
재조합 베쿨로바이러스 벡터의 새로운 가능성

김지영¹ · 김현주¹ · 홍성갑²

¹연세대학교 · ²목원대학교

A Novel Possibility of Recombinant Baculovirus Vector

Ji-Young Kim¹, Hyun Joo Kim¹, Seong-Karp Hong²

¹Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, South Korea.

²Division of Bio and Health Sciences, Mokwon University, Daejeon, South Korea.
E-mail : karp@mokwon.ac.kr

요 약

재조합 베쿨로바이러스는 polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) 유전자로 구성된다. 본 재조합 베쿨로바이러스 벡터는 세포주와 조직에 감염시켰고 그 결과 다른 벡터 시스템에 비교하여 재조합된 유전자의 전이와 유전자 발현에 있어서 새로운 가능성이 발견되었다. 본 재조합 베쿨로바이러스 시스템의 유전자의 전이와 발현의 효율은 타 벡터시스템 보다 우수하였다.

ABSTRACT

Recombinant baculovirus vector is composed of genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). This recombinant baculovirus vector was transfected into cell lines and tissues and then found out a novel possibility in view of gene transfer and gene expression in comparison to other vector systems. Efficacy of gene transfer and gene expression of this recombinant baculovirus vector was higher than any other vector system.

Keyword

baculovirus, protein transduction domain, vesicular stomatitis virus G

I. INTRODUCTION

Baculoviruses are the most prominent viruses known to affect the insect population. They are double-stranded, circular, supercoiled DNA molecules in a rod-shaped capsid. More than 500 baculovirus isolates have been identified. Wild-type baculoviruses exhibit both lytic and occluded life cycles that develop independently throughout the three phases of virus replication [1].

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. Baculoviruses contain circular double-stranded genome ranging from 80–180 kbp.

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, we constructed several recombinant baculovirus vector systems and compared in view of gene transfer and expression of several genes in cell lines.

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°C in a humidified atmosphere of 95% air/ 5% CO₂ [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'-GATCTAGAAGCAGCGACAGAGCGAAGAAGGACGG TATTAAC-3' and (bottom strand) 5' -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCATAATTG ACAGCT-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' [3]. The *EcoRV/BglI* EGFP fragment of the PCR product and the *EcoRV/BglI* Tat fragment of pCR 2.1-Tat were subcloned into the *NdeI/BamHI* 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 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 (Biomedica, USA), then viewed on an fluorescence microscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

III. RESULTS AND CONCLUSIONS

several novel recombinant baculovirus vector systems were constructed in this study. These vectors were included genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). 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 compared expression of recombinant baculovirus vectors (Bac-AFP-Luc, BacG-AFP, Bac-SV40-Luc, and BacG-SV40-Luc) in infected HepG2 cell line and Hur7 cell line reproducing with AFP (Fig. 1A). Also we compared expression of recombinant baculovirus vectors (Bac-SV40-Luc and BacG-SV40-Luc) in infected A549, A172, and HeLa cell lines reproducing without AFP (Fig. 1B).

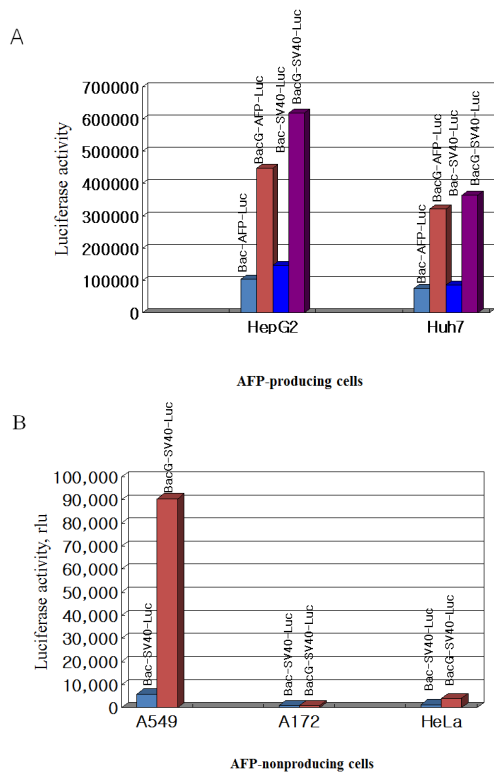


Fig. 1. Comparison of Expression of luciferase by recombinant vaculovirus vectors in infected cell lines reproducing with or without AFP. (A: Bac-AFP-Luc, BacG-AFP, Bac-SV40-Luc, and

BacG-SV40-Luc in infected HepG2 cell line and Hur7 cell line reproducing with AFP; B: Bac-SV40-Luc and BacG-SV40-Luc in A549, A172, and HeLa cell lines reproducing without AFP.

Expression of recombinant baculovirus vectors of BacG-AFP and Bac-SV40-Luc were higher than those of Bac-AFP-Luc and BacG-SV40-Luc, respectively, in infected HepG2 cell line and Hur7 cell line reproducing with AFP. Expression of recombinant baculovirus vector of BacG-SV40-Luc was higher than that of Bac-SV40-Luc in only A549 cell lines reproducing without AFP.

On the other hand, expression of EGFP by recombinant vaculovirus vectors (Bac-AFP, BacG-AFP, Bac-SV40, BacG-SV40) were compared in infected cell lines (HepG2, Hur7, A549, A172, and HeLa) (Fig. 2).

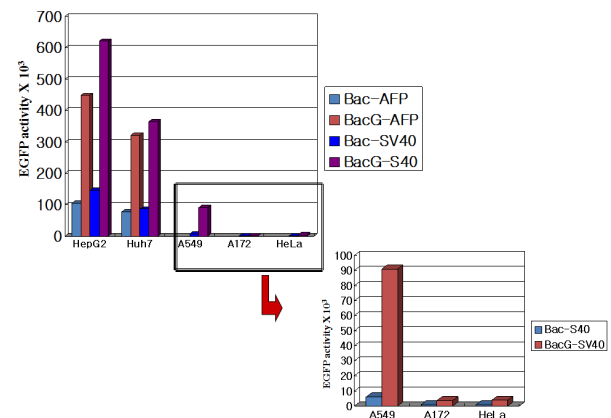


Fig. 2. Expression of EGFP by recombinant vaculovirus vectors (Bac-AFP, BacG-AFP, Bac-SV40, BacG-SV40) in infected cell lines (HepG2, Hur7, A549, A172, and HeLa).

Expression of EGFP by recombinant baculovirus vectors of BacG-AFP and BacG-SV40 were represented higher than those of Bac-AFP-Luc and Bac-SV40-Luc, respectively, in infected HepG2, Hur7, and A549, cell lines.

From this study, we recognized that several novel recombinant baculovirus vector systems were more excellent than any other vector system in view of gene transfer and gene

expression of several recombinant vector genes in cell lines.

ACKNOWLEDGMENTS

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0024912) and a faculty research grant of Yonsei University College of Medicine for 2014(6-2014-0045).

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