유용한 유전자들로 재구성된 베큘로바이러스 벡터

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Baculovirus Vector Reconstructed with Useful Genes

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요 약

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

ABSTRACT

Recombinant baculovirus was 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.

Keyword

baculovirus, 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 useful 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).

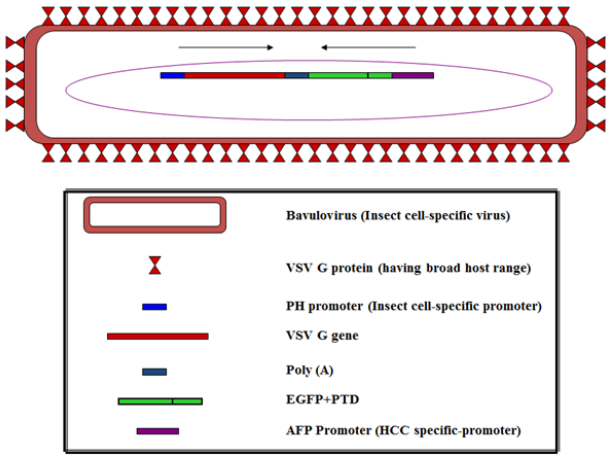

Fig. 1. Reconstruction of vaculovirus vectors with useful genes.

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

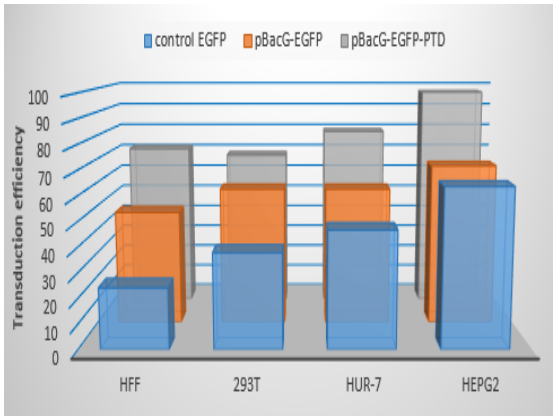

Fig. 2. Determination of tranduction effeciency of cell lines by reconstructed baculovirus (pBac-EGFP and pBacG-EGFP-PTD) in infected cell lines (HFF, 293T, Hur7, and HepG2).

Transduction efficiency of EGFP of reconstructed baculovirus (pBacG-EGFP-PTD) were higher than reconstructed baculovirus (pBacG-EGFP and control EGFP) in infected cell lines HFF, 293T, Hur7, and HepG2, 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.

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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.