재조합 베큘로바이러스 벡터 시스템의 신 구축

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Novel Construction of Recombinant Baculovirus Vector System

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요 약

본 연구실에서는 새로운 베큘로바이러스 벡터 시스템을 구축하였다. 즉 본 벡터 시스템은 polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), protein transduction domain (PTD)을 코딩하는 유전자들로 구성 되어있다. 이렇게 새로이 제작된 베큘로바이러스 벡터 시스템과 대조군의 벡터 시스템과 효율과 발현율을 비교하였다. 그 결과 본 연구실에서 제작된 베큘로바이러스 벡터 시스템이 다른 대조군의 벡터 시스템에 비해 효과적임을 확인할 수 있었다

ABSTRACT

We constructed novel recombinant baculovirus vector system. This vector system contained coding genes for polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). We compared efficacy and rate of expression of this novel recombinant baculovirus vector system with other control vector system. From this result, we confirmed that this novel recombinant baculovirus vector system was superior to other control vector system.

Keyword

Recombinant, baculovirus, protein transduction domain, vector

Ⅰ. INTRODUCTION

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]. The baculovirus expression vector system is particularly advantageous and have been developed with advances in cell culture and molecular biology methods. Baculovirus system has become one of the most versatile and powerful eukaryotic vector systems for recombinant protein expression. The HIV-1 PTD of Tat has potentially enhanced utility relative to other PTDs owing to its smaller size of 11 aa. This short Tat peptide, YGRKKRRQRRR (residues 47-57) is sufficient for the intracellular transduction and subcellular localization [2, 3]. 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 [1].

In this study, we constructed a novel recombinant baculovirus vector system and compared efficacy and rate of its expression with other control vector system.

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℃ in a humidified atmosphere of 95% air/ 5% CO_2 [4].

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'-GATCTAGAAGCAGCGACAGAGGCGAAGAAGGACGG TATTAACT-3' and (bottom strand) 5' -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCATAATTG ACAGCT-3' [4]. 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' [4]. 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.

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 (Biomedia, USA), then viewed on an fluorescencemicroscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

E. Flow cytometry

The cultures were harvested with trypsin 24 h after infection and transduction, then washed and resuspended in PBS supplemented with 1% fetal bovine serum. The data collection was performed by FACS Calibur flow cytometry (Becton Dickinson, USA).

III. RESULTS AND CONCLUSIONS

We constructed novel recombinant baculovirus vector system. This vector system contained coding genes for polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD).

We compared efficacy and rate of expression [Fig. 1] of this novel recombinant baculovirus vector system with other control vector system. From this result, we confirmed that pBac-EGFP-PTD, a novel recombinant baculovirus vector system, was superior to pBac-EGFP, a control vector system.

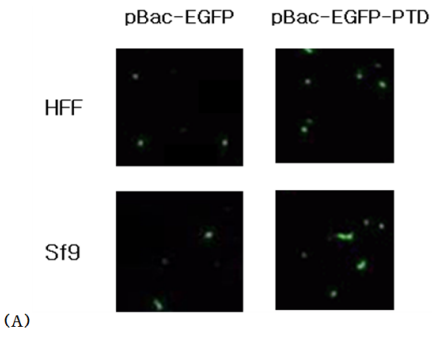

Fig. 1. Expression of EGFP from recombinant baculovirus cotained PTD and non-PTD genes (A: comparision by flourecense microscope, B: comparision by FACS analysis)

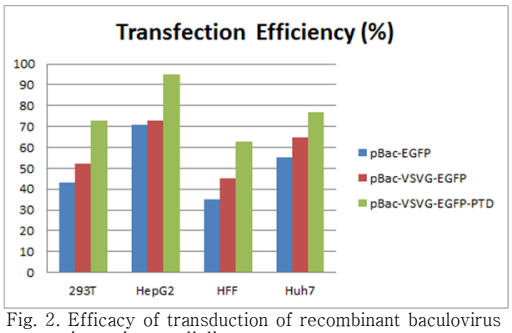

Fig. 2. Efficacy of transduction of recombinant baculovirus vector in various cell lines.

Transfection efficiency of pBac-VSVG-EGFP-PTD ,another novel baculovirus vector system, was higher than pBac-EGFP or pBac-VSVG-EGFP in 293T, HepG2, HFF, and Hur7 cell lines

In this results, we confirmed this novel recombinant baculovirus vector system was superior to other control vector system.

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