Review

Application of cationic liposomes for delivery of nucleic acids

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A B S T R A C T

Nucleic acid-based bioactive substances have recently emerged as a new class of next-generation therapeutics, but their development has been limited by their relatively weak delivery into target cells. Cationic liposomes have been studied as a means to enhance the stability of nucleic acid therapeutics in the bloodstream and improve their cellular delivery. As nucleic acid therapeutics, siRNA and plasmid DNA have been extensively tested for delivery using cationic liposomes. This review discusses recent progress in the application of cationic liposomes for the delivery of nucleic acid therapeutics.

1. Introduction

As our understanding of molecular pathogenesis has improved, nucleic acid therapeutics have received attention as a modality for modulating the cellular expression levels of specific genes. Unlike chemical drugs that function by binding to target proteins, nucleic acid therapeutics can modulate the pathogenic cellular machinery by controlling the expression levels of functional proteins. For this modulation of target protein expression level to occur, however, the nucleic acid therapeutics must enter the cell membrane and reach the cytoplasm.

Various nucleic acid-based molecules have been studied as next-generation therapeutics, including plasmid DNA, antisense oligodeoxynucleotides (AS-ODN), small interfering RNA (siRNA), and micro RNA (miRNA). These molecules share various physicochemical properties, are larger than chemical drugs, and carry highly negative charges [1–3], limiting their cellular delivery. For nucleic acid therapeutics to be successful, therefore, new delivery systems should be developed in parallel with the identification of appropriate target proteins.

Plasmid DNA, which was among the earliest nucleic acids considered for therapeutic purposes, has long been studied in the context of gene therapy [4]. In this context, researchers...
have focused on viral vectors, which can confer high transfection efficiencies and continuous modulation of gene expression. However, the tragic death of Gelsinger during a clinical trial using an adeno-viral vector brought home the chilling notion that viral materials may not be entirely safe [5]. Furthermore, viral vectors have several disadvantages as pharmaceutical candidates, such as the inconvenience of needing to design a vector for each target molecule, and a lack of knowledge regarding the dose dependency of cellular expression.

Non-viral vectors typically have a lower transfection efficiency than viral vectors, but they have been extensively studied because they are considered far safer. Nanoparticulate delivery systems, in which cationic lipid nanoparticles are loaded via the negative phosphate groups of nucleic acids, are a major class of non-viral vectors that show high productivity and loading efficiency [6–8]. Nanoparticulate systems for carrying nucleic acids may be topologically classified as lipid- or polymer-based nanoparticles, and each is called a ‘lipoplex’ or ‘polyplex’ after it interacts with nucleic acids. The cellular delivery of these complexes is believed to occur via endocytosis followed by endosomal escape into the cytoplasm [9].

As a delivery system of nucleic acid, cationic liposomes have advantages. First, the cationic liposomes are biodegradable after administration in vivo. The presence of endogenous enzymes can breakdown the lipid components of the liposomes. The unparalleled biocompatibility of liposomes among various nanocarriers resulted in the use of cationic liposomes for delivery of various siRNAs for in vivo studies [10]. The lipid composition-dependent modulation of surface charge density can control the interaction forces with negatively charged nucleic acids. The inclusion of pegylated lipids or functional lipids can make possible the diverse surface modification of liposomes. Moreover, the inclusion of lipophilic chemical drugs in the lipid bilayers of cationic liposomes can provide co-delivery of anticancer drug and therapeutic nucleic acids [11,12]. Given the advantages, cationic liposomes have been studied for delivery of various nucleic acids such as plasmid DNA, antisense oligonucleotides and siRNA [13,14].

2. Factors that affect the delivery efficiencies of nucleic acids

For the development of nucleic acid therapeutics using cationic liposomes, one prerequisite is that the nucleic acids must be appropriately delivered to target cells and reach the appropriate subcellular compartment (e.g., the cytoplasm or nucleus). The delivery efficiencies of cationic liposomes are known to be affected by the types of cationic and helper lipids, and their compositions.

2.1. Cationic lipids used for liposomes

Cationic lipids, which are the core components of nanoparticles, have a common structure of a positively charged head group and one or two hydrophobic tail region(s) made of hydrocarbon chains or steroid structures. Felgner and colleagues reported the synthesis of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride with a monovalent cationic head and two hydrocarbon tails, and its use to prepare small unilamellar liposomes [15]. They transfected DNA-entrapped lipoplexes into mouse L cells and demonstrated that the cationic lipids neutralized the negatively charged DNA, giving the cationic lipoplexes a better chance to interact with negatively charged cell membranes. Since then, various cationic lipid and lipid-based nanoparticles have been designed and evaluated for the cellular delivery of nucleic acids, including DNA, siRNA, miRNA, and AS-ODN.

These new cationic lipids have been identified by a combination of library techniques and rationale-based prediction. Screening of a lipid-like material library yielded a cationic lipid of ten carbons and two alkyl chains that was found to be more effective than other candidates [16]. The screened cationic lipid was studied for the systemic administration of apolipoprotein B-specific siRNA to cynomolgus monkeys at a dose of 6.25 mg/kg, and the expression of apolipoprotein B in liver tissues was reported to be reduced by more than 50% over 2 weeks.

More recently, Mevel and colleagues [17] synthesized various cationic lipids and tried to find a cationic liposome composition that was effective for plasmid DNA delivery. Among the newly synthesized cationic lipids, N,N'-dioctadecyl-N-(4,8-diaza-10-aminodecanoyl) glycine amide formulated into cationic nanolipoplexes showed the highest transfection efficiency for plasmid DNA. Furthermore, N,N'-dioctadecyl-N-(4,8-diaza-10-aminodecanoyl) glycine amide was shown to deliver plasmid DNA to OVCAR-3 and HeLa cell lines more efficiently than the transfection reagent, Lipofectamine 2000.

The rationale-based prediction of new cationic lipids [18], in contrast, has been based on the hypothesis that cationic lipids can interact with natural anionic lipids of the endothelial membrane after endocytosis, and cone-shaped lipids will induce destruction of the bilayer membrane. To design cationic lipids capable of improving transfection efficiency, the authors controlled the lipid head groups, hydrocarbon domains and linkers. Using the designed lipids, they showed that nucleic acid–lipid particles composed of 1,2-diolinoxyly-3-dimethylaminopropane-based cationic lipids showed gene-silencing effects when used to encapsulate siRNA at doses of 0.01 mg/kg in mice and 0.3 mg/kg in cynomolgus monkeys.

A recent structure–activity relationship study revealed that a subtle difference in lipid structure could yield distinct differences in transfection efficiency [19]. The authors designed and synthesized 1,4,7,10-tetraazacyclododecan cyclo-based and imidazolium-containing cationic lipid having different hydrophobic regions (e.g., cholesterol and dioxygenin, respectively). The two cationic lipids were shown to induce effective gene transfection in HEK293 cells.

2.2. Helper lipids used for liposomes

Neutral lipids have also been routinely used as helpers for cationic liposomes. For example, the neutral lipid, 1,2-dioleoylsn-glycerol-3-phosphoethanolamine (DOPE), is known to be involved in endosomal escape after the endocytosis of lipoplexes [20], and cholesterol (an endogenous lipid) can be inserted between the lipid bilayers to increase the rigidity of nanoparticles [21]. To increase in vivo stability, a very general
Intratumoral injection of the cationic liposome complex- therapeutic efficacy of the lipoplexes was tested in mice, tumor cells were decreased as compared to control group.

For plasmid DNA to be functionally translated within a cell, the plasmid DNA must undergo effective intracellular trafficking into the cytoplasm, and from there into the nucleus. Plasmid DNA encoding interleukin 12, a cytokine with antitumor activity, was complexed with cationic liposomes and tested for its in vivo anticancer effects in a metastatic lung cancer mouse model [26]. The studied cationic liposomes were composed of all-trans-retinoic acid (to enhance the antitumor effects), DOTAP and cholesterol (molar ratio, 3:1:2) and complexed with interleukin 12-encoding plasmid DNA. After two intravenous injections with plasmid DNA (1.2 mg/kg/mouse), the numbers of tumor nodules and plasmid DNA decreased as the weight ratio of DC-Chol to plasmid DNA increased, with the highest efficiency observed at a ratio of 3:1. A recent study reported the possible dependency of different endocytic pathways on cationic liposome compositions [25]. Lipoplexes consisting of plasmid DNA plus DC-Chol or DOPE-based cationic liposomes were shown to enter the cells preferentially via raft-mediated endocytosis, whereas lipoplexes that included 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) or dioleoylphosphocholine-based cationic liposomes were taken up by non-specific fluid-phase macropinocytosis.

3. Cationic liposomes for complexation with various nucleic acids

3.1. Plasmid DNA lipoplexes

For plasmid DNA to be functionally translated within a cell, the plasmid DNA must undergo effective intracellular trafficking into the cytoplasm, and from there into the nucleus. Plasmid DNA encoding interleukin 12, a cytokine with antitumor activity, was complexed with cationic liposomes and tested for its in vivo anticancer effects in a metastatic lung cancer mouse model [26]. The studied cationic liposomes were composed of all-trans-retinoic acid (to enhance the antitumor effects), DOTAP and cholesterol (molar ratio, 10:0.5:0.5) and complexed with interleukin 12-encoding plasmid DNA. After two intravenous injections with plasmid DNA (1.2 mg/kg/mouse), the numbers of tumor nodules and tumor cells were decreased as compared to control group.

In another study, cationic liposomes consisting of 3\(\beta\)-[N-(N',N'-dimethylamino-ethane) carbamoyl] cholesterol (DC-Chol) and DOPE are considered representative liposomes for efficient gene delivery. For plasmid DNA delivery, the most efficient molar ratio of DC-Chol to DOPE was found to be 1:2 [24]. The transfection efficiency of plasmid DNA decreased as the weight ratio of DC-Chol to plasmid DNA increased, with the highest efficiency observed at a ratio of 3:1. A recent study reported the possible dependency of different endocytic pathways on cationic liposome compositions [25]. Lipoplexes consisting of plasmid DNA plus DC-Chol or DOPE-based cationic liposomes were shown to enter the cells preferentially via raft-mediated endocytosis, whereas lipoplexes that included 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) or dioleoylphosphocholine-based cationic liposomes were taken up by non-specific fluid-phase macropinocytosis.

3.2. Oligonucleotide lipoplexes

An oligonucleotide is a short nucleic acid polymer of <50 bases. An AS-ODN is a single strand of DNA or RNA that binds to a complementary mRNA sequence. Since AS-ODNs can downregulate certain RNAs and suppress target protein expression, they are considered to have potential as nucleic acid-based medicines. To develop oligonucleotide-based therapeutics, however, the instability of oligonucleotides in physiological environments and their insufficient cellular uptake must be overcome.

Zhang and colleagues [28] developed cationic liposomes composed of dimyristoyl 1,2-diacyl-3-trimethylammonium-propane, phosphatidylcholine, and cholesterol for systemic delivery of AS-ODNs against Raf-1 protein serine/threonine kinase (a known target signaling protein for cancer treatment). They observed that systemic administration of AS-ODNs complexed to cationic liposomes reduced Raf-1 protein expression in liver and tumor tissues, and inhibited the growth of PC-3 tumors in mice.

In another study, Bcl2-specific AS-ODNs were complexed to protamine and cationic liposomes composed of DC-Chol, phosphatidylcholine, and polyethylene glycol-distearylphosphatidylethanolamine [29]. The liposomes significantly increased the cellular uptake of Bcl-2 AS-ODNs, resulting in significant downregulation of Bcl-2 protein levels. Notably, the study introduced a new method of forming lipoplexes using a multi-inlet microfluidic hydrodynamic focusing system, which yielded a smaller mean size and narrower size distribution compared to lipoplexes prepared with the conventional mixing method.

AS-ODNs and cationic liposomes were studied for the treatment of atopic dermatitis [30]. Interleukin-13-targeting AS-ODNs were complexed to cationic liposomes composed of DOTAP and sodium cholate, and applied topically to skin lesions of mice with atopic dermatitis. This treatment dose-dependently alleviated the atopic dermatitis, with maximal inhibition observed in response to 200 µg of interleukin-13 AS-ODNs.

CpG ODNs, which are synthetic single-stranded DNAs known to function as a vaccine adjuvant [31], have also been delivered using cationic liposomes. A Th1-mediated immune response is promoted by the interaction of CpG ODNs with toll-like receptor 9, and CpG ODNs reportedly possesses antitumor activity. Cationic liposomes have been formulated to efficiently deliver CpG ODNs to boost immune responses or treat cancers. CpG ODNs were complexed to cationic liposomes composed of N-{1-(2,3-dioleoyloxy)propyl}-N,N,N-trimethylammonium chloride, or N-{1-(2,3-dioleoyloxy) propyl}-N,N,N-trimethylammonium chloride and cholesterol. The intranasal administration of cationic liposome-complexed CpG ODNs was found to prevent pulmonary metastasis, suppress proliferation of tumor cells in the lung, and prolong the survival time of mice more efficiently than naked CpG ODNs. Furthermore, the complexation of CpG ODNs with cationic liposomes composed of N-{1-(2,3-dioleoyloxy)propyl}-N,N,N-trimethylammonium chloride
and cholesterol exhibited antitumor activity via the activation of natural killer cells [32].

Lipoplexes composed of CpG ODNs and cationic liposomes have been tested for their ability to prevent melioidosis, an infectious disease caused by Burkholderia pseudomallei [33]. CpG ODNs complexed to cationic liposomes were administered to mice at a dose of 100 µg per animal, and the mice were challenged 30 days later with B. pseudomallei. The results revealed that DOTAP liposomes complexed to CpG ODNs prevented B. pseudomallei infection more effectively than DOPC liposomes complexed to CpG ODNs.

3.3. SiRNA lipoplexes

The RNA interference (RNAi) pathway allows siRNAs and miRNAs to negatively regulate protein expression [34]. SiRNAs are double-stranded RNAs of 21–23 nucleotides that induce the silencing of homologous target mRNAs. For their action, double-stranded siRNAs separate into two single-stranded RNAs: the passenger and guide strands. The passenger strand is degraded by argonaute-2, while the guide strand is incorporated into an RNAi-induced silencing complex, which binds the mRNA complementary to the guide strand and cleaves it. SiRNAs appear to have great potential to treat diverse diseases, as they can easily downregulate various target mRNAs regardless of their location (i.e., in the nucleus or the cytoplasm), and their specific binding suggests that they will induce fewer side effects than conventional chemical drugs. As a novel nucleic acid-based strategy, siRNA-based treatments have several advantages over conventional chemical drugs. However, some challenges must be overcome to facilitate the development of siRNA-based therapeutics, including the need to identify proper target genes and develop optimized delivery systems.

A number of researchers have sought to enhance the cellular delivery and gene-silencing efficiencies of siRNA using cationic liposomes. For example, cationic liposomes composed of DC-6-14, DOPE, and cholesterol were formulated to deliver siRNAs specific for firefly luciferase [35]. Transfection was improved when the cationic lipids and siRNA were mixed with constant and intense agitation, indicating that the method of loading the siRNA onto cationic liposomes could modulate the efficiency of transfection.

The therapeutic applications of siRNA lipoplexes vary depending on the target protein. To treat immunological disease, siRNAs specific for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were complexed to cationic liposomes containing 1,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane, 1,2-distearyl-sn-glycerol-3-phosphocholine (DSPC) and cholesterol [36]. Mice were treated with the complexes (5 mg/kg siRNA), and after 4 days, they showed ~40% less GAPDH production in macrophages and dendritic cells from the peritoneal cavity, and ~60% GAPDH expression in spleen derived antigen-presenting cells.

In other work, heavy chain ferritin specific-siRNAs were complexed to cationic liposomes and locally administered to human glioma U251-bearing mice [37]. Intratumoral injection of ferritin-specific siRNAs complexed to cationic liposomes composed of DC-Chol and DOPE reduced tumor growth to a degree comparable to that seen with carmustine (a DNA-alkylating agent that is primarily used in glioma therapy).

Argonaute-2-specific siRNAs, which have been shown to induce apoptosis, were delivered using PEGylated cationic liposomes composed of DC-6-14, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, DOPE, and 1,2-distearyl-sn-glycero-3-phosphoethanolamine-N-(methoxy(polyethylene glycol)-2000) [38]. For treatment of Lewis lung carcinoma-bearing mice, the complexes were intravenously injected into tumor cell-inoculated mice (1 mg/kg every other day for five doses). The repeated administration of these complexes reduced the expression of argonaute-2 in tumor tissues and significantly suppressed tumor growth.

3.4. miRNA lipoplexes

miRNAs are short (~22 mer) noncoding RNAs that are found in eukaryotic cells [34] and act as biological regulators by binding to complementary mRNA sequences. An miRNA is transcribed from its encoding nuclear gene in the form of a primary miRNA, which is several hundred nucleotides long. The RNase III enzyme, Drosha, processes the primary miRNA into a pre-miRNA (~70 nucleotides long) that carries a characteristic hairpin loop. The pre-miRNA then moves to the cytoplasm, where the RNase III enzyme, Dicer, produces mature miRNAs and passenger strands. Finally, the mature miRNAs are incorporated into the RNAi-induced silencing complex for degradation of their target miRNAs.

Cationic liposomes consisting of 1,2-di-octadecenyl-3-trimethylammonium propane, cholesterol, and D-α-tocopheryl polyethylene glycol 1000 succinate were shown to efficiently deliver pre-miRNA-133b, resulting in a 2.3-fold increase in the expression of mature miRNA-133b and an 1.8-fold decrease in Mcl-1 protein in A549 non-small lung cancer cells, compared with cells that received the control siPORT NeoFX transfection agent [39]. ICR mice that received cationic liposomes with pre-miRNA-133b (1.5 mg/kg) by tail-vein injection showed ~52-fold higher mature miRNA-133b expression in lung tissues compared to mice that received scrambled pre-miRNA-containing cationic liposomes.

Another recent study [40] demonstrated that systemic delivery of cationic liposomes carrying tumor-suppressor miRNA had the potential to treat cancer. Cationic liposomes containing 1,2-dioleoyl-3-trimethylammonium-propane, cholesterol, and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] were complexed to miRNA-34a or the miRNA-143/145 cluster. MiRNA-34a is a component of the p53 transcriptional network and regulates cancer stem cell survival, and was thus selected as a tumor suppressor, while the miR-143/145 cluster is known to suppress the expression of KRAS2 and its downstream effector, Ras-responsive element binding protein-1. Intravenous delivery of miRNA-34a or miRNA-143/145 using cationic liposomes inhibited tumor growth in both a subcutaneous xenograft model and an orthotopic pancreatic cancer xenograft model.

4. Recent progress in cationic liposomes

Although various cationic lipid-based delivery systems have been used for the in vitro and in vivo delivery of nucleic acids, there are still challenges that must be overcome, including the
low delivery efficiency, cytotoxicity, non-specific tumor targeting, and low anti-tumor activity. In an effort to address these limitations, researchers have developed improved cationic lipids by combining novel synthesized lipids, targeting ligands, and/or anti-tumor drugs.

4.1 Engineered lipid-based systems

The engineering of new lipids has been investigated as a means to improve the delivery efficiencies of nucleic acids. For example, researchers synthesized the cholesterol-derivative cationic lipid, 3-β(N-(N',N'-dimethyl, N'-hydroxyethyl amino-propane) carbamoyl) cholesterol iodide (DMHAPC-Chol), and showed that it could promote the delivery of vascular endothelial growth factor (VEGF)-specific siRNAs into tumor cells [41]. Structurally, the lipid has a biodegradable carbamoyl linker and a hydroxyethyl group in its polar amino head moiety. Cationic liposomes composed of DMHAPC-Chol and DOPE in equimolar proportion delivered VEGF siRNA to A431 and MDA-MB-231 cells and exhibited >90% effective silencing of VEGF protein expression. In another study, a cholesterol-based polycationic liposomal formulation in which the hydrophilic part of spermine was conjugated with one or two cholesterol residues was developed for the delivery of siRNA [42]. Liposomes consisting of synthetic polycationic lipids and DOPE were shown to silence the expression of enhanced green fluorescent protein (EGFP) in EGFP-expressing HEK293 cells.

In addition to cholesterol derivatives, arginine-based cationic lipids have also been studied for siRNA delivery. Poly-L-arginine lipid derivatives formulated with poly-L-arginine-conjugated polyethylene glycol lipids, DOTAP, DOPE, and cholesterol were synthesized for enhanced delivery of siRNA [43]. The liposomes successfully reduced the expression of green fluorescent protein (GFP) and showed low cytotoxicity in H4II-E and HepG2 cells. In other work, the arginine derivative, N,N-distearyl-N-methyl-N-2-(N'-arginy1) aminoethyl ammonium chloride, was used for cationic liposome formulation with cholesterol [44]. The cationic liposomes were complexed to c-Myc siRNA and intravenously administered to B16F10 melanoma-bearing mice (1.2 mg/kg once a day for three days), resulting in the sensitization of B16F10 tumors to paclitaxel.

Another study suggested the use of arginine-based DiLA² lipids as a cationic lipid component for apolipoprotein B-specific siRNA delivery [45]. Following intravenous administration to mice (ED₅₀, 0.1 mg/kg), cationic liposomes prepared from DiLA² and DOPE showed the potential to suppress apolipoprotein B mRNA expression in the liver. After single systemic dose, the maximal reduction (~80%) in the levels of target mRNA was observed at day 2 post-dose, and >50% reduction of target mRNA was sustained up to day 9 post-dose.

Alkaline amino acid-based cationic liposomes have been studied for their potential to enhance the stability of cationic liposomes in serum [46]. Lysinylated cholesterol, histidinylated cholesterol, and arginylated cholesterol were tested, and lysinylated cholesterol and arginylated cholesterol lipid based-cationic liposomes showed more efficient transfection plasmid DNA in serum-containing media.

Conjugates of spermine with cholesterol or long-chain hydrocarbons have been formulated into liposomes. Spermine-tagged cationic lipids and DOPE (1:1 ratio) were complexed to EGFP-encoding plasmid DNA or RNA for electropulsing into immature dendritic cells or dendritic cell progenitors [47]. Intravenous administration of the nucleic acid-pulsed dendritic cells to tumor-bearing mice was found to induce the production of antitumor cytokines, suggesting that cationic liposomes could be used to generate nucleic acid-pulsed dendritic antitumor vaccines.

Recently, a biomimetic thioether lipid library was screened using thiol-yne “click” chemistry, which conjugates cationic thioether amine lipids with two hydrophobic alkyl thiols [48]. One DOPE-containing lipid formulation was found to increase the uptake of GFP-specific siRNAs in various cell types.

Since cationic liposomes typically show relatively high cytotoxicities, various strategies have been proposed to reduce their toxicity and enhance their in vivo delivery of siRNA. To this end, researchers have coated the cationic lipoplexes with non-toxic and biodegradable anionic polymers, such as poly-L-glutamic acid sodium salt, poly(acrylic acid) sodium salt, dextran sulfate sodium salt, algic acid sodium salt, hyaluronic acid sodium salt, heparin sulfate sodium salt, and carboxymethylcellulose sodium salt [49]. Among these anionic polymers, polyglutamate did not have any obvious toxicity over a wide range of sizes, and the coated cationic liposomes showed enhanced delivery of siRNA in the liver and lung tissues compared to uncoated lipoplexes (Fig. 1a).

4.2 Ligand modifications for targeted delivery

Although cationic liposomes have the potential to deliver nucleic acids in vivo, their delivery to the specific target site remains a major challenge. To enhance the distribution of nucleic acid-bearing cationic liposomes to target tissues, researchers have modified the liposome surfaces with peptides and small molecules.

For example, Arg-Gly-Asp (RGD) peptide-modified liposomes were studied for their ability to enhance the delivery of nucleic acids to integrin receptor-expressing cells [50]. RGD-modified cationic liposomes loaded with p-glycoprotein-specific siRNAs showed higher delivery to integrin receptor-expressing human breast cancer MCF7/A cells, resulting in significant silencing of p-glycoprotein. Consistent with this, molecular imaging revealed a higher distribution of RGD-modified cationic liposomes and siRNA lipoplexes in MCF7/A tumor tissues compared to adjacent normal tissues in a mouse model. In a recent study, liposomal surfaces were modified with both cyclic RGD and octaarginine to utilize the integrin receptor-binding effects of cyclic RGD and the cell-penetrating effects of octaarginine (Fig. 1b) [51]. The dual-ligand modified cationic liposomes showed increased cellular uptake into integrin αvβ3-expressing cells and more effective transfection of luciferase-encoding plasmid DNA.

The conjugation of lipids to bioactive small molecules (e.g., folic acid) has been investigated for the targeted delivery of nucleic acids. For example, a lipoplex formed by the non-covalent association of folic acid to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine was tested for its potential to enhance the stability of cationic liposomes in serum [46]. Lysinylated cholesterol, histidinylated cholesterol, and arginylated cholesterol were tested, and lysinylated cholesterol and arginylated cholesterol lipid based-cationic liposomes showed more efficient transfection plasmid DNA in serum-containing media.
ethylphosphocholine:cholesterol liposome significantly enhanced the transfection efficiency of plasmid DNA encoding thymidine kinase and inhibited TSA and SCC7 cell growth in vitro. These folate-associated lipoplexes showed higher antitumor efficacy in mice bearing SCC7 tumor xenografts. In another approach, folic acid-tagged cationic liposomes were complexed with calf thymus DNA for activation of macrophages, and showed higher delivery of DNA to folate receptor-expressing cells as compared to plain cationic liposomes without folic acid. In tumor-bearing mice, folate-tagged lipoplexes induced the production of interferon-γ and interleukin-6 and prolonged survival compared to that in mice treated with folate-free lipoplexes.

Glycyrrhetinic acid has been used to target hepatocellular carcinoma cells, based on a study showing that protein kinase C, a binding target of glycyrrhetic acid, is expressed more highly on the surface of hepatocellular carcinoma cells compared to adjacent non-tumor liver cells. A glycyrrhethinic acid–polyethyleneglycol–cholesterol conjugate was synthesized and used to formulate cationic liposomes with DOTAP and cholesterol. These liposomes showed higher capacities to form complexes with GFP-expressing plasmid DNA and enhance the transfection of plasmid DNA into hepatocellular carcinoma cells, compared to control cationic liposomes that lacked glycyrrhetinic acid.

Since mannose receptors are found on macrophages, mannose has been used to modify cationic liposomes for macrophage delivery. To suppress osteoclastogenesis, which is induced by activated macrophages, mannosylated cationic liposomes were complexed with the double-stranded oligonucleotide, NFκB decoy. Mannose-cationic liposome/NFκB decoy complexes efficiently induced NFκB activation and inhibited the production of tumor necrosis factor-α. In another study, macrophage-targeting NFκB decoys loaded in mannosylated cationic liposomes were used to prevent lipopolysaccharide-induced lung inflammation. After intratracheal administration to a rat model of lung inflammation, manose-tagged cationic liposome/NFκB decoy complexes significantly downregulated the expression of NFκB and reduced the release of tumor necrosis factor-α and interleukin-1β.

Anisamide-modified cationic liposomes were studied for their ability to confer targeted delivery of oligonucleotides to sigma receptor-expressing cells. Splice-switching oligonucleotides (SSOs) are single-strand oligonucleotides that bind to a splice site or splicing enhancer, blocking access to the endogenous splicing machinery and generating an alternate version of the mature mRNA. In a mouse model of lung metastasis, the systemic administration of anisamide-modified cationic lipoplexes loaded with Bcl-x SSO reduced tumor growth.

Dexamethasone is known to have a nucleus-targeting function. To enhance the nuclear transport of plasmid DNA,
dexamethasone was conjugated with PAMAM dendrimers and mixed with DOPE (1:1) to form liposomes. The PAMAM-dexamethasone/DOPE-based cationic liposomes enhanced the expression of plasmid DNA in HEK293 cells and showed reduced cytotoxicity compared to polyethylenimine (m.w. 25,000).

Overall, the modification of targeting ligands can help achieve specific targeting and avoid non-specific distribution to the liver and other tissues. From the perspective of commercialization, however, ligand-tailoring technologies still face many hurdles, including the need for more streamlined manufacturing processes and improved quality control.

### 5. Current challenges to clinical applications

Despite recent progress, lipoplexes of cationic liposomes and nucleic acids still suffer from several limitations. One is their low stability in the bloodstream, which is caused by the characteristics of cationic lipids. Until they reach their target sites and cells, cationic lipid components can interact with serum proteins, potentially disrupting the integrity of liposomal structures or forming aggregates that are too large to be taken up by cells. To increase the in vivo stability of lipoplexes in the blood, researchers often include PEG-conjugated lipids and cholesterol as components of the cationic liposomes. Another limitation is the relatively weak delivery of nucleic acids into target cells. For anti-cancer therapy, enhanced retention and permeability may serve as an initial driving force for the delivery of lipoplexes to tumor tissues. Once in the tumor tissues, the effective recognition of tumor cells and intracellular delivery should proceed.

Among the potential cationic lipid-encapsulated nucleic acid therapeutics, the current leader is Atu027 of Silence Therapeutics, which consists of AtuRNAi (targeting protein kinase N3) and AtuPLEX™ (based on cationic lipids). Atu027 was demonstrated to be safe and has completed a Phase I study for the treatment of solid cancer. Currently, researchers are planning a Phase Ib study in which Atu027 will be combined with chemotherapy.

### 6. Conclusion

Numerous cationic lipid-based vehicles have been developed for nucleic acid therapeutics, and substantial progress has been made toward improving their efficacy in vivo. Several clinical trials of siRNA-based therapeutics are ongoing, suggesting that nucleic acids may emerge as new class of therapeutics in the near future. Their success will rely on the development of effective delivery systems. Despite recent progress, however, we still need to continue developing a variety of safe, stable, and effective cationic lipid-based carriers that are capable of delivering nucleic acids to the proper target sites and cells.

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## References


