재조합 베큘로바이러스벡터와 대조 벡터의 비교

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Comparison of Recombinant Baculovirus Vector Systems and Control Vector System

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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) 유전자로 구성된 재조합 베큘로바이러스를 제작하였다. 본 재조합 베큘로바이러스 시스템은 여러가지 세포주와 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 효능과 안전성면에서 우수하였다.

ABSTRACT

A recombinant baculovirus vector systems were 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). These recombinant baculovirus vector system were transfected into various cell lines and tissues and confirmed gene transfer and expression of these vector systems with only control vector system. From the result, gene transfer and gene expression of recombinant baculovirus vector systems were superior in terms of efficacy and safety than in the control vector system.

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

Ⅰ. INTRODUCTION

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 [1].

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 [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 diverse recombinant baculovirus vector systems and compared efficacy and safety in view of transduction and expression of several genes 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 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

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 recombinant baculovirus vector (pCMV-EGFP) and contol vector (pUPII-eGFP) in NIH/3T3 and MBT-2 cell lines (Fig. 1). The fusion proteins containing recombinant baculoviral vector were monitored during expression of the entire protein due to green fluorescence. EGFP protein of recombinanat baculovirus containing pCMV-EGFP showed higher efficacy of expression than that of control vector (pUPII-eGFP) in only NIH/3T3 cell line, even though recombinant baculovirus vector (pCMV-EGFP) and contol vector (pUPII-eGFP) in MBT-2 cell line were similar.

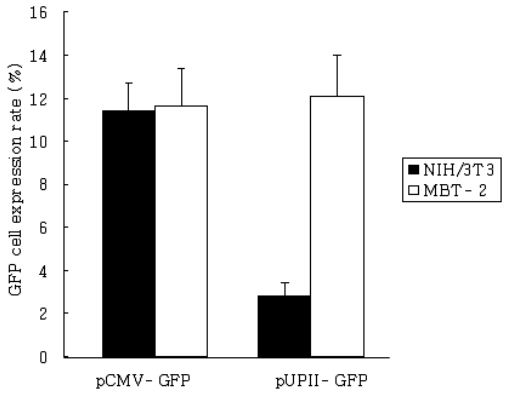

Fig. 1. Difference of Expression of EGFP by control UPII promoter in NIH/3T3 and MBT-2 cell line.

Expression of EGFP of pBac-VSVG-EGFP-Tat was higher than control vector in murine bladder tissues (A and B), 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.

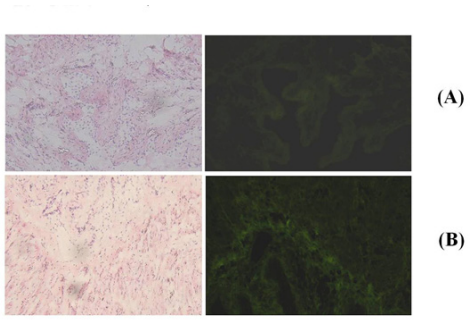

Fig. 2. Expression of EGFP in murine bladder injected by recombinant baculovirus vector-liposome complex.

From this result, we confirmed this novel several recombinant baculovirus vector systems were superior to mere control vector system in view of transduction and expression of several recombinant vector genes in cells and murine tissues.

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