Baculovirus 벡터내 재구성된 유전자의 전이와 발현

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Transfection and Expression of Reconstructed Genes within Baculoviral Vectors

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요 약

Baculovirus는 원래 알팔파 루퍼 (looper)로부터 분리되었으며 154 개의 오픈 리딩 프레임 (ORF)을 가진 134-kbp 게놈을 포함하고 있다. 주요 캡시드 단백질 VP39는 약간의 단백질과 함께 p6.9 단백질로 DNA를 감싸는 뉴클레오 캡시드(21 nm x 260 nm)로 형성된다. 그것들은 막대 모양의 캡시드 안에 이중 가닥의 고리 모양의 슈퍼 코일 DNA 분자이다. 야생형 baculovirus는 용균 및 폐색 된 생명주기를 모두 나타내며 바이러스 복제의 3 단계에 걸쳐 독립적으로 발달한다. 재조합 baculovirus는 광범위한 포유류 세포 유형에서 벡터를 전달하고 재조합 단백질을 발현 할 수 있다. 특히, 이들 baculovirus 벡터에 우세한 선별 마커를 포함시킴으로써 많은 세포에서 다양한 재조합 유전자를 발현시킬 수 있다. 본 연구의 배큘로 바이러스 벡터는 cytomegalovirus (CMV) 프로모터, uroplakin II promoter, polyhedron promoter, 수포 구내염 바이러스 G (VSVG), 녹색 형광 단백질 (EGFP), 단백질 전달 도메인 (PTD) 유전자 등으로 재구성되었다. 이러한 재구성 된 벡터를 다양한 세포 및 세포주에 감염시켰다. 우리는 다른 재조합 벡터와 비교하여 이러한 재조합 벡터의 전이 및 발현을 조사하는 수행하였다. 본 연구에서, 우리는 이 재조합 벡터의 형질 감염 및 발현이 어떤 대조군 벡터보다 더 높은 효능을 갖는다는 것을 알았다. 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 was originally isolated from the alfalfa looper and contains a 134-kbp genome with 154 open reading frames (ORF). The major capsid protein VP39 together with some minor proteins forms the nucleocapsid (21 nm x 260 nm) that encloses the DNA with p6.9 protein. They are double-stranded, circular, supercoiled DNA molecules in a rod-shaped capsid. Wild-type baculoviruses exhibit both lytic and occluded life cycles that develop independently throughout the three phases of virus replication. Recombinant baculoviruses can transfer their vectors and express their recombinant proteins in a wide range of mammalian cell types. Especially, inclusion of a dominant selectable marker in these baculoviral vectors can express diverse recombinant genes in many cells. Baculoviral vectors were reconstructed with cytomegalovirus (CMV) promoter,uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) gene and so on. These reconstructed vectors were infected into various cell and cell lines. We performed transfection and expression of these recombinant vectors comparison with other control vectors. From this study, we knew that transfection and expression of these recombinant vectors have higher efficacy than any 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, enhanced green fluorescent protein, protein transduction domain, vesicular stomatitis virus G

Ⅰ. INTRODUCTION

The history of the discovery of baculoviruses is intimately related to the development of the silk industry that occurred in China as early as 5000 years ago. 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, genes of cytomegalovirus (CMV) promoter, uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) gene and so on were reconstructed. This reconstructed vector was infected into various cell lines and tissues. We investigated gene transfer and gene expression of this reconstructed vector in comparison to other vectors and recognized that this reconstructed vector was higher effective than any other 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′-GATCTAGAAGCAGCGACAGAGGCGAAGA AGGACGGTATTAACT-3′ and (bottom strand) 5′ -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCA TAATTGACAGCT-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′-AGAATCCGCTAGCGCT ACCGGTCGCCACCCATGG -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

In this study, baculovirus vector was reconstructed with various and indispensable genes. These vectors were included genes of (CMV) promoter, uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) gene, 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) (Fig. 1).

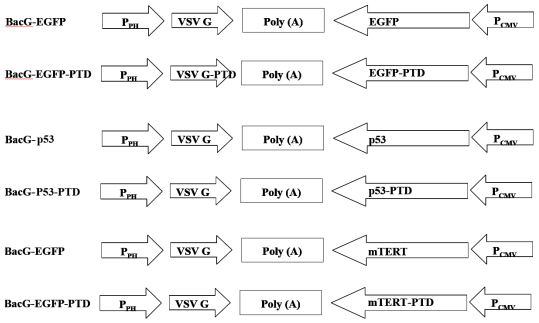

Fig. 1. Recombined baculovirus vectors constructed with diverse genes including (CMV) promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD), and p53.

We reconstructed 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 (293T, NIH293, HepG2, HFF, Hur7, and SNU46) (Fig. 2).

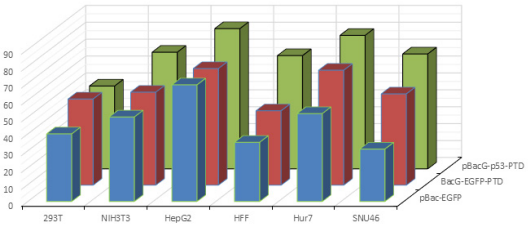

Fig. 2. Determination of gene expression of cell lines by reconstructed baculovirus (pBac-EGFP, pBacG-EGFP-PTD, and pBacG-P53-PTD) in infected cell lines (293T, NIH293, HepG2, HFF, Hur7, and SNU46).

Gene expression efficiency of recombined baculovirus vector with pBacG-P53-PTD were higher in cell lines 293T, NIH293, HepG2, HFF, Hur7, and SNU46. Among them, gene expression efficiency of recombined baculovirus vector was higher in HepG2 cell line than in any other cell lines.

In this study, we recombined baculovirus vectors with genes of (CMV) promoter, uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) gene, and so on. These recombined vectors were infected into various cell lines. We investigated gene transfer and gene expression of this recombined vector in comparison to control vector and recognized that reconstructed baculo virus vector with pBacG-P53-PTD was higher effective than any other vector.

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