PEGylated Nano-hybrid Adenovirus: Retargeting Adenovirus to Tumor Cells

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1. INTRODUCTION

Adenoviruses are commonly used in gene therapy because of efficient infection in many cell types. However adenoviruses have several limitations including inflammation, immune responses, and non-specific gene transfer.

Here we report PEGylated adenovirus to overcome the inherent problems associated with viral gene therapy. The surface of adenoviruses was PEGylated with FOL-PEG conjugate. PEG on the surface of adenoviruses, can reduce immune response against viral capsid protein. Folate immobilized on the distal chain end of PEG can function as a retargeting moiety to folate receptor overexpressing cells.

Gene expression of FOL-PEG-Adenovirus was significantly enhanced against a folate receptor over-expressing cell line (KB cells) compared to a folate receptor deficient cell line (A549 cells).

2. MATERIALS AND METHODS

2.1. Conjugation of ADV-PEG-FOL and ADV-PEG

Folate(FOL) was activated with DCC and NHS (1:2:2) in DMSO and NH_2-PEG-COOH(MW3400) was added afterward. After 4hr, the reactants were precipitated with acetone and centrifuged at 2600rpm to eliminate free folate. Then, FOL-PEG-COOH conjugate in the supernatant was dialyzed against deionized water (MW cut-off=1000) and freeze dried.

Adenovirus(ADV) was harvested from HEK293 cell by virus harvest procedure (Ambion) and purified with $CsCl_2$ gradient ultracentrifugation.

FOL-PEG-COOH conjugate was activated with EDC, NHS (1:10:10) in 5% sucrose-KPBS (pH8.1) and adenovirus (PEG: adenovirus=10^5:1, molar ratio) was added. After 4hr at room temperature, ADV-PEG-FOL conjugate was dialyzed against 3% sucrose-PBS (MW cut-off =50,000) at 4°C. For control, PEG-NHS was conjugated to adenovirus (PEG: adenvirus = 10^5:1, molar ratio).

The degree of PEGylation was measured by fluorescamine assay. Virus size and surface charge were detected by DLS.

2.2. Target specific transfection.

Adenovirus(ADV) expressing GFP protein was used for assay. KB and A549 cell lines were seeded on a 6 well-plate at cell density of $3x10^5$cells/well. After one day, culture media was replaced to 2% FBS media and $2x10^9$ particles of ADV, ADV-PEG and ADV-PEG-FOL were added to each plate. After two days, the cells were treated with 1% Triton-X 100 for cell lysis and centrifuged. The supernatant was used by fluorometer for detection of GFP expression(excitation 488nm and emission 520nm).

2.3. Immunoassay

RAW 264.1(macrophage cell line, $1x10^6$/well) was subcultured on a 6 well-plate. After one day, ADV, ADV-PEG and ADV-PEG-FOL($2x10^{10}$ pts) were added to each plate and incubated for 1day.
Interleukin-6 level in culture media was determined by ELISA.

3. RESULTS and DISCISSION

As a result of PEGylation, about 26% of viral surface amines were conjugated to the terminal carboxyl group of FOL-PEG. After PEGylation, size of viral particle was increased from 73.4nm (ADV) to 159.6nm (ADV-PEG) ~169.9nm (ADV-PEG-FOL). Surface charge of viral particles was also changed from -20mV to -7mV (ADV-PEG) and -4mV (ADV-PEG-FOL).

Gene expression levels of ADV, ADV-PEG and ADV-PEG-FOL were significantly different in KB and A549 cell lines. For FOL receptor deficient cell line A549, both of ADV-PEG and ADV-PEG-FOL showed low GFP expression level against control adenovirus. On the other hand, transfection efficiency of ADV-PEG-FOL was much higher than that of control adenovirus for KB cell line(Figure 1)

Innate immune responses are also important to efficiency of gene transfection using viral vector. Immune response was examined by measuring released cytokine(Interleukin-6; IL-6) level using macrophage cell line. The PEGylation of adenovirus attenuated innate immune responses. IL-6 level for adenovirus-treated cells was about two fold than that in non-treated control cell. However, IL-6 levels for the cells treated with PEGylated adenoviruses (ADV-PEG and ADV-PEG-FOL) were similar to that for non-treated control cells (Figure 2)

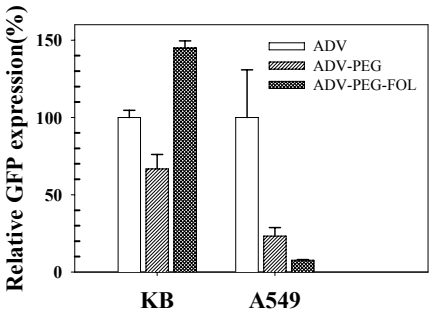

Figure 1. Target specific gene delivery of folate-PEG conjugated adenovirus

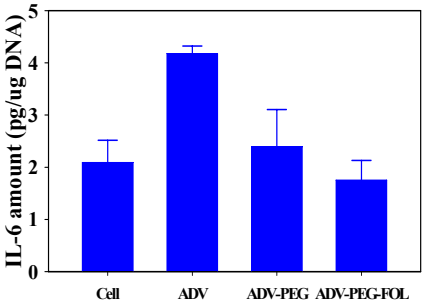

Figure 2. Reduced immune response of PEGylated adenovirus in RAW264.1(macrophage cell line)

4. CONCLUSION

We have demonstrated that adenovirus can be retargeted to specific tumor cells. By PEGylation with attaching a targeting moiety, innate immune responses were attenuated. Folate-PEG conjugated adenovirus is safe, efficient, and potential carrier for target-specific therapeutic gene delivery.

5. REFERENCES

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