# 재조합 베큘로바이러스벡터의 효과적 발현

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# Effective Expression of Recombinant Baculovirus Vector Systems

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요 약

polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) 유전자가 포함된 재조합 베큘로바이러스를 구축하였다. 본 재조합 베큘로바이러스 시스템은 인간 섬유아세포와 여러 가지 조직에 감염하여 시험하였고 재조합된 유전자의 전달과 유전자 발현을 대조 벡터시스템과 비교하였다. 본 연구의 결과로 제작된 재조합 베큘로바이러스 시스템은 유전자의 전달과 발현에 있어서 대조 벡터시스템 보다 더욱 효과적이고 안전적이었다..

ABSTRACT

A 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 human foreskin fibroblast cells and various tissues and investigated gene transfer and expression of these vector systems with control vectors. From the study, these recombinant baculovirus vector systems were more effective and safe than control vector in view of gene transfer and expression

Keyword

baculovirus, enhanced green fluorescent protein, human foreskin fibroblast, 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 [1]. TBaculoviruses 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 [2], they are not known to be capable of replication in mammalian

or other vertebrate animal cells. Baculoviruses contain circular double-stranded genome ranging from 80–180 kbp.

Baculovirus expression in insect cells represents a robust method for producing recombinant glycoproteins [3], [4]. Baculovirus-produced proteins are currently under study as therapeutic cancer vaccines with several immunologic advantages over proteins derived from mammalian sources [5]. 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, we constructed a recombinant baculovirus vector system and compared efficacy of gene transfer and expression in cells and murine tissues.

## II. MATERIALS AND METHODS

### 2.1. 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% $CO_2$ [6].

### 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'-GATCTAGAAGCAGCGACAGAGGCGAAGAAGGACGGTATTAACT-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' [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.

### 2.3. 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 $\mu$M. 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).

### 2.5. Western blot analysis

The Sf9 cells were infected with Bac-EGFP, Bac-EGFP-Tat m.o.i. (multiplicity ofinfection) 10, in 6-well plates. After 48 h, the cells were lysed in a Laemmli buffer (125 mM Tris, 2% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue, pH 6.8) and heated to 100℃ for 5 min prior to electrophoresis. All the samples were run on SDS-10% polyacrylamide gel electrophoresis. The VSV-G-specific monoclonal antibody was obtained from Roche Molecular Biochemicals. The presence of the VSV-G protein was detected using an ECL Western blotting analysis system (Amersham Bioscience, Sweden).

## III. RESULTS AND CONCLUSIONS

We constructed novel 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 expression of VSVG of recombinant baculovirus cotaining Tat and without Tat (A: EGFP expressed by VSVG using flourecense microscope, B: EGFP expressed by each vector using Western blot hybridization) in Fig. 1. The fusion proteins containing recombinant baculoviral vector were monitored during expression of the entire protein due to green fluorescence (Fig. 1A). EGFP protein of recombinanat baculovirus containing pBacG-EGFP-Tat showed higher efficacy of expression than that of recombinanat baculovirus containing pBacG-EGFP (without Tat).

(A)

(B)


Fig. 1. Expression of VSVG of recombinant baculovirus cotaining Tat and without Tat (A: EGFP expressed by VSVG using flourecense microscope, B: EGFP expressed by each vector using Western blot hybridization).

It represented that expressed EGFP of pBac-VSVG-EGFP-Tat was higher than that of pBac-EGFP in murine pancreas, lung, and kidney tissues, respectively (Fig. 2). This mean transduction and expression efficiency of recombinant baculovirus containing pBacG-EGFP-Tat was higher than that of pBacG-EGFP (without Tat) when these was transfected into animal tissues.

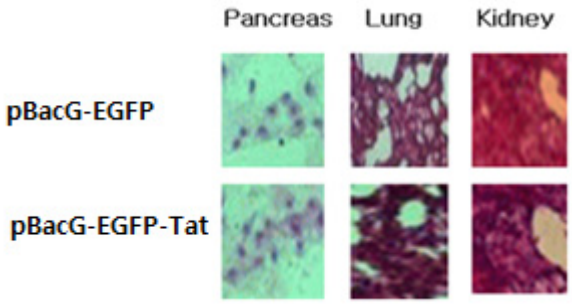


Fig. 2. Expression of EGFP of recombinant baculoviruses cotaining Tat and without Tat genes infected into mouse organs (pancreas, lung, and kidney)

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

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## REFERENCES

[1] G. F. Rohrmann, “Baculovirus Molecular Biology (2nd ed.),”. Bethesda: National Center for Biotechnology Information pp.1, 2011.

[2] C. Hofmann, V. Sandig, G. Jennings, M. Rudolph, P. Schlag, M. Strauss, "Efficient Gene Transfer into Human Hepatocytes by Baculovirus Vectors,". *PNAS* vol. 92, pp.10099-10103, 1995.

[3] F. Altmann, E. Staudacher, I. B. Wilson, L. Marz, "Insect cells as hosts for the expression of recombinant glycoproteins," *Glycoconjugate J.* vol. 16, pp. 109–123, 1999.

[4] T. Kost, and J. P. Condreay, "Recombinant baculoviruses as expression vectors for insect and mammalian cells," *Current Opinion in Biotechnology* vol. 10, pp.428–433, 1999.

[5] D. J. Betting, X. Y. Mu, K. Kafi, D. McDonnel, F. Rosas,, D. P. Gold, J. M. Timmerman, "Enhanced immune stimulation by a therapeutic lymphoma tumor antigen vaccine produced in insect cells involves mannose receptor targeting to antigen presenting cells," *Vaccine* vol. 27, pp.250 - 259, 2009.

[6] J. S. Yoon, Y. T. Jung, S. K. Hong, S. H. Kim, M. C. Shin, D. G. Lee, W. S. Min, S. Y. Paik, "Charateristics of HIV-Tat protein transduction domain," *J. Microbiol.* vol. 42, pp. 328-335, 2004.