor Suppression Gene Inserted

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요 약

재조합 배큘로 바이러스는 배양 된 곤충 세포에서 이종 유전자를 발현하는데 널리 사용된다. 재조합 배큘로 바이러스는 광범위한 포유류 세포 유형에서 재조합 단백질의 발현을위한 유전자 전달 벡터로서 작용할 수있다. 바큘로 바이러스 시스템은 안전성, 대규모 및 높은 수준의 유전자 발현 관점에서 중요한 이점을 갖는다. 본 연구에서는 pOPINEneo-3C-GFP 벡터로부터 재구성 된 바큘로 바이러스 벡터를 사이토 메갈로 바이러스 (CMV) 프로모터, 강화 된 녹색 형광 단백질 (EGFP) 및 p53과 *NcoI* 및 *XhoI*로 재조합시켰다. 이러한 재조합 벡터를 다양한 세포 및 세포주에 감염시켰다. 이와 같이 개발 된 바큘로 바이러스 벡터는 재조합 유전자의 전이 및 발현을 통상적 인 벡터와 비교하여 분석 하였다. 이러한 결과는 바큘로 바이러스 벡터가 대조군 벡터보다 전이 및 전이에서 더 높은 효율을 갖는다는 것을 시사한다. 본 연구는 과학 기술부, 한국 정보 기술 진흥 기금 (MSIP)이 후원하는 한국 연구 재단 (NRF)을 통해 중견 연구원 프로그램 (NRF-2016R1A2B4016552)을 통해 지원되었다.

ABSTRACT

Recombinant baculoviruses are widely used to express heterologous genes in cultured insect cells. Recombinant baculoviruses can serve as gene-transfer vectors for expression of recombinant proteins in a wide range of mammalian cell types. Baculovirus system has significant benefits in view of safety, large-scale, and high level of gene expression. In this study, baculoviral vectors which were reconstructed from pOPINEneo-3C-GFP vector, were recombined with cytomegalovirus (CMV) promoter, green fluorescent protein (GFP), and p53 with *NcoI* and *XhoI*. These recombinant vectors were infected with various cells and cell lines. The baculovirus vector thus developed was analyzed by comparing the metastasis and expression of the recombinant genes with conventional vectors. These results suggest that the baculovirus vector has higher efficiency in metastasis and expression than the control vector. This work was supported by a grant from Mid-Career Researcher Program(NRF-2016R1A2B4016552) through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning(MSIP).

키워드

baculovirus, pcDNA3.1, protein transduction domain, vesicular stomatitis virus G

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Ⅰ. Introduction

Recombinant baculoviruses are widely used to express heterologous genes in cultured insect cells and insect larvae. For large-scale applications, the baculovirus expression vector system is particularly advantageous. The major difference between the naturally occurring in vivo infection and the recombinant in vitro infection is that the naturally occurring polyhedrin gene within the wild-type baculovirus genome is replaced with a recombinant gene or cDNA. These genes are commonly under the control of polyhedrin and p10 promoters. In the late phase of infection, the virions are assembled and budded recombinant virions are released. However, during the very late phase of infection, the inserted heterologous genes are placed under the transcriptional control of the strong polyhedrin promoter [1]. Baculoviruses are essentially nonpathogenic to mammals and plants. and have been reproducibly scaled up for the large-scale production of biologically active recombinant products [1].

There are several advantages of using baculovirus expression system over *E. coli* system, such as improved solubility, ability to incorporate post-translational modifications, and higher yields for secreted proteins. For customers who prefer a eukaryotic expression system, are exploring lower-cost alternatives to mammalian expression system but do not want to compromise on the overall quality of their recombinant protein Recombinant baculoviruses can serve as gene-transfer vectors for expression of recombinant proteins in a wide range of mammalian cell types. More over, by inclusion of a dominant selectable marker in the viral vector, cell lines can be derived that stably express recombinant genes. The baculoviruses are a family of large rod-shaped viruses that can be divided to two genera: nucleopolyhedroviruses (NPV) and granuloviruses (GV). While GVs contain only one nucleocapsid per envelope, NPVs contain either single (SNPV) or multiple (MNPV) nucleocapsids per envelope. The enveloped virions are further occluded in granulin matrix in GVs and polyhedrin for NPVs. Moreover, GV have only single virion per granulin occlusion body while polyhedra can contain multiple embedded virions [2]. Baculoviruses have very species-specific tropisms among the invertebrates with over 600 host species having been described. Immature (larval) forms of moth species are the most common hosts, but these viruses have also been found infecting sawflies, mosquitoes, and shrimp. Although baculoviruses are capable of entering mammalian cells in culture [3], they are not known to be capable of replication in mammalian or other vertebrate animal cells.

Baculovirus expression in insect cells represents a robust method for producing recombinant glycoproteins [4, 5]. Baculovirus-produced proteins are currently under study as therapeutic cancer vaccines with several immunologic advantages over proteins derived from mammalian sources [6]. They have a restricted range of hosts that they can infect that is typically restricted to a limited number of closely related insect species. Because baculoviruses are not harmful to humans, they are considered a safe option for use in research applications.

In this study, baculoviral vectors which were reconstructed from pOPINEneo-3C-GFP vector, were recombined with cytomegalovirus (CMV) promoter, green fluorescent protein (GFP), and p53 with *NcoI* and *XhoI*. These recombinant vectors were infected with various cells and cell lines. The baculovirus vector thus developed was analyzed by comparing the metastasis and expression of the recombinant genes with conventional vectors. These results suggest that the baculovirus vector has higher efficiency in metastasis and expression than the control vector.

II. Materials and methods

2.1. cell culture

The human hepatoma cell line, Huh7, the human cervical carcinoma line, HeLa, and the human glioblastoma, A172, were grown in Dulbecco's modified Eagle medium (DMEM; Gibco BRL, USA), supplemented with 10% FBS and 4 mM glutamine. The cultures were maintained at 37℃ in a humidified atmosphere of 95% air/ 5% CO_2 [7].

2.2. Construction of vector expressing fusion protein

In order to construct the plasmid with the HIV-1 Tat fused to the C-terminus of EGFP (pEGFP-Tat), two oligonucleotides were synthesized and annealed to generate a double stranded oligonucleotide encoding the 11 amino acids from the basic domain

of the HIV-1 Tat. The sequences were (top strand) 5'-GATCTAGAAGCAGCGACAGAGGCGAAGAAGG ACGGTATTAACT-3' and (bottom strand) 5' -ATCTT CGTCGCTGTCTCCGCTTCTTCCTGCCATAATTGAC AGCT-3' [6]. The double stranded oligonucleotide was inserted into pCR *2.1* (Invitrogen, USA) in order to generate pCR 2.1-Tat. Second, the EGFP gene sequence was amplified using PCR from pEGFP-N1 (Invitrogen, USA), with the sense primer 5'-AGAATCCGCTAGCGCTACCGGTCGCCACCCATG G -3' and antisense primer 5'-GAAGATCTCTTGTAC AGCTCGTCCAT-3' [3]. The *Eco*RV/*Bgl*II EGFP fragment of the PCR product and the *Eco*RV /*Bgl*II Tat fragment of pCR 2.1-Tat were subcloned into the *Nde*I/*Bam*HI sites of pET-15b Clontech, USA), generating pEGFP-Tat. The ABI Prism automated sequencing method was used to confirm the nucleotide sequences of all the PCR products.

2.3. Transduction of the fusion protein

When the cells were 70% confluent, the culture medium was replaced with fresh medium containing 10% FBS, and the EGFP or EGFP-Tat added to the growth medium, to a final concentration of 0.5 μ M. The cells were then sampled at the times shown or after at least 10 min.

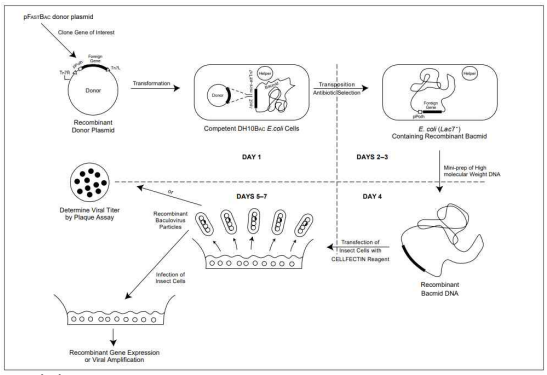
2.4. Fluorescence microscopy

The cultured cells were grown on glass coverslips, and then infected with the recombinant baculovirus (m.o.i. 10) for 1 h. Forty eight hour after infection, the cells were washed three times with 1 ml of PBS (pH 6.4), sealed in GelMount (Biomedia, USA), then viewed on an fluorescencemicroscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

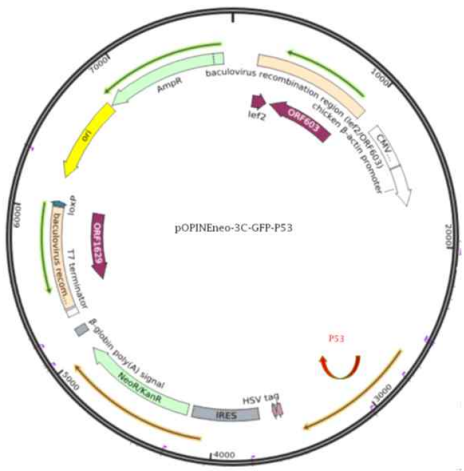
III. Results and conclusions

This baculovirus vector was reconstructed with various and indispensable genes from pcDNA3.1 vector. These vectors were included genes of cytomegalovirus (CMV) promoter,uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD), and so on. A peptide (RKKRRQRRR), derived from the HIV-1 Tat basic domain fused to the C-terminus of EGFP as recombinant fusion proteins, was produced to facilitate the visualization of the PTDs in cell cultures.

We reconstructed with diverse genes including polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, uroplakin II promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD), and p53 (Fig. 1). and these baculoviral vectors identified by expression of EGFP within these vectors



(A)



(B)

Figure 1. Recombined baculoviral vectors and infected cells with these vectors. Generation of recombinant baculoviruses and gene expression with the BAC-TO-BAC expression system (A). Baculoviral vectors constructed with diverse genes including (CMV) promoter, polyhedron promoter, green fluorescent protein (GFP), and p53 genes (B).

We recombined various and indispensable baculovirus vectors with pBac-EGFP, pBacG-EGFP-PTD, pBacG-p53, and pBacG-p53-PTD including (CMV) promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced

green fluorescent protein (EGFP), protein transduction domain (PTD), and p53.

Comparsion of expression of pTERT-p53 and pCMV-p53 represents that promoter TERT operates p53 gene in NIH3T3 cacer cell line (Fig. 2).

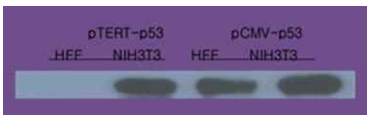
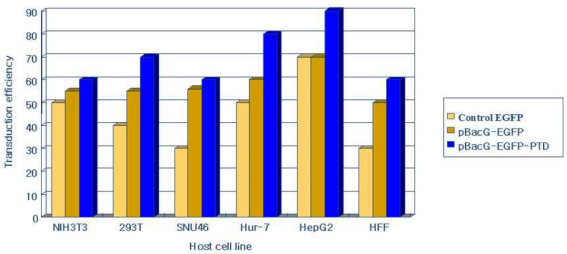


Figure 2. Comparsion of expression of pTERT-p53 and pCMV-p53 in NIH3T3 cacer cell line.

Transduction efficacy of luciferase of recombinant baculoviral vectors containing BacG-EGFP-PTD into cancer cell lines of NIH3T3, 293T, SNU 46, HepG2, and HFF, respectively. This result suggest that BacG-EGFP-PTD vector has more efficient transduction into cancer cell lines than other control vectors (Fig. 3).



MOI = 100

Figure 3. Transduction efficacy of luciferase of recombinant baculoviral vectors containing BacG-EGFP-PTD into cancer cell lines of NIH3T3, 293T, SNU 46, HepG2, and HFF, respectively.

In this study, baculoviral vectors which were reconstructed from pOPINEneo-3C-GFP vector, were recombined with cytomegalovirus (CMV) promoter, green fluorescent protein (GFP), and p53 with *NcoI* and *XhoI*. These recombinant vectors were infected with various cells and cell lines. The baculovirus vector thus developed was analyzed by comparing the metastasis and expression of the recombinant genes with conventional vectors. These results suggest that the baculovirus vector has higher efficiency in metastasis and expression than the control vector.

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