siRNA delivery to lung-metastasized tumor by systemic injection with cationic liposomes

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Abstract

Context: Cationic liposomes can efficiently deliver siRNA to the lung by intravenous injection of cationic liposome/siRNA complexes (lipoplexes).

Objective: The aim of this study was to examine a formulation of cationic liposomes for siRNA delivery to lung metastasis of breast tumor.

Materials and methods: For the preparation of cationic liposomes, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or dimethyldioctadecylammonium bromide (DDAB) as a cationic lipid and cholesterol (Chol) or 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) as a neutral lipid were used. In vitro and in vivo gene silencing effects by cationic lipoplexes were evaluated after transfection into stably luciferase-expressing human breast tumor MCF-7-Luc cells and after intravenous injection into mice with lung MCF-7-Luc metastasis, respectively. Intracellular localization of siRNA after transfection into MCF-7 cells by cationic lipoplexes and biodistribution of siRNA after intravenous injection of cationic lipoplexes into the mice with lung metastasis were examined by confocal and fluorescent microscopy analyses, respectively.

Results: In in vitro transfection, DOTAP/DOPE and DDAB/DOPE lipoplexes of luciferase siRNA strongly suppressed luciferase activity in MCF-7-Luc cells, but DOTAP/Chol and DDAB/Chol lipoplexes did not, although DOTAP/Chol and DDAB/Chol lipoplexes exhibited higher cellular uptake than DOTAP/DOPE and DDAB/DOPE lipoplexes. When their cationic lipoplexes were intravenously injected into mice with lung MCF-7-Luc metastasis, siRNAs were mainly accumulated in the lungs; however, the reduced luciferase activities in the lung-metastasized tumors were observed only by injections of DOTAP/Chol and DOTAP/DOPE lipoplexes, but not by DDAB/Chol and DDAB/DOPE lipoplexes.

Conclusions: DOTAP-based liposomes might be useful as an in vivo siRNA delivery carrier that can induce gene silencing in lung-metastasized tumors.

Keywords

Breast tumor, cationic liposome, gene knockdown, lung metastasis, siRNA delivery

Introduction

Cancer is the major cause of disease-related death; the majority of these deaths are a result of cancer metastatic growth (Taketo, 2011). Metastasis is the most deadly aspect of cancer due to the difficult treatment and the spread of the cancer to the lung, liver, brain and other key organs. In particular, the lung is a common site of metastasis due to its anatomic and functional structure, and is highly vascular and rich in oxygen, providing not only pathways for metastatic seeding, but also a nutrient-rich environment for neoplastic growth (Zheng & Fernando, 2010). Tumor metastases in the lung are found in 20–54% of deceased cancer patients (Hess et al., 2006). Surgical resection is a widely accepted procedure for treating lung metastases. Chemotherapy, in particular, is helpful in preventing metastasis; however, chemotherapy is often associated with serious side effects. Therefore, novel systemic therapies of tumor metastasis are needed (Krishnan et al., 2006; Nguyen et al., 2009).

RNA interference (RNAi) is a powerful gene-silencing process that holds great promise in the field of cancer therapy. Synthetic small interfering RNAs (siRNAs), which are small double-stranded RNAs, are substrates for the RNA-induced silencing complex. However, there are challenges associated with the in vivo delivery of siRNA, such as enzymatic instability and low cellular uptake. In siRNA delivery, non-viral vectors have been more commonly used than viral ones. Of all the carriers, lipid-based formulations such as cationic liposomes are currently the most widely validated means for systemic delivery of siRNA to the lung.

Cationic liposomes can efficiently deliver siRNA to the lung by intravenous injection of cationic liposome/siRNA complexes (lipoplexes). This is because electrostatic interactions between positively charged lipoplexes and negatively
charged erythrocytes cause agglutination (Eliyahu et al., 2002), and the agglutinates contribute to high entrapment of lipoplex in the highly extended lung capillaries (Simberg et al., 2003). Among the cationic liposomes, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)-based cationic liposomes have been reported as a system for delivering siRNA to the lung (Sioud & Sorensen, 2003; Sorensen et al., 2003). In a clinical setting, the DOTAP/cholesterol lipoplex of plasmid DNA encoding the tumor suppressor gene TUSC2/FUS1 has been exploited for the treatment of non-small cell lung cancer (NSCLC) patients (Lu et al., 2012). Although liposomal or nanoparticle formulae for the systemic delivery of siRNA into lung-metastasized tumors have been reported by several groups (Kenjo et al., 2013; Li et al., 2008; Shim et al., 2013; Yang et al., 2012), there are still few reports on their application for the delivery of siRNA to lung-metastasized tumor.

In this study, to examine a formulation of cationic liposomes for siRNA delivery to lung-metastasized tumor, we selected DOTAP or dimethyldioctadecylammonium bromide (DDAB) as a cationic lipid, and cholesterol (Chol) or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as a neutral lipid, and prepared cationic liposomes for evaluation of their in vitro and in vivo gene silencing effects. DDAB-based liposomes have been often used as a carrier of plasmid DNA, but not siRNA. Here, we found that the in vitro silencing effects by cationic lipoplexes do not closely correspond with the in vivo silencing effects, and the in vivo knockdown efficiency was strongly affected by the type of cationic lipid.

Materials and methods

Materials

1,2-Dioleoyl-3-trimethylammonium-propane methyl sulfate salt (DOTAP) was obtained from Avanti Polar Lipids Inc. (Alabaster, AL). Dimethyl dioctadecylammonium bromide (DDAB, product name DC-1-18) were obtained from Sogo Pharmaceutica Co., Ltd. (Tokyo, Japan). 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was obtained from NOF Co. Ltd. (Tokyo, Japan). Cholesterol (Chol) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of the finest grade available.

Cell culture

Human breast cancer MCF-7-Luc (TamR-Luc#1) cells stably expressing firefly luciferase (pGL3) were donated by Dr. Kazuhiro Ikeda (Division of Gene Regulation and Signa Transduction, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan). The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% heat-inactivated FBS, 100 µg/ml kanamycin and 0.5 mg/ml G418 at 37 °C in a 5% CO2 humidified atmosphere.

siRNA

The siRNAs targeting nucleotides of firefly pGL3 luciferase (Luc siRNA), and non-silencing siRNA (Cont siRNA) as a negative control were synthesized by Sigma Genosys (Tokyo, Japan). The siRNA sequences of the Luc siRNA were as follows: sense strand: 5’-GUGGAUUUCGAGUCGUCUUA A-3’ and antisense strand: 5’-AAGACGACUCGAAUCCCA AU-3’. In Cy5.5-labeled Luc siRNA (Cy5.5-siRNA), Cy5.5 dye was conjugated with the 5’-end of the sense strand. The siRNA sequences of the Cont siRNA were as follows: sense strand: 5’-GUACCGCAGUGCUACUGUAUC-3’ and antisense strand: 5’-UACCAUGACGUGCGGGUCGU-3’. Alexa Fluor®488-labeled AllStars Negative Control siRNAs (AF-siRNA) were obtained from Qiagen (Valencia, CA).

Preparation of liposome and lipoplex

Cationic liposomes were prepared with formulae as shown in Table 1 by a thin-film hydration method, as previously reported (Hattori et al., 2013). The particle size distributions of cationic liposomes were measured by the cumulant method using a light-scattering photometer (ELS-Z2, Otsuka Electronics Co., Ltd., Osaka, Japan) at 25 °C after diluting the dispersion to an appropriate volume with water. The ζ-potentials were measured using ELS-Z2 at 25 °C after diluting the dispersion to an appropriate volume with water. The size of cationic liposomes was adjusted to approximately 100 nm.

To prepare cationic liposome/siRNA complex (cationic lipoplex), cationic liposome suspension was mixed with siRNA by vortex-mixing for 10 s at charge ratios (±) of 1/1, 2/1, 3/1 and 4/1, and left for 15 min at room temperature. The theoretical charge ratio (±) of cationic liposome to siRNA was calculated as the molar ratio of siRNA phosphate to theoretical charge ratio (±) of cationic liposome.

Gel retardation assay

After preparation of the cationic lipoplexes at various charge ratios (±), the lipoplexes of 1 µg of siRNA were analyzed on a...
18% acrylamide gel for siRNA in Tris-borate-EDTA (pH 8.0) buffer and were visualized by ethidium bromide staining, as previously reported (Yoshizawa et al., 2008).

Luciferase activity
MCF-7-Luc cells were prepared by plating in a 6-well plate 24 h prior to each experiment. For transfection, each lipoplex of 50 pmol Luc siRNA or Cont siRNA was diluted in 1 ml of medium supplemented with 10% FBS and then the mixture was added into the cells. Forty-eight hours after the transfection, luciferase activity was measured as counts per sec (cps)/µg protein using the luciferase assay system (Pica Gene, Toyo Ink Mfg. Co. Ltd., Tokyo, Japan) and BCA reagent (Pierce, Rockford, IL), as previously reported (Hattori & Maitani, 2005).

Flow cytometric analysis
MCF-7-Luc cells were prepared by plating on 6-well culture dishes 24 h prior to each experiment. Cationic liposomes were mixed with 50 pmol AF-siRNA at a charge ratio (±) of 4/1. The cationic lipoplexes were diluted in 1 ml of medium containing 10% FBS, and the mixture was added to the cells. After 3-h incubation, the dish was washed two times with 1 ml of PBS to remove any unbound lipoplexes. The amount of AF-siRNA in the cells was determined by examining fluorescence intensity on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA), as previously described (Yoshizawa et al., 2008).

Confocal microscopy
MCF-7-Luc cells were plated on 35-mm culture dishes. The cationic liposomes were mixed with 50 pmol AF-siRNA at a charge ratio (±) of 4/1. The cationic lipoplexes were diluted in 1 ml of medium supplemented with 10% FBS, and the mixture was added into the cells. After 3-h transfection, acidic compartments like endosomes were labeled with Lysotracker® Red DND-99 (Invitrogen, Carlsbad, CA), as previously described (Yoshizawa et al., 2008).

Lung-metastasized tumor-bearing mice
Animal experiments were conducted with ethical approval from our institutional animal care and use committee. To generate the mice with lung-metastasized tumor, MCF-7-Luc cells (1 × 10⁶) were intravenously injected into the tail vein of female BALB/c nu/nu mice (8 weeks of age, CLEA Japan, Inc., Tokyo, Japan). To confirm tumor implantation, the lungs were excised 9 days after the injection and immediately frozen. The tumor sections (16 µm thick) were stained with hematoxylin and pure eosin (H&E staining; Muto Pure Chemicals Co., Ltd., Tokyo, Japan).

Biodistribution of cationic lipoplexes in mice
Cationic lipoplexes of 50 µg of Cy5.5-siRNA were intravenously administered via lateral tail veins into mice with lung metastasis at 7–9 days after tumor cell challenge. One hour after injection, the mice were sacrificed, and the tissues were frozen on dry ice and sliced at 16 µm. The localization of Cy5.5-siRNA was examined using an Eclipse TS100-F microscope (Nikon, Tokyo, Japan).

In vivo gene suppression
The cationic lipoplexes of 50 µg of Cont siRNA or Luc siRNA were intravenously injected into mice with lung metastasis at 7–9 days after tumor cell challenge. Before and 48 h after the injection, luciferase activity in lung-metastasized tumors was quantitatively measured by in vivo imaging analysis. For in vivo imaging analysis, D-luciferin (potassium salt) dissolved in PBS (3 mg/mouse) was intravenously injected into the mice and then the mice were subsequently anesthetized with 1.5% isoflurane (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). In vivo bioluminescence imaging was performed using a NightOWL LB981 NC100 system (Berthold Technologies, Bad Wildbad, Germany). A grayscale body-surface reference image was collected using a NightOWL LB981 CCD camera. The images were analyzed using the IndiGo2 software provided with the in vivo imaging system (Berthold Technologies, Oak Ridge, TN). Luciferase activity was measured as cps per lung-metastasized tumor, and calculated as change (%) during 48 h after injection of cationic lipoplex.

Statistical analysis
The statistical significance of differences between mean values was determined by Student’s t-test. A p value of 0.05 or less was considered significant.

Results and discussion
Particle size and ζ-potential of cationic lipoplexes
Cationic liposomes were prepared for evaluation of their possibility for use as an siRNA delivery vector. DOTAP/Chol liposomes are often used to transfect plasmid DNA into normal lungs (Song et al., 1997; Templeton et al., 1997); however, there are still few reports on the application of DOTAP/Chol liposomes for the delivery of siRNA to lung-metastasized tumor. In our preliminary study, we found that DDAB/Chol lipoplexes of plasmid DNA could induce high gene expression in the lungs, comparable to that of DOTAP/Chol lipoplexes, when intravenously injected into normal mice (Supplemental Figure S1). However, to the best of our knowledge, DDAB-based liposomes have not been well studied as a carrier of siRNA. In neutral helper lipid of cationic liposomal formulation, it has been reported that neutral lipid significantly affects the interaction of cationic lipoplexes with erythrocytes in blood circulation after intravenous injection (Sakurai et al., 2001b). Therefore, in this study, we used DOTAP or DDAB as a cationic lipid, and
DOPE or Chol as a neutral lipid, and prepared cationic liposomes for siRNA delivery to lung-metastasized tumor. In DOTAP-based cationic liposomes, DOTAP/Chol liposome consisted of DOTAP and Chol at a molar ratio of 1/1, and DOTAP/DOPE liposome consisted of DOTAP and DOPE at a molar ratio of 1/1 (Table 1). In DDAB-based cationic liposomes, DDAB/Chol liposome consisted of DDAB and Chol at a molar ratio of 1/1, and DDAB/DOPE liposome consisted of DDAB and DOPE at a molar ratio of 1/1 (Table 1).

First, to determine the optimal charge ratio (±) of cationic liposome and siRNA for preparation of cationic lipoplex, we monitored the association of siRNA with cationic liposomes by gel retardation electrophoresis (Figure 1). Naked siRNA was detected as bands on acrylamide gel. Beyond a charge ratio (±) of 4/1, no migration of siRNA was observed for DOTAP/Chol, DOTAP/DOPE and DDAB/Chol lipoplexes, although slight migration of siRNA was observed for DDAB/DOPE lipoplex. These findings suggested that the cationic lipoplexes were almost always formed at a charge ratio (±) of 4/1. In subsequence experiments, we thus decided to use cationic lipoplexes formed at a charge ratio (±) of 4/1.

Next, we measured the particle size and ζ-potential of cationic liposomes and lipoplexes. The sizes of DOTAP/Chol, DOTAP/DOPE, DDAB/Chol and DDAB/DOPE liposomes were 108, 110, 148 and 89 nm, respectively, and the ζ-potentials were +48, 51, 54 and 64 mV, respectively. When DOTAP/Chol, DOTAP/DOPE, DDAB/Chol and DDAB/DOPE liposomes were mixed with siRNA at a charge ratio (±) of 4/1, the sizes were 500, 205, 164 and 172 nm, and the ζ-potentials were about +43, 52, 22 and 57 mV, respectively. Among the lipoplexes, DOTAP/Chol lipoplex was relatively large in size.

DOPE has been widely used as a co-lipid for cationic liposome-mediated transfection of siRNA and plasmid DNA (Aissaoui et al., 2011). The role of DOPE is not fully understood, but it might affect the structural transition of cationic liposomes at the acidic pH of late endosomes in cells (Mochizuki et al., 2013). Furthermore, DOPE in cationic liposomes has been reported to induce the formation of the hexagonal phase, facilitating the release of nucleic acids from the complexes in endosomes (Zuhorn et al., 2005). From these findings, DOTAP/DOPE and DDAB/DOPE lipoplexes might be able to induce high gene suppression in tumor cells.

**Cellular association and gene knockdown efficiency**

Next, we examined the gene silencing effects by cationic lipoplexes using a luciferase assay system with MCF-7-Luc cells. DOTAP/DOPE and DDAB/DOPE lipoplexes of Luc siRNA exhibited high suppression of luciferase activity; however, the DOTAP/Chol and DDAB/Chol lipoplexes could not induce effective suppression (Figure 2).

Furthermore, to investigate whether cationic lipoplexes could be taken up well by the cells, we examined the cellular association by flow cytometric analysis. DOTAP/Chol and DDAB/Chol lipoplexes exhibited higher cellular association than DOTAP/DOPE and DDAB/DOPE lipoplexes, respectively (Figure 3A and B), indicating that the inclusion of cholesterol in a formulation of cationic liposome resulted in good uptake by the cells, but could not induce a gene silencing effect.

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**Endosomal localization**

To clarify why DOTAP/DOPE and DDAB/DOPE lipoplexes showed higher gene knockdown efficiency than DOTAP/Chol and DDAB/Chol lipoplexes, we investigated the endosomal localization of siRNA at 3 h after transfection by cationic lipoplexes (Figure 4). For co-localization with endosome stained by Lysotracker, we found that the fluorescent signals of AF-siRNA transfected by DOTAP/DOPE and DDAB/DOPE lipoplexes were often not co-localized with regions showing acidic compartments, compared with those by

Figure 1. Effect of the charge ratio (±) of cationic lipoplex to siRNA on the association of siRNA with cationic liposomes. Charge ratio (±) indicates the molar ratios of DOTAP or DDAB nitrogen to siRNA phosphate. Cationic lipoplexes of siRNA at various charge ratios (±) were analyzed by 18% acrylamide gel electrophoresis.

Figure 2. Gene suppression in MCF-7-Luc cells by cationic lipoplexes. Cationic lipoplexes formed at a charge ratio (±) of 4/1 were added to MCF-7-Luc cells at 50 nM siRNA, and the luciferase assay was carried out 48 h after incubation. Each column represents the mean ± SD (n = 3). **p < 0.01, compared with Cont siRNA.
Figure 3. Cellular association 3 h after transfection of DOTAP- (A) and DDAB-based cationic lipoplexes (B). Cationic lipoplexes of AF-siRNA were formed at a charge ratio (±) of 4/1. The association of cationic lipoplex with MCF-7 cells was determined on the basis of AF488 fluorescence by flow cytometry.

Figure 4. Endosomal localization of AF-siRNA through cationic lipoplex-mediated delivery. Cationic lipoplexes of AF-siRNA were prepared at a charge ratio (±) of 4/1, and then transfected into MCF-7 cells. Green signals show the localization of AF-siRNA, and red signals the localization of Lysotracker red DND-99. Arrows indicate the localization of siRNA not co-localized with acidic compartments in the cells. Scale bar = 20 μm.
DOTAP/Chol and DDAB/Chol lipoplexes (Figure 4). This finding indicates that DOTAP/DOPE and DDAB/DOPE lipoplexes were efficiently released from endosomes after internalization, followed by a pH drop. Cationic lipoplexes are needed to escape from endosomes after cellular internalization for the induction of gene knockdown. Therefore, DOTAP/DOPE and DDAB/DOPE lipoplexes might improve the escape from endosomes after endocytosis by the fusogenic activity of DOPE.

Biodistribution of siRNA after injection of lipoplex

To generate the mice with lung-metastasized tumor, MCF-7-Luc cells were intravenously injected into the tail vein of mice, and tumor metastasis in the lungs was confirmed by bioluminescence and H&E staining (Supplemental Figure S2A and B). To investigate the biodistribution of siRNA after the injection of cationic lipoplexes into mice bearing lung metastasis, we intravenously injected cationic lipoplexes of Cy5.5-siRNA into the mice, and observed the biodistribution of siRNA at 1 h after the injection of the lipoplexes by fluorescent microscopy (Figure 5). When naked siRNA was injected, accumulation was strongly observed only in the kidneys, indicating that the naked siRNA was quickly eliminated from the body by filtration in the kidneys. Upon injections of cationic lipoplexes, all of the cationic lipoplexes exhibited the accumulation of siRNA mainly in the lung. For DOTAP/DOPE and DDAB/DOPE lipoplexes, Cy5.5-siRNA was also observed in the kidneys. From this finding, the inclusion of DOPE in a liposomal formulation might destabilize the lipoplex in blood circulation, and siRNA dissociated from cationic lipoplexes in blood accumulates in the kidneys.

Biodistribution of Cy5.5-siRNA at 1 h after intravenous administration by cationic lipoplexes into mice with lung-metastasized MCF-7-Luc tumor. Green signals indicate localization of Cy5.5-siRNA. Scale bar = 100 μm.

DOTAP/Chol lipoplex of plasmid DNA was demonstrated to have high transfection efficiency in the lung by intravenous injection (Hattori et al., 2013), and it caused electrostatic interaction between positively charged lipoplexes and negatively charged erythrocytes soon after intravenous injection. DOTAP/Chol, DOTAP/DOPE, DDAB/Chol and DDAB/DOPE lipoplexes of siRNA may also cause electrostatic interaction between the lipoplexes and erythrocytes after intravenous injection, and their agglutinates contributed to the high entrapment of lipoplexes in the highly extended lung capillary (Simberg et al., 2003).

Gene suppression in vivo

Finally, to investigate whether cationic lipoplexes of siRNA could suppress the expression of a targeted gene in lung-metastasized tumor, we chose to target the luciferase gene, and evaluated the knockdown efficiency by assaying the level of luciferase activity at 48 h after intravenous injection of cationic lipoplex of Cont or Luc siRNA into the mice (Figure 6). The injection of DOTAP/Chol or DOTAP/DOPE lipoplex could induce significant suppression of the luciferase level in the lung-metastasized tumors (Figure 6A and B); however, injection of DDAB/Chol or DDAB/DOPE lipoplex did not affect luciferase activity in the tumors (Figure 6C and D). It has been reported that in vivo transfection of plasmid DNA into normal mice, DOTAP/Chol lipoplex exhibited higher transfection activity in the lung than DOTAP/DOPE lipoplex (Templeton et al., 1997). After intravenous injection, 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA)/Chol lipoplex stably bound to erythrocytes without much loss of transfection activity, whereas DOTMA/DOPE lipoplex...
reduced the transfection activity by the binding and subsequent fusion with erythrocytes (Sakurai et al., 2001a). However, in our study, DOTAP/DOPE lipoplex showed similar gene knockdown efficiency in lung-metastasized tumor by siRNA to DOTAP/Chol lipoplex. To clarify the discrepancy between previous and our results, further study will be needed to examine whether DOTAP/DOPE lipoplex of siRNA could fuse with erythrocytes.

The effect of cationic lipid structure on siRNA transfection efficiency has been previously reported (Semple et al., 2010). The head groups and linker groups of cationic lipids were shown to influence the in vivo siRNA delivery efficiencies. Furthermore, the extent of unsaturation of hydrocarbon chains of cationic lipid is known to affect siRNA delivery efficiency (Heyes et al., 2005). DDAB and DOTAP have saturated and unsaturated hydrocarbon chains of cationic lipid, respectively. The reason why DDAB/Chol and DDAB/DOPE lipoplexes could not induce gene silencing effect compared with DOTAP/Chol and DOTAP/DOPE lipoplexes was not clear, but the difference of saturation in hydrocarbon chains of cationic lipid might be one of the factors producing a difference of in vivo gene silencing effect between DOTAP- and DDAB-based liposomes.

In comparison of transfection efficiency between plasmid DNA and siRNA, DOTAP/Chol and DDAB/Chol lipoplexes of plasmid DNA induced high gene expression in the lungs of normal mouse by intravenous injection (Supplemental Figure S1). In contrast, DOTAP/Chol lipoplex of siRNA exhibited gene suppression in lung-metastasized tumors, but DDAB/Chol lipoplex of siRNA did not (Figure 6). It was not clear why the DDAB/Chol lipoplex could not induce the gene knockdown in lung-metastasized tumors, but we speculate that the DDAB/Chol lipoplex might be transfected into lung endothelial cells rather than lung-metastasized tumor cells.

In our results, the neutral lipid of cationic liposomes strongly affected the in vitro gene silencing effect, but not cationic lipid. In contrast, the cationic lipid affected the in vivo gene silencing effect rather than neutral lipid. These findings suggest that the in vivo silencing effect by cationic lipoplexes was strongly affected by the type of cationic lipid. From these findings, DOTAP-based liposomes might be useful as an in vivo siRNA delivery carrier that can induce gene silencing in lung-metastasized tumors. However, the further optimization of cationic lipids in liposomal formulations might require improvement of the in vivo knockdown efficiency in lung-metastasized tumor.

In this study, we examined a formulation of cationic liposome for systemic delivery of siRNA into lung-metastasized tumor. DOTAP-based cationic lipoplexes accumulated in lung-metastasized tumor after intravenous injection, and could induce a gene silencing effect. From our results, DOTAP-based lipoplexes are outstanding tools for siRNA delivery to lung-metastasized tumor. Further study should be performed to examine the therapeutic applications by a gene silencing effect in metastatic tumor.

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Declaration of interest

The authors declare no conflicts of interest or financial interests in any product or service mentioned in this article, including grants, employment, gifts, stock holdings, honoraria, consultancies, expert testimony, patents and royalties.

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Supplementary material available online.

Supplemental Figures S1 and S2.