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Enhanced Tumor-targeted Gene Delivery by Immunolipoplexes Conjugated with the Humanized *Anti*-TAG-72 Fab' Fragments

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Cationic immunoliposomes were prepared by conjugation of Fab' fragments of the recombinant humanized monoclonal antibody (HuCC49) against tumor-associated glycoprotein (TAG)-72 to sterically unilamella liposomes. The cationic immunoliposomes are composed of cationic lipid (*O,O'*-dimyristyl-*N*-lysyl aspartate, DMKD), cholesterol, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene-glycol)$_{2000}$] (DPPE-PEG-maleimide) with a molar ratio of 0.5:0.47:0.03. Plasmid DNA was effectively condensed by addition of transferrin (Tf) during the formation of *anti*-TAG-72 PEG-immunolipoplexes (PILPs). These *anti*-TAG-72 PILPs were able to adhere to the surface of TAG-72-overexepressing LS174T human colon cancer cells more effectively than conventional liposomes, thereby facilitating gene delivery *in vitro*. Furthermore, intravenous administration of the *anti*-TAG-72 PILPs into the tumor-carrying mice exhibited efficient localization of the reporter gene in the tumor tissues.

Key Words : Immunolipoplexes, Humanized monoclonal antibody, Tumor-associated glycoprotein-72, Targeted gene delivery

Introduction

Targeted gene therapy has become a viable possibility for the treatment of diseases such as cancer. The efficacy of gene therapy places a considerable dependence on the delivery system which can efficiently and selectively deliver the gene to the target site with minimal side effect. To date, many gene delivery vehicles have not been amenable to systemic administration due to their lack of targeting ability and rapid degradation within the bloodstream.[1] Therefore, ideal delivery vehicle would be one that could be administered systemically and selectively targeted to tumor cells, wherever they occur in the body.[2] A hydrophilic polymer, polyethylene glycol (PEG), has been shown to create a steric shield around the surface of a liposome or protein, thereby conferring protection from nonspecific opsonization by plasma proteins, which can enhance the circulation time of liposome or protein in the blood.[3] The application of this modification (PEGylation) to nonviral gene delivery systems has drawn considerable interest.[4,5] Advantages of PEGylation have been described elsewhere, including improvement of systemic circulation of lipoplexes (or polyplexes) and reduction of *in vivo* toxicity of the polyplex.[6]

Liposome-based gene delivery systems have been widely studied as an alternative to virus-based gene delivery vehicles. However, the *in vivo* gene transfer capabilities of lipoplexes have been found to be generally less effective than that of viral systems.[7] The effectiveness of a liposome-based gene delivery system can be achieved by modifying the liposomal surface through addition of targeting molecules, such as antibodies or antibody fragments, so that they selectively deliver their cargo to tumor cells.[8] In this study, we have developed a new formulation of cationic immunoliposomes, in which the tumor specific antibody was conjugated *via* PEG and maleimide phospholipid for effective delivery of genes to target cells *in vivo*. These immunoliposomes consist of cationic lipid *O,O'*-dimyristyl-*N*-lysyl aspartate (DMKD), cholesterol, and phospholipid with PEG-conjugated DPPE-PEG$_{2000}$-Mal (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene-glycol)$_{2000}$]) for coupling of antibody Fab' fragments.

Tumor-associated glycoprotein 72 (TAG-72) is a glycoprotein that is often found on the surface of many cancer cells, such as ovary, breast, colon, and pancreatic cells, and TAG-72 was purified and used as antigen to produce monoclonal antibodies.[9] In our cationic immunoliposome, Fab' fragments of the humanized *anti*-TAG-72 monoclonal antibody, HuCC49, were used instead of whole antibodies to reduce Fc receptor-mediated reticuloendothelial system (RES) uptake.[10,11] Conjugation *via* thiol residues of the Fab' fragments permits the retention of the antigen-binding activity of recombinant humanized monoclonal antibody. Furthermore, the *anti*-TAG-72 PEG-immunolipoplexes (PILPs) were prepared by addition of iron-binding blood plasma glycoproteins, transferrin (Tf). Tf-modified PILPs were more effective in gene transfection in the cell culture than plain lipoplexes without transferrin. Of importance, the *anti*-TAG-72 PILPs containing human transferrin showed significantly

enhanced transfection efficiency of the reporter gene in the colon cancer cell-xenografted nude mice.

Experimental Section

Cell Cultures and Plasmid. CHO cell line (AKA 120-0.32) that is stably transfected with plasmid (pdCMV-dhfr-AKA/Hzk) encoding humanized *anti*-TAG-72 monoclonal antibodies (HuCC49) was used as previously described.[13] The cells were maintained in MEM-α (Gibco, Carlsbad, CA) supplemented with 10% dialyzed fetal bovine serum and 100 μM methotrexate (Sigma, St Louis, MO). Human colon adenocarcinoma LS174T cells (CL-188; ATCC, Manassas, VA), human colorectal adenocarcinoma WiDr cells (CCL-218; ATCC), and human breast carcinoma MCF-7 cells (HTB-22; ATCC) were cultured in minimal essential medium (Gibco) supplemented with 10% heat-inactivated bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C under 5 % CO_2. The firefly luciferase under the cytomegalovirus (CMV) promoter (pAAV-CMV-Luc) was used as described in the previous study.[13] The plasmids were propagated in DH5α strain of *E. coli* under LB media supplemented with ampicillin. The plasmids were purified using the Mega plasmid purification kit (QIAGEN, Valencia, CA) and quantified spectrophotometrically (A_{260}/A_{280} values approximately 1.95).

Preparation of Humanized Antibody CC49 (HuCC49 IgG) and Fab' Fragments. AKA 120-0.32 cells were cultured in 2 L of SFM II media (Gibco) for 5 days and the culture media were harvested by filtration through a 0.45-μm filter (Nunclon, Rochester, NY). The filtered CHO cell media were applied to a HiTrap protein-G column (GE healthcare) and the eluate was collected in 1-mL fractions. The fractions were assayed for identification of the humanized antibody CC49 (HuCC49 IgG) using the detergent compatible protein assay reagent kit (Bio-Rad, Hercules, CA) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

$F(ab')_2$ fragments of the antibodies (HuCC49) were produced using an Immunopure IgG_1 $F(ab')_2$ preparation kit (Thermo, Rockford, IL). In brief, the HuCC49 antibodies were dialyzed using a Dialysis Cassettes (Slide-A-Lyzer 10 K, Thermo) against 20 mM sodium acetate buffer (digestion buffer, pH 4.5). The dialyzed antibodies were concentrated with an Ultra-Amicon 30 K (Millipore, Billerica, MA), resuspended in 0.5 mL of digestion buffer and added to 0.25 mL of immobilized pepsin slurry (Thermo). The solution was then incubated with a serum separator tube (Thermo) for 4 h at 37 °C in a shaker water bath. The reaction solution was diluted with 4 mL binding buffer and then loaded on an Immunopure protein-A column (Pierce, Rockford, IL). The eluted fractions containing $F(ab')_2$ were detected by a standard protein assay (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA). The $F(ab')_2$ fragments (110 kDa) were concentrated using Centricon-50 (Millipore) and then resuspended in 1 mL phosphate-EDTA buffer (100 mM sodium phosphate, 5 mM EDTA, pH 6.0). To cleave the disulfide bonds of the $F(ab')_2$ fragments, 2-mercaptoethylamine-HCl was added (0.05 M of final concentration) to the $F(ab')_2$ solution and the mixture was incubated for 90 min at 37 °C. The resulting Fab' fragments were purified through a Sephadex G-25M column (PD-10 column; GE healthcare) pre-equilibrated with acetate-EDTA buffer (100 mM anhydrous sodium acetate, 88 mM sodium chloride and 1 mM EDTA, pH 6.5) and quantified using a standard protein assay. The Fab' fragments (55 kDa) were concentrated using Centricon-10 (Millipore), resuspended in acetate-EDTA buffer and kept under nitrogen atmosphere at 4 °C prior to coupling to liposomes. The purity of Fab' fragments was assessed by SDS-PAGE.

Detection of TAG-72 Expression in Cancer Cells by HuCC49 Fab' Fragments. *In vitro* immunogenicity of the purified HuCC49 Fab' fragments was evaluated by immunofluorescence staining of LS174T, WiDr, and MCF-7 cells. These tumor cell lines were grown on 13-mm cover slips (Nunclon) in 20-well plates for 20 h. The cells were fixed with 2% paraformaldehyde and then incubated in the presence of the purified HuCC49 Fab' fragments (1:250 dilution) for 60 min at room temperature. After treatment with fluorescein isothiocyanate (FITC)-conjugated goat *anti*-human IgG antibody (Sigma) for 45 min at room temperature, the cells on cover slips were examined under a fluorescence microscope (BX61-32FDIC, Olympus, Tokyo, Japan).

Preparation of *anti*-TAG-72 PEG-cationic immunoliposomes and *anti*-TAG-72 PEG-immunolipoplexes (PILPs). Antibody-conjugated PEG-cationic immunoliposomes were prepared as described previously.[14] In brief, cationic lipid (*O,O'*-dimyristyl-*N*-lysyl aspartate, DMKD, Avanti Polar Lipids, Alabaster, AL), cholesterol, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethyleneglycol)2000] (DPPE-PEG-maleimide, Avanti Polar Lipids) were mixed in a molar ratio of 0.5:0.47:0.03 in chloroform: methanol (2:1, v/v). The chloroform and methanol was evaporated under a stream of N_2 gas. Vacuum desiccation for a minimum of 2 h ensured removal of the residual organic solvent. The dried lipid film (total 20 mmol of lipids) was hydrated with 1 mL of HEPES buffer (20 mM, pH 7.4). The hydrated lipids were sonicated with a bath sonicator twice for 1 min with a 5-min interval, which produced small unilamellar liposomal vesicles. The liposome solution was then extruded through 800-, 400-, 200- and 100-nm pore size polycarbonate membranes (Millipore). HuCC49-Fab' fragments were covalently conjugated to maleimide groups at the termini of the DPPE-PEG_{2000}-Mal chains by co-incubation of 600 μg Fab' and 20 μmol of phospholipid at 55 °C for 30 min.[15] Unconjugated antibodies were removed from the liposomes by CL-4B gel filtration chromatography (GE healthcare). These prepared antibody-conjugated liposomes were stored at 4 °C until use.

A schematic representation of the preparation strategy of *anti*-TAG-72 PILP is shown in Figure 1. Briefly, 40 μg of pDNA in 100 μL of 20 mM HEPES buffer (pH 7.4) was mixed with 10 μL of transferrin (Tf) solution (1 μg/mL,

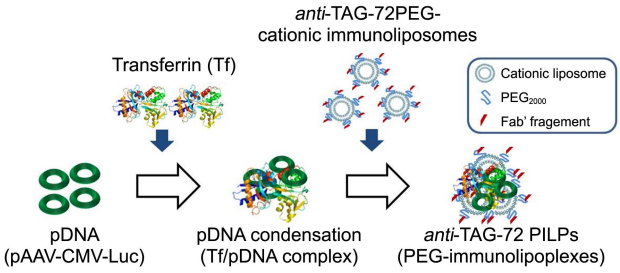

Figure 1. Schematic representation of the preparation strategy of *anti*-TAG-72 PILPs (PEG-immunolipoplexes).

Sigma) and incubated for 15 min at room temperature with frequent rocking. 100 μL of 2 mM liposome solution (*anti*-TAG-72 PEG-cationic immunoliposomes) was added and incubated for 20 min at room temperature. The particle size and zeta potential were measured using a Zetasizer 3000HS (Malvern, UK). The measurement was performed under the conditions of 25 °C and 90° scattering angle.

DNase I Protection Assay. DNase I (1 unit per μg of DNA; Sigma) was added to 10 μL of *anti*-TAG-72 PEG-immunlipoplexes solution containing 2 μg pDNA and the mixtures were incubated at 37 °C for 1 h. 2 μL of 0.5 M EDTA and 2 μL of 1% Triton X-100 was added to stop DNase I reaction, and the mixture was further incubated for 20 min at room temperature to release DNA from the PILPs. Finally, the reaction mixtures were run on 1% agarose gel and visualized by UV illumination.

Cellular Binding of *anti*-TAG-72 PILPs. *Anti*-TAG-72 PEG-cationic immunoliposomes containing Lissamine Rhodamine B-dioleoyl phosphatidylethanolamine (Rho-DOPE, 0.1 mol % of total lipid, Avanti Polar Lipids) were used to evaluate specific cellular binding of the *anti*-TAG-72 PILPs. *Anti*-TAG-72 PILPs were added to LS174T, WiDr, and MCF-7 cells (2×10^5 cells) in a 20-well plate and incubated for 30 min at 37 °C. For the competition assay of *anti*-TAG-72 PILPs, a 20-fold excess of free *anti*-TAG-72 Fab' fragment antibody (3 μg per well respectively) was added to each cell before the cells were transfected with the same *anti*-TAG-72 PILPs. The treated cells were thoroughly washed with phosphate buffered saline (PBS, pH 7.4) and observed by a inverted fluorescence microscopy (AxioVert200, Carl Zeiss, Oberkochen, Germany) .

***In vitro* Transfection of Luciferase Gene Using *anti*-TAG-72 PILP.** LS174T, WiDr, or MCF-7 cells were seeded per well (5×10^4 cells) in a 20-well plate. After 20 h, the medium containing 10 % FBS was replaced with serum-free medium and the cells were transfected with *anti*-TAG-72 PILPs, or lipoplexes of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)/cholesterol or *O,O'*-dimyristyl-*N*-lysyl aspartate (DMKD)/cholesterol (6:1, N/P ratio; 10 μL) carrying the luciferase gene (1 μg pAAV-CMV-Luc per well) as previously described.[16] The cells were initially incubated with each liposome in the presence of 0, 10 or 60% serum for 4 h and then further incubated in a fresh 10% serum containing the media for 20 h. The transfected cells were washed twice with PBS (pH 7.4) and lysed using 200 μL of lysis buffer (1% Triton X-100, 1 mM dithiothreitol and 2 mM EDTA) at room temperature for 2 h. The cell lysates were centrifuged in a microcentrigufe at 12,000 × g for 2 min at 4 °C to pellet debris. Luciferase activity in the lysates was evaluated with a luciferase assay kit (Promega, Madison, WI). The relative luminescence units were determined using a Sirius single tube luminometer (Berthold, Bad Wildbad, Germany). The protein concentrations in the lysates were measured with the detergent compatible protein assay kit. The measurements were expressed as relative luminescence units of luciferase per milligram of total cellular protein.

***In vivo* Gene Delivery into Tumor-carrying Mice.** Nude mouse xenografts were prepared by subcutaneous injection of LS174T cells (2.5×10^6 cells) on the lower back of 6-week-old female athymic nude mice (BALB/cAnNCrjBgi-nu; ORIENT BIO Inc., Gyeonggi-do, Korea). Fifteen days later, the tumor-carrying mice (n = 4, average 200 mm^3 of tumor volume) were slowly injected intravenously with a solution (250 μL) containing *anti*-TAG-72 PILPs encapsulating pAVV-CMV-Luc or DMKD/cholesterol lipoplexes of pAVV-CMV-Luc (6:1, N/P ratio) (40 μg DNA per each mouse). To assess the luciferase expression, major organs (tumor, heart, kidney, liver, lungs and spleen) were removed 48 h post-transfection and homogenized in the lysis buffer. Luciferase activities in the lysates were measured as described above. The statistical significance of the experimental results was analyzed by the Student's *t*-test. Statistical significance was set at $P < 0.001$.

Results and Discussion

Specificity of Humanized Antibody CC49 (HuCC49) Fragment (Fab') to TAG-72. First, we examined the target specificity of the Fab' fragment of the humanized *anti*-TAG-72 monoclonal antibodies (HuCC49) against TAG-72 expression in the LS174T colon adenocarcinoma cells by immunofluorescence cell staining using the HuCC49 Fab'

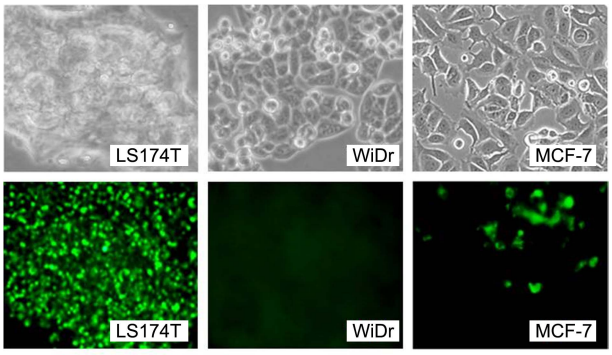

Figure 2. Specificity of *anti*-TAG-72 HuCC49 Fab' fragments to TAG-72 antigens on cancer cells. Expression of TAG-72 was monitored by immunofluorescence staining of LS174T (TAG-72-positive), WiDr (TAG-72-negative) or MCF-7 (TAG-72-mild positive) cells using the HuCC49 Fab' and goat *anti*-human FITC-IgG. Bright field (top) and fluorescence (bottom) microscopic photographs were of the same filed at a magnification of × 40.

fragments and Fab'-specific FITC-labeled secondary antibody. Specific binding of HuCC49 Fab' fragments were observed only in LS174T and MCF-7 (TAG-72-positive) but not in WiDr cells (TAG-72-negative) by immunofluorescence cell staining (Fig. 2), which is consistent with the previous result.[17] The results clearly showed that the HuCC49 Fab' fragments specifically target the antigen TAG-72 present on the surface of the TAG-72-positive colon cancer cells.

Physical Characteristics of *anti*-TAG-72 PILPs. To assess physical characteristics of the *anti*-TAG-72 PEG-immunolipoplexes (*anti*-TAG-72 PILPs), their sizes and zeta potentials were measured and compared with those of plain lipoplexes (DMKD/Chol). As shown in Figure 3(a), the mean diameter of immunolipoplexes were significantly decreased to 120.3 ± 7.4 nm compared with the mean particle size of the plain lipoplexes (305.2 ± 12 nm). The zeta potential of immunolipoplexes (8.9 ± 3.8 mV) were also decreased, as compared with those of plain lipoplexes (35.2 ± 6.8 mV). The reduced values of size and zeta potential of *anti*-TAG-72 PILPs are known to be beneficial for passive extravasation of lipoplexes.[18] In the preparation of *anti*-TAG-72 PILPs, plasmid DNA was effectively condensed by the addition of transferrin (Tf) harboring the positive charge, which contributes to the formation of the smaller pDNA complexes prior to the formation of *anti*-TAG-72 PILPs complex (Fig. 1). Thus, inclusion of transferrin into *anti*-TAG-72 PILPs did result in a significant decrease in the zeta potential and mean diameter of the lipoplexes.

To investigate the role of *anti*-TAG-72 PILPs in protecting the complexed pDNA from being attacked by degradative enzymes *in vivo*, an *in vitro* DNase I protection assay was performed with *anti*-TAG-72 PILPs prepared with or without transferrin. Nuclease treatment was able to remove any pDNA, either free or bound to the exterior of liposomes. As shown in electrophoresis with ethidium bromide staining (Fig. 3(b)), the pDNA encapsulated within the *anti*-TAG-72 PILPs was effectively protected from DNase I and the free pDNA was completely degraded by DNase I treatment. The amount of encapsulated pDNA was visualized by UV illumination after treatment of the *anti*-TAG-72 PILPs with detergent Triton X-100. The pDNA encapsulation efficiency was increased with transferrin in the *anti*-TAG-72 PILPs (Fig. 3(b), lane 4 and 6), as compared with *anti*-TAG-72 PILP without transferrin (Fig. 3(b), lane 3 and 5). These results imply that positively charged transferrin is likely to condense more pDNA and subsequently form the smaller lipoplexes than the lipoplexes without transferrin.

Specific Binding of *anti*-TAG-72 PILPs to TAG-72 Positive Cancer Cells. The *anti*-TAG-72 PILPs containing fluorescent dye-conjugated phospholipid (Rho-DOPE) were used to evaluate specific binding to the surface of tumor cells expressing TAG-72. *Anti*-TAG-72 PILPs were added to

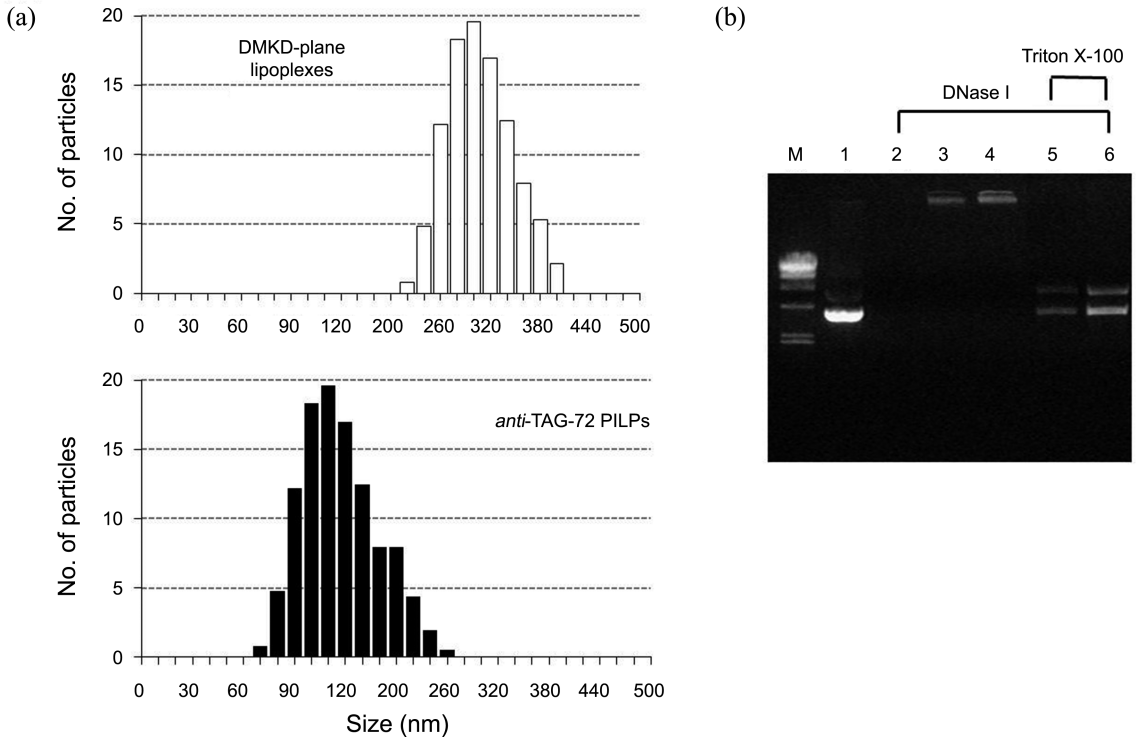

Figure 3. Physical characteristics and DNase I protection assay of the *anti*-TAG72 PILPs. (A) Size and charge of DMKD-plain lipoplexes and *anti*-TAG-72 PILPs were measured using a Zetasizer 3000HS. The opened-bar and the closed-bar represents DMKD-plain lipoplexes and *anti*-TAG-72 PILPs, respectively. (B) Plasmid-encapsulating *anti*-TAG-72 PILPs were treated with DNase I for 30 min at 37 °C (lanes 2-6). The same *anti*-TAG-72 PILPs were lysed by treatment with Triton X-100 for 20 min at room temperature to release pDNA (lanes 5-6). Aliquots of the samples were run on 1.0% agarose gel and visualized by UV illumination after EtBr staining. Lane M, λ/Hind III DNA molecular-weight markers; lane 1, untreated naked pDNA (2 μg); lane 2, naked pDNA treated with DNase I; lane 3 and 5, *anti*-TAG-72 PILPs without transferrin; lane 4 and 6, *anti*-TAG-72 PILPs with transferrin.

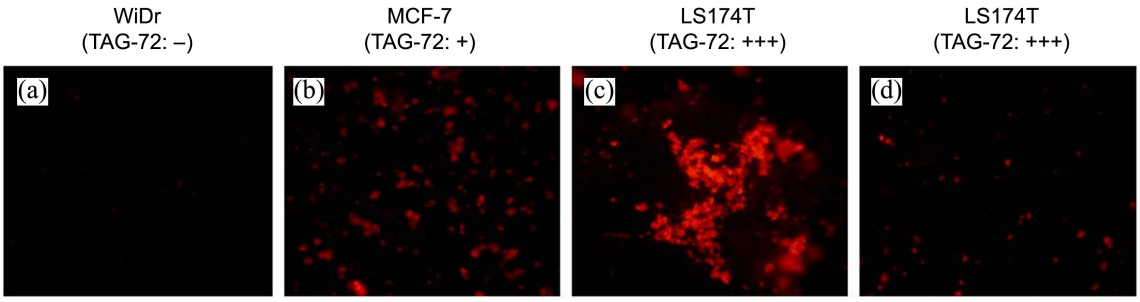

Figure 4. Cell-specific binding of *anti*-TAG72 PILPs. To evaluate their cellular binding affinity, *anti*-TAG-72 PILPs containing DOPE-rhodamine (0.1 mol %) were incubated with cells (each 2×10^5 cells); (a) WiDr cells, (b) MCF-7 cells, (c) LS174 T cells, and (d) LS174T cells that were pretreated with a 20-fold excess of free *anti*-TAG-72 Fab' for 30 min at 37 °C. The cellular binding of the *anti*-TAG-72 PILPs were observed by fluorescence microscopy.

the TAG-72-positive LS174T cells, MCF-7 cells or the TAG-72-negative WiDr cells. The *anti*-TAG-72 PILPs were able to effectively bind to LS174T cells (Fig. 4(c)), but not to WiDr cells (Fig. 4(a)). The *anti*-TAG-72 PILPs were also able to moderately bind to human breast cancer cells, MCF-7 (Fig. 4(b)), which express TAG-72 less than LS174T cells. Moreover, the cellular association of the *anti*-TAG-72 PILPs to the TAG-72-positive LS174T cells was completely blocked by addition of a 20-fold excess of free *anti*-TAG-72-Fab' fragments before treatment with the *anti*-TAG-72 PILPs (Fig. 4(d)). These results indicate that the *anti*-TAG-72 PILPs specifically target to TAG-72 in the human colon adenocarcinoma cells expressing TAG-72.

***Anti*-TAG-72 PILP Facilitates Gene Delivery *in vitro* and *in vivo*.** The *in vitro* transfection efficiency of *anti*-TAG-72 PILPs encapsulating the reporter gene (pAAV-CMV-Luc) encoding the firefly luciferase was evaluated with LS174T and WiDr cells. For gene delivery to LS174T cells, the *anti*-TAG-72 PILPs were more effective than conventional cationic liposomes such as DOTAP- or DMKD-based liposomes. The *anti*-TAG-72 PILPs exhibited effective transfection of the reporter gene to LS174T cells and not to WiDr cells, confirming the TAG-72-mediated specific gene-transfer capability of these immunolipoplexes (Fig. 5(a)). Moreover, their specific gene transfection efficiency was retained even in the presence of 60% serum.

To examine their tumor-specific gene-transfer capability, the *anti*-TAG-72 PILPs containing the luciferase reporter gene were injected intravenously into nude mice bearing LS174T tumor cell xenografts. As expected, at 48 h post-administration of the *anti*-TAG-72 PILPs, the LS174T tumor tissues displayed a higher level of luciferase expression than any other major organs. By contrast, DMKD-based cationic lipoplexes exhibited the highest gene expression in the lungs, which are not relevant to tumor expression (Fig. 5(b)). Thus, the *anti*-TAG-72 PILPs are able to deliver the gene to the site of tissues containing the tumor cells expressing TAG-72.

In this study, we conjugated Fab' fragments of the humanized *anti*-TAG-72 antibodies (HuCC49) to liposomes for tumor-directed gene delivery. The Fab' fragments retained the capability of specific binding to TAG-72 expressed in

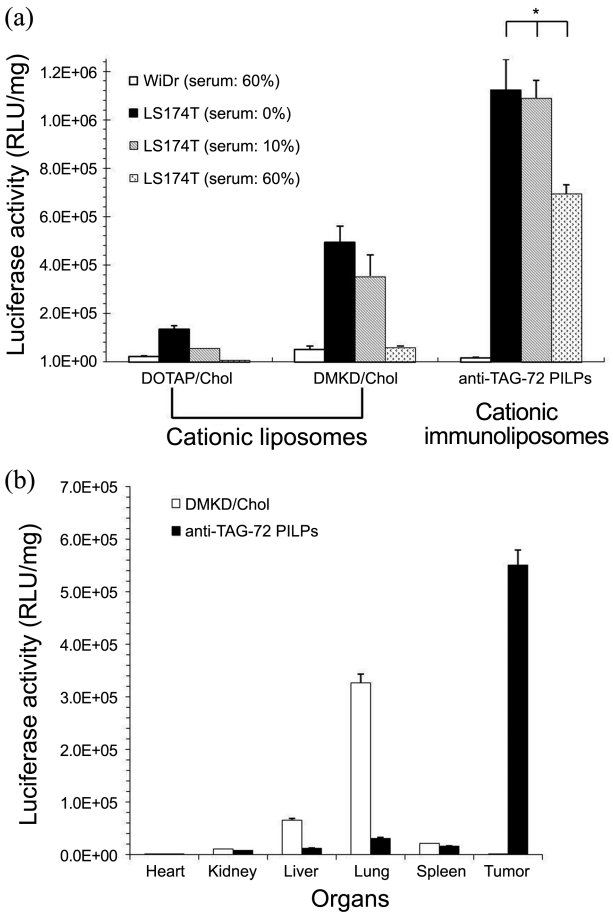

Figure 5. *In vitro* and *in vivo* gene delivery mediated by *anti*-TAG-72 PILPs. (a) LS174T and WiDr cells were transfected for 4 h with various formulations of *anti*-TAG-72 PILPs, encapsulating 1 μg of pAAV-CMV-Luc in the presence of 0, 10 or 60% serum. Luciferase activity in the transfected cells was assayed after incubation for an additional 20 h. Each bar represents the mean ± S.D. for three separate luciferase assay experiments. Statistical analysis was performed by Student's t-test; $*P < 0.001$ versus expression by DOTAP- or *O,O'*-dimyristyl-*N*-lysyl aspartate (DMKD)-based liposomes in LS174T cells. (B) At the 15th day post-subcutaneous inoculation of LS174T cells (2.5×10^6 cells), tumor-carrying mice were injected intravenously with *anti*-TAG-72 PILPs or DMKD lipoplexes containing pAAV-CMV-Luc (40 μg pDNA per mouse, $n = 4$). Major organs and tumor tissues were removed 48 h post-transfection, and luciferase activity in the organs were measured. Each bar represents the mean ± S.D.

LS174T tumor cells. The Fab' fragments were conjugated by disulfide linkage to PEG termini exposed on the liposomal surface. This type of linkage between the Fab' molecules and liposomes can have two meaningful advantages; 1) effective outward exposition of the antigen binding domain of the Fab', and 2) enhanced half life of the immunolipoplexes in the blood circulation. Targeted liposomal delivery system must suffice a reasonable plasma circulation time to allow access to a tumor site.

In conclusion, a novel type of immunolipoplexes was designed for the *in vivo* gene delivery to colon cancer cells. The small and uniform *anti*-TAG-72 PILPs coupled to Fab' fragments of genetically engineered 'humanized' antibodies against TAG-72 antigens were able to specifically bind to TAG-72 antigens expressed in LS174T colon cancer cells. The resulting *anti*-TAG-72 PILPs exhibited more efficient *in vitro* and *in vivo* targeted-gene delivery to the cancer cells than conventional cationic liposomes. Thus, the *anti*-TAG-72 PILP-mediated gene transfer technology allows a gene targeting to the distant sites following the intravenous administration. This approach provides a promising tool of systemic delivery of anticancer gene therapy reagents to the sites of tumors, which may have a potential for clinical application in human colon cancer treatment.

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