pcDNA3.1 벡터에서 재구성된 재조합 Baculovirus 벡터의 효능

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Efficacy of Recombinant Baculovirus Vector Reconstructed in pcDNA3.1 Vector

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요 약

Baculovirus 발현 시스템은 박테리아 발현 시스템, 특히 복잡한 번역 후 변형을 필요로 하는 것과 비교하여 다량의 재조합 단백질을 생성하는 빠르고 비용 효율적인 방법을 포함하는 많은 알려진 장점을 갖는다. 특히 재조합 baculovirus는 광범위한 포유류 세포 유형에서 벡터를 전달하고 재조합 단백질을 발현 할 수 있다. 본 연구에서는 pcDNA3.1로부터 재구성된 baculovirus 벡터를 사용하였는데 이 벡터는 cytomegalovirus (CMV) 프로모터, uroplakin II promoter, polyhedron promoter, 수포 구내염 바이러스 G (VSVG), 녹색 형광 단백질 (EGFP), 단백질 전달 도메인 (PTD) 유전자 등 다양한 유전자들로 재조합 되어 개발되었다. 이러한 재구성 된 벡터를 다양한 세포 및 세포주에 감염시켰다. 이렇게 개발된 baculovirus 벡터는 재조합된 유전자들의 전이성 및 발현성을 기존의 일반적인 벡터와 비교하여 분석하였다. 본 연구결과로 이렇게 개발된 baculovirus 벡터는 기존의 대조군 벡터보다 전이성 및 발현성면에서 더 높은 효율을 갖는다는 것을 확인하였다. 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

Baculovirus expression systems have many known advantages including fast and cost-effective methods to generate large amounts of recombinant proteins in comparison to bacterial expression systems, particularly those requiring complex post-translational modifications. Especially, recombinant baculoviruses can transfer their vectors and express their recombinant proteins in a wide range of mammalian cell types. In this study, baculoviral vectors which were reconstructed from pcDNA3.1 vector, were recombined with cytomegalovirus (CMV) promoter,uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). 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

Baculovirus expression systems have many known advantages including fast and cost-effective methods to generate large amounts of recombinant proteins in comparison to bacterial expression systems, particularly those requiring complex post-translational modifications Baculoviruses have double-stranded, circular, and supercoiled DNA molecules in a rod-shaped capsid. They are the most prominent viruses known to transfer the genes. Recombinant baculoviruses are widely used to express heterologous genes in cultured insect cells [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 pcDNA3.1 vector, were recombined with cytomegalovirus (CMV) promoter,uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). 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 efficienc 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'-GATCTAGAAGCAGCGACAGAGGCGAAGAAG

GACGGTATTAACT-3' and (bottom strand) 5' -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCATAATTGACAGCT-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'-AGAATCCGCTAGCGCTACCGGTCGCCACCCATGG -3' and antisense primer 5'-GAAGATCTCTTGTACAGCTCGTCCAT-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 μM. 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

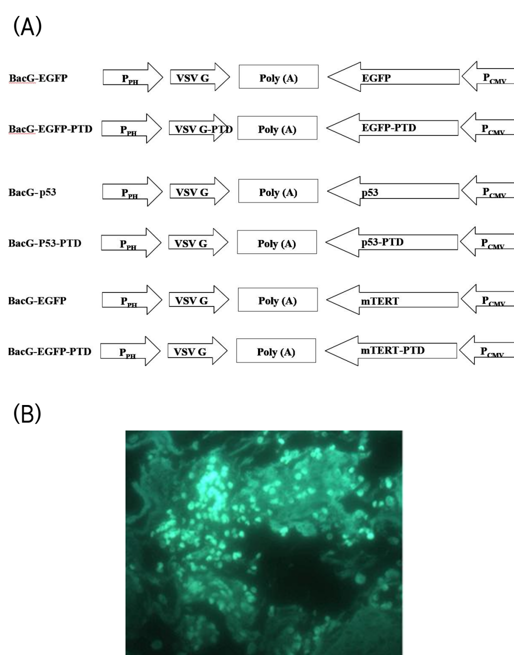

Figure 1. Recombined baculoviral vectors and infected cells with these vectors. Baculoviral vectors constructed with diverse genes including (CMV) promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD), and p53 (A) and infected cells by baculoviral vectors with including EGFP-PTD 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.

Transduction efficiency of reconstructed baculovirus vectors (pBac-EGFP, pBacG-EGFP-PTD, and pBacG-P53-PTD) were compared in infected cell lines (sf9, HFF, HepG2, Hur7, 293T, and NIH3T3) (Fig. 2).

Figure 2. Transduction efficiency of recombinant baculoviral vectors (pBac-EGFP, pBacG-EGFP-PTD, and pBacG-P53-PTD) in infected cell lines (sf9, HFF, HepG2, Hur7, 293T, and NIH3T3).

Survival rate of recombinant baculoviral vectors (pBac-EGFP, pBacG-EGFP-PTD, and pBacG-P53-PTD) in infected sf9 cell. Most recombinant baculoviral vectors represented high survival rate and safety in sf9 cell.

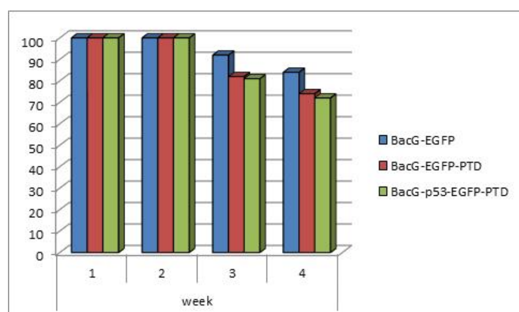

Figure 3. Survival rate of recombinant baculoviral vectors (pBac-EGFP, pBacG-EGFP-PTD, and pBacG-P53-PTD) in infected sf9 cell.

In this study, baculoviral vectors which were reconstructed from pcDNA3.1 vector, were recombined with cytomegalovirus (CMV) promoter,uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). 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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