Inhibition Effect of Human Cytomegalovirus Replication by Peptide nucleic acids (PNA)

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ABSTRACT

Human cytomegalovirus (HCMV) provokes fatal infections in AIDS patients that have deficient immune functions and patients that have cellular immune responses repressed after bone marrow transplantation. A new candidate for therapeutic against HCMV is needed because conventional treatments as ganciclovir, acyclovir, cidofovir, and foscarnet cytosine used currently are improper due to their side effects and advent of resistant HCMV. In this study, peptide nucleic acids (PNAs) against UL54 (DNA polymerase) and UL97 (phosphotransferase) that were essential in replication of HCMV were applied in inhibition of replication of HCMV. From the results of this study, 4 PNAs $_{PNA}$UL97-1, $_{PNA}$UL97-2, $_{PNA}$UL54-3, and $_{PNA}$UL54-4) showed 3.7, 3.1, 1.7, and 1.6 folds of inhibition effect against replication of HCMV in the human fibroblast cells. These PNA ssuggest a novel possibility as therapeutic against HCMV.

key word: HCMV, PNA, UL97, UL54

I. Introduction

Peptide Nucleic acids (PNA) is a basically natural mechanism of gene silencing that is widely conserved in the world of multicellular organisms and is thought to have evolved as a defence strategy against viruses and transposable genetic elements. The molecular mediators of PNA are nucleotides analogues as its chemical structure that induce the sequence specific degradation of homologous DNA or RNA. These PNAs have rapidly developed into a powerful experimental tool for manipulating gene degradation. Since exogenous PNAs expression allows the targeted knock-down of virtually any gene, even in mammalian systems, PNA methodology has become broadly applied to many areas, not just for the study of gene functions.

Human cytomegalovirus (HCMV), the prototype of the Betaherpesvirus family is an important pathogen in the transplantation patients, this may have been due to technical limitations, since cell culture infection systems typically rely on growth-arrested primary fibroblasts, which in turn are difficult to transfect efficiently. Therefore, we are willing to optimize transfection protocol of PNA into human foreskin fibroblast (HFF) cells using the Qiagen transfection kit.

II. Materials and Methods

1) Design of PNAs specific for Human cytomegalovirus

PNA	Sequences (5'->3')	Position in HCMV genome
PNAUL54-3	CCC GAA GAA ACG CAA C	81149-81164
PNAUL54-4	GCA GAT ACT GTA GCC G	81122-81137
PNAUL97-1	GTC CAC GGC ATA ACA AAT CTT	141470-141450
PNAUL97-2	GCA TAC ACG ACA CTG GTG ATT	142773-142753
PNAUL97-3	CCA CGG CAT AAC AAA T	142399-142384
PNAUL97-4	ATA CAC GAC ACT GGT G	142771-142756

2) CMV culture and titration

3) Transfection efficiency of PNA
(1) Cytopathic effect
(2) FACS
(3) Real time RT-PCR

III. Results

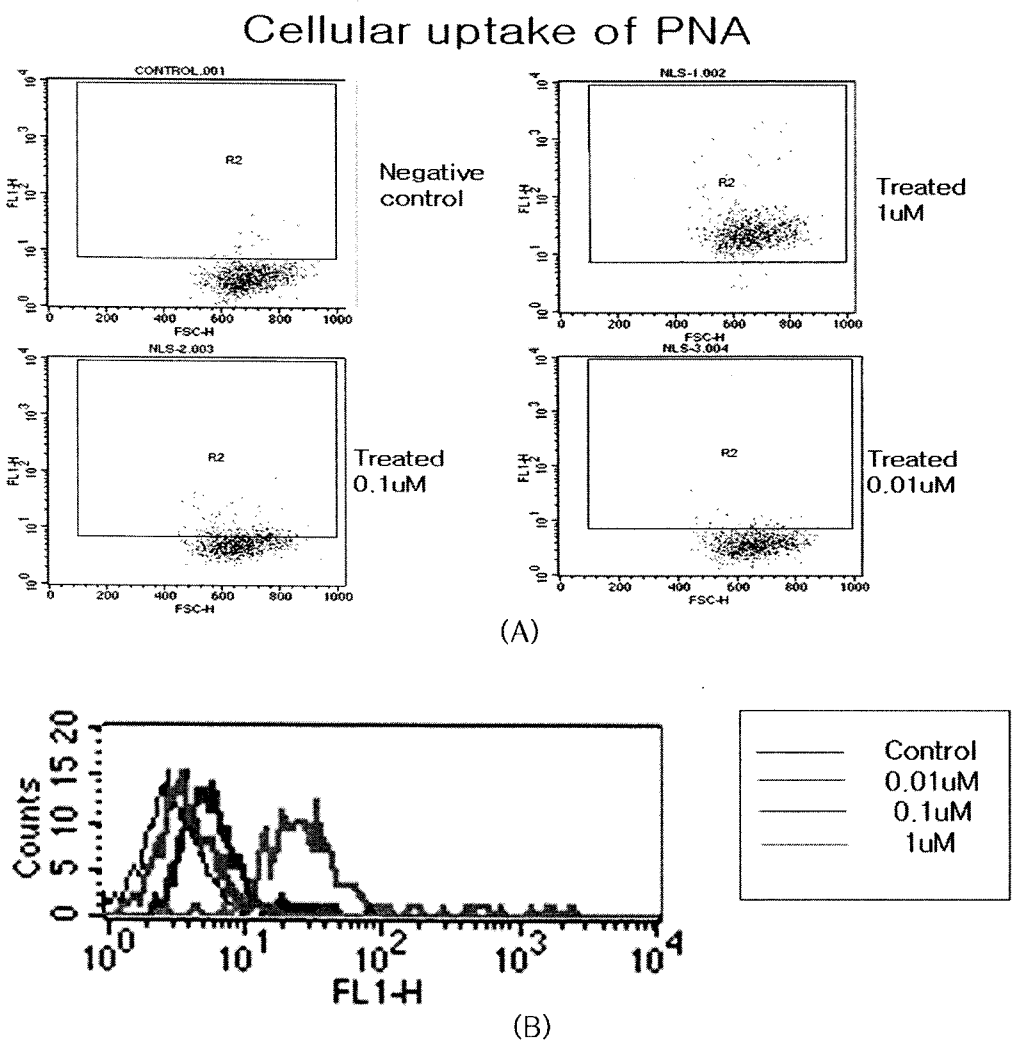

Fig. 1. Determination of uptake concentration of PNA-IFTC into human foreskin fibroblast cells by FACS analysis.

A: Cellular uptake of PNA

B: Infected human fibroblast cell by cytomegalovirus

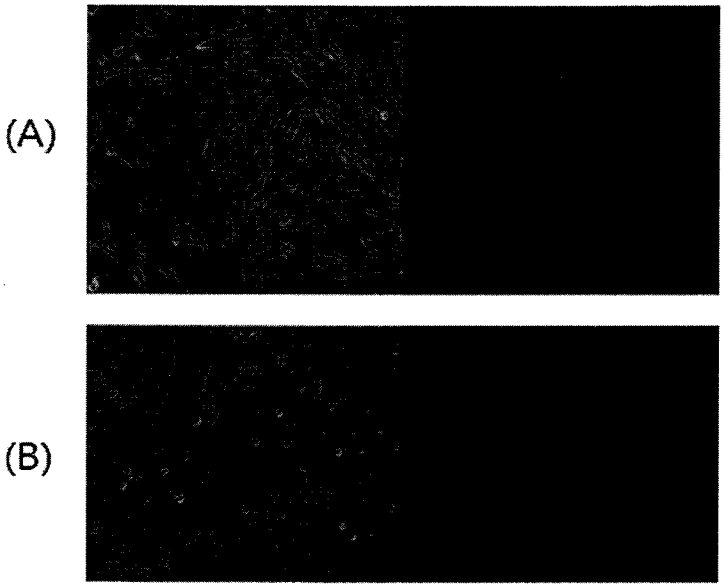

Fig. 2. Analysis by CPE and IFA after HCMV infected into HFF cells
(A) transfection (1 uM) (B) Mock

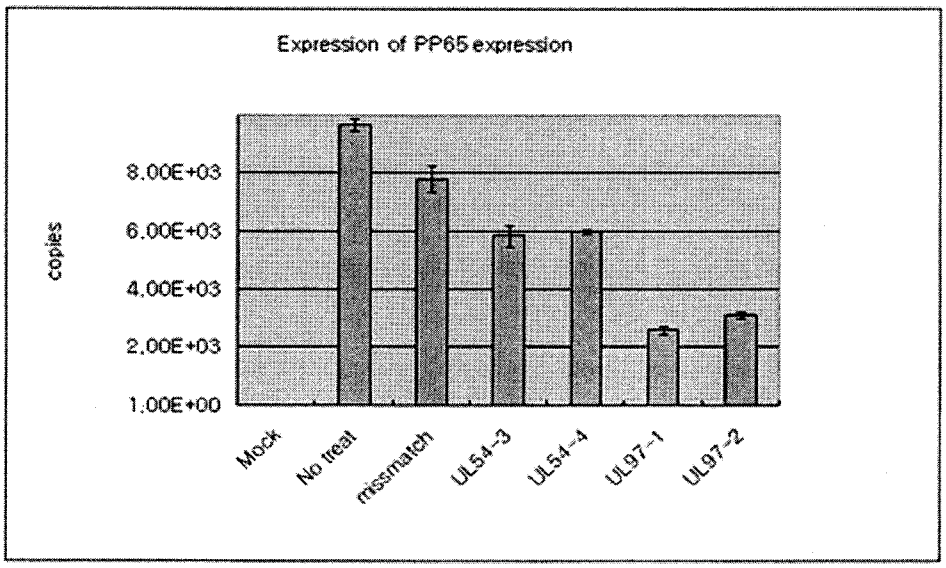

Fig. 3. Inihibition effect of PNA against CMV by real-time RT-PCR

IV. Conclusion

From the results of this study, 4 PNAs $_{PNA}$UL97-1, $_{PNA}$UL97-2, $_{PNA}$UL54-3, and $_{PNA}$UL54-4) showed 3.7, 3.1, 1.7, and 1.6 folds of inhibition effect against replication of HCMV in the human fibroblast cells. These PNA ssuggest a novel possibility as therapeutic against HCMV.

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