
새로운 재조합 베쿨로바이러스벡터의 유전자전이와 유전자발현

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Gene Transfer and Gene Expression of Novel Recombinant Baculovirus Vector System

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요 약

베쿨로바이러스 시스템이 제조되었는데 이것은 polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) 유전자를 재조합한 것이다. 본 재조합벡터 시스템은 인간 섬유아세포에 적용하여 시험하였고 재조합된 유전자의 전이와 유전자 발현을 대조 벡터시스템과 비교하였다. 본 연구로부터 새롭게 제작된 본 베쿨로바이러스 시스템이 유전자의 전이와 유전자 발현 면에서 대조 벡터시스템 보다 고효율을 나타내었다.

ABSTRACT

Several baculovirus vector systems recombined with coding genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) were constructed. These recombinant baculovirus vector systems were applied into human foreskin fibroblast cells and compared the effects of gene transfer and gene expression of these recombinant baculovirus vector systems with control vector system. From this study, it showed that these novel recombinant baculovirus vector systems were superior efficacy to control vector system in view of gene transfer and gene expression.

Keyword

baculovirus, enhanced green fluorescent protein, protein transduction domain, vesicular stomatitis virus G

I. INTRODUCTION

Baculoviruses are widely used to express heterologous genes in insect cells cultured. The baculovirus expression vector system is particularly advantageous for many application field and specialized media, transfection reagents, and vectors that have been developed in response to recent advances in insect cell culture and molecular biology methods. Since 1983, baculovirus system is one of the most powerful eukaryotic vector systems for recombinant protein expression [1]. Baculovirus system has significant benefits in view of safety, large-scale, and high level of

gene expression.

PTDs, Specific proteins, have been identified as carriers for the efficient delivery of proteins that do not permeate living cells [2]. Although the mechanism is unknown, transduction occurs in receptor- and transporter-independent manners, which appears to target the lipid bilayer directly [3]. PTDs include the peptides derived from the basic domain of HIV-1 Tat, the homeodomain of *Drosophila Antennapedia* and the HSV VP22 transcription factor. The short Tat peptide, YGRKKRRQRRR (residues 47-57) is sufficient for the intracellular transduction and subcellular

localization [4, 5]. This domain can deliver a wide variety of proteins, ranging in size from 15 to 120 kDa, across the plasma membrane by a mechanism referred to as protein transduction [4].

In this research, we constructed a recombinant baculovirus vector system containing PTD and compared efficacy of gene transfer and expression in human foreskin fibroblast (HFF).

II. MATERIALS AND METHODS

A. cell culture

The insect cell line, Sf9, was grown in Grace's medium (Invitrogen, USA), supplemented with 10% fetal bovine serum (FBS; HyClone, USA). 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°C in a humidified atmosphere of 95% air/ 5% CO₂ [2].

B. 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'-GATCTAGAAGCAGCGACAGAGCGAAGAAGGACGG TATTA ACT-3' and (bottom strand) 5'-ATCTTCGTCGCTGCTCCGCTTCTTCTGCCATAATTG ACAGCT-3' [2]. 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'-AGAATCCGCTAGCGCTACCGGTGCGCCACCCATGG -3' and antisense primer 5'-GAAGATCTCTGTACAGCTCGTCCAT-3' [2]. The *EcoRV*/*Bgl*II EGFP fragment of the PCR product and the *EcoRV*/*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.

C. Transduction of the fusion protein into cells

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.

D. 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 (Biomedica, USA), then viewed on an fluorescence microscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

III. RESULTS AND CONCLUSIONS

We constructed a recombinant baculovirus vector system. 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. A control EGFP expression vector was also constructed by inserting the coding sequence for EGFP into the pET15b expression vector.

We compared through analysis of FACS of mock and HFF infected by recombinant baculovirus containing vesicular stomatitis virus G, VSVG, and EGFP in Fig. 1.

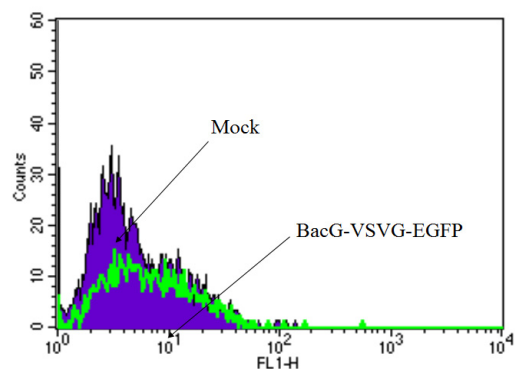


Fig. 1. Analysis of FACS of HFF infected by recombinant baculovirus containing vesicular stomatitis virus G, VSVG, and EGFP

We confirmed transfection efficiency of pBac-VSVG-PTD-EGFP was higher than pBac-VSVG-EGFP in human foreskin fibroblast

(Fig. 2).

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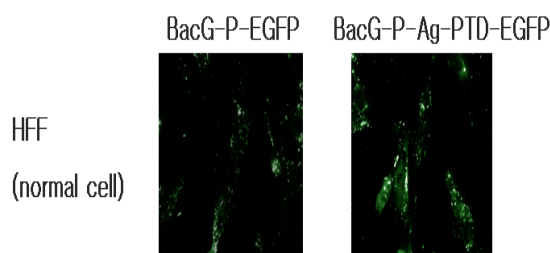


Fig. 2. Expression of EGFP of recombinant baculoviruses containing PTD and without PTD genes infected into human foreskin fibroblast.

From this results, we confirmed this novel recombinant baculovirus vector system was superior to mock or the other control vector system.

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