Inhibition of Human Cytomegalovirus Replication using Peptide Nucleic Acids with Polyethylenimine

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ABSTRACT

To control replication of human cytomegalovirus (HCMV) effectively, inhibitors of peptide nucleic acids (PNA) with a gene delivery agent, PEI (polyethylenimine) against HCMV were applied. The transfection of these PNA inhibitors with PEI agent into host cells showed synergic inhibition effect of HCMV replication. These inhibition effect was confirmed by methods of RT-PCR, CPE, real-time-PCR, and Western blot.

key word: human cytomegalovirus, peptide nucleic acids, polyethylenimine

I. Introduction

Human cytomegalovirus (HCMV) is an fatal pathogen in post-bone marrow transplants (BMT) with suppressed cellular immunity. There is an urgent need for effective and safe therapeutics of HCMV infections. PNA is a basically natural mechanism of gene silencing that is widely conserved in the world of multicellular organisms and is used as a defence tool against viruses. PEI was the second polymeric transfection agent discovered after poly-l-lysine. PEI condenses DNA into positively charged particles, which bind to anionic cell surface residues and are brought into the cell via endocytosis. Once inside the cell protonation of the amines results in an influx of counter-ions and a lowering of the osmotic potential. The transfection of PNA inhibitors with PEI agent into host cells showed synergic inhibition effect of HCMV replication. These inhibition effect was confirmed by methods of RT-PCR, CPE, real-time-PCR, and Western blot.

II. Materials and Methods

1) Design of PNAs specific for Human cytomegalovirus

Table 1. Nucleic acids of HCMV used as inhibition of replication

HCMV genes	Sequences (5'->3')	Position
UL54-3	GTT GCG TTT CTT CGG G	81164-81149
UL54-4	CGG CTA CAG TAT CTG C	81137-81122
UL97-3	ATT TGT TAT GCC GTG G	142384-142399
UL97-4	CAC CAG TGT CGT GTA T	142756-142771

2) CMV culture and transfection

HFF cell line (ATCC CRL-1635) was maintained in DMEM supplemented with 10% of FBS at 37°C in 5% CO2. HCMV AD169 strain (ATCC VR-538) used in this study was stored at -70°C. HFF cells (8 x 104cell/ml) in 12-well plates were transfected with 20 nmol/l each of the siRNAs using the transfection reagent according to the manufacturer's instructions.

3) Assay

(1) Cytopathic effect

(2) RT-PCR
(3) Real time
(4) Western blot

III. Results

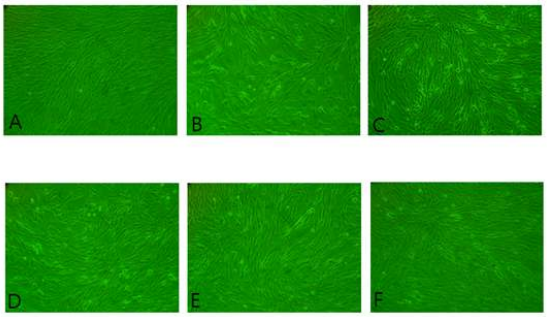

Fig 1. Transfection effect of PNA with PEI into cell. Negative contro (A) virus-infected cells, positive control (B), $_{PNA}$UL54-4 (C), $_{PNA}$UL54-3 (D), $_{PNA}$UL97-2 (E), $_{PNA}$UL97-1 (F)

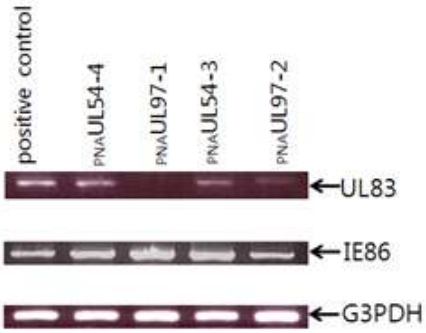

Fig. 2. Inhibition by PNAs against HCMV using RT-PCR

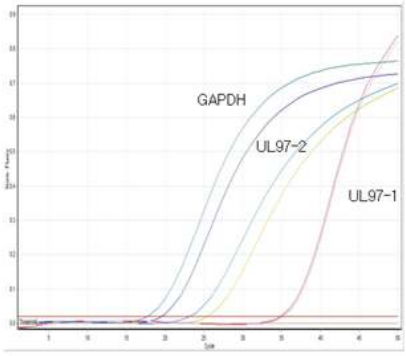

Fig. 3. Inhibition against UL97-1 and UL97-2 of HCMV using realtime PCR

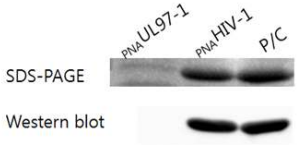

Fig. 4. Inhibition against replication of HCMV using Western blot

IV. Conclusion

In this study, PNAs such as $_{PNA}$UL97-1, $_{PNA}$UL97-2, $_{PNA}$UL54-3, and $_{PNA}$UL54-4) with PEI inhibited effectively against replication of HCMV in the human fibroblast cells. These PNA with PEI could be a novel tool as therapeutic agent against HCMV infection.

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