Nanoparticles for siRNA-Based Gene Silencing in Tumor Therapy

Anish Babu, Ranganayaki Muralidharan, Narsireddy Amreddy, Meghna Mehta, Anupama Munshi, and Rajagopal Ramesh*

***Abstract*— Gene silencing through RNA interference (RNAi) has emerged as a potential strategy in manipulating cancer causing genes by complementary base-pairing mechanism. Small interfering RNA (siRNA) is an important RNAi tool that has found significant application in cancer therapy. However due to lack of stability, poor cellular uptake and high probability of loss-of-function due to degradation, siRNA therapeutic strategies seek safe and efficient delivery vehicles for *in vivo* applications. The current review discusses various nanoparticle systems currently used for siRNA delivery for cancer therapy, with emphasis on liposome based gene delivery systems. The discussion also includes various methods availed to improve nanoparticle based-siRNA delivery with target specificity and superior efficiency. Further this review describes challenges and perspectives on the development of safe and efficient nanoparticle based-siRNA-delivery systems for cancer therapy.**

***Index Terms*— Cancer, gene silencing, liposome, nanoparticle, polymer, siRNA.**

I. Introduction

IT IS estimated that 1 685 210 of new cancer cases will be diagnosed in the U.S. in 2016; among these cases, 595 690 deaths are predicted [1]. The high mortality rate due to cancer highlights the urgent need for newer therapeutic modalities that can effectively forestall cancer progression. Over the past several years, researchers have made great achievements in elucidating cancer cell growth regulatory pathways and associated protein expressions leading to the identification of many druggable targets at the molecular level [2]–[5]. RNA interference technology (RNAi) has been highly successful in exploring these druggable targets [6], [7], [8], but the real advantage of RNAi is that it can even target disease-causing genes that are considered "non-druggable" [9], [10]. As an advanced therapeutic approach, RNAi garners special attention from cancer researchers [11]. Widely recognized as a powerful RNAi tool, small interfering RNA (siRNA) can specifically silence the target genes in a cell [12]. A siRNA-based targeted gene silencing approach may allow for the development of gene-specific drugs against certain genes that are otherwise difficult to manipulate, using small molecule drugs or antibodies. In cancer therapy, silencing specific genes may result in global knockdown of proteins involved in cancer cell growth, proliferation, metastasis, and/or multi-drug resistance. Recent studies have identified several cancer-related genes as potential targets for siRNA-based therapy.

However, the therapeutic activity of naked siRNAs cannot be harnessed, as they have very short *in vivo* half-lives and rapid body clearance [13]. The half-life of siRNAs in the circulation is affected mainly by interaction with opsonin proteins and premature degradation by nucleases. SiRNAs in the circulation may also fail to extravasate the endothelial tissue and reach the tumor milieu. The extracellular matrix (ECM) forms the next barrier, where the siRNAs can diffuse poorly through interaction with the cell membranes. Thus, numerous biological barriers hinder the siRNA molecules administered *in vivo* from reaching the desired tissue and delivering their therapeutic activity [14]. Even if the siRNA reaches the target tissue, there are additional hurdles at a cellular level that adversely affect the cellular entry and stability. The important issue there is the negative charge of the siRNA, which causes repulsion from the similarly charged cell membrane; the result is poor cell uptake. If the siRNA manages to enter the cell, it must tackle with the endosomes and escape the harsh acidic environment. For these reasons, advanced delivery strategies are required to overcome the biological barriers of siRNA to specifically reach the target cell and to promote its therapeutic action by gene silencing.

A recent review highlighted the siRNA delivery barriers and described various strategies to overcome these biological barriers for successful gene delivery to the target cell [15]. To explore the potential of siRNA therapeutics to the fullest, suitable carriers are required for siRNA delivery across these biological barriers [16], [17]. A decade ago, viral vectors were the most common gene delivery vehicles in cancer therapy [18], [19]. However, viral vectors are known to cause immune-related problems and mutagenesis, both of which jeopardize the safety of those vectors in gene therapy [19]. Recently, nanoparticles or nanostructured carriers have increasingly been adopted as reliable non-viral siRNA delivery systems [17]. The advantages of using nanoparticles for siRNA delivery, particularly at a systemic level, include i) the prolonged half-life

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A. Babu, R. Muralidharan, and N. Amreddy are with the Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA, and also with the Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA.

M. Mehta and A. Munshi are with the Department of Radiation Oncology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA, and also with the Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA.

*R. Ramesh is with the Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA, and also with the Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA, and also with the Graduate Program in Biomedical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA (e-mail: rajagopalramesh@ouhsc.edu).

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TABLE I
RECENTLY COMPLETED AND ONGOING CLINICAL TRIALS* USING siRNA FOR CANCER THERAPY

Intervention/ SiRNA target	**Disease**	**Stage of trial**	**Delivery vehicle/ Specifications**	**Sponsor/ Collaborator**	**Clinicaltrial.gov Identifier number**
EphA2	Advanced Cancers	Phase 1 (Recruiting)	Liposome (DOPC neutral liposome)	M.D. Anderson Cancer Center and Cancer Research Institute of Texas	NCT01591356
Proteasome si RNA and tumor antigen RNA-transfected dendritic cells	Metastatic Melanoma	Phase I (Completed)	Not specified	Scott Pruitt	NCT00672542
siRNA against CML fusion gene	Chronic Myeloid Leukemia	Completed	Pseudo Viral (SV40) Particles	Hadassah medical organization	NCT00257647
APN401 factors in immune cells	Melanoma, Kidney and Pancreatic cancer, solid tumor	Phase I Active	Not specified	Comprehensive Cancer Center of Wake Forest University and NCI	NCT02166255
Atu027	Advanced Solid Tumors	Phase I (Completed)	Lipid nanoparticles	Silence Therapeutics GmBH	NCT00938574
CALAA-001/M2 subunit of ribonuclease reductase	Solid Tumor, Cancer	Phase I (Terminated)	Cyclodextrin Contained Polymer	Calando Pharmaceuticals	NCT00689065
anti KRASG12D siRNA (siG12D) LODER	Pancreatic Ductal Adenocarcinoma Pancreatic Cancer	Phase I	Biodegradable Polymeric Matrix	Silenseed, Ltd.	NCT01188785
TKM-080301/PLK I	Cancers with Hepatic Metastases	Phase I Completed	Lipid Nanoparticles	NCI	NCT01437007
DCR-MYC	Hepatocellular Carcinoma	Phase 2 (Recruiting)	Lipid Nanoparticles	Dicerna Pharmaceuticals, Inc.	NCT02314052
DCR-MYC	Multiple Myeloma, Lymphoma, Pancreatic Neuroendocrine Tumors, Solid Tumors	Phase I (Recruiting)	Lipid Nanoparticles	Dicerna Pharmaceuticals, Inc.	NCT02110563

*Data retrieved from www.clinicaltrials.gov on 07/10/2016

of siRNA in blood, ii) improved pharmacokinetics, and iii) preferential targeting of tumor tissues by an Enhanced Permeation and Retention (EPR) effect. It is important to note that many siRNA-based drugs have undergone recent clinical trials [20], and several are ongoing for cancer gene therapy (Table I).

In this review, we discuss various nanoparticle delivery systems that have been explored in siRNA-based cancer therapy. Based on our experience of over a decade, we highlighted the applications of liposomes and lipid-based nanoparticles for gene delivery in cancer therapy. The importance of polyethylene glycol (PEG) modification in the context of

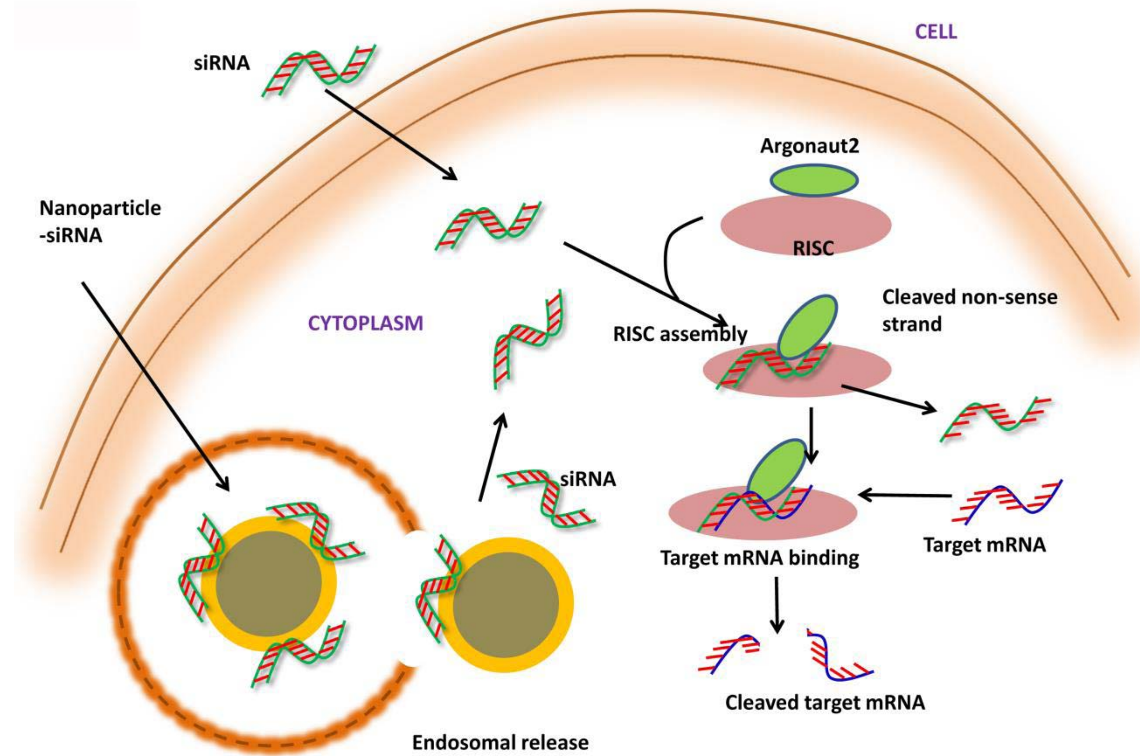

Fig. 1. Mechanism of RNAi by siRNA in host cells. siRNA is either delivered using a vehicle or entered cells via non-specific mechanism undergoes a series of processing events resulting in specific mRNA silencing by complementary sequence binding.

liposome-based gene delivery is explained in detail. Further, cationic liposome-based targeted gene delivery in cancer therapy is discussed. In addition to liposomes/lipid nanoparticles, popular gene delivery vehicles and new strategies in polymer-based and inorganic nanoparticles that we have developed are mentioned, with a focus on siRNA-based cancer gene therapy.

II. siRNA-Based Gene Silencing and Its Role in Cancer Therapy

The RNAi mechanism occurring through short RNAs is a multi-stage process [21]. RNAi acts as a natural defensive mechanism of host cells when foreign genetic material, such as viral genes or transposable elements, seeks entry. Upon cytoplasmic entry, double-stranded oligonucleotides encounter DICER, a ribonuclease, leading to cleavage into short nucleotide fragments (∼20 bp). In the next stage, these short RNA fragments are separated into individual stands of RNAs called passenger strands and guide strands. The guide strand (complementary to target mRNA) is then recruited by RNA-induced silencing complex (RISC), while the passenger strand is degraded. The RISC allows the guide strand to pair with mRNA and degrade the mRNA strand, catalyzed by the Argonaute protein family, causing specific gene silencing [22]. Fig. 1 shows the simplified mechanism of RNAi upon siRNA entry/delivery into host cells. The specificity of therapeutic siRNA relies on careful design of its complementary sequences against target mRNA. Using multiple siRNA sequences together in gene silencing applications would help to reduce any unanticipated off-target effects *in vivo*.

Overexpression of certain oncogenes or mutations in DNA alters the molecular events and transforms normal cells to tumorigenic cells. Rationally designed siRNA can specifically modulate the expression of those cancer-causing genes by homology-based pairing and post-transcriptional silencing. Since RNAi is specific and efficiently silences the target mRNAs, resulting in a global knockdown of oncogenic proteins, it may be possible to treat any stage of cancer. Recent studies explored a number of cancer-causing genes [23], and several have been identified as potential targets for siRNA-based therapy [24], [25]. Thus, RNAi has affected cancer therapy by downregulating tumorigenic genes, resulting in pro-apoptotic and anti-metastatic effects, while having negligible effects on healthy tissues. The specificity and therapeutic efficiency of RNAi can be increased with the help of nanoparticle carriers, as per many recent reports in both *in vitro* and *in vivo* cancer models [26], [27], [28], [29]. Though no major clinical breakthroughs have yet been achieved for RNAi, it is hoped that, with the help of advanced delivery strategies, RNAi might achieve the potential for clinical success in the not-too-distant future.

III. Nanoparticle Delivery Systems for siRNA

Nanoparticles have immense potential to modify the fate of siRNA upon *in vivo* administration. The advantages of nanoparticles, such as size and shape tunability, capacity to

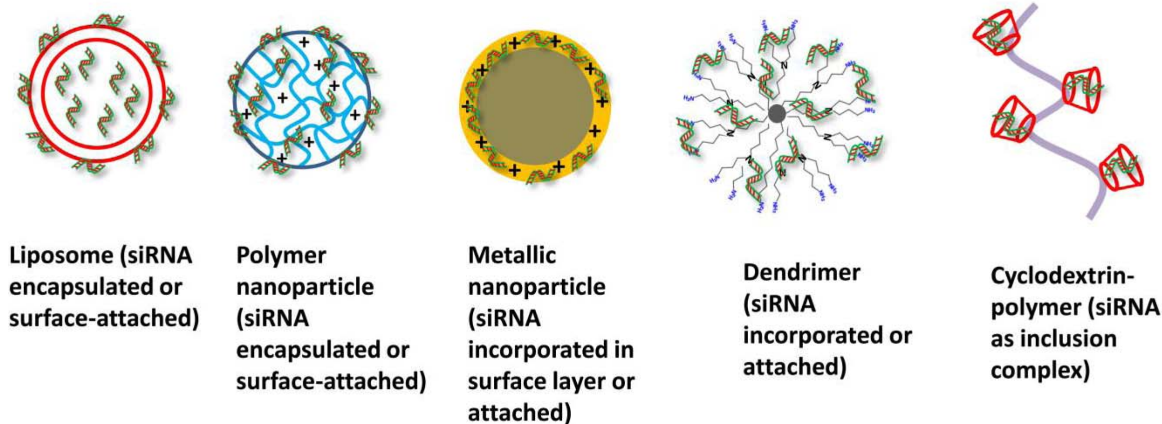

Fig. 2. Liposome and nanoparticle systems with different architectures used in gene therapy. Note that each of these systems uses different methods for siRNA loading. siRNA is encapsulated, surface-attached or loaded as inclusion material.

protect the siRNA from degradation, ability to control the surface characteristics, and their biological behavior, favor the siRNA delivery across the biological barriers. A broad range of nanoparticle systems are currently under investigation for siRNA-based gene silencing in cancer therapy [30]. Nanoparticles carry siRNA as inclusions in their core, or as surface-attached molecules *via* either covalent or non-covalent forces. Fig. 2 shows a cartoon of nanoparticles that are commonly used for siRNA delivery and different methods of siRNA loading.

The susceptibility of surface-attached siRNAs to degradation may be avoided by chemical modification [31] or additional layer(s) of neutral polymers, such as PEG. In the next section, we summarize various nanoparticle systems, including liposomes, polymers, and inorganic nanoparticles, that are commonly used for siRNA-based gene therapy for cancer.

IV. Liposomes and Lipid Nanoparticles for siRNA Delivery

Lipid-based delivery systems are one of the most promising and extensively studied siRNA delivery systems for systemic applications, due to their biodegradability and biocompatibility. The development of different formulation strategies using a number of different lipids generated a wide variety of lipid nanoparticles (LNPs). The gene delivery efficiency depends on the size and charge of the liposomes, degree of PEGylation, type of targeting ligand, and targeting tissue. To achieve the maximum accumulation of siRNA in the targeted tissue, the siRNA cargo should first be protected in the blood stream during circulation.

There are several methods to achieve a stable siRNA complexation. The simplest method is to mix preformed cationic liposomes with a siRNA solution at a desired ratio to form lipoplexes [32]. These siRNA-lipid complexes do not face the electrostatic barrier when entering the cells, and are easily endocytosed by the plasma membrane. Kim *et al*. demonstrated liposome complexes prepared by mixing siRNA with DOTAP/cholesterol containing Apolipoprotein A or recombinant human ApoA-1 [33]. A similar method was employed by Sato *et al*., who made a complex of Ubc13-siRNA and galactosylated liposomes, and showed knockdown of hepatic gene expression [34]. Studies from our lab demonstrated that DOTAP:chol lipid nanoparticles could deliver tumor suppressor genes MDA7/IL-24, p53, and FUS1, and selectively reached the tumor sites without any toxicity [35], [36], [37]. Treatment with DOTAP-chol-TUSC2 resulted in gene expression and alterations in TUSC2-regulated pathways. A Phase I clinical trial was conducted using a DOTAP:chol-TUSC2 tumor suppressor gene [38].

Grafting PEG chains onto the surface of siRNA/liposome complexes protects from reticuloendothelial system (RES) evasion and increases blood circulation time [39]. Due to the steric and electrostatic nature of PEG, the stealth liposomes delay the binding of opsonin proteins on its surface and the nanoparticles are able to circulate for long time, allowing them to accumulate in the tumor milieu in relatively high quantity. On the other hand systemic administration of naked nanoparticles is prone to rapid opsonin recognition and clearance from circulation as depicted in Fig. 3

Also the steric and electrostatic nature of PEG allows the interaction between the liposomes and endosomal membranes is blocked and prevents the endosomal release [16]. The length and density of the PEG also strongly affect the circulation time and tumor accumulation. For instance, PEGylated liposomes with shorter PEG are negatively charged; these will be taken up into the macrophages and reduce the circulation time [40]. On the other hand, very long PEG will reduce the cellular uptake and the endosomal escape medium-sized PEGs are commonly used [41]. A higher molar ratio of PEG to total lipid gives higher surface coverage, which provides more steric shielding and creates a stealth property.

Studies demonstrated that a single dose of PEGylated nanocarriers showed a longer circulation time [42], [43]. The repeated dose of PEGylated nanocarriers is rapidly cleared from circulation due to the ABC (accelerated blood circulation) phenomenon, which affects the therapeutic efficacy, in a time-dependent manner [42], [44], [45]. Suzuki *et al*. observed that the third dose of doxorubicin in PEGylated lipo-

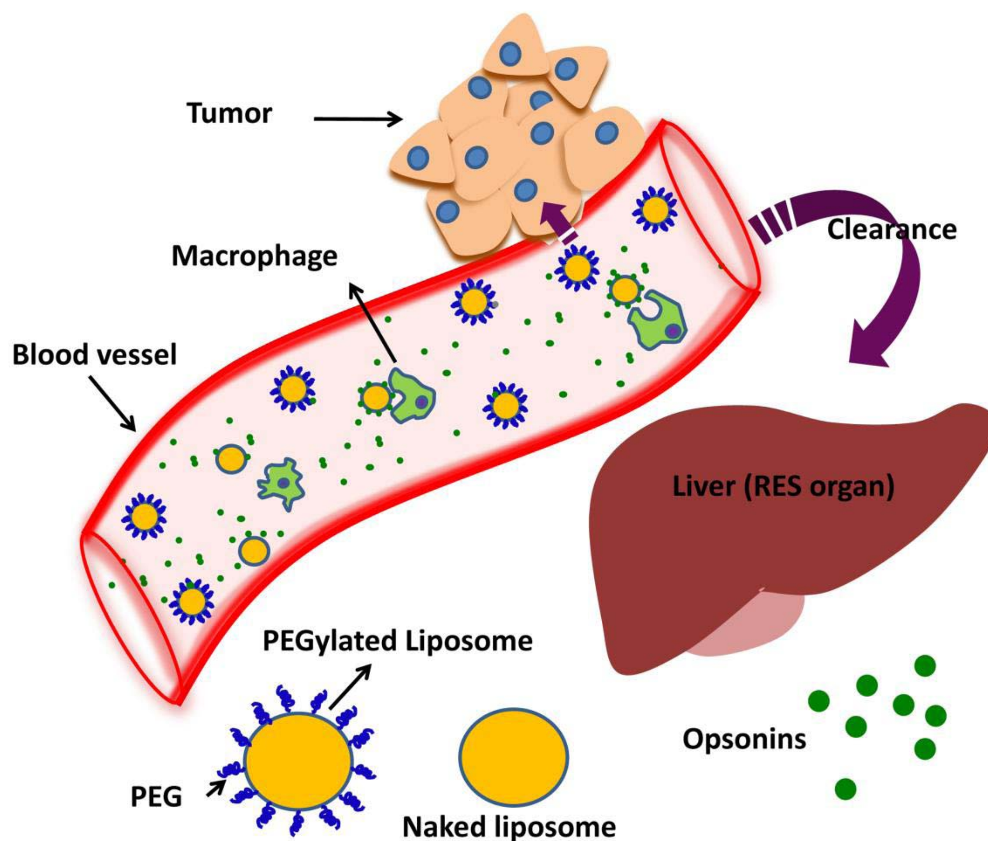

Fig. 3. Schematic illustration of the fate of PEGylated and naked liposomes in circulation. The presence of PEG layer in sterically inhibit the binding of opsonin proteins in blood thus delay the recognition of macrophages for bioclearance and prolong the blood circulation time. This enhances the chance of PEGylated-liposomes to repeatedly reach tumor site and accumulation into the tumor. Naked liposomes are easy target for opsonin proteins and thereby macrophages allowing rapid clearance from circulation into RES organs (e.g.: Liver).

somes (Doxil) remained in the blood circulation for an extended period [46]. They concluded that Doxil was taken up by the B cells and hindered the production of IgM, resulting in a less pronounced ABC phenomenon.

Post-insertion of PEG-conjugated lipids into preformed liposomes is an ideal method of "stealthing" the liposomal gene carrier system for enhanced stability and therapeutic efficiency of its payload. Wang *et al*. achieved 90% of gene knockdown for CD47 by using the post-insertion method [47]. Carmona *et al*. used the alternative strategy of covalent coupling of PEG chains using oxime bonds into preformed siRNA/liposome complexes, which are stable at neutral pH and decompose at pH 5.5 [48]. Another strategy involves the use of modified pH-sensitive PEG, which binds to liposomes through ionic interactions [49]. They demonstrated that the polymer-based liposomal complexes consist of positively charged liposomal core DOPE dioleoylphosphatidylethanolamine and cholesterol P (HEMA-lysine-modified cholesterol), to which negatively charged folate-PEG-P(HEMA-His-co-MAAc) is linked. Methacrylic acid becomes protonated at the endosome, which results in the release of PEG from the lipid core. This allows the positively charged liposomal membranes to fuse with endosomes.

Chono *et al*. adopted another strategy of condensing siRNA and hyaluronic acid, using the cationic polypeptide protamine to obtain a net negatively charged complex [50]. Cationic liposomes (DOTAP/cholesterol) were added to coat the complex *via* charge–charge interactions. This was further modified using DSPE-PEG-anisamide by post-insertion. Li *et al*. synthesized a membrane/core nanoparticle by coating a lipid bilayer on calcium phosphate nanoparticle and trapping the siRNA in the core, which was further modified by post-insertion of PEG to improve delivery potential [51].

Hyperbranched polyglycerols form an alternative to PEG in preparing stealth liposomes [52]. These structures have several hydroxyl groups that are freely available for post-modification with ligands. In addition, it has been shown that protein adsorption on corresponding modified monolayers is reduced in comparison to PEG-modified liposomes. A rapid method to synthesize a new class of lipidoids was introduced recently [53]. They observed successful gene silencing even at very low siRNA dose. Moreover, low-dose gene silencing reduced the amount of carrier material injected intravenously. Silencing multiple genes with a single intravenous administration was possible; five hepatocellular gene targets were silenced simultaneously in a mouse model. Codelivery of pDNA and siRNA was developed using lipid-like materials [54]. 1,3,5-Triazine-2,4,6-trione (TNT) lipid derivatives were used to codeliver pDNA and Tie2 siRNA, which resulted in simultaneous gene expression and silencing [54]. This could

be of great interest for the future treatment of diseases that are related to the disordered function of several genes.

New strategies to engineer the cationic lipids to improve the siRNA delivery are being developed. Briefly, phospholipids have three regions: 1) cationic head, 2) hydrophobic hydrocarbon backbone, and 3) a linker. One or more regions can be altered to improve the transfection efficiency. Introducing different alkyl chains in the same lipid with varying lengths in the hydrophobic domain have been shown to enhance the transfection efficiency without any toxic effects [55]. Hybrid nanosystems are developed by combining lipid and polymers to improve the encapsulation efficiency of RNA. These lipopolyplexes have been demonstrated to reduce undesirable toxicity.

V. Targeted siRNA Delivery for Cancer Therapy: Emphasis on Liposomes

It has been demonstrated that targeting enhances the circulation time and cellular uptake, decreases systemic toxicity, and delivers the nucleic acid therapeutics effectively [56]. The targeting moieties include receptor substrates, cell-penetrating peptides, and monoclonal antibodies. These moieties are largely used for targeted siRNA delivery using liposomes or nanoparticles. Moreover, surface modifications have been shown to increase the cellular uptake of liposomes; the modifications increase the complexity during blood circulation and tumor penetration [57]. PEGylated lipids can be functionalized to provide a site for conjugating a targeting moiety that will cleave from the liposome surface in the tumor microenvironment.

When formulating the targeted systems, there are two primary strategies: active targeting or passive targeting. Passive targeting depends on the size and physical property of the delivery vehicle for long circulating liposomes. Active targeting requires the covalent linking of a targeting moiety, which will bind to the receptor on the surface of the tumors. The amount of PEG and targeting ligand varies for each case. Typically, for example, RGD-modified lipoplexes were prepared using 10% RGD peptides (cRGDPEG5k) to target integrin receptors overexpressed in cancer cells [58].

Folic acid is one of the most studied targeting ligands, due to its high affinity towards the receptor; as many cancers overexpress folate receptors [59]. Folate is usually conjugated to amino-PEG and is incorporated into the bilayer of the liposomes. Previous studies from our laboratory showed that DOTAP:chol can efficiently deliver DNA and siRNA both *in vitro* and *in vivo*. DOTAP:chol liposomes were modified with folic acid molecules (FNP) for targeted siRNA delivery in folate receptor-overexpressing lung cancer cells [56]. FNP was used to encapsulate siRNA molecules specific for RNA binding protein HuR (HuR-FNP) in cancer cells. Our studies demonstrated the preferential uptake of HuR-FNP by the FR-overexpressing cancer cells, with minimal cytotoxicity to the normal cells. Moreover, the gene silencing efficiency of HuR-FNP was significantly higher compared to its non-targeted counterpart. Further study revealed that HuR-FNP induced apoptosis and arrested cell growth in G1 phase of cell cycle and inhibited cell migration [56]. Our *in vivo* pilot studies showed that intravenous injections of HuR-FNPs were selectively taken up by the tumor, without causing any toxicity to the surrounding organs [Data unpublished]. Since transferrin receptors play a significant role in cell growth and the transferrin receptor (TFR) is overexpressed on malignant tissues, the delivery of therapeutic compounds via transferrin receptor-mediated endocytosis will lead to enhanced therapeutic effects [60]. Recent studies in our laboratory showed that surface functionalization with transferrin on DOTAP:chol was promising for the development of a functionally active delivery system. Our *in vitro* studies demonstrated the significance of TfR-mediated uptake of siRNA with improved selective cytotoxicity. Increased cellular uptake was observed with transferrin-modified liposomes, compared with that of its non-targeted counterpart, demonstrating the importance of targeted delivery systems. To explore the distribution level of siRNA-loaded NPs in the tumor and in various organs, we first evaluated the distribution of NPs in the animal using indo cyanine green (ICG). The ICG-loaded NPs were injected intravenously and the animals were imaged at different time points using IVIS SPETRUM. The NIR signal was initially observed throughout the body. Four hours after injection, most of the signal was observed in the liver. At 24 h post-injection, a clear signal was observed in the tumor. These studies demonstrate that our NPs were stable in the circulation and were able to carry the payload (here, ICG) to the target tissue in mouse tumor models. Further, our *in vivo* efficacy study based on delivering siRNA to TfR-overexpressing tumors demonstrated a significant reduction in tumor volume [Data unpublished].

Current advancements in siRNA delivery systems for cancer therapy have reached far beyond the traditional liposomes or lipid nanoplexes. A wide variety of polymer-based and inorganic nanoparticles are currently being investigated for gene delivery applications. The following sections summarize some of the polymer and inorganic nanoparticle systems that have shown promise in becoming future gene delivery vehicles for cancer therapy.

VI. Polymeric Nanoparticles in siRNA Delivery

Chitosan is a biopolymer that has been widely explored for the delivery of nucleic acid therapeutics [61]. Using chitosan is highly advantageous because of its biocompatibility, mucoadhesive property, strong cationic nature, and ability to form nanoparticles by simple complexation with anionic polynucleotides. The presence of a large number of free amino groups in chitosan is beneficial for the conjugation of ligands or antibodies for targeted delivery of the payload. The formation of a siRNA-chitosan nanoparticle complex is dependent on the proper ratio of free amino groups in chitosan to phosphate groups in siRNA, known as the N/P ratio [62]. The N/P ratio determines the strength of the nanoparticle-siRNA complex and facilitates controlled release during siRNA delivery. Howard *et al*. determined that maintaining the proper N/P ratio is essential for particle size tunability in low molecular weight chitosan-siRNA nanoparticles [63]. Crosslinkers, such as tripolyphosphate, thiamine pyrophosphate, and hyaluronic

acid, form ionic bonds with chitosan and stabilize the structure of nanoparticles. Crosslinking allows for strong siRNA incorporation into chitosan nanoparticles, but slows down the siRNA release beyond the simple charge-based interaction between the siRNA and chitosan polymer.

Although chitosan is advantageous in many ways, its use as a promising siRNA delivery vehicle has been realized by structure modification with additional functional groups or with other components that improve its physicochemical characteristics. Chitosan modifications with carboxymethyl groups have been attempted to improve hydrophobicity and transfection efficiency with siRNA. A pH-sensitive polymer, carboxymethyl chitosan assisted pH-sensitive delivery of siRNA from a liposome formulation [64]. In a typical strategy, a chitosan derivative known as glycol chitosan was hydrophobically modified with 5β-cholanic acid for siRNA/drug codelivery into cancer models [65]. The researchers demonstrated that sequential delivery of doxorubicin and BCl2 siRNA with remarkably long-term and enhanced anti-cancer efficacy was possible with 5β-cholanic acid-modified glycol chitosan nanoparticles. Chitosan also forms hybrid nanosystems with other polymers; these hybrid systems displayed improved transfection efficiency for siRNA gene silencing in cancer therapy [66]–[68].

Polyethylene imine (PEI) is a synthetic polymer that has been successfully used as a siRNA delivery vehicle in gene silencing applications. Both linear and branched PEIs are being explored for gene delivery. The strong cationic nature of PEI from its numerous protonable amine groups allows it to form noncovalent complexes with siRNA. However, this complex formation between PEI and siRNA is dependent on PEI's molecular weight and the N/P ratio. Though synthetic, the water solubility and high transfection efficiency are important features of PEI that makes it a feasible transfection reagent *in vitro*. The endosomal entrapment of siRNA during transfection can be overcome by the inclusion of PEI, which creates a proton sponge effect in endosomes, allowing the siRNA to escape from the harsh acidic conditions of endo-lysosomes. Thus, in many studies, PEI is included as a primary or secondary component in the nanoparticle structure. This ultimately improves the transfection efficiency of siRNA.

Although PEI is known to elicit non-specific toxicity, depending on its polymer length and/or branches, combination with other biocompatible polymers makes it feasible for use as an excellent gene delivery tool. For example, in a typical cancer therapy application, PEI was modified with dioleoylphosphatidylethanol-amine (DOPE) and used as a carrier for P-gp siRNA and doxorubicin against breast cancer cells [69]. This hybrid system was successful in siRNA-mediated P-gp gene silencing and sensitized the drug-resistant MCF-7 breast cancer cells to doxorubicin. Another study demonstrated vascular endothelial growth factor (VEGF)-targeted siRNA delivery using a PEI-PEG-APRPG peptide nanoparticle complex [70]. Here, APRPG peptide acted as an angiogenic vessel homing peptide targeting VEGF. Subramanian *et al.* reported that, by conjugating epithelial cell adhesion molecule (EpCAM) aptamer to PEI-siEp (siRNA) complex, specific silencing of EpCAM was achieved and cancer cell proliferation was selectively inhibited [71]. Another study also reported VEGF-targeted siRNA delivery by synthesizing bioreducible PEI (SS-PEI) polymer for treating liver cancer *in vivo*. This PEI-based siRNA nanoparticle system downregulated VEGF and inhibited liver tumor growth [72]. The acceptability of PEI for cancer gene therapy applications is increasing, due to modifications to improve its biocompatibility.

Cyclodextrin is an excellent alternative to traditional siRNA delivery systems. Cyclodextrin has a hydrophobic inner cavity and hydrophilic exterior, which enables it to form inclusion complexes with a variety of payloads. The small size, safety profile, and cationic nature of cyclodextrin make it an attractive delivery system for siRNA. However, for better transfection efficiency, adamantane, a hydrophobic molecule, is used as an inclusion in the cyclodextrin cavity [73]. By conjugating transferrin to the adamatane-cyclodextrin inclusion complex, targeted delivery of gene therapeutics was achieved against cancer cells [74]. A cyclodextrin-polymer-based nanoparticle was the first targeted siRNA therapeutic that underwent clinical trials [74]. This nanoparticle system consisted of cyclodextrin, PEG, and human transferrin (Tf) as targeting ligand (CALAA-01). CALAA-01 successfully delivered the M2 subunit of ribonucleotide reductase (RRM2) siRNA to transferrin receptor overexpressing cancer in human subjects. This phase I clinical trial gave valuable information regarding the safety profile of CALAA-01 in humans [75]. Further studies using cyclodextrin-siRNA nanoparticles have focused mainly on therapeutic efficacy *in vitro*.

Recently, Fitzgerald *et al.* demonstrated that an anisamide-conjugated cyclodextrin-(PEG) adamantane-siRNA complex targeted the sigma receptor in prostate cancer cells [76]. Another study also explored a cyclodextrin-siRNA(s) complex to treat prostate cancer cells, and achieved specific targeting of target genes NF-kB and SRF [77]. These examples indicate that cyclodextrin-based nanoparticles hold great promise in cancer gene therapy.

Polylactic acid-co-glycolic acid (PLGA)-based nanoparticles have been used in pharmaceutical and healthcare products applications since their FDA approval for clinical use in 1969. PLGA nanoparticles have been highly researched for chemotherapeutic delivery for cancer. However, gene delivery applications of PLGA nanoparticles in anti-cancer gene therapy have only recently become clear. Its high loading efficiency, sustained release, and efficient protection of siRNA from degradation are important features that are advantageous for siRNA delivery [78]. Due to high capacity in drug/gene loading, recent studies have utilized multiple therapeutic components in PLA/PLGA nanoparticles for cancer therapy. Recently, we reported a nanoparticle system with a polylactic acid core carrying cisplatin (CDDP) and siRNA/plasmid DNA attached electrostatically to chitosan-coated PLA nanoparticles for cisplatin-resistant ovarian cancer cells [79]. This approach significantly increased the drug sensitivity of cisplatin-resistant ovarian cancer cell lines. In a similar approach, a nanoparticle system was designed for the codelivery of paclitaxel (PTX) and Stat-3 siRNA to drug-resistant lung cancer cells [80]. PTX was loaded in a PLGA core, and siRNA was adsorbed onto the PEI covering of PLGA nanoparticles. This strategy

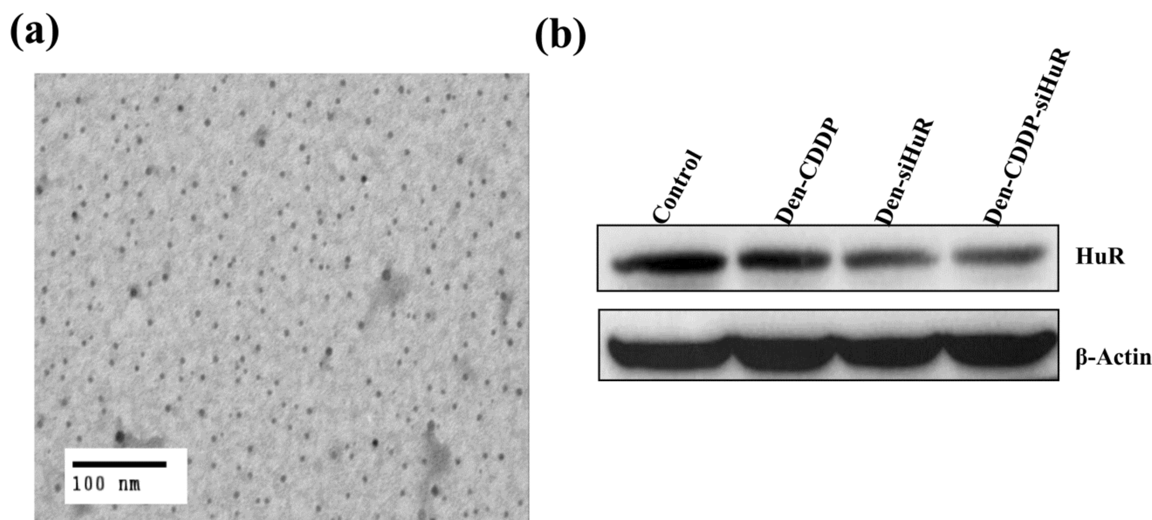

Fig. 4. PAMAM dendrimer codelivers siRNA and anti-cancer drug: **(a)** TEM image of PAMAM dendrimer (Den) after PEG/PEI modification. **(b)** Western blot images showing the efficiency of Dendrimer-siRNA based gene silencing effect in corresponding HuR protein expression, when the siRNA is used alone (Den-siHuR) or in combination with CDDP (Den-CDDP-siHuR) in lung cancer cells.

permitted the simultaneous action of Stat-3 siRNA and PTX, which chemosensitized the lung cancer cells.

One of the challenges in PLGA nanoparticle formulation is the slow release of encapsulated siRNA and, thus, low efficiency. However, newer technology, like particle replication in non-wetting templates (PRINT), has been applied to design PLGA-based nanoparticles with more efficient siRNA delivery. Recently, Hassan *et al*. reported a PRINT-based PLGA/siRNA nanoparticle with lipid coating for cancer gene therapy [81]. They achieved high efficiency in siRNA loading and uniform-sized nanoparticles by employing the PRINT technique, which enabled significant knockdown of target genes in prostate cancer *in vitro*.

Dendrimers are a special class of polymeric nanoparticles that are synthesized step-by-step to obtain typical architecture like a branched tree. They can be precisely synthesized and their size and surface properties can be controlled to carry siRNA. The multiple surface functional groups (-OH, -COOH, -NH_2) in dendrimers are advantageous, because they allow for the conjugation of various ligands, including small molecules (folic acid, biotin, transferrin), cell-penetrating peptides, and antibodies against tumor-associated antigens. This adaptability fosters nanoparticle delivery specifically to the desired tissue.

Among the polymer-based dendrimers, poly amidoamine (PAMAM) nanoparticles stand out, due to their biocompatibility and their ability to interact with cellular components. PAMAM dendrimers have gained considerable recent attention in delivering siRNA molecules to cancer cells for therapeutic purposes [82]. Since PAMAM is known for its cationic nature, nucleic acid materials interact with PAMAM *via* electrostatic forces and condense to form spherical nanoparticles. Another advantage is that PAMAM helps in the cytoplasmic entry of siRNA that it carries by endosomal escape, facilitated by its buffering capacity. Advancements in dendrimer technology paved the way for developing commercial gene delivery products, such as Superfect and Polyfect (Qiagen, Valencia, CA, USA).

Many recent modifications have been made to PAMAM dendrimers to increase their potency in cancer gene therapy. Modification of PAMAM dendrimers with PEG has been shown to reduce the toxicity and improve the efficiency in gene delivery [83]. In a typical study, PEG-PAMAM/VEGF siRNA dendriplexes showed efficient gene silencing and inhibited vascular-like formation (angiogenesis) in retinal vascular endothelial cells [84]. Nam *et al*. evaluated an arginine-grafted, bioreducible poly(cystaminebisacrylamide-diaminohexane), called ABP, and PAMAM (PAM-ABP) for delivering anti-VEGF siRNA to various cancer types, including hepatocarcinoma, lung adenocarcinoma, and fibrosarcoma, *in vitro* [85]. Interestingly, their PAM-ABP dendrimer showed better gene silencing efficiency than did PEI/siRNA polyplexes. These studies show that PAMAM dendrimers are promising gene delivery systems for cancer therapy.

In our laboratory, while exploring the advantages of PAMAM dendrimers, the common anti-cancer drug cisplatin (CDDP) was encapsulated by employing a hydrolysis method. We co-encapsulated HuR siRNA (antiproliferative) through electrostatic interaction (PAMAM-CDDP-siHuR). This combinatorial dendrimer-based system was evaluated for therapeutic efficacy in H1299 and A549 non-small cell lung cancer cells and compared with normal lung fibroblast MRC9 cells. Fig. 4 shows the transmission electron micrograph (TEM) of PEG/PEI modified PAMAM dendrimer carrying siRNA and the western blot diagram depicting the gene silencing effect in HuR protein expression when used alone or codelivered with CDDP. Our results demonstrated that PAMAM-CDDP-siHuR nanoparticles had a combined therapeutic effect by specific HuR gene silencing and CDDP mediated DNA damage induction, resulting in cell growth inhibition in lung cancer cell lines, while minimizing toxicity to normal cells.

VII. Inorganic Nanoparticles for Gene Therapy

Inorganic nanoparticles serve as an important siRNA delivery platform in cancer gene therapy. The small size and

high surface-area-to-volume ratio of inorganic nanoparticles are advantageous in loading siRNA with high efficiency. Since these nanoparticles have hard cores, siRNAs are often attached to surfaces of the nanoparticles *via* covalent or non-covalent bonds. There have been a wide range of inorganic nanoparticles, of which iron oxide, gold, silica, and QDots are commonly used for gene delivery applications.

Magnetic iron oxide nanoparticles are known to be an efficient carrier of therapeutic nucleic acids *in vitro* and *in vivo*. These nanoparticles can be prepared in smaller sizes by simple chemical methods [86], [87]. The surfaces of magnetic nanoparticles can be modified with different types of polymers that could help covalently or non-covalently attach siRNA or DNA and protect them from degradation. Coating magnetic iron oxide nanoparticles with polymers, such as chitosan or PEI, confers a net positive surface charge, which helps in electrostatically interact with siRNA [88], [89]. Moreover, such polymer coatings present multiple binding sites for ligands. In addition to siRNA carrying ability, iron oxide nanoparticles present super paramagnetism (SPIO) when an external magnetic field is applied and this property aids in targeted image-guided delivery (magnetofection) of therapeutic siRNA. Scherer *et al*. reported the transfection of DNA with superparamagnetic nanoparticles coated with PEI by applying an external magnetic field *in vitro* and *in vivo* [90]. The targeted transfection occurred in a shorter time than the time required for other non-viral vectors. Later, Schillinger *et al*. demonstrated the potential of magnetofection in delivering small interfering RNA (siRNA) into HeLa cells [91]. When administered *in vivo*, these SPIONs were specifically targeted to the tumor milieu, guided by an external magnetic field, and thereby reduced the accumulation and toxicity of siRNA in healthy tissues [92].

Targeting ligands and magnetic guidance act together as a dual targeting mechanism for SPIO nanoparticles and enhance the therapeutic efficacy of siRNA gene silencing in cancer cells. Recently, human breast cancer cells were transfected with shRNA for NOTCH-1 using an iron oxide/PEI/shRNA nanoparticle complex [93]. The shRNA-loaded magnetic nanoparticle was preferentially taken into breast cancer cells with high efficiency, and inhibited cell proliferation and increased apoptosis and cell death. Thus, magnetic iron oxide nanoparticles can be exploited as an efficient non-viral delivery system for targeted gene therapy, as well as for cancer diagnosis, by utilizing their magnetic resonance properties.

Gold nanoparticles (AuNPs) recently emerged as a reliable siRNA carrier system. As it is an inert metal gold is non-toxic, and forms fine nanoparticles, and could be easily functionalized for safe and efficient gene delivery. Covalent or electrostatic methods can be used to conjugate siRNAs onto the surface of gold. The presence of free thiol groups can be exploited for such modifications of gold nanoparticles, allowing targeted molecules or stimuli-responsive materials for controlled release of siRNA to be attached. Giljohann *et al.* attached polyvalent siRNA molecules to the surface of AuNPs *via* thiol groups; the half-life and stability was increased many times over that of free siRNA [94]. This siRNA-AuNP complex resulted in significant knockdown of the targeted gene in an *in vitro* cell model.

Adding a PEI coating to the AuNP core cationizes nanoparticles, making them an attractive siRNA delivery system, as reported by Song *et al*. [95]. PEI-capped AuNPs bound to siRNA firmly by electrostatic interaction. The siRNA delivered by this system downregulated GFP expression in MDA-MB-435 cells. Further, targeting Polo-like kinase 1 (PLK1) gene siRNA-PEI-capped AuNPs achieved significant gene knockdown and enhanced cell apoptosis [95]. These findings indicated that AuNPs with cationic polymer modifications act as an excellent gene delivery system, without exhibiting any toxicity on its own.

Gold nanoparticles can also be functionalized to become stimuli-responsive to facilitate efficient siRNA delivery. For example, amine-functionalized gold nanoparticles complexed with siRNA-PEG molecules having disulfide linkages showed efficient delivery of siRNA in cytoplasm in responsive to a glutathione-reducing environment [96]. The released siRNA also showed efficient target gene knockdown, while the carrier caused negligible cytotoxicity.

Recently, the photothermal properties of gold nanoparticles have been utilized in siRNA delivery, as reported in [97] and [98]. Controlled release of siRNA captured to poly-L-Lysine (PLL) epilayer to gold nanoshell structure is accomplished by near infrared (NIR) laser irradiation at 800 nm, which is near the resonance wavelength of gold nanoshell [97]. They observed efficient green florescent protein (GFP) gene silencing in H1299 cells when the gold nanoshell-PLL structure delivered either single-stranded antisense DNA or siRNA specific to the GFP gene. The application of NIR radiation at a certain energy helps in local heating and cavitation of endosomes, through which siRNA can escape into the cytoplasm [98]. These structural, stimuli-responsive, and photothermal properties of gold nanoparticle-based carriers are advantageous in cancer gene therapy applications.

Silica nanoparticles form a district class of therapeutic carriers for cancer therapy, due to their flexibility, non-toxicity, and high drug-loading capacity. Silica nanoparticles have a porous structure with a negative surface charge. Therefore, the surface should be cationized in order to encapsulate siRNA or DNA. The pore sizes of mesoporous silica nanoparticles (MSN; $\sim$3 nm) are advantageous in incorporating small drug molecules or siRNA, but not large molecules, like DNA. However, researchers sought large cationic polymers, such as PEI [99] or poly(2-dimethylaminoethyl methacrylate) [100], to modify MSNs for effective binding of siRNA and to act as facilitators of the proton sponge mechanism for endosomal escape.

Recent studies in anti-cancer therapy exploited the structural advantage of MSNs in the codelivery of anti-cancer chemotherapeutics and siRNA [101], –[103]. Meng *et al*. utilized MSN for the codelivery of p-Glycoprotein (Pgp) siRNA and doxorubicin to overcome drug resistance in a cancer cell line *in vitro* [102]. They functionalized the MSN with phosphonate groups for doxorubicin inclusion in pores and helped PEI to attach to the surface of MSN for siRNA binding. This dual therapeutic system increased the chemosensitivity of

cancer cells to doxorubicin by specific siRNA-based knockdown of Pgp. The same group later reported that, by using PEI-PEG modified MSN for codelivery of doxorubicin and Pgp siRNA, a drug-resistant breast cancer xenograft model was sensitized to doxorubicin [103]. Furthermore, for the purposes of cancer treatment, Li *et al*. synthesized MSN with a highly efficient siRNA delivery capacity [104]. Instead of traditional method of attaching siRNA in the outer cationic layer, they encapsulated the therapeutic siRNAs in the pores of MSNs. Further, they modified the MSN-siRNA core system with a PEI-fusogenic peptide complex to achieve targeted delivery. These findings suggest a highly effective siRNA-based anti-cancer therapeutic effect, both *in vitro* and *in vivo*.

Moller *et al*. reported that siRNA incorporation was significantly increased upon modification of MSNs with block copolymer containing positively charged amino acids and oleic acids [105]. The nanoparticle system showed enhanced delivery of siRNA and efficient endosomal escape conferred by positively charged amino acids and oleic acid blocks. The transfection efficiency was as high as 90% in KB cells, even with a low concentration of MSN carrying siRNA (2.5 μg MSN containing 32 pM siRNA) in 100 μl per well. Many such strategies for achieving enhanced therapeutic efficacy for siRNA are being investigated with MSNs. These strategies might be translatable to cancer treatment.

Semiconductor quantum dots (Qdots) are another group of inorganic nanoparticles that can be used in siRNA-based gene therapy for cancer. These nanoparticles are small, bright, photostable, fluorescent spherical nanocrystals that exhibit size-dependent fluorescence properties that are attractive for diagnostic imaging. These Qdots can be functionalized by hydrophobic organic molecules, and the nucleic acid molecules can be attached to the surface *via* electrostatic and disulfide bonds. The main advantage of using Qdots for gene delivery is that the Qdots can act as a theranostic system to provide both siRNA-based gene silencing and diagnostic imaging. In addition, the specific gene delivery mechanism can be visualized and genes can be tracked to reduce off-target effects. Chen *et al*. developed Qdots-based multicolor probes to monitor and track gene knockdown studies [106]. They cotransfected siRNA and QDots using standard transfection techniques, and observed that cellular fluorescence, from photostable QDots correlated with level of gene silencing. The researchers were able to collect cells with a uniform level of gene silencing using fluorescence-activated cell sorting (FACS). They were also able to show multiplexed levels of gene knockdown in same cell by using two different colors of QDOTs and two siRNAs.

QDots encapsulated in amphiphilic PEI (amPEI) have recently been explored for multiple purposes, including gene delivery [107]. Qdots were encapsulated in amPEIs. On the PEI surface, siRNA was attached with electrostatic interaction. These QDamPEI-siRNA particles were used for gene delivery, cell-specific labeling, and ratiometric oxygen sensing. Findings revealed transfection efficiency higher than conventional gene transfections. Another group demonstrated the use of Qdots in cellular tracking of siRNA and as a self-trafficking transfection agent for siRNA [108]. Here, Qdots and HER2/neu siRNA were encapsulated in chitosan nanoparticles surface-labelled with HER2 antibody targeted to HER2-overexpressing SKBR3 breast cancer cells. The siRNA was able to knockdown the target gene and was simultaneously tracked using QDot fluorescence. Recently, newer strategies have emerged to improve the transfection efficiency and tracking ability of siRNA using different QDots with a view toward theranostic applications in cancer [109]–[111]

Though a number of nanoparticle carriers have been developed by taking advantage of the availability of the vast material/biomaterial resources, there are challenges yet to overcome such as off-target effects, toxicity and poor efficiency. However, proper testing of these systems in suitable models and by learning from their pitfalls will inspire the design of better gene delivery vehicles. As newer technologies are available new class of nanoparticle carriers are always being tested by scientists for improved delivery of nucleic acid therapeutics, alone, or in combination with other drugs/imaging agents for cancer therapy. Most of them have sought interesting features in creating formulations that have targeting capability and show low toxicity to normal tissues. Some of them are proof-of concept designs, yet have shown potential in carrying multiple therapeutics/diagnostic agents for cancer therapy. Table II shows some of the most recent studies, especially focused on siRNA delivery representing the current trends in cancer nanotechnology.

VIII. siRNA Tracking and Analysis

In order to understand the fate of delivered siRNA many fluorescence based techniques have been developed recently. One such method is based on fluorescence resonance energy transfer (FRET) technique. In a typical study an upconversion nanoparticle (UCN; silica/NaYF4:Yb,Er) was used as an energy donor to the fluorescent dye (BOBO-3) conjugated siRNA attached to the nanoparticle surface [120]. An NIR laser was used to excite the UCN to allow energy transfer to the BOBO-3 dye conjugated to siRNA. This technique has been used as a method to monitor release and biostability of siRNA in live cells. In a different approach FRET-based imaging was used by Jarve *et al.* in tracking and understanding the siRNA integrity in intracellular environment [121]. They used fluorescein and tetramethyl rhodamine dual-labelling on opposing strands of siRNA, to study the RNAi efficiency and the non-destructive and non-invasive degradation state of siRNA in cells. Upon transfection degradation of a double-labelled siRNA results in spatial separation of the dyes and a breakdown of FRET, this distinguishes intact or degraded siRNAs in cells as a measure of RNAi efficiency. To study the organ specific delivery and to monitor the gene silencing efficiency of payload siRNA, a fluorescent dye that have both imaging and targeting capability was conjugated to polymer nanoparticles by Press *et al.* [122]. The nanoparticle has the polymethiene NIR-dye conjugated to its polymer backbone that acted as an escort system to monitor the uptake and clearance of the nanoparticle-siRNA complex in rat liver and kidney. In a different strategy Liu *et al.* encapsulated red fluorescent protein (RFP) in chitosan nanoparticles that carry siRNA [123]. In this study the fate of the siRNA delivery by

TABLE II
REPRESENTATIVE EXAMPLES OF CURRENT TRENDS IN siRNA BASED CANCER THERAPY USING SPECIALIZED NANOMATERIALS

Nanoparticle material	**Therapeutics**	**Disease Target/Model**	**Notable features of the study**	**Reference**
Functional polyesters	siRNA to ubiquitin B	Lung cancer	Cell type specificity of drug carrier: lung cancer *versus* normal cells	112
Anisamide-targeted gold nanoparticle	siRNA to RelA unit of Nuclear Factor k B	Prostate cancer	Targeting sigma-1 or sigma-2 receptors overexpressed in prostate cancer cells for siRNA delivery	113
Intercalation mediated nucleic acid nanoparticles	Model siRNAs (eg; Hela Luc-reporter genes)	Hela cell Model (Proof-of-concept)	Using intercalation as additional force to electrostatic and hydrophobic interactions in siRNA nanoparticle carrier for enhanced delivery	114
Dextran coated-Super-paramagnetic iron oxide	Polo like Kinase-1 siRNA	Pancreatic ductal adenocarcinoma	Multifunctional nanoparticle with (a) uMUC1 targeted peptide EPPT1, (b) membrane translocation peptide, (c) SPION for MRI imaging, and (D) siPLK1 for gene silencing demonstrated theragnostic application in PDAC	115
Monoarginie-Chol-DOPE small lipid nanoparticle)	siRNA against kinesin spindle protein	Various cancer cell lines and Prostate tumor	Liposomes showed advantages of small size, lack of toxicity, neutral surface charge, and highly efficient siRNA complex formation for cancer therapy	116
Lipid-Polycation-Hyaluronic acid(LPH)-PolyMetformin nanoparticle	Vascular Endothelial Growth factor-siRNA/ Metformin	Lung cancer	Antitumor activity of metformin and the capacity of Polymetformin to aid therapeutic siRNA carrier combination for cancer therapy	117
NIR-fluorescent polymer nanoparticle	V-Raf murine sarcoma viral oncogene homolog B (BRAF) siRNA	Anaplastic thyroid cancer	NIR imaging (based on polymer carrier)-guided siRNA delivery for personalized treatment of advanced malignancies	118
Plasmonic gold nanoparticle	Polo like kinase-1 siRNA	Pancreatic cancer	NIR pulsed laser triggered controlled release of siRNA. Also required significantly less material than conventional gene delivery vehicles for trasfection	119

chitosan nanoparticles was tracked by RFP fluorescence *in vitro* and *in vivo*.

To understand the efficiency of siRNA-carrier material interaction and the stability of siRNA, dye-quenching assays have been developed. For example, SYBR-Green dye gives enhanced fluorescence upon intercalation with siRNA, whereas the dye quenches when siRNA condense upon interaction with the carrier material [124]. Similarly, while developing fluorescence based siRNA quantification assay for nanocarriers in cells, the phenomena of fluorescence quenching of dye-labelled siRNA upon interaction with different nanocarriers have been reported by Vader *et al* [125]. In their study they have observed the dye-quenching phenomenon and correlated with the condensation level or release mechanism of siRNA from nanoparticle-complex upon cell uptake. Alabi *et al.* utilized a FRET-based approach in studying the formation, stability and disassembly of nanoparticle-siRNA complex [126]. Based on the principle that if the siRNAs labelled with two different FRET efficient dyes are in free form (as they are apart) the FRET-mechanism will be absent, whereas the siRNAs condensed in delivery vehicle can lead to efficient energy transfer (as they are in close proximity) resulting in enhanced FRET fluorescence. Altogether, development of many such tools and assays has been very helpful in assessing the transfection efficiency of RNAi, and in detection, quantification and analysis of extracellular and intracellular siRNA.

IX. CHALLENGES AND PERSPECTIVES

SiRNA-based gene silencing has progressed rapidly as a potential gene therapy strategy for cancer management. Challenges, including poor stability of siRNA *in vivo*, chances of off-target effects due to poor selection of specific sequences while designing siRNA, cellular barriers like endosomal degradation, and competition from natural RNAi components in cells, must still be resolved. Nanotechnology has revolutionized the delivery mechanism of labile therapeutics, in particular, nucleic acid-based drugs. Liposomes and nanoparticle systems promise rapid bench-to-bedside transition of nucleic acid therapeutics by overcoming many hurdles in their delivery to the target.

However, nanoparticle siRNA delivery systems also face many obstacles, from their synthesis to major physiological hurdles, including rapid clearance and non-specific toxicity. Further progress in this area is required. The challenges in consistency in nanoparticle synthesis have been partly addressed by the development of newer technologies, such as microfluidics and PRINT. Materials for nanoparticle synthesis should be chosen carefully, as many reports suggest material-related toxicity and off-target effects due to unknown causes. Although the PEG modification of nanoparticles improves particle stability and half-life in *in vivo* systems, many questions have raised about its immune-related safety and pharmaceutical characteristics, including drug loading efficiency and targeting. To improve the *in vivo* stability and to overcome immune-related problems, newer strategies of nanoparticle modification using materials, such as gangliosides, pullulan, synthetic polymers like polyglycerols, vinyl, poly(2-Oxosaline), or poly amino acid-based lipids, have been proposed as alternatives to PEG modification. A variety of molecules, including DNA-, RNA- and peptide-based aptamers, antibodies, and small molecules, are under investigation to address the problems in targeted delivery of therapeutics. This will certainly enhance the target specificity of siRNA therapeutics and minimize the toxicity towards healthy tissue.

As cancers present with ever-increasing heterogeneity, siRNA-based therapy is increasingly being explored in combination with chemo- [127], [128], immune- [129]–[131], radiation- [132]–[134], and photodynamic therapies [135]–[137]. While gene silencing using siRNA improves the sensitivity of drug resistant cancer to chemotherapeutics [127] siRNA mediated downregulation of immunosuppressive genes enhances the sensitization of cancer cells towards T cell-mediated killing [129]. Reports also suggest that siRNA based silencing of specific genes enhances radiosensitization of cancer cells [133], [134]. In contrast, downregulation of certain genes protect normal tissues from harmful effect of radiation [132]. RNAi has also shown its impact in enhancing photodynamic therapy as a result of increased sensitization induced by silencing specific genes that causes photodynamic resistance in cancer cells [135]–[137]. Nanoparticle drug delivery systems have tremendously improved such combination therapy strategies, at least in preclinical models. Worldwide, researchers seek to quickly translate many of these potential strategies to the clinic setting with the help of advanced nanoparticle delivery systems.

X. Conclusion

RNAi-based gene silencing therapy has undergone a revamp resulting from efficient *in vivo* delivery strategies using liposomes and nanoparticles. The current review presented some commonly used siRNA delivery systems and their advantages in cancer gene therapy. Based on our experience with liposomes and other nanoparticle systems, we have also described some of our efforts in siRNA delivery targeting cancer. Liposomes are biodegradable, have flexible physico-chemical properties, and have the potential for rapid translation to the clinic setting. New strategies to improve the stability and targeting efficiency of liposomal siRNA carriers are under investigation. Polymeric nanoparticles have the unique ability to load siRNA with high efficiency, provide slow and controlled release, and allow for effective intracellular trafficking of siRNAs. They are easy to manipulate for codelivery of siRNA(s) and chemotherapeutics or other anti-cancer agents. Inorganic nanoparticles are rigid and non-biodegradable. Nevertheless, many inorganic nanoparticles are multifaceted and are being explored in both imaging and therapeutics. Despite these advantages, some drawbacks must be addressed, such as non-specific interaction with healthy tissues, off-target effects, and unwanted cytotoxicity.

The use of minimal delivery materials with increased loads of therapeutic compounds is recommended, as this process can reduce the material-related toxicity and other unwanted effects. However, advanced engineering in nanoparticle design and the current pace of discovering new druggable targets strengthens the areas of potential development. It is hoped that nanoparticle-based siRNA therapy will gain better recognition in the near future and become a conventional mode of cancer therapy.

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