재조합 베큘로바이러스벡터의 유전자전달과 발현의 효과

사영희* · 홍성갑*

*연세대학교 · **목원대학교

Efficacy of Gene Transfer and Expression of Recombinanat Baculovirus Vector System

Young-Hee Sa* · Seong-Karp Hong**

*Yonsei University College of Medicine **Mokwon University

E-mail : karp@mokwon.ac.kr

요 약

polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) 유전자가 포함된 새로운 재조합 베큘로바이러스를 제조하였다. 본 재조합 베큘로바이러스 시스템은 293T, HepG2, HFF, Hur7 세포에 감염하여 시험하였고 재조합된 유전자의 전이와 유전자 발현을 대조 벡터시스템과 비교하였다. 본 연구로부터 새롭게 제작된 재조합 베큘로바이러스 시스템이 감염에 의한 유전자의 전달과 해당 유전자 발현에 있어서 대조 벡터시스템 보다 우수한 효과를 나타내었다.

ABSTRACT

Novel baculovirus vector systems including 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 transfected into diverse cells of 293T, HepG2, HFF, and Hur7 cells and compared the effects of gene transfer and expression of these vector systems with control vector. From the result, we confirmed that these recombinant baculovirus vector systems were more excellent than control vector in efficacy of gene transfer and expression

Keyword

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

Ⅰ. INTRODUCTION

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. The most studied baculovirus is Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV). The virus was originally isolated from the alfalfa looper (a lepidopteran) 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.

BV acquires its envelope from the cell membrane and requires a glycoprotein, gp64, to

be able to spread systemic infection. This protein forms structures called peplomers on one end of the budded virus particle but is not found on ODV (although several other proteins are only associated with the ODV form). Some differences also exist in the lipid composition of the viral envelope of the two forms. While the BV envelope consists of phosphatidylserine, ODV contains phosphatidylcholine and phosphatidylethanolamine.

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 Sf9, human foreskin fibroblast (HFF), HepG2, and Hur7 cells and stability of these recombinant baculoviruses systems in mouse.

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 [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'-GATCTAGAAGCAGCGACAGAGGCGAAGAAGGACGG TATTAACT-3' and (bottom strand) 5' -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCATAATTG 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'-AGAATCCGCTAGCGCTACCGGTCGCCACCCATGG -3' and antisense primer 5'-GAAGATCTCTTGTACAGCTCGTCCAT-3' [2]. 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.

III. RESULTS AND CONCLUSIONS

Three recombinant baculovirus vector systems were constructed. 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.

Transduction efficacy of recombinant recombinant baculovirus cotaining BacG-EGFP-PTD-Ag into Sf9, HFF, HepG2, and Hur7 cells, respectively, represents in Fig. 1.

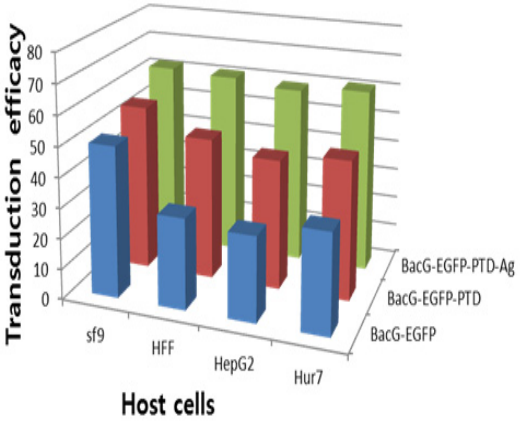

Fig. 1. Transduction efficacy of recombinant recombinant baculovirus cotaining BacG-EGFP-PTD-Ag into Sf9, HFF, HepG2, and Hur7cells, respectively.

Among three recombinant baculovirus systems, BacG-EGFP show 50, 30,, 28, and 33 percent of transduction of efficacy in Sf9, HFF, HepG2, and Hur7 cells, respectively. BacG-EGFP-PTD show 55, 47, 43, and 46 percent of transduction of efficacy in Sf9, HFF, HepG2, and Hur7 cells, respectively. BacG-EGFP-PTD-Ag show 62, 61, 59, and 61 percent of transduction of efficacy in Sf9, HFF, HepG2, and Hur7 cells, respectively.

Transduction of efficacy of recombinant baculovirus (BacG-EGFP-PTD-Ag) was superior to any other recombinant baculovirus in Sf9, HFF, HepG2, and Hur7 cells.

We investigated survival rate of three recominant baculoviruses in mice upto 4 weeks (Fig. 2). Survival rate (number) of mouse by BacG-EGFP infection was 10 upto 3 weeks but 8 in 4 weeks. BacG-EGFP-PTD was 10 upto 2 weeks but 9 in 3 and 4 weeks and BacG-EGFP-PTD-Ag was also 10 upto 2 weeks but 9 in 3 and 4 weeks. Recombinant baculovirus (BacG-EGFP-PTD-Ag) was most stable than the other two recombinant baculoviruses.

In this study, we confirmed this recombinant baculovirus vector system (BacG-EGFP-PTD-Ag) was superior to other control vector systems.

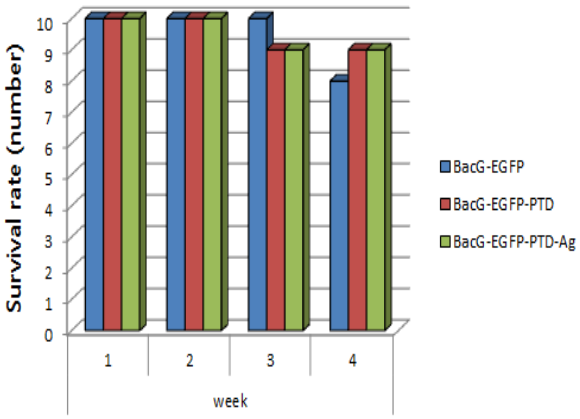

Fig. 2. Stability of recombinant baculoviruses cotaining PTD and without PTD genes infected into mouse.

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