Effects of cationic liposomes on in vitro and in vivo transduction mediated by adenoviral vectors

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ABBREVIATIONS

Ad: adenovirus

CAR: coxsackie adenovirus receptor

Chol: cholesterol

CMV: cytomegalovirus

DMKE: O, O'-dimyristyl-glutamyl-lysine

FACS: fluorescence-activated cell sorter

Gal-Cer: D-galactosyl-β1-1'ceramide

GFP: green fluorescence protein

Luc: luciferase

MCPS: mononuclear cell phagocytic system

MOI: multiplicity of infection

MTT: 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide

PBS: phosphate-buffered saline

PFU: plaque forming unit

RLU: relative light unit

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ABSTRACT

Adenoviral vectors have been widely used in gene therapy trials because of their efficiency in gene transfer. However, their application is limited by their requirement for cancer cells to express appropriate receptors such as coxsackie adenovirus receptor (CAR) *in vitro* and by non-specific viral uptake in the liver. To overcome both limits, in this study adenoviral vectors were complexed with cationic liposomes, a non-viral gene carrier non-specific to cell types and then tested in varied cancer cell lines and mice carrying tumor models. Adenoviral vectors encoding green fluorescence protein (GFP) or luciferase (Luc) were mixed with O,O′-dimyristyl-glutamyl-lisine (DMKE), DMKE/cholesterol (Chol), or DMKE/Chol/D-galactosyl-β1-1'ceramide (Gal-Cer) liposomes to form adenovirus(Ad)-liposome complexes. Cancer cell lines expressing CAR were effectively infected by adenoviral vectors without cationic liposomes, which was proved by fluorescence-activated cell sorter (FACS) analysis of GFP expression. Adenoviral transduction to CAR-deficient B16BL6 and MCF-7 cancer cells was significantly enhanced by cationic liposome complexation to adenoviral vectors. Meanwhile, the cationic liposomes

variably affected *in vitro* adenoviral gene transduction to CAR-expressing HeLa, HepG2, and SNU739 cells. *In vivo* adenoviral transduction of luciferase gene was imaged by luminometer (IVIS^{TM}). In order to evaluate biodistribution of adenoviral vectors, adenovirus or Ad-DMKE liposome complexes were intravenously administered to C57BL/6 mice bearing CAR-deficient B16BL6 mouse melanoma cells. The adenovirus complexed with the DMKE liposomes exhibited 200-fold lower hepatic transduction than adenovirus alone. Meanwhile, gene expression in tumor tissues was insignificantly affected by the cationic liposomes. The DMKE/Chol and DMKE/Chol/Gal-Cer liposomes were more effective in reduction of adenoviral hepatotropism than the DMKE liposomes in the same mouse model. Adenovirus and the Ad-liposome complexes were also administered to mice carrying CAR-positive tumors. When systemically administered to BALB/c nu/nu mice carrying CAR-positive HeLa cells, Ad-vectors were readily accumulated in the mononuclear cell phagocytic system (MCPS) in the liver and spleen. However, adenovirus complexed with DMKE/Chol/Gal-Cer liposomes exhibited no transgene expression in the liver and spleen. This study suggests that the cationic liposomes complexed with Ad-vectors enhance *in vitro* adenoviral transduction to CAR-deficient cells as well as reduce hepatotropism of Ad-vectors.

Key word: adenovirus, gene therapy, cationic liposomes, coxsackie adenovirus receptor (CAR), MCPS (mononuclear cell phagocytic system)

Ⅰ. *INTRODUCTION*

Use of recombinant human adenoviral vectors continues to show increasing promise as gene therapy vehicles, expecially for cancer gene therapy, due to several important attributes, such as its excellent gene transfer efficiency to numerous dividing and non-dividing cell targets. In addition, adenoviral vectors (Ad-vectors) are rarely linked to any severe diseases in immunocompetent humans, providing a rationale for further development of these vehicles (1). Entry of adenoviral vectors into most target cells is mediated by the initial binding of the adenoviral fiber to the coxsackie adenovirus receptor (CAR) on the cell membrane (2). CAR expression and efficiency of adenoviral transgene expression have been correlated in a number of studies, suggesting that adenoviral binding and entry into target cells is a critical step in achieving successful adenoviral gene expression (3). However, one of the hurdles confronting gene transfer by adenoviral vectors is their inefficient transduction to target cells lacking sufficient expression of CAR; such cells include many advanced cancer cells, skeletal muscle cells, smooth muscle cells, peripheral blood cells, hematopoietic stem cells, dendritic cells, and so on (4). A high dose of vectors is required to achieve efficient gene transfer to these cell types. This in turn increases unwanted side effects, such as vector-associated immunogenic toxicities (4).

Another hurdle confronting Ad-vector-mediated gene transfer is their nonspecific distribution in tissues after *in vivo* gene transfer because of the relatively broad expression of CAR. This property imposes an increased risk of toxicity due to vector dissemination to non-targeting cells (4). Also, large numbers of virus are still removed by the mononuclear cell phagocytic system (MCPS) of the liver after systemic administration, resulting in reduction of vector plasma circulation time (5-8). Therefore, after intravenous injection to

mice, adenoviral vectors are involved in accumulation in mouse liver.

Based on a clear understanding of Ad-vector cell recognition, the development of CAR-independent Ad-vectors has rationally focused on the fiber protein, the primary determinant of Ad-vector tropism (1). Strategies to eliminate hepatotropism, based on modification of particular viral capsid proteins such as fiber and penton base (9) have been reported.

Instead of modification of adenoviral vector, surface modification of Ad-vectors with non-viral gene-transferring systems has been considered as another option for reduction of adenoviral hepatotropism. Complexing adenoviral vectors with cationic liposomes or polymers has been shown to be effective in increasing adenoviral transgene expression by facilitating adenovirus binding to the cell surface via rather non-specific charge interaction, expecially on CAR-deficient cell lines (10-13). Ad-liposome complexes also resulted in reduced immunogenicity of adenovirus (14).

Numerous studies on non-viral gene transfer utilizing liposomal vectors have shown that lipid composition is important in determining the efficacy of liposome-mediated gene transfer (15). This is likely to be due to effects on the physical stability, intracellular uptake, endosomal release, and nuclear localization of liposome-plasmid DNA complexes, all of which affect the ultimate transgene expression (16). The complexes between liposomes and adenoviral vectors are known to form cationic liposomes coating on the negatively charged surface of adenovirus particles by electronic interactions similar to those in liposome-plasmid DNA complexes (16). Therefore, lipid compositions may be also an important parameter determining transduction efficiency of Ad-liposome complexes.

Consistent with these considerations, in this study the Ad-liposome complexes were prepared with DMKE (O,O'-dimyristyl-glutamyl-lisine)-based liposomes and then utilized to infect various types of cancer cells with varied

amount of CAR expression. The Ad-liposome complexes were systemically administered to an animal model to evaluate the effect of cationic liposomes on biodistribution and *in vivo* transduction efficacy of Ad-vectors. This study will provide important information regarding how to resolve the two hurdles, inefficient transduction to CAR-deficient cells and hepatotropism of Ad-vectors.

Ⅱ. *MATERIALS AND METHODS*

1. Materials

D-galactosyl-β1-1′-ceramide was purchased from Avanti Polar Lipid (Alabaster, USA). Cholesterol was purchased from Sigma (St. Louis, USA). DMKE were chemically synthesized by Dr. Doo-Ok Jang (Department of Chemistry, Yonsei University, Korea). Accupower® RT premix was purchased from Bioneer (Daejeon, Korea). C57BL/6 mice and BALB/c nu/nu mice were obtained from Orient Bio (Sungnam, Korea). The recombinant Ad-CMV-GFP (replication defective adenovirus type 5) was kindly provided by Dr. Chae-Ok Yun (Department of Medicine, Yonsei University, Seoul, Korea). The recombinant Ad-CMV-Luc was kindly provided by Dr. Tae-Sub Lee (Korea Cancer Center Hospital, Seoul, Korea). The viral stock were kept frozen at -80℃ until use. The plasmid, pEGFP-N1 encoding green fluorescent protein driven by the CMV promoter was purchased from Clontech Laboratories (Palo Alto, USA).

2. Cell lines and cell culture

Human hepatocellular carcinoma SNU-739 was purchased from Korean Cell Line Bank (Seoul, Korea). Human breast adenocarcinoma MCF-7, human cervical adenocarcinoma HeLa, human hepatocellular carcinoma HepG2 and mouse melanoma B16BL6 were purchased from the American Type Culture Collection (Manassas, USA). MCF-7 and HepG2 cells were maintained as monolayer cultures in DMEM (Gibco, Carlsbad, USA). SNU-739 cells were cultureed in RPMI 1640, B16BL6 and HeLa cells were cultured in MEM (Gibco, Carlsbad, USA) medium. The all media were supplemented with 10% fetal bovine serum, 100 units/㎖ penicillin and 100 ㎍/㎖ streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37℃.

3. Preparation of cationic liposomes

DMKE and cholesterol (Chol) were mixed at a molar ratio of 60:40. Gal-liposomes consisting of DMKE, Chol and D-galactosyl-β1-1′ ceramide (Gal-Cer) were mixed at a molar ratio of 60:35:5 (Table 1). The lipids (DMKE, Chol, and Gal-Cer) were dissolved in chloroform and methanol mixture (2:1, v/v). The organic solvent was evaporated under a stream of N_2 gas. Vacuum desiccation for 2 hours ensured removal of the residual organic solvent. Then, dried lipid film formed was hydrated in 1 ㎖ distilled water with vigorous vortexing. After hydration, the dispersion was sonicated. Each suspension was extruded through polycarbonate membranes of a 100 nm pore size at 60℃ using an extruder (Avanti Polar Lipids, Alabaster, USA). The lipid concentration was adjusted to 1 ㎎/㎖. All liposomes were stored at 4℃ until use.

Table 1. Composition of cationic liposomes

Compositions / Liposomes	DMKE	Cholesterol	Gal-Cer
DMKE (100 molar ratio)	100%	0%	0%
DMKE/Chol (60:40 molar ratio)	60%	40%	0%
DMKE/Chol/Gal(60:35:5 molar ratio)	60%	35%	5%

4. RT-PCR analysis for CAR expression on the surface of cancer cells

Total cellular RNA was extracted from cells. The cDNA against the extracted RNA was synthesized with oligo (dT) primers and Accupower® RT premix (Bioneer, Daejeon, Korea) according to the manufacture's instructions, giving a final volume of 50 ㎕ of cDNA preparation. The PCR amplification was carried out with 2 ㎕ of cDNA preparation and a specific primer pair of CAR (Table 2). GAPDH was used as an internal standard (Table 2). Twenty ㎕ of a PCR reaction solution was prepared using Accupower® RT premix: 1 ㎕ (10 pmole) of each sense and antisense primer, 1.5 ㎕ of cDNA sample and 16.5 ㎕ of distilled water. The mixture was submitted to 25 cycles of amplification. In each cycle, denaturation was done at 94℃ for 20 sec, annealing of primers to target cDNA was carried out 61℃ for 30 sec, and extension was done at 72℃ for 1 min, followed by a final 10 min elongation at 72℃. The PCR products were visualized by ultraviolet illumination after electrophoresis in 1% TBE agarose gel and staining with ethidium bromide.

Table 2. Primers for RT-PCR and their PCR product sizes

Name	Type	Sequence	Product size
CAR	Sense	5'-GCCTTCAGGTCGCAGATGTTAC-3'	567 bp
	Anti-sense	5'-TCGCACCCATTCGACTTAGA-3'	
GAPDH	Sense	5'-GTCAACGGATTTGGTCGTATT-3'	631 bp
	Anti-sense	5'-AGTCCTTCTGGGTGGCAGTGAT-3'	

5. *In vitro adenoviral transduction*

The recombinant adenoviral Ad-CMV-GFP was an E1 and E3-deleted type 5 adenoviral vector (Figure 1). The CMV represents the cytomegalovirus promoter and arrows show their direction of transcription. The deleted E1 region had a GFP expression cassette. Adenoviral vectors were propagated in 293 cell lines. The vectors were then diluted to a titer of $24.\times10^{12}$ vp/㎖. Cancer cells were seeded in 24-well culture plates at a density of 5×10^{4} cells/well and were used when 70-80% confluent.

Complexes of Ad-vectors and cationic liposomes were made by mixing Ad-CMV-GFP and the prepared liposomes in 100 ㎕ of serum free medium, followed by incubation for 30 min at room temperature. Cancer cells were washed with phosphate-buffered saline (PBS) and the prepared Ad-liposome complexes were then added with 400 ㎕ of serum free medium. After 4 h incubation in a CO_2 incubator at 37℃, the cells were washed with PBS to remove the complexes, and 1 ㎖ of fresh 10% serum-containing medium was added to the cells. The transduced cells were then incubated for an additional 48 h before assessing GFP expression.

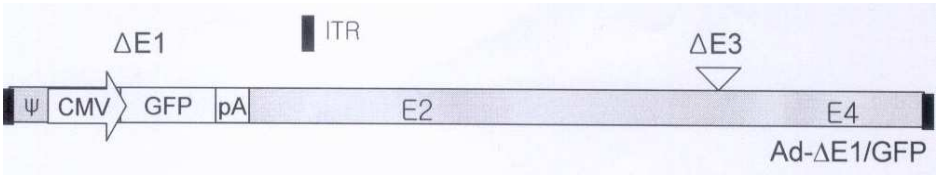


Figure 1. Genomic structure of Ad-CMV-GFP (replication defective adenovirus type 5)

6. Analysis of in vitro GFP expression

The transduced cells were washed twice with PBS and fixed with 300 ㎕ of 2% paraformaldehyde for 5 min at 4℃. The cells were washed twice with PBS again, harvested with a trypsin-EDTA solution, and then resuspended in 400 ㎕ medium with 100 ㎕ FBS. After centrifugation for 5 min at 1,500 rpm at 4℃, the supernatant was discarded and the fixed cells resuspended in 500 ㎕ PBS. GFP expression in the suspended cells was directly determined in a FACS Calibur machine (Beckton Dickinson, San Jose, USA). Ten thousand fluorescent events per sample were acquired. Expression of GFP in the transduced cells was also viewed with a fluorescence microscopy and photographed (magnification: ×100).

7. Cytotoxicity assay of cationic liposomes

Cytotoxicity of cationic liposomes complexed to Ad-vectors was determined by the MTT assay. Cancer cells were plated in 96-well plates (1×10^4 cells/well) in 0.1 ㎖ medium supplemented with 10% FBS and cultured for 24 h. The cells were treated with the various concentrations of cationic liposomes-adenovirus complexes and then cultured for 24 h. Fity ㎕ of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St Louis, USA) solution (1 ㎎/㎖) was added to the cells, which were further incubated for 4 h. After the media containing MTT were removed, 150 ㎕ of DMSO was added to solubilize the MTT-formazan product in the cells. The absorbance at 540 nm was measured with a microplates reader (Molecular Devices, Sunnyvale, USA).

8. In vivo gene transduction and analysis of transgene expression

A HeLa xenograft model was prepared by subcutaneous inoculation of 5×10^6 HeLa cells in 100 ㎕ of medium to 5-week-old female nude mice. A B16BL6 xenograft model was also prepared by subcutaneous inoculation of 5×10^6 B16BL6 cells in 100 ㎕ of medium to 5-week-old female C57BL/6 mice. An individual tumor-bearing mouse in groups was intravenously injected with 3×10^8 pfu of Ad-CMV-Luc or Ad-CMV-Luc complexed with cationic liposomes (120 ㎍ of lipids) in a final volume of 150 ㎕ of 2.5% w/v glucose-saline. At 6 h and 24 h post injection, the mice were anesthetized with isoflurane. Then, the same mice were intraperitoneally injected with D-luciferin substrate dissolved in sterile PBS (30 ㎎/㎖) and imaged with a luminometer.

The animals were placed in a light-tight chamber and a gray-scale reference image was obtained under the low-level illumination. The florescence and photons emitted from the mice, transmitting through the tissues, were collected with a cooled charged-coupled device (CCD) camera (Xenogen, Alameda, USA). The acquisition time was ranged for 3 min. The results were analyzed using Living Image® software (Xenogen, Alameda, USA). The body temperature was maintained throughout imaging using a 37℃ platform in the chamber. After administration of luciferin substrate, the mice were imaged for a period of time ranging from 1 sec to 3 min, depending on signal intensity. Raw values of luciferase expression were recoded as photons of light emitted per second.

After photographed, the liver, lungs, spleen, hearts, kidney, intestine, stomach and tumor tissues were harvested. The harvested organs were washed with cold saline twice and homogenized in a lysis buffer (0.1 M Tris-HCl, 2 mM EDTA, and 0.1% Triton X-100, pH 7.8) using a Heidolph tissue tearor

(Scientific Support, Hayward, USA). The tissue mixtures were centrifuged at 14,000 rpm for 2 min at 4℃. Ten ㎕ of supernatant was subjected to measurement of luciferase activity in a luminometer with a luciferase kit (Propmega Biosciences, San Luis Obispo, USA). To calculate the relative light unit (RLU), the protein concentration of the sample was also measured with the DC protein assay kit (Bio-Rad, Hercules, USA).

Ⅲ. *RESULTS*

1. CAR expression on the surface of cancer cells

cDNA was synthesized from total RNA extracted from various cell lines by reverse transcriptase. cDNA sequences of CAR and GAPDH were amplified with the designated primer oligonucleotides mentioned earlier (Table 2). The size of each PCR product was as follow; CAR, 567 bp; GAPDH, 631 bp. The PCR products were electrophoresed in 1% TBE agarose gel and visualized by ultraviolet illunmination after staining with ethidium bromide. CAR mRNA level was high in HeLa, Hep G2 and SNU-739 cell lines (Figure 2). In contrast, the receptor was not expressed in B16BL6 and MCF-7 (Figure 2). Hence, HeLa for CAR-expressing cell line and B16BL6 for CAR-deficient cell line were selected for further *in vitro* and i*n vivo* trasnduction studies with Ad-vector and Ad-liposome complexes as cancer models.

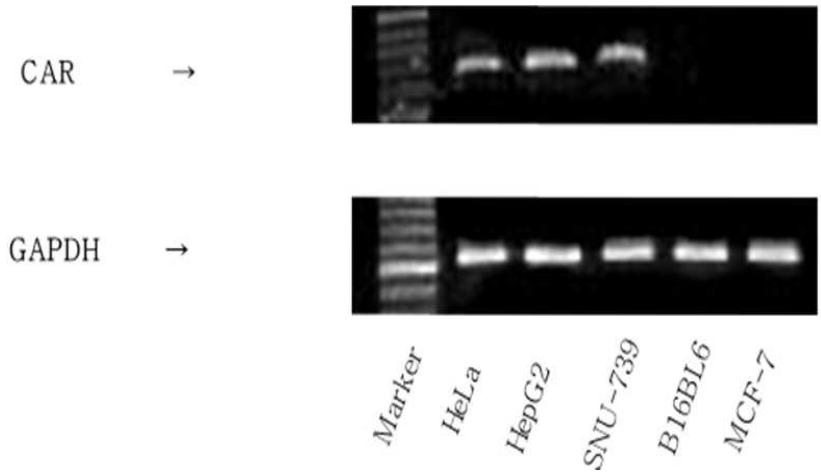


Figure 2. Detection of CAR expression in varied cancer cell lines by RT-PCR. cDNAs of CAR and GAPDH (a control of synthesized RNA quality) were amplified with their specific primers. PCR products were separated on 1% agarose gels and stained with ethidium bromide. Lane 1, 100 bp marker; lane 2, HeLa; lane 3, HepG2; lane 4, SNU-739; lane 5, B16BL6; lane 6, MCF-7.

2. *In vitro* transduction mediated by adenoviral vector and adenovirus-liposome complexes

In order to compare transduction efficiency of Ad-vectors and Ad-liposome complexes in CAR-expressing and CAR-deficient cancer cells, HeLa cells and B16BL6 cells were transduced with the two gene-transferring systems. First of all, the optimal MOI (multiplicity of infection) of adenoviral vectors was determined for effective gene transduction. After 4 h transduction and additional 48 h incubation, GFP expression in the transduced cells was viewed with a fluorescence microscopy and by the FACS analysis. Addition of the higher amount of Ad-vectors exhibited the more efficient GFP expression (Figure 3). According to the result of GFP expression in HeLa cells (Figure 3A), nearly all cells were transduced in the presence of the 100 MOI of Ad-vectors. Meanwhile, CAR-deficient B16BL6 cells were not effectively transduced with Ad-vectors (Figure 3 B). These results clearly showed that GFP expression mediated by Ad-vectors was dependent upon expression of CAR on the cell surface and 100 MOI (5×10^6 pfu) of Ad-vector was an optimal titer for transduction to CAR-expressing cancer cells.

Three different types of cationic liposomes were prepared as described in Table 1: DMKE liposomes, DMKE/Chol liposomes, and DMKE/Chol/Gal-Cer liposomes. Varied amount of these cationic liposomes were complexed with a fixed amount of Ad-vector (100 MOI). HeLa cells and B16BL6 cells were transduced by Ad-vectors and the prepared Ad-liposome complexes to investigate effects of cationic liposomes on adenoviral gene transduction and to determine the optimal ratio of liposome and Ad-vectors for efficient *in vitro* and *in vivo* transduction. Liposome complexation little affected the transduction efficiency of Ad-vectors in HeLa cells (Figure 4A). According to the FACS analysis, the GFP expression mediated by Ad-vectors was slightly higher than

that by Ad-liposome complexes. Among the cationic liposomes, transduction interference by DMKE/Chol liposomes was slightly higher than those by the other formulations.

In contrast, adenoviral transduction in CAR-deficient B16BL6 cells was enhanced by complexation with cationic liposomes. Especially, the adenovirus complexed with the DMKE liposomes (2 ㎍ lipid/㎖) was able to transduce 26-fold higher number of cells than the adenovirus only. The highest GFP expression was obtained in the presence of 0.5～2 ㎍/㎖ of DMKE liposomes. The highest GFP expression with the DMKE/Chol liposomes and the DMKE/Chol/Gal-Cer liposomes was seen at 0.5 and 4 lipid ㎍/㎖, respectively.

According to transduction results in both cell lines, the DMKE liposomes (0.5～1 ㎍/㎖) insignificantly affect the adenoviral transduction in CAR-positive cells, but significantly enhanced the transduction in CAR-negative cells.

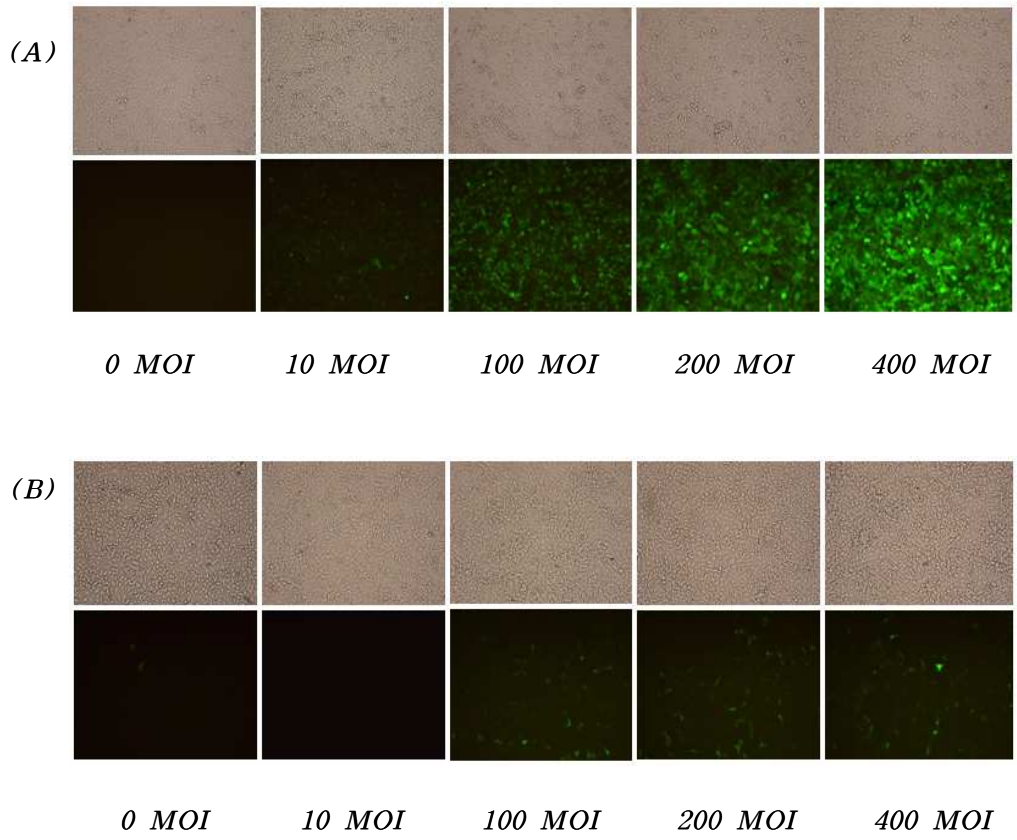
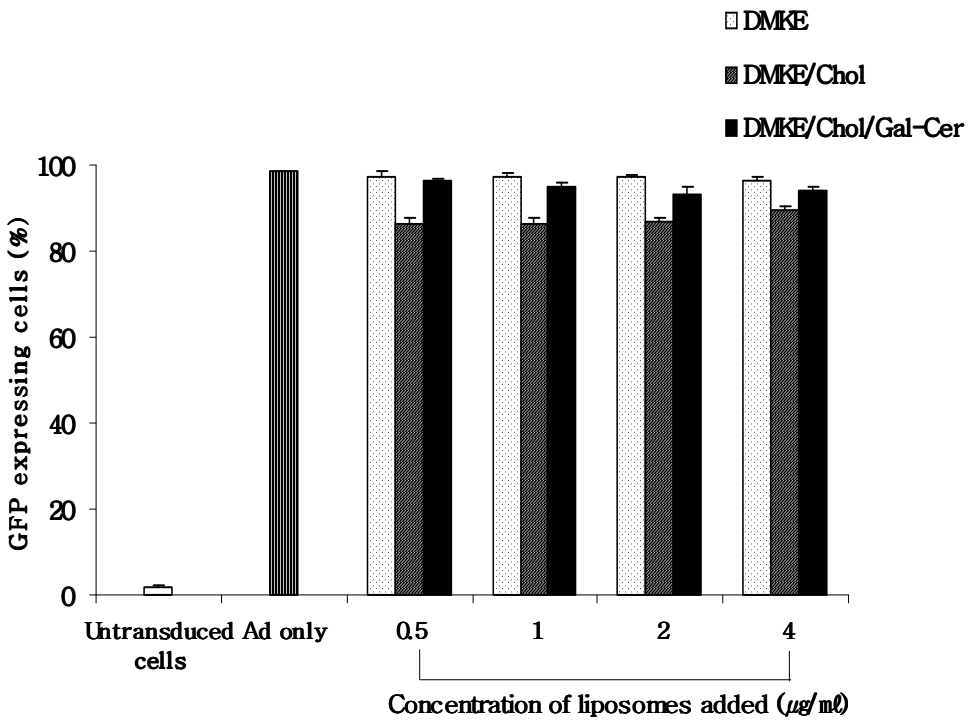


Figure 3. GFP expression by Ad-vectors in HeLa and B16BL6 Cells. Various MOIs of Ad-vectors were added to HeLa cells (A) and B16BL6 (B), transduced for 4 h, and incubated for additional 48 h. GFP expression of the cells was examined under a light microscopy (upper low) and a fluorescence microscopy (lower low).

(A) HeLa


DMKE
DMKE/Chol
DMKE/Chol/Gal-Cer
GFP expressing cells (%)
100
80
60
40
20
0
Untransduced cells
Ad only
0.5
1
2
4
Concentration of liposomes added (μg/㎖)

(B) B16BL6

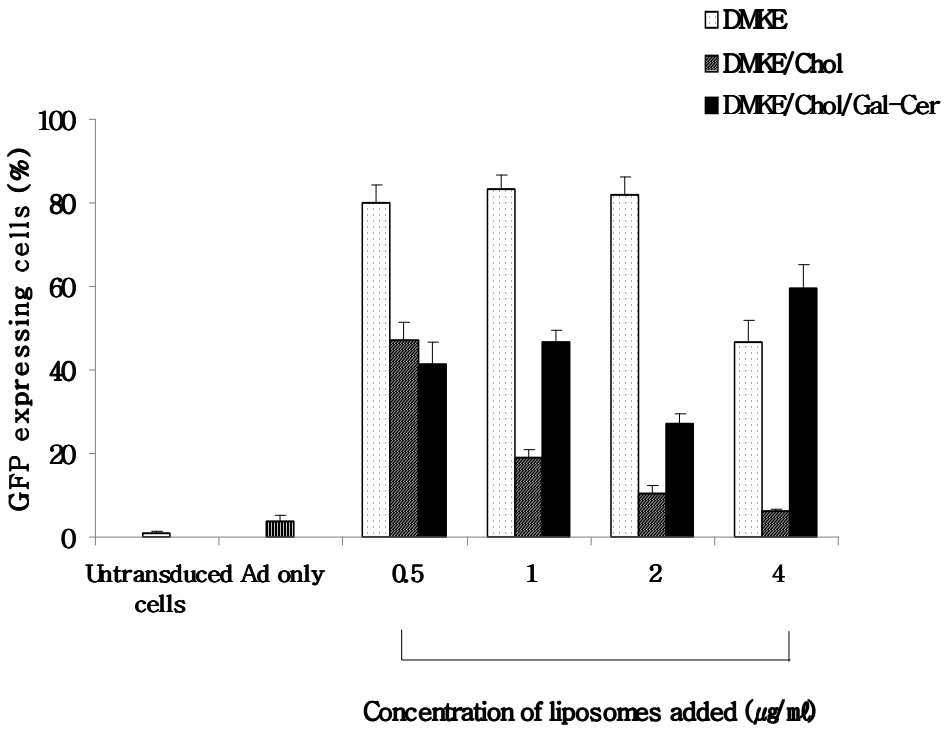


Figure 4. Effects of amount and composition of cationic liposomes complexed to Ad-vectors on adenoviral transduction in HeLa and B16BL6 cells. Numbers of HeLa (A) and B16BL6 (B) cells expressing GFP were counted by FACS analysis 48 h post transduction with Ad-vectors or Ad-liposome complexes.

3. Cytotoxicity of cationic liposomes complexed to Ad-vectors

B16BL6 cells were incubated in the presence of varied concentrations of DMKE, DMKE/Chol, and DMKE/Chol/Gal-Cer liposomes to measure their cell toxicities. As described elsewhere, the cationic liposomes were cytotoxic at the relatively higher concentration (>20 ㎍ lipid/㎖) (Figure 5). However, at the range of the optimized lipid concentration for *in vitro* transduction (<2 ㎍ lipid/㎖), the DMKE liposomes did not severely damage the cultured B16BL6 cells, exhibiting over 90% cell viability. The DMKE liposomes appeared to be more cytotoxic than the DMKE/Chol and DMKE/Chol/Gal-Cer liposomes.

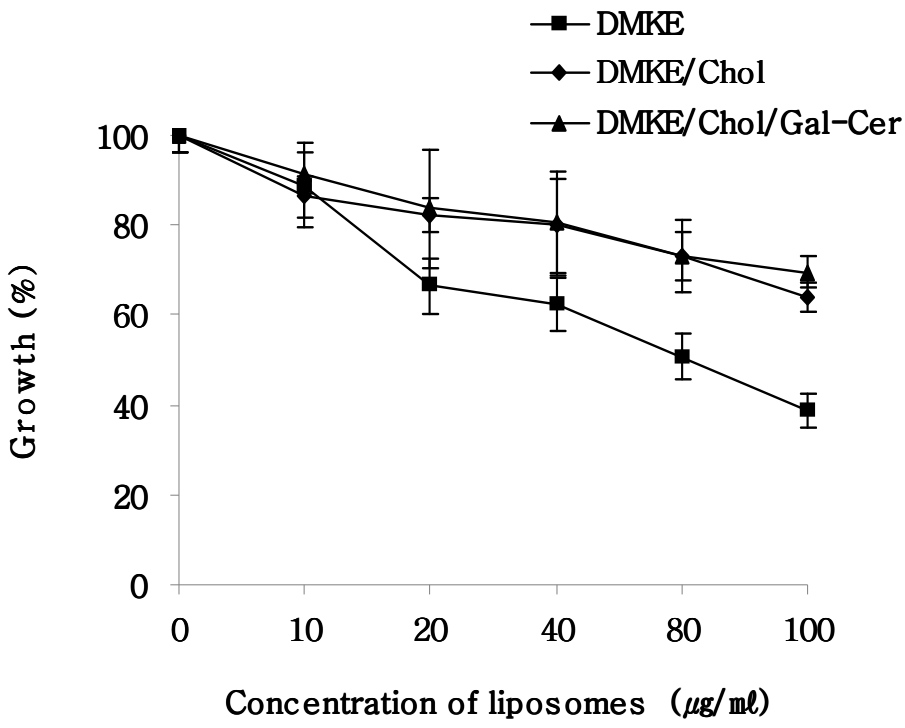


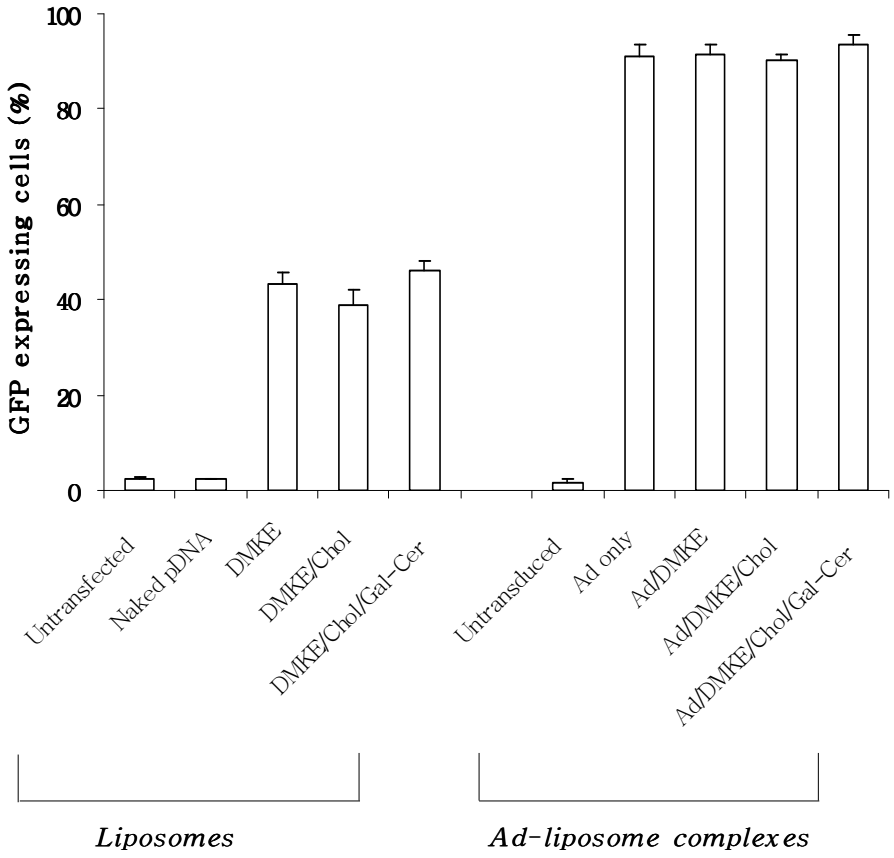
Figure 5. Cytotoxicity of various cationic liposomes complexed with Ad-vectors. B16BL6 cells seeded in 96-well plates (1×10^4 cell/well) were incubated with varied doses of liposomes pre-complexed with 1×10^6 pfu Ad-CMV-GFP for 2 h. Cell viability was measured by the MTT assay.

4. In vitro transfection by cationic liposomes and transduction by Ad-liposome complexes

GFP expression mediated by liposome transfection was compared with that by Ad-liposome complexes. After 4 h transfection or transduction and then additional 24 h or 48 h incubation, GFP expression in HeLa cells was measured by FACS analysis. The cells were transfected with 1 ㎍ pDNA complexed 6 ㎍ of liposomes (1:6 wt ratio) or transduced with 100 MOI of Ad-vector complexed 1 ㎍/㎖ of liposomes.

Generally GFP expression by lipidic transfection was lower than that by adenoviral transduction (Figure 6). As shown previously (Figure 4), DMKE, DMKE/Chol or DMKE/Chol/Gal complexation did not affect transduction efficiency of Ad-vectors in HeLa cells (91.0±2.57, 91.5±1.94, 90.4±0.90, and 93.7±1.68%, respectively) (Figure 6A). However, adenoviral transduction to B16BL6 cells was significantly elevated by complexation with cationic liposomes, specially DMKE liposomes (Figure 6B). Addition of cholesterol or galactosyl ceramide to the DMKE liposomes reduced the transfection efficiency of the cationic liposomes and transduction efficiency of Ad-liposome complexes. This result clearly shows that cationic liposomes can help Ad-vectors to transduce to CAR-deficient cells.

(A) HeLa


GFP expressing cells (%)
100
80
60
40
20
0
Untransfected
Naked pDNA
DMKE
DMKE/Chol
DMKE/Chol/Gal-Cer
Untransduced
Ad only
Ad/DMKE
Ad/DMKE/Chol
Ad/DMKE/Chol/Gal-Cer
Liposomes
Ad-liposome complexes

(B) B16BL6

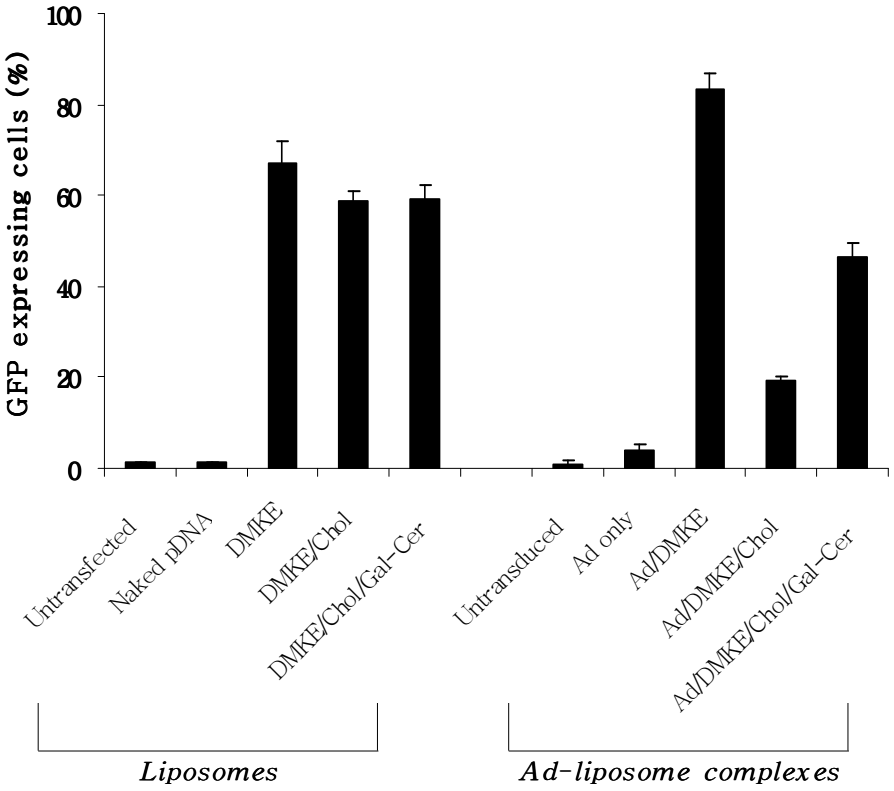


Figure 6. In vitro transfection by cationic liposomes and transduction by Ad-liposome complexes in HeLa and B16BL6 cells. One ㎍ of pDNA complexed to 6 ㎍ of DMKE, DMKE/Chol, or DMKE/Chol/Gal-Cer liposomes at 1:6 wt ratio, or 5×10^6 pfu of Ad-vector complexed to 1 ㎍/㎖ of DMKE, DMKE/Chol, or DMKE/Chol/Gal-Cer liposomes were added to HeLa (A) and B16BL6 cells (B). The cells were incubated for 4 h and additional 24 h for transfection or 48 h for transduction. GFP expression in the cells was measured by FACS analysis.

5. In vitro gene transduction mediated by Ad-liposome complexes in varied cell lines

Liposome-complexed adenoviral vectors have shown particularly effective in CAR-deficient cell lines (11, 14). Thus, in this study, effects of cationic liposome complexation on transduction efficiency of Ad-vectors were investigated in various cancer cells, CAR-positive or CAR-negative. After transduction by Ad-liposome complexes, GFP expression in the cells was measured by FACS analysis. The cells were transduced under an optimized condition of 5×10^6 pfu Ad-vector complexed to 1 ㎍/㎖ of DMKE, DMKE/Chol or DMKE/Chol/Gal-Cer cationic liposomes.

In CAR-deficient B16BL6 and MCF-7 cell lines, approximately 3% and 11% of the respective cells were transduced with Ad-vectors alone (Figure 7). DMKE or DMKE/Chol/Gal-Cer liposome complexation increased the adenoviral transduction by 5.5-fold and 27-fold in MCF-7 and B16BL6, respectively. Generally DMKE liposomes was the most effective in enhancement of adenoviral transduction in CAR-deficient cells among the prepared cationic liposomes.

On the whole, Ad-vectors were able to transduce the GFP gene to CAR-expressing cells more effectively than CAR-deficient cells. Under the same transduction condition, the percentages of cells transduced by Ad-vectors alone were approximately 25%, 44%, and 91% in HepG2, SNU-739, and HeLa cells. As shown previously (Figure 4), cationic lipid complexation to Ad-vectors little affected the adenoviral transduction to HeLa cells, but negatively affected the transduction to HepG2 cells. Interestingly, the same formulation enhanced the transduction to SNU-739 cell, one of CAR-positive cells. Complexation with the DMKE liposomes exhibited approximately 21%, 92%, and 96% of transduction in HepG2, HeLa, and SNU-739 cells,

respectively. Under the same experimental conditions, the DMKE/Chol liposomes and DMKE/Chol/Gal-Cer liposomes also exhibited the similar effect with the DMKE liposomes.

These results indicate that cationic liposome complexation generally enhances adenoviral gene transfer to CAR-deficient cells and affect adenoviral gene transfer to CAR-expressing cells positively or negatively, depending on cell types.

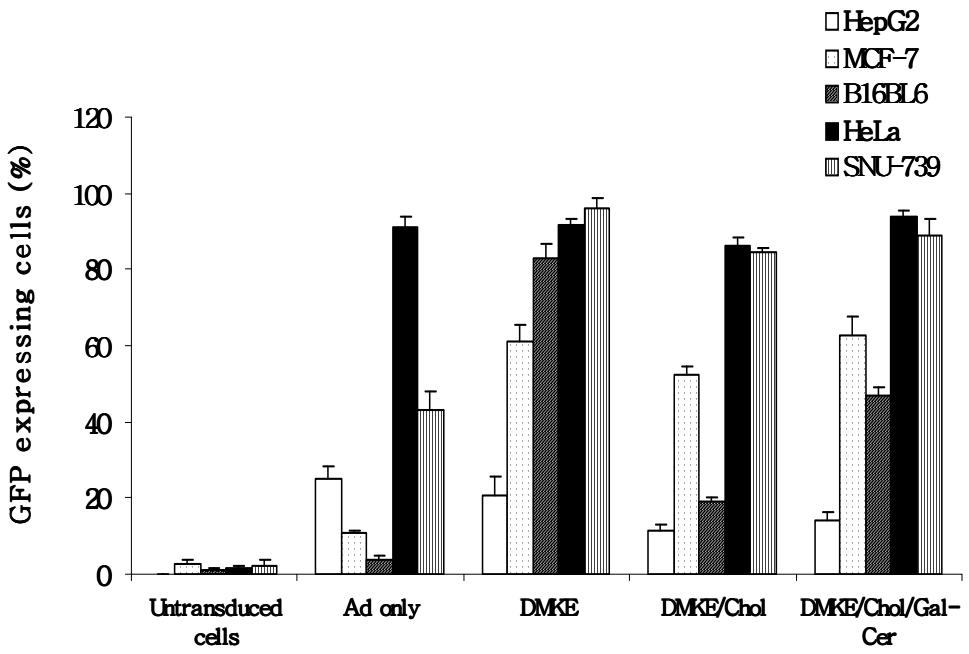


Figure 7. In vitro transduction by Ad-vectors and Ad-liposome complexes in various types of cancer cells. Ad - vectors (5×10^6 pfu) complexed with 1 ㎍/㎖ of DMKE, DMKE/Chol and DMKE/Chol/Gal liposomes were added to various types of cancer cells. The cells were incubated for 4 h and then additional 48 h. GFP expression in the cells was measured by FACS analysis.

6. In vivo transduction by Ad-vectors and Ad-liposome complexes

In order to verify effect of cationic liposome complexation on *in vivo* gene transduction activities of adenoviral vectors, Ad-liposome complexes were intravenously administered into B16BL6 tumor-bearing C57BL/6 mice and HeLa tumor-bearing BALB/c nc/nc nude mice via tail vain. The major organs of the mononuclear cell phagocytic system (MCPS) including the liver and spleen were examined for luciferase transgene expression following tail vain injection of Ad-liposome complexes or Ad-vectors. The transduced mice were intraperitoneally injected with D-luciferin substrate dissolved in sterile PBS 6 h and 24 h post injection, and then imaged with a luminometer. The fluorescence and photons emitted from the mice, transmitting through the tissues, were collected with a cooled charge-coupled device (CCD) camera (Xenogen, Alameda, USA)

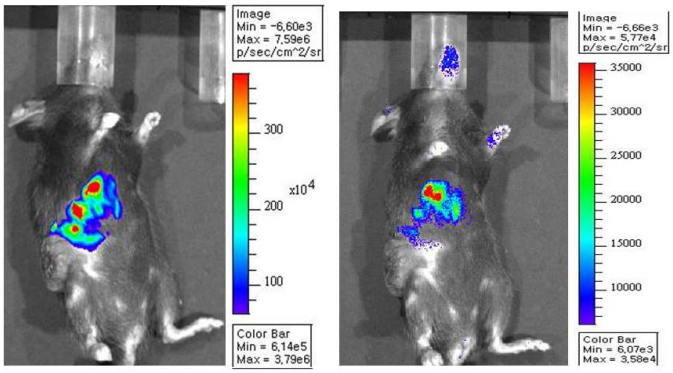
As a result, in C57BL/6 mice carrying B16BL6 tumors, the adenoviral vectors were predominantly localized in liver tissues (Figure 8). Meanwhile, the mice treated with three different types of Ad-liposomes complexes (DMKE, DMKE/Chol, and DMKE/Chol/Gal-Cer liposomes) exhibited significantly lower luciferase expression levels in the liver and spleen than the ones treated with Ad-vectors alone (Figure 8). The reduction of hepatic and splenic gene expression by cationic liposome complexation were not directly related with enhancement of transduction in the other organs. Only tumor tissues exhibited a slightly enhanced transgene expression by the Ad-DMKE liposome complexes (Figure 9). The DMKE/Chol and DMKE/Chol/Gal-Cer liposomes not only significantly removed hepatictropism of the adenoviral vectors, but also reduced transduction to tumor tissues (Figure 9).

In HeLa tumor-bearing nc/nc nude mice, the adenoviral vectors were also

predominantly localized in liver tissues 6 h, but the transgene expression faded away in 24 h post injection (Figure 10). The reduction of transgene expression by cationic liposome complexation did not yield enhanced transduction to any other organs including tumors.

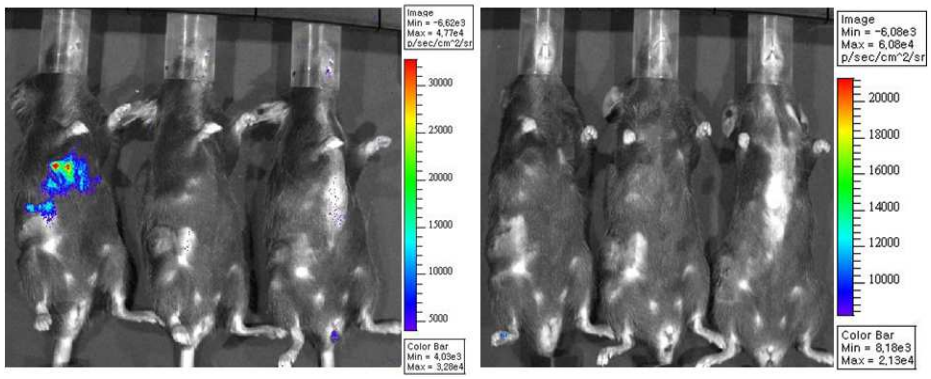
The treated mice were sacrificed 24 h post injection and luciferase gene expression in the major organs was viewed with a luminometer. Generally Ad-vectors exhibited higher luciferase expression in internal organs including the liver and spleen (Figure 11). The DMKE/Chol/Gal-Cer liposomes lowered the hepatic transduction and transduction to other organs as well. These results indicate that hepatic transduction by Ad-vectors can be reduced by cationic liposome complexation, but the current formulation of the complexes is not good enough to redistribute the residual viral complexes to other organs, specially to tumor tissues.

A



6 h post injection *24 h post injection*

B



mouse 1 mouse 2 mouse 3 **mouse 4 mouse 5 mouse 6**

6 h post injection *24 h post injection*

Figure 8. In vivo transduction by Ad-CMV-Luc and Ad-CMV-Luc complexed with cationic liposomes in a B16BL6 mouse model. Ad-CMV-Luc (3×10^8 pfu) (A) and Ad-CMV-Luc complexed with DMKE liposomes (mouse 1, mouse 4), DMKE/Chol liposomes (mouse 2, mouse 5), or DMKE/Chol/Gal-Cer (mouse 3, mouse 6) (120 μg lipid : 3×10^8 pfu) (B) were intravenously injected into B16BL6 tumor-bearing C57BL/6 mice via tail vein. The mice were intraperitoneally injected with D-luciferin (3 mg/mouse) 6 h and 24 h post injection. Six minute later, the treated mice were imaged by a CCD IVIS^{TM} system.

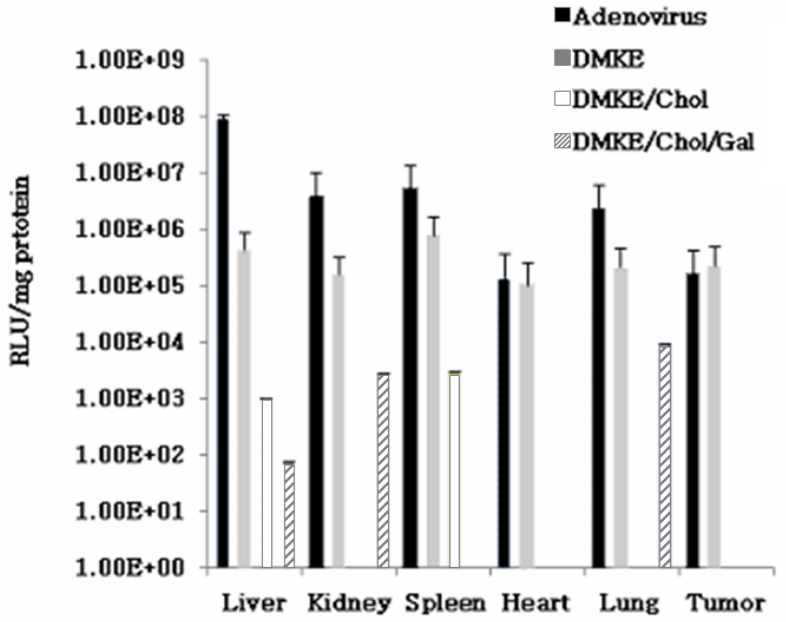
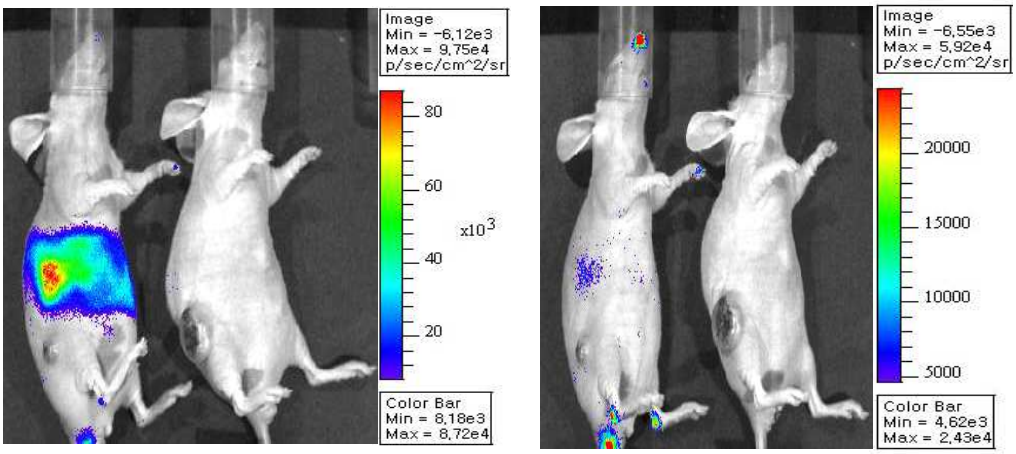


Figure 9. In vivo transgene expression by Ad-CMV-Luc and Ad-CMV-Luc complexed with various formulations of cationic liposomes in major organs of mice carrying B16BL6 tumors. Ad-CMV-Luc (3×10^8 pfu) or Ad-CMV-Luc with DMKE, DMKE/Chol and DMKE/Chol/Gal-Cer liposomes (120 μg lipid : 3×10^8 pfu) were intravenously injected into B16BL6 tumor-bearing C57BL/6 mice via tail vein. Major organs were collected 24 h post injection and the luciferase expression in the organs was calculated to RLU of luciferase per milligram of protein.



mouse 1 *mouse 2* *mouse 3* *mouse 4*

6 h post injection *24 h post injection*

Figure 10. In vivo transduction by Ad-CMV-Luc and Ad-CMV-Luc complexed with cationic liposomes in a HeLa mouse model.

Ad-CMV-Luc (3×10^8 pfu) (mouse 1, mouse 3) or DMKE/Chol/Gal-Cer liposomes complexed with Ad-CMV-Luc (120 μg lipid : 3×10^8 pfu) (mouse 2, mouse 4) were intravenously injected into HeLa tumor-bearing BALB/c nc/nc nude mice via tail vein. The mice were intraperitoneally injected with D-luciferin (3 mg/mouse) 6 h and 24 h post injection. Six minute later, the treated mice were imaged by a CCD IVIS^{TM} system.

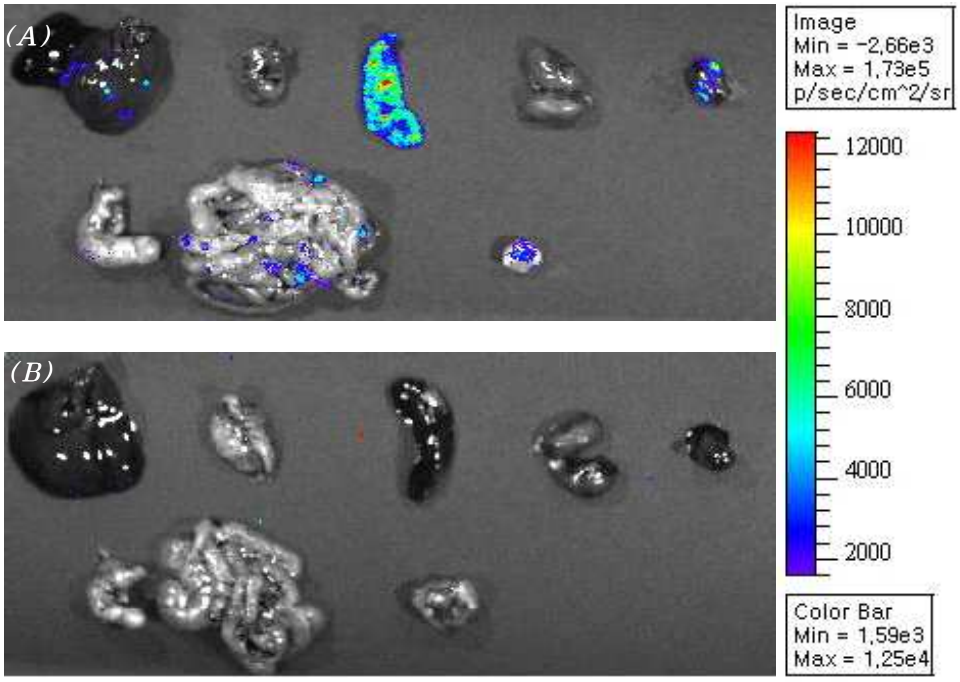


Figure 11. In vivo transgene expression by Ad-CMV-Luc and DMKE/Chol/Gal-Cer liposomes complexed with Ad-CMV-Luc in major organs of mice carrying HeLa tumors. Ad-CMV-Luc (3×10^8 pfu) (A) or Ad-CMV-Luc with DMKE/Chol/Gal-Cer liposomes (120 μg lipid : 3×10^8 pfu) (B) were intravenously injected into HeLa tumor-bearing BALB/c nu/nu nude mice via tail vein. The mice were intraperitoneally injected with D-luciferin (3 mg/mouse) 24 h post injection. Six minute later, the treated mice were imaged by a CCD IVIS^{TM} system.

Ⅳ. *DISCUSSION*

Despite a variety of extensive efforts to improve human adenovirus serotype 5 as a therapeutic agent, this vector exhibits innate drawbacks of CAR dependency and hepatotropism. Of particular concern is that many clinically important tissues, including several cancer types, are refractory to Ad5 infection due to low CAR expression. Indeed, down-regulation of CAR has been reported for several tumor types, including glioma, ovarian cancer, lung cancer, breast cancer and others (17-19). As a consequence of limiting CAR levels in many clinically relevant target cells, a high Ad-vector dosage is often required for *in vivo* efficacy. Since over 95% of systemically administered adenoviral particles are sequestered in the liver via hepatic macrophage (Kupffer cells) (20) and hepatocyte (21) uptake, therapeutically relevant adenoviral doses often results in vector-related liver toxicity (22-26). Thus, Ad-vectors exhibiting CAR-independency with low hepatotropism will be helpful for maximal transduction of low-CAR targets at the lowest possible vector dose.

In this study, Ad-vectors were complexed with cationic liposomes, resulting in efficient CAR-independent *in vitro* transduction and low-hepatotropism when systemically applied. According to the *in vitro* data with CAR-deficient B16BL6 cells, complexation with the DMKE-based cationic liposomes ablated CAR-dependent transduction capability of Ad-vectors. Ad-vectors compelxed with the cationic liposome exhibited significantly elevated transgene expression in CAR-deficient B16BL6 cells. This clearly shows that the DMKE-based cationic liposomes are able to enhance *in vitro* infectivity of Ad-vectors to CAR-deficient cancer cells. These results suggest that cationic liposome complexation provides CAR-independent tropism for Ad-vectors.

In contrast, in CAR-expressing cells, cationic liposome complexation

exhibited a variety of outcomes, little effect on transduction to HeLa cells, inhibitory effect on transduction to HepG2 cells as described elsewhere(16), and positive effect on transduction to SNU-739 cells. This may imply that initial interaction modes of the lipid-coated surface of Ad particles with the CAR-expressing surface of cancer cells are varied depending on the nature of cancer cell surface, such as CAR density, electronegativity, and so on. The different initial interaction modes may result in varied efficiency of internalization of adenoviral particles into cytoplasm.

The amount and composition of cationic liposomes were major parameters governing transduction efficiency of Ad-vectors complexed with cationic liposomes. The concentrations of cationic liposomes affected the level of adenoviral transduction efficiency. In CAR-expressing HeLa cells, while addition of the DMKE liposomes upto 4 ㎍/㎖ had no effect on adenoviral transduction, DMKE/Chol and DMKE/Chol/Gal-Cer liposomes exhibited sudden reduction at the same concentration. In CAR-deficient B16BL6 cells, the DMKE liposomes enhanced adenoviral transduction stably from 0.5 to 2 ㎍/㎖ of lipid concentration. Meanwhile, the DMKE/Chol and DMKE/Chol/Gal-Cer liposomes showed maximal enhancement of adenoviral transduction at 0.5 and 4 ㎍/㎖, respectively. According to these results, the DMKE-liposomes were the most effective in mediating adenoviral transduction in all cell lines tested. The optimized concentration of liposomes was 1 ㎍/㎖ of liposomes for 5×10^6 pfu of adenovirus.

Intravenous administration of Ad-vectors results in accumulation in the liver, spleen, heart, lung and kidneys of mice, although theses tissues may not necessarily be the highest in CAR expression (27-29). Instead, anatomic barriers, the structure of the vasculature and the degree of blood flow in each organ probably contribute to their biodistribution, in addition to non-specific viral particle uptake by macrophages. This is true with regard to the liver in

particular, which sequesters the majority of systemically administered adenoviral particle via hepatic macrophage uptake (20) as well as hepatocyte transduction (21). This process leads to cytokine release, inflammation and liver toxicity (22, 24, and 30). Thus, the nature of adenovirus-cell interactions dictating the fate of systemically applied Ad-vector has to be elucidated in detail and indesirable interactions should be avoided or eliminated to enhance transduction to desired target cells.

To this end, attempts to reduce the hepatotropism of Ad5 vectors have been based on the assumption that CAR-based interactions are required for liver uptake *in vivo*. Complexation with cationic liposome has been attempted to inhibit hepatocyte and/or liver Kupffer cell uptake using Ad5 vectors. Recent studies focused on Adenoviral vector biodistribution have demonstrated that Kupffer cell and hepatocyte uptake *in vivo* are largely CAR-independent (26, 31).

In this regard, three different cationic liposomes complexed to adenoviral vectors were systemically administered in B16BL6 tumor-bearing C57BL/6 mice. The mice administered with adenoviral vectors alone exhibited a relatively higher luciferase expression in the liver and other organs as well. As previously reported, this may be resulted from Kupffer cell and hepatocyte uptake in the liver. Meanwhile, complexation with DMKE-based cationic liposomes yielded remarkably different biodistribution patterns. The hepatic transduction of adenoviral vectors was greatly reduced by addition of the DMKE cationic liposomes. Unfortunately, the reduction of hepatic transduction was not compensated in any other organs including tumors. In fact, the complexation with the DMKE liposomes showed 200-fold lower transgene expression in the liver while only slightly higher expression in tumors. It is conceivable that the different surface nature of Ad-liposomes complexes may provide a different biodistribution pattern. The adenoviral particles are

electronegatively charged while the Ad-liposomes complexed are electropositively charged. Therefore, their opsonization in blood will be different from each other, which will result in different patterns of circulation in the body. At least, the *in vivo* data in this study clearly also show that complexation with the DMKE-based cationic liposomes can reduce the hepatotropism.

A number of studies have reported that Ad-liposome complexes can increase transduction efficiency in cell lines that are resistant to adenoviral infection (32-34). To my knowledge, this is the first study to look at *in vivo* transduction by Ad-liposome complexes. The DMKE-based liposomes complexed to Ad-vectors were able to reduce *in vivo* hepatotropism of Ad-vectors. In addition, complexation with the cationic liposomes also displayed CAR-independent transduction by Ad-vectors in the tested cancer cells resistant to adenoviral infection. This implied that the DMKE-based cationic liposomal membranes coating adenoviral particles may provide more effective interactions with host cells. However, at this moment the liposomal formulation prepared for this study is not appropriate for systemic delivery of Ad-vectors to any intended organs or tissues, such as tumors. In order to develop an Ad-liposome complex system for target-specific transduction, the liposomal system has to be further optimized in terms of lipid composition, surface charge, and particle size. Conjugation of targeting ligands, cell-specific antibodies and peptides, will be also helpful to delivery adenoviral particles to target cells.

Ⅴ. *REFERENCES*

1. Nakayama M, Both GW, Banizs B, Tsuruta Y, Yamamoto S, Kawakami Y, Douglas JT, Tani K, Curiel DT, Glasgow JN (2006) An adenovirus serotype 5 vector with fibers derived from ovine atadenovirus demonstrates CAR-independent tropism and unique biodistribution in mice. Virology 350 (1), 103-115.
2. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finber, RW (1997) Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275 (5304), 1320-1323.
3. Buttgereit P, Weineck S, Ropke G, Marten A, Brand K, Heinicke T, Caselmann WH, Huhn D, Schmidt-Wolf IGH (2000) Efficient gene transfer into lymphoma cells using adenoviral vectors combined with lipofection. Cancer Gene Therapy 7 (8), 1145-1155.
4. Mizuguchi H, Hayakawa T (2004) Targeted adenovirus vectors (Review). Human gene therapy 15 (11), 1034-1044.
5. Einfeld DA, Schroeder R Roelvink, PW, Lizonova A, King CR, Kovesdi I, Wickham TJ (2001). Reducing the native tropism of adenovirus vectors requires removal of both CAR and integrin interaction. The Journal of Virology 75 (23), 11284-11291.
6. Leissner P, Legrand B, Schlesinger Y, Hadji DA, van Raaij M, Cusack S, Pavirani A, Mehtali M (2001) Influence of adenoviral fiber mutations on viral encapsidation, infectivity and in vivo tropism. Gene therapy 8 (1), 49-57.
7. Alemany R, Curiel DT (2001) CAR-binding ablation does not change biodistribution and toxicity of adenoviral vectors. Gene therapy 8 (17), 1347-1353.

8. Wang Y, Hu JK, Krol1 A, Li YP, Li, CY, Yuan F (2003) Systemic dissemination of viral vectors during intratumoral injection. Molecular cancer therapy 2(11), 1233-42.
9. Wickham TJ (2000). Targeting adenovirus. Gene Therapy 7 (2), 110-114.
10. Byk T, Haddada H, Vainchenker W, Louache F (1998) Lipofectamine and related cationic lipids strongly improve adenoviral infection efficiency of primitive human hematopoietic cells. Human gene therapy 9 (17), 2493-502.
11. Fasbender AI, Zabner J, Chillon M, Moninger TO, Puga AP, Davidson BL, Welsh MJ (1997) Complexes of adenovirus with polycationic polymers and cationic lipids increase the efficiency of gene transfer in vitro and in vivo. Journal of Biological Chemistry 272 (10), 6479-6489.
12. Lee SG, Yoon 너, Kim CD, Kim K, Lim DS, Yeom YI, Sung MW, He o DS, Kim NK (2000) Enhancement of adenoviral transduction with polycationic liposomes in vivo. Cancer gene therapy 7 (10), 1329-35.
13. Toyoda K, Na k ane H, Heistad DD (2001) Cationic polymer and lipids augment adenvirus-mediated gene transfer to cerebral arteries in vivo. Journal of Cerebral Blood Flow & Metabolism 21 (9), 1125-31.
14. Yotnda P, Chen DH, Chiu W, Piedra PA, Davis A, Templeton NS, Brenner MK (2002) Bilamellar cationic liposomes protect adenovirus from preexisting humoral immune responses. Molecular Therapy 5 (3), 233-241.
15. Mok KW, Lam AM, Cullis PR (1999) Stabilized plasmid-lipid particles: factors influencing plasmid entrapment and transfection properties. Biochimica et Biophysica Acta (BBA)/Biomembranes 1419 (2), 137-150.
16. Lee EM, Hong SH, Lee YJ, Kang YH, Choi KC, Choi SH, Kim IH, Lim SJ (2004) Liposome-complexed adenoviral gene transfer in cancer cells expressing various levels of coxsackievirus and adenovirus receptor. Journal of Cancer Research and Clinical Oncology 130 (3), 169-177.

17. Miller, C.R., Buchsbaum, D.J., Reynolds, P.N., Douglas, J.T., Gillespie, G.Y., Mayo MS, Raben D, Luriel DT (1998) Differential susceptibility of primary and extablished human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. Cancer Research 58 (24), 5738-5748.
18. Hemminki A, Alvarez RD (2002) Adenoviruses in oncology : a viable option? Bio Drugs 16 (2), 77-87.
19. Bauerschmitz G.J, Barker SD, Hemminki A (2002) Adenoviral gene therapy for cancer: from vectors to targeted and replication competent agents (Review). Int. Journal of Oncology 21 (6), 1161-1174.
20. Tao N, Gao GP, Parr MJ, Baradet T, Wilson JM, Barsoum J, Fawell SE (2001) Sequesteration of adenoviral vector by Kupffer cells leads to a nonlinear dose response of transduction in liver. Molecular therapy 3 (1), 28-35.
21. Connelly S (1999) Adenoviral vectors for liver-directed gene therapy. Current Opinion in Molecular Therapeutics 1 (5), 565-572.
22. Peeters MJ, Patijn GA, Lieber A, Meuse L, Kay MA (1996) Adenovirus-mediated hepatic gene transfer in mice: comparison of intravascular and biliary administration. Human Gene Therapy 7 (14), 1693-1699.
23. Sulivan DF, Dash S, Du H, Hiramatsu N, Aydin F, Kolls J, Blanchard J, Baskin G, Gerber MA (1997) Liver-directed gene transfer in non-human primates. Human Gene Therapy 8(10), 1195-1206.
24. Worgall S, Wolff G, Falck-Pedersen E, Crystal, RG (1997) Innate immune mechanisms dominate elimination of adenoviral vectors following in vivo administration. Human Gene Therapy. 8 (1), 37-44.
25. Alemany, R., Suzuki, K, Curiel, D.T., (2000) Blood clearance rates of adenovirus type 5 in mice. Journal of General Virology 81 (Pt, 11),

2605-2609.

26. Shayakhmetov DM, Li ZY, Ni S, Lieber A (2004) Analysis of adenovirus sequestration in the liver, transduction of hepatic cells, and innate toxicity after injection of fiber-modified vectors. The Journal of Virology 78 (10), 5368-5381.
27. Fechner H, Haack A, Wang H, Wang X, Eizema K, Pauschinger M, Schoemaker RG, Veghel RV, Houtsmuller AB, Schultheiss HP, Lamers JMJ, Poller W (1999) Expression of coxsackie adenovirus receptor and alphav-integrin dose not correlate with adenovector targeting in vivo indicating anatomical vector barriers. Gene Therapy 6 (9), 1520-1535.
28. Reynold P, Dmitriev I, Curiel D (1999) Insertion of an RGD motif into the HI loop of adenovirus fiber protein alters the distribution of transgene expression of the systemically administered vector. Gene Therapy 6 (7), 1336-1339.
29. Wood M, Perrotte P, Onishi E, Harper ME, Dinney C, Pagliaro L, Wilson DR (1999) Biodistribution of an adenoviral vector carrying the luciferase reporter gene following intravesical or intravenous administration to a mouse. Cancer Gene Therapy 6 (4), 367-372.
30. Lieber A, He CY, Meuse L, Schowalter D, Kirllova I, Winther B, Kay MA (1997) The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. The Journal of Virology 71 (11), 8798-8807.
31. Liu Q, Zaiss AK, Colarusso P, Patel K, Haljan G, Wickham TJ, Muruve DA (2003) The role of capsid-endothelial interaction in the innate immune response to adenovirus vectors. Hum Gene Therapy 14 (7), 627-643.
32. Toyoda K, Ooboshi H, Chu Y, Fasbender A, Davidson BL, Welsh MJ, Heistad DD (1998) Cationic polymer and lipids enhance adenovirus-mediated gene transfer to rabbit carotid artery. Stroke 29 (10), 2181-2188.

33. Ryuke Y, Mizuno M, Natsume A, Yoshida J (2000) Transduction efficiency of adenoviral vectors into human glioma cells increased by association with cationic liposomes. Neurologia medico-chirurgica 40 (5), 256-260.

34. Fukuhara H, Hayashi Y, Yamamoto N, Fukui T, Nishikawa M, Mitsudo K, Tohnai I, Ueda M, Mizuno M, Yoshida J (2003) Improvement of transduction efficiency of recombinant adenovirus vector conjugated with cationic liposome for human oral squamous cell carcinoma cell lines. Oral Oncology 39 (6), 601-609.

국문 요약

양이온성 리포솜이 아데노바이러스성 벡터의 세포내 및 체내 유전자 전달에 미치는 영향

연세대학교 대학원
임상병리학과
이 연경

아데노바이러스 벡터는 유전자 발현효율이 좋아 유전자 치료에 널리 사용되고 있다. 하지만 이러한 아데노바이러스 벡터는 세포감염을 위해서 CAR (coxsackie adenovirus receptor)의 발현을 필요로 하며, 특히 체내에서는 간, 비장 등의 장기에 비특이적으로 축적되는 단점을 가지고 있다. 따라서 본 논문에서는 이러한 단점을 극복하기 위해, 다양한 조성의 양이온성 리포솜과 아데노바이러스 벡터를 결합(complexation)시켜 세포내 그리고 체내 유전자전달(transduction)양상을 확인하고자 하였다. 양이온성 리포솜은 DMKE(O, O'-dimyristyl-glutamyl-lisine), DMKE/Chol(cholesterol) (60:40 molar ratio), DMKE/Chol/Gal-Cer (galactosyl ceramide) (60:35:5, 몰비율) 세 가지로 제조되었다. 일반적으로 CAR를 발현하지 않는 B16BL6 세포와 MCF-7 세포에서는 양이온성 리포솜이 아데노바이

러스 벡터의 유전자전달활성을 향상시켰다. 반면에 CAR를 발현하여 아데노바이러스에 의해 유전자전달이 비교적 잘되는 HeLa, HepG2, SNU739 세포에서는 다양한 영향을 주었다. 양이온성 리포솜이 HeLa 세포에서의 유전자전달에 거의 영향을 주지 않았거나 HepG2 세포에서는 유전자전달효율을 감소시켰던 반면에 SNU793 세포에서는 유전자전달효율을 향상시켰다. B16BL6 세포가 이식된 C57BL/6 동물 모델에 아데노바이러스 벡터만을 투여한 결과 간조직에서 가장 높은 유전자발현을 볼 수 있었다. 반면에 양이온성 리포솜을 결합한 아데노바이러스 복합체를 투여한 경우에는 간에서의 유전자발현이 최고 200배의 이상의 감소하였다. 이러한 간에서 유전자발현 감소에도 불구하고 암조직을 포함한 여타 조직에서의 유전자발현은 증가되지 않았다. DMKE/Chol, DMKE/Chol/Gal-Cer 리포솜 역시 간에서의 유전자발현을 억제하였을 뿐 아니라 다른 장기에서 유전자발현 또한 감소하였다. 또한 HeLa 세포가 이식된 누드마우스에서도 아데노바이러스는 간과 비장에서 높은 유전자 발현을 유도하였다. 같이 투여된 DMKE/Chol/Gal-Cer 리포솜 역시 간과 비장에서 유전자 발현을 억제하였다. 본 연구를 통해 아데노바이러스 벡터에 양이온성 리포솜을 결합시킴으로써 대상세포의 CAR발현에 상관없이 효과적인 유전자발현을 유도할 수 있으며, 생체 내에서는 간과 비장으로의 축적을 감소시킬 수 있었다.

핵심어: 아데노바이러스성 벡터, 유전자 치료, 양이온성 리포솜, coxsackie adenovirus 수용체(CAR), MCPS(mononuclear cell phagocytic system)

감사의 글

학부 졸업 후 공백기를 거쳐 다시 대학원이라는 곳에 발 들여 놓은 것이 엊그제 같은데 벌써 2년이라는 시간이 훌쩍 지나가 버렸습니다. 2년 동안 많은 것을 배우고 느끼게 해주었던 시간이었습니다.

바쁘신 와중에도 논문 시작부터 교정까지 지도해주시고 신경 써주신 박용석 교수님께 깊이 감사드립니다. 관심과 애정을 가지시고 지도해주신 양용석 교수님, 오옥두 교수님, 김종배 교수님, 김태우 교수님, 이혜영 교수님께 진심으로 감사드립니다. 또한 실험실 전반에 걸쳐 관심을 가지고 지도해주신 임병혁 선생님께 감사드립니다. 실험에 필요한 기자재 및 조언을 아낌없이 주신 원자력 병원의 최태현 선배님과 밤늦은 시간까지 같이 실험에 도움을 주신 삼성 의료원 문철 선배님, 외국에서 바쁘신 와중에도 친절히 질문에 답해주신 근식 오빠, 2년간 대학원 생활을 하면서 여러모로 가르쳐주고 조언 해주신 진숙언니, 인수 오빠, 규상 오빠, 방혜은 선생님, 현철 오빠, 은아 언니, 은주 선배, 연임 선배, 관훈 선배, 처음 입학해서 어리버리한 후배 잘 챙겨주고 논문 쓸 때 도움 많이 준 인호선배, 다른 할 일 많은데도 불구하고 부탁할 때마다 거절 없이 도와준 정석, 후배지만 힘들 때마다 내 옆에서 친구처럼 같이 고민해주고 귀 기울여 주던 경혜, 힘든 대학원 생활에서 언제나 웃음과 도움을 주었던 착한 후배들 정례, 혜은, 실험하러 갈 때 마다 자신들의 일인 것처럼 3인 1조가 되어 휴일도 잊어버린 채 실험에 도움을 준 수희, 종찬, 수다친구 도완, 착한 형엽 오빠, 상정, M&D 선생님들, 실험실에서 뭐든지 열심인 화연, 모두에게 감사드립니다.

동기 없이 시작한 대학원 생활이었지만 외롭지 않고 힘들지만 이겨낼 수 있었던 것은 모두 선배님 후배님들의 관심과 위로 덕분이었습니다. 무엇보다도 항상 기도로 저를 보살펴 주시며 든든한 후원자가 되어주신 저의 부모님과 사랑하는 동생 수진 에게 깊은 감사의 마음을 전하고 싶습니다.