재조성된 베큘로바이러스 벡터의 유전자 전이와 발현

김지영[1] · 김현주[1] · 사영희[1] · 홍성갑[2]
[1]연세대학교 ·[2]목원대학교

Gene Transfer and Expression of Newly Reconstructed Baculovirus Vectors

Ji-Young Kim[1], Hyun Joo Kim[1], Young-Hee Sa[1], Seong-Karp Hong[2]
[1]Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, South Korea.
[2]Division of Bio and Health Sciences, Mokwon University, Daejeon, South Korea.
E-mail : karp@mokwon.ac.kr

요 약

베큘로바이러스 벡터가 cytomegalovirus (CMV) promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD)의 유전자로 재조성 되었다. 이렇게 재조성된 베큘로바이러스 벡터는 다양한 세포주와 조직에 감염시켰다. 우리는 이 재조성된 벡터와 다른 대조 벡터를 비교하여 유전자의 전이와 유전자 발현을 비교하였다. 결론적으로 이 재조성된 베큘로바이러스 벡터의 유전자의 전이와 발현의 효율이 대조 벡터 보다 우수한 효율을 나타내었다.

ABSTRACT

Baculovirus vectors were reconstructed using cytomegalovirus (CMV) promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) genes. These reconstructed vector was transfected into various cell lines and tissues. We compared this reconstructed vector with other control vectors in view of gene transfer and gene expression. In conclusion, we confirmed that gene transfer and expression of these reconstructed vectors was higher efficient than any other control vector.

Keyword

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

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].

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.

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.

We reconstructed with useful genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). This reconstructed vector was infected into various cell lines and tissues. We investigated gene transfer and gene expression of this reconstructed vector in comparison to other vectors and recognized that this reconstructed vector was higher effective than any other control vector.

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 [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'-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' [3]. 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

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

Ⅲ. RESULTS AND CONCLUSIONS

Baculovirus vector was reconstructed 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 reconstructed with diverse genes including polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) (Fig. 1).

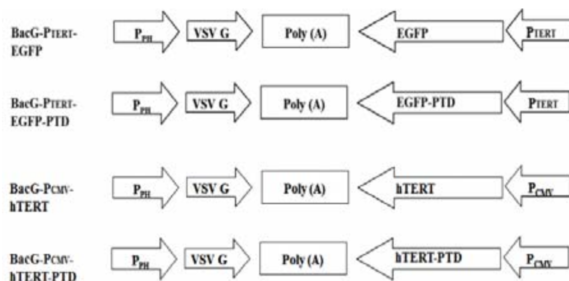

Fig. 1. Reconstructed baculovirus vectors with diverse genes.

We reconstructed four baculovirus vectors with pBac-EGFP, pBacG-EGFP-PTD, pBacG-hTERT, and pBacG-hTERT-PTD.

Transduction efficiency of reconstructed baculovirus vectors (pBac-EGFP and pBacG-EGFP-PTD) were compared in infected cell lines (NIH293, 293T, SNU46, Hur7, HepG2, and HFF) (Fig. 2).

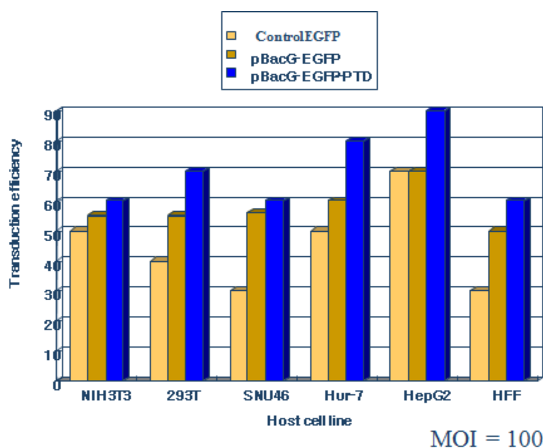

Fig. 2. Determination of tranduction effeciency of cell lines by reconstructed baculovirus (pBac-EGFP, pBacG-EGFP-PTD, and control EGFP) in infected cell lines (NIH293, 293T, SNU46, Hur7, HepG2, and HFF).

Transduction efficiency of reconstructed baculovirus vector with pBacG-EGFP-PTD were higher than other baculovirus vectors (pBacG-EGFP and control EGFP) in infected cell lines NIH293, 293T, SNU46, Hur7, HepG2, and HFF, respectively.

In this study, we reconstructed with useful genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). This reconstructed vector was infected into various cell lines and tissues. We investigated gene transfer and gene expression of this reconstructed vector in comparison to other vectors and recognized that this reconstructed vector was higher effective than any other control vector.

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 (2016R1A2B4016552) and a faculty research grant of Yonsei University College of Medicine for 2015(6-2015-0070).

REFERENCES

[1] "Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques" pp. 1, 2014.

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

[3] 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.

[4] 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.

[5] 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.

[6] 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.

[7] 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.