Targeted Nanocarrier Based Systems for the Treatment of Lung Cancer

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Abstract

In Hawai'i, lung cancer is among the top cancers diagnosed and a leading cause of death. Despite current understanding and modern surgery, radiology, and chemotherapy techniques, the survival of those suffering from lung cancer remains low. Current anticancer drugs have poor tumor tissue selectivity and toxicity issues that contribute to their overall low efficacy, detrimental effects to normal tissues, and drug resistance. A potential way of mitigating cancer is through RNA interference (RNAi) by the delivery of small interfering RNA (siRNA) to target select proteins or genes involved in cancer progression, known as oncoproteins or oncogenes, respectively. However, the clinical utility of delivering unformulated siRNA has been hindered due to poor cell penetration, nonspecific effects, rapid degradation, and short half-life. As an alternate for conventional chemotherapy, nanoparticles (AKA nanocarriers) may be designed to localize within the tumor environment and increase targeted cell internalization, thus reducing systemic adverse effects and increasing efficacy. Nanoparticles play important roles in drug delivery and have been widely studied for cancer therapy and diagnostics, termed collectively as theranostics. Nanoparticles composed of natural and artificial polymers, proteins, lipids, metals, and carbon-based materials have been developed for the delivery of siRNA. Cancer targeting has been improved by nanoparticle surface modification or conjugation with biomolecules that are attracted to or stimulate therapeutic agent release within cancer tissues or cells. In this mini-review article, we present recent progress in nanocarrier-mediated siRNA delivery systems that include lipid, polymer, metallic and carbon-based nanoparticles for lung cancer therapy.

Introduction

Cancer is a leading cause of death in Hawai'i and is characterized as a set of diseases that involve abnormal cell growth that may initiate in one location and then metastasize, or invade other tissues in the body.[1] Despite the recent advances in diagnosis and treatment, lung cancer is among the most common cancers diagnosed on the islands only following prostate and breast cancers in both men and women, respectively.[1,2] The Native Hawaiian and Pacific Islander populations that originate from Hawai'i, Guam, Samoa, or other Pacific islands are among the fastest-growing populations in the United States (U.S.) and have increased cancer rates than Asian Americans.[3] Due to different rates of smoking habits, lung cancer rates in Samoan men are about 30% higher than those in Native Hawaiian men.[3] Worldwide, in 2016 there were approximately 58,000 new cancer cases and nearly 17,000 cancer deaths among Asian Americans, Native Hawaiians, and Pacific Islanders.[3] In Hawai'i, about 6,500 Hawai'i residents are diagnosed with invasive cancer and more than 2,000 die from the disease each year.[4]

Overall nationally, an estimated 221,200 lung cancer cases were detected and diagnosed in 2015 that made up approximately 13% of all cancer diagnoses.[5] The lung cancer 5-year survival rate is 54% when the disease is still localized within the lungs, however only 15% of cases are diagnosed at early stages.[6] For metastasized tumors, the 5-year survival rate is 4%.[6] The overall lung cancer survival rate is much lower than other leading cancer causes.[6] Non-small cell lung cancer (NSCLC) occurs when malignant cells form in the tissues of the lung and can be classified as adenocarcinoma, carcinoid tumor, large cell carcinoma, pleomorphic, salivary gland carcinoma, squamous cell carcinoma, and unclassified carcinoma.[2] NSCLC accounts for 85% of the total cases of lung cancer, where 75% of these cases at diagnosis are metastatic.[7] Small-cell carcinoma, also known as oat-cell carcinoma, is an aggressive cancer that begins in the bronchi and rapidly spreads, or metastasizes, throughout the body. Small-cell carcinoma occurs most often in smokers, almost exclusively, and represents approximately 15% of lung cancers in the U.S.[8,9]

Standard lung cancer treatment entails combinations of surgery, chemotherapy, and radiation therapy. Although early detection and treatment make a significant difference in life expectancy, many lung cancer patients are diagnosed with the advanced or metastatic disease.[10] Surgical resection may be considered the most effective strategy to cure NSCLC; however, surgery is not possible in every case.[11] Current NSCLC anticancer drugs have poor tumor tissue selectivity and toxicity issues that contribute to their overall low efficacy and detrimental effects to normal tissues.[12] Chemotherapy options often have issues with multidrug resistance due to overexpression of drug-resistance genes that drive mechanisms to stop cancer cell death and increase the expression of cellular pumps that remove the anti-cancer drugs from the cells. Multidrug resistance leads to higher chemotherapy doses that in turn, lead to more risk for severe adverse effects. New treatment options for lung cancer include the drugs afatinib (Gilotrif), ramucirumab

(Cyramza), and bevacizumab (Avastin).[13] Afatinib, approved by the U.S. Food and Drug Administration (FDA) in 2013, targets the epidermal growth factor receptor (EGFR) and receptor tyrosine-protein kinase erbB-2, AKA Her2/neu, via irreversible covalent inhibition to inhibit metastasis and tumor growth in EGFR mutant positive NCSLC patients.[14-16] Mutations in the expression or activity of receptor tyrosine kinases EGFR and ErbB-2 causes cancer.[17] Ramucirumab, FDA approved in 2014, is a monoclonal antibody that acts as an angiogenesis inhibitor by binding the vascular endothelial growth factor 2 (VEGFR2).[18] However, conventional delivery of these therapies leads to the potential intolerable side effects and risk of recurrence due to resistance.

More effective pharmacological interventions for cancer therapy are necessary because surgery and radiotherapy are not viable options in some patients and chemotherapy results in low response rates with detrimental adverse effects. A well-designed delivery system that can deliver anticancer therapeutics specifically to cancerous cells should be developed to avoid adverse effects and to increase efficacy.

Cancer Targeting siRNA Therapeutics

Several methods have been proposed to control protein markers that are overexpressed in cancer.[19] These methods include small molecule inhibitors or antibody biologics. A method for downregulating protein expression that targets the mRNA level, before protein transcription even begins, is RNA interference (RNAi). RNAi is a naturally occurring protein regulating process that has a high degree of specificity and the potential to silence mRNA and associated protein expression.[20] A method for eliciting RNAi is mediated through the delivery of small-interfering RNAs (siRNAs) to the cell cytoplasm of target cells. Therapeutic siRNAs are synthetic double-stranded RNA of 21-23 base pairs that can be designed to suppress target mRNA sequences, in a process known as post-transcriptional gene silencing. To exert the therapeutic effect, the siRNA must travel to the cell cytoplasm to be attached to the multi-protein RNA-induced silencing complex (RISC).[21] Intracellular siRNA, specifically targeted to a particular mRNA for degradation, undergoes RNAi processing in the cell to induce short-term silencing of protein coding mRNA.[22] The siRNAs, as a class of therapeutic agents, are capable of efficient knockdown of targeted oncoproteins and oncogenes, as well as those proteins that play a role in multidrug resistance, and therefore have great potential for the treatment of lung cancer or other diseases.[23]

Nanotechnology in Drug Delivery

Nanotechnology has greatly impacted the field of medicine which in part has catapulted preclinical siRNA delivery systems into a new era. Drug half-life, retention, and targeting efficiency can be increased along with a subsequent reduction in adverse effects by incorporating nanotechnology-based therapeutic delivery systems. A brief history of the breakthroughs in cancer nanomedicine, or the therapeutic application of nanoparticle drug delivery systems, is shown in Figure 1.[24] A few chemotherapeutic nanoparticle formulations have been approved by the FDA, namely liposomal doxorubicin (Doxil) and Nab-paclitaxel (Abraxane).[25-29] Bind Biosciences, Inc. demonstrated nanoparticles containing a combination of chemotherapeutic and prostate-specific membrane antigens (PSMA) that outperform either drug alone at diminishing lesions of the lung and tonsillar regions and that the formulation greatly lowered the required dose.[30,31] Calando Pharmaceuticals established the foremost clinical evidence of RNAi using a polymeric nanoparticle delivery system known as CALAA-01.[32,33]

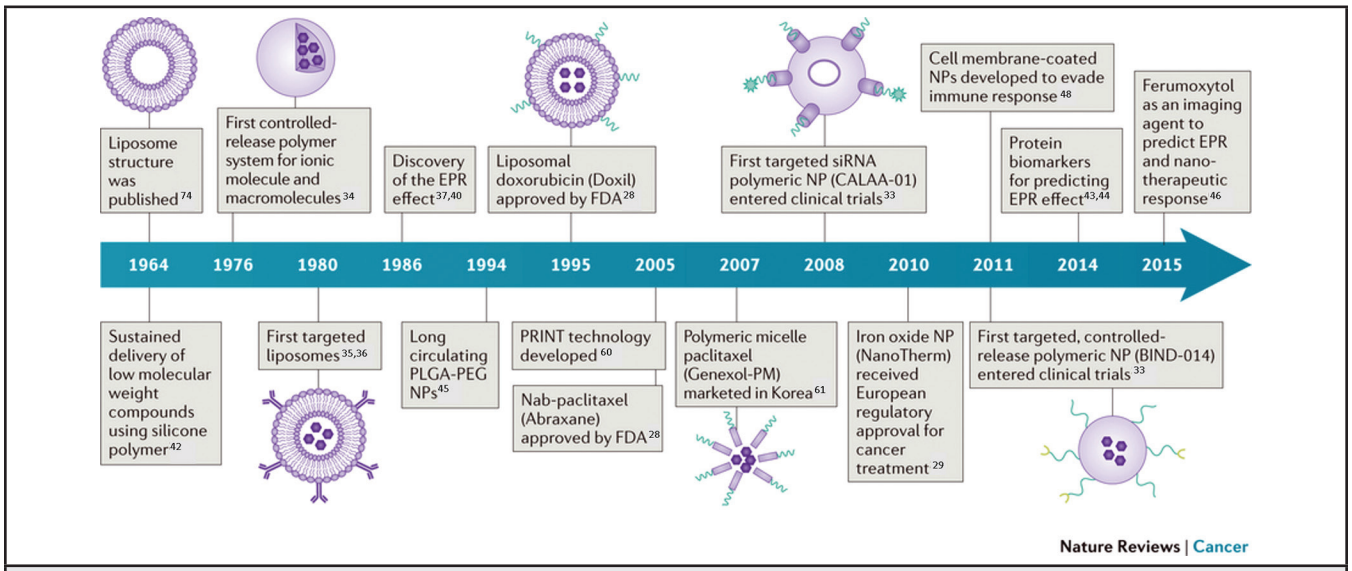

Figure 1. A timeline showing the history of major cancer nanomedicine breakthroughs.
Figure adapted with permission from Nature Publishing Group: Nature Reviews Cancer, 2017.[24]

As opposed to conventional chemotherapy delivery where the drug is administered systemically by i.v. injection and shows various drug concentrations in all tissues of the body, lung cancer targeting strategies using nanocarriers may achieve controlled release and are classified as having either passive or active targeting directly to cancer tissue.[34-36] Passive cancer targeting is possible through the enhanced permeation and retention (EPR) effect that has been exploited in many cancer drug delivery systems since its identification in the late 1980's.[37-40] Nanocarrier circulation times and accumulation levels in tumor tissues are influenced by the properties and characteristics of the nanocarriers, such as the polymer or lipid, surface molecules, particle size, or surface charge.[41-46]

Strategies to improve delivery to tumor sites by including microenvironment homing mechanisms including tumor penetrating peptide and stimuli responsive functional surface nanocarrier modifications have been explored.[47] In addition to conjugating the polymer polyethylene glycol (PEG) to the surface of the nanoparticles, known as PEGylation, cell membrane-coated nanoparticles have been developed to evade immune responses by tricking the immune system.[48] Active targeting involves molecular targeting agents, such as surface bound ligands, to specifically target the biomarkers or receptors on cancer tissues or cells that may provoke uptake of nanocarriers.[49-51]

Nanocarrier-based siRNA Delivery Systems for Lung Cancer

Naked siRNA is prone to degradation, has a shorter plasma half-life, rapid renal clearance, and limited permeability across cell membranes, making clinical efficacy unlikely.[52,53] A variety of nanocarrier systems in early cancer therapeutic clinical studies have shown enhanced efficacy and reduced side effects.[30,54] Cationic lipid based systems have emerged as the most attractive for siRNA delivery. However the use is limited due to poor transfection efficiency and toxicity *in vivo*.[55] Natural polymer based delivery systems are biocompatible and biodegradable with high physiological tolerance and low immunogenicity.[56,57] Rigid nanoparticles are composed of inorganic metals or of carbon-based materials.[58] Here, we focus on siRNA nanocarriers that have undergone laboratory experimentation published recently.

Examples of Polymer-based siRNA Nanocarriers

Polymers used for siRNA nanocarriers include naturally occurring ones, such as chitosan, or synthetics ones, such as polyethylenimine (PEI). Polymeric nanocarriers have received much attention in the area of siRNA delivery because of their biocompatibility and versatile modifiability.[32,54,59-61]

Yan, et al, developed a combinatorial functional polyester library to identify formulations that have highly efficient delivery of siRNA to A549 (ATCC® CCL-185™) human epithelial lung carcinoma for potential lung delivery of siRNA.[62] They found that two types of polyplex nanoparticles that contained PEG 2000 DMG modified lipid or Pluronic F-127 nonionic surfactant on their surface increased their serum stability and decreased their surface charge. This study showed that inhalation of the surface modified polyplex nanoparticles had resulted in significantly more nanoparticles localized within the lungs and significant gene downregulation in the A549 orthotropic lung tumors than when delivered by i.v. administration.

Another study that evaluated nanocarriers in an A549 lung cancer cell line was conducted by Seifi-Najmi, et al, who demonstrated the preparation and characterization of varying combinations of doxorubicin, High Mobility Group At-Hook 2 (HMGA2) siRNA, or combination siRNA/doxorubicin entrapped within carboxymethyl dextran trimethyl chitosan nanocarriers.[63] Nanoparticles loaded with doxorubicin combined with HMGA2 siRNA outperformed the other formulations in treated A549 cell assays including lessened cancer cell viability, alteration of pro-cancer markers, induction of apoptosis, and inhibition of migration.

Cisplatin is an anti-cancer chemotherapy drug, also known as a cytotoxic or antineoplastic drug, that interferes with DNA replication by crosslinking DNA, thus preventing cell division by mitosis. However, cellular resistance to cisplatin therapy is commonly observed. A mediator of cisplatin sensitivity in human cancer cells at the mitotic checkpoint is the mitotic arrest deficient-2 (Mad2) protein.[64] Nascimento, et al, designed chitosan polysaccharide (sugar polymer) nanoparticles containing surface conjugated EGFR targeting ligand that entrapped Mad2 siRNA in combination with cisplatin and determined them to be safe and efficacious in cisplatin resistance and sensitive NSCLC cell culture models.[65,66] Their rationale was if the RNAi that downregulated the essential mitotic checkpoint gene Mad2, the cells would become more sensitive to cisplatin based chemotherapeutics and therefore lead to increased cellular death. EGFR targeted nanoparticles presented a steady and favored tumor targeting capability with fast blood plasma clearance to penetrate and localize within the tumor tissue for up to 4 days.[67] These targeted nanoparticles showed a six-fold advanced tumor targeting ability when related to non-targeted chitosan nanoparticles.[67]

Another siRNA and chemotherapeutic combination study was conducted by Xu, et al, who designed a pH-responsive PEI nanoparticle containing doxorubicin and Survivin siRNA for potential inhalation therapies for metastatic lung cancers.[68] Survivin is a protein that inhibits caspase activation, which causes negative regulation of programmed cell death, or apoptosis; thus it belongs to the family of proteins that inhibit apoptosis.[69] Therefore, siRNA directed towards survivin expression will remove this negative regulation, allowing for normal cell death of cancerous cells. Doxorubicin release from these PEI nanocarriers was pH dependent, where the release was higher in acidic tumor microenvironments. The doxorubicin and Survivin siRNA was delivered in vitro and was shown to impart cellular death in B16F10 cancer cell culture lines. Using cancer mouse models that had B16F10 tumors, locally delivered siRNA loaded doxorubicin coupled PEI nanoparticles by inhalation resulted in the accumulation of doxorubicin and Survivin siRNA within the lung tissue and airways, where a

substantial quantity of doxorubicin and siRNA were found in tumor tissues, with a low amount of doxorubicin and siRNA observed within normal lung tissues. Furthermore, the Survivin siRNA doxorubicin conjugated PEI nanoparticles presented superior antitumor effectiveness when compared to individual doxorubicin or Survivin siRNA delivery.

Srikar, et al, produced tri-block nanoparticles that were composed of enzymatically degradable gelatin nanoparticles with cetuximab-siRNA molecules conjugated to its surface and entrapped with the tyrosine kinase inhibitor, gefitinib.[70] Delivery of siRNA to chemotherapeutic resistant KRAS mutated NSCLC cells via a targeted strategy was believed to foster cell sensitization to tyrosine kinase inhibitors, leading to increased cellular death. This study demonstrated targeted proto-oncogenes with nanoparticle therapies.

Metformin is a well-known diabetic medication that has been studied as an anti-cancer drug. Its mechanism is believed to rely on the ability to reduce insulin resistance and indirectly lower levels of insulin, a known cancer cell growth promoter, as well as through direct inhibition of cancer cell growth.[71] Zhao, et al, prepared a polymer conjugate of Metformin, coined PolyMetformin through dicyandiamide conjugation to linear PEI.[72] The positive charge of PolyMetformin facilitated the entrapment of the negatively charged siRNA into a core-membrane structured lipid-polycation-hyaluronic acid nanoparticle as shown in Figure 2. Vascular endothelial growth factor (VEGF) is a signaling protein that stimulates blood vessel formation, and if expressed by cancer cells can cause tumor growth and metastasis.[73] LPH-PolyMetformin nanoparticles facilitated VEGF siRNA delivery in a human lung cancer xenograft, leading to hindrance of tumor tissue growth. Without RNAi, the lipid-polycation-hyaluronic acid-PolyMetformin nanoparticles were able to induce antitumor efficacy similarly to Metformin. PolyMetformin was combined with siRNA to further improve the therapeutic activity of an anti-oncogene and oncoprotein therapy.

Examples of Lipid-based siRNA Nanocarriers

Several siRNA loaded lipid nanoparticle delivery systems have undergone evaluation as cancer therapies within clinical trials. However none have specifically targeted lung cancer. Lipid-based siRNA nanocarriers include liposomes, lipid complexes, and solid lipid nanoparticles.[74] Phase I clinical trials of stable nucleic acid lipid particles (SNALP) encompassing siRNA's directed towards serine/threonine-protein kinase (PLK1), a mitosis regulating gene, for cancer therapy was initiated by Tekmira Pharmaceuticals Corporation (NCT01262235, BC, Canada).[75,76] The Anderson Cancer Center (Texas, USA) initiated a Phase I clinical trial where siRNAs encapsulated within a neutral liposome composed of 1,2-dioleoyl-sn-glycero-3-phosphatydylcholine for oncoprotein Eph2 suppression (NCT01591356) for delivery for the treatment of advanced cancers.

Lung cancer and mesothelioma tumorigenesis, or the onset of tumor growth, has been associated with the receptor EphA2 overexpression. A liposomal cisplatin formulation, known as Lipoplatin™ , has been administered against cisplatin resistant cancers. To further enhance the sensitivity of lung cancer cells to Lipoplatin, Lee, et al, combined receptor EphA2 siRNA with Lipoplatin and targeted them to tumor cells.[77] This group demonstrated that silencing EphA2 significantly enhanced the cellular sensitivity of lung tumor and malignant pleural mesothelioma cells to Lipoplatin.[77]

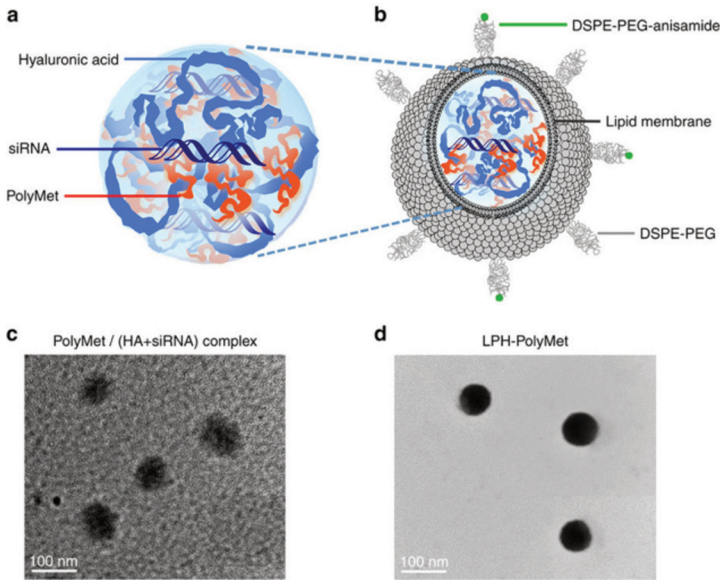

Figure 2. Schematic illustration and representative TEM images of PolyMet nanoparticles. Anionic HA + siRNA mixture was condensed by cationic PolyMet into negatively charged PolyMet (HA+ siRNA) complex (a, c). DOTAP/cholesterol cationic liposomes were added to the complex to form lipid coating, then DSPE-PEG and DSPE-PEG-anisamide were used to prepare a liposome using the post-insertion method to form LPH-PolyMet nanoparticles (b,d). By Zhao, et al, 2016. Used under Creative Commons Attribution 4.0.[72]

Human antigen R (HuR), a RNA binding protein, has been shown to be overexpressed in various cancers, has demonstrated its role in several oncoprotein expression regulations, and has been linked to overall resistance and poor-prognosis. Muralidharan, et al, hypothesized that cancer cell-targeted inhibition of HuR would suppress oncoproteins that would thus result in effective lung cancer therapy.[78] To examine this proposition, folate receptor-α (FRA)-targeted DOTAP:Cholesterol lipid nanoparticles carrying HuR siRNA (HuR-FNP) against human lung cancer cells were prepared and tested for stability release as shown in Figure 3. The prepared particles had a particle size of approximately 100 nm, adequately protected the siRNA from degradation, and displayed good release profiles (Figure 3b-d). HuR-FNP was shown to induce apoptotic cell death in H1299 cells that resulted in noteworthy growth inhibition and higher cell cytotoxicity.[78]

Examples of Metallic- and Carbon-based siRNA Nanocarriers

Iron oxide nanoparticles, carbon nanotubes, gold nanoparticles, and quantum dots have been developed for siRNA delivery as lung cancer therapies and diagnostic agents. Iron oxide nanoparticles theranostics have the ability to be used as a drug delivery system and a contrast agent for magnetic resonance imaging (commonly known as MRI) through the selective delivery of therapeutics agents to target sites. Carbon nanotubes have been used as drug delivery carriers because they can enter cells. Gold nanoparticles are interesting drug or siRNA delivery systems because they can be easily formed into a desired shape or size, they have exclusive surface plasmon resonance, and their surfaces may be modified through conjugation of thiolated targeting molecules.[58] Quantum dots are nanoparticulate materials having semiconductive nature which may be incorporated into living cells or tissue for experimental purposes but have been proposed for therapeutic applications as well.[79]

Lee, et al, determined whether chitosan-deoxycholic acid nanoparticles containing perfluoropentane and iron oxide can be used as an siRNA delivery system with the use of ultrasound exposure as shown in Figure 4.[80] The results show that the polymer coated iron oxide nanoparticles were able to successfully promote siRNA uptake, leading to significant apoptosis 3 days following ultrasound treatment.

Li, et al, developed a single vehicle l-arginine and hydroxypropyl-cyclodextrin quantum dot nanoparticulate combination drug delivery system containing siRNA directed towards B-cell lmphoma 2 (Bcl-2), which is a regulator of apoptosis, combined with carboplatin, doxorubicin, and paclitaxel for treatment in an A549 lung cancer cell line.[81] When compared to treatments consisting of only the free chemotherapeutics, the use of siRNA and chemotherapeutic combination loaded quantum dot nanocarriers exerted a 3 to 4 times increase in A549 cell cytotoxicity, implying improved treatment efficacy for the combination of siRNA and chemotherapeutic. These multifunctional quantum dot nanocarriers may potentially be a worthwhile method for delivering siRNA and chemotherapeutics for the lung cancer combination therapies and due to their fluorescent properties, may also serve as a diagnostic agent.

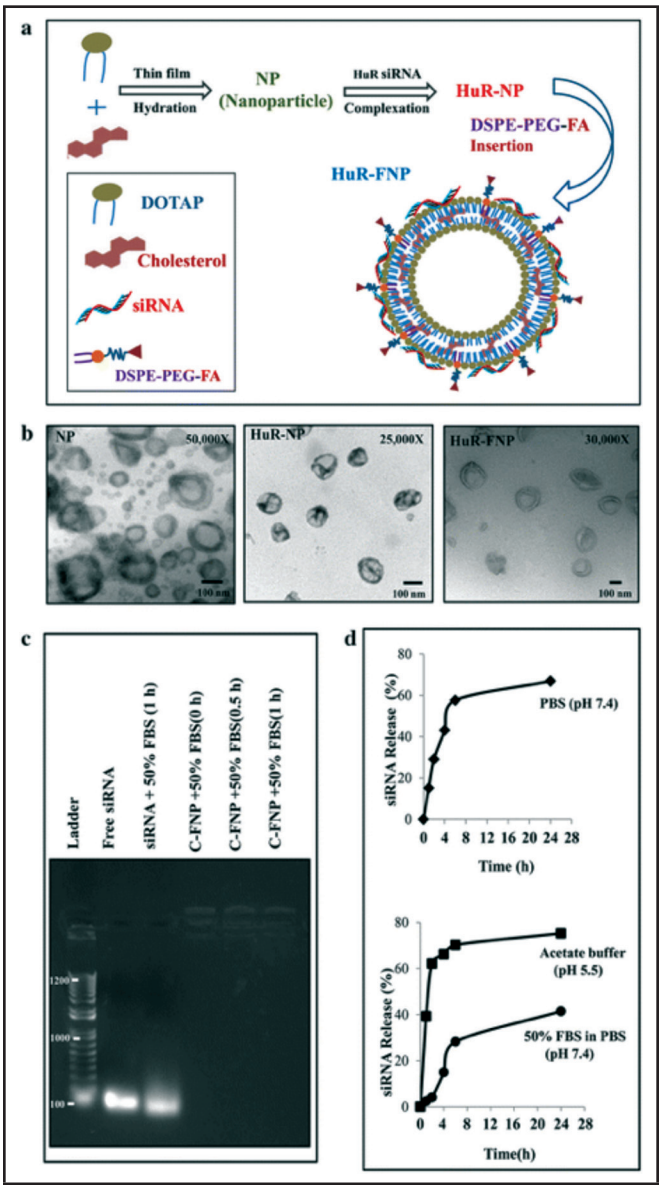

Figure 3. Synthesis and physicochemical characterization of siRNA-FNP. **a** Scheme showing HuR-FNP preparation. **b** TEM image of NP, HuR-NP, and HuR-FNP. Scale bar denotes 100 nm. **c** Agarose gel electrophoretogram showing siRNA protection by FNP at different time (0, 0.5, and 1 hr) points of incubation compared to naked siRNA exposed to serum for 1 hr. Free siRNA not exposed to serum was used as an internal marker. **d** siRNA release profile over time from siRNA-FNP in PBS (pH 7.4) measured by Quanti-iT Picogreen Assay (top figure); and from fluorescently labeled siRNA (siGLO)-FNP in acetate buffer (pH 5.5) and in 50% FBS containing PBS (pH 7.4) (bottom figure). By Muralidharanet, al, 2016. Used under Creative Commons Attribution 4.0.[78]

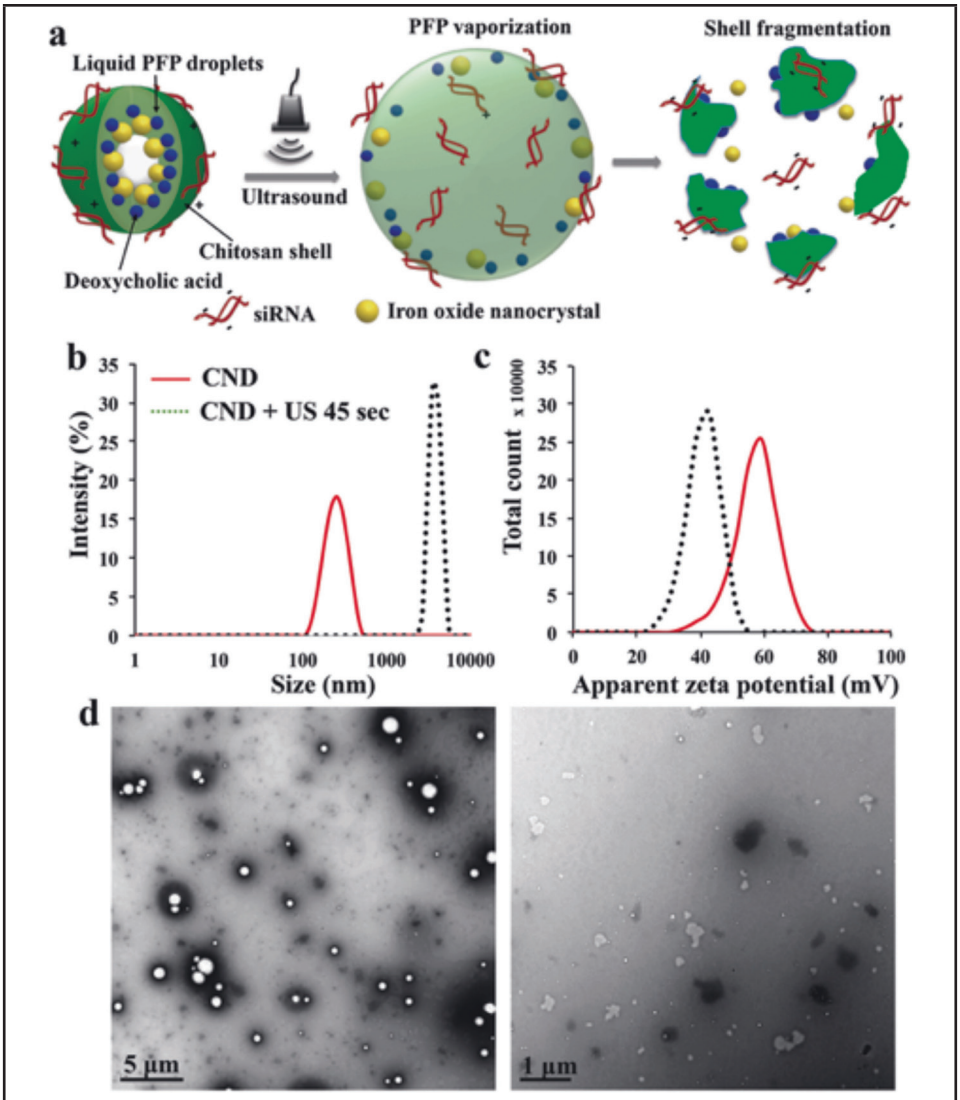

Figure 4. **a** Schematic representation of chitosan-deoxycholic acid coated perfluoropentane nanodroplets (CNDs). **b** Hydrodynamic diameters of CNDs before (solid line) and after (dotted line) exposure to ultrasound for 45 s in 10% glycerol in water. **c** Zeta potential of CNDs before and after ultrasound exposure. **d** TEM images of CND before (left) and after (right) ultrasound exposure for 45 s. By Lee, et al, 2017. Used under Creative Commons Attribution 4.0.[80]

Kamrani Moghaddam, et al, studied the ability of siRNA loaded hexagonal selenium nanoparticles (HSNM-siRNA) to prevent EGFR signaling in human NSCLC.[82] Plain hexagonal selenium nanoparticles and HSNM-siRNA were each separately used to treat NSCLC cell lines, and then onco-gene and -protein expression levels were evaluated. HSNM-siRNA was shown to downregulate the EGFR signaling gene expression and increase in a number of apoptotic cells.[82]

Mi, et al, developed a porous silicon-based nanocomposite material that simultaneously delivered chemotherapeutic agents and siRNA to the lungs after i.v. injection.[83] The silicon microparticles entrapped B-Raf proto-oncogene serine/threonine kinase siRNA-loaded liposomes and contained docetaxel entrapped polymeric nanoparticles conjugated to their surface. A synergistic antitumor effect was demonstrated when siRNA/docetaxel nanocarriers were used to treat melanoma cell cultures and also showed synergistic efficacy in vivo using a melanoma lung metastasis mouse model. The siRNA/docetaxel nanocarriers displayed higher accumulation in the lungs of the mouse model that exhibited metastatic melanoma lesions.

Wu, et al, designed multi-functionalized, integrated theranostic folate-conjugated reducible polyethylenimine passivated carbon dots (fc-rPEI-Cdots) which were used to encapsulate EGFR and cyclin B1 siRNA.[84] These particles were capable of emitting visible blue photoluminescence and siRNA intracellular delivery. In vitro cell culture studies suggested that the developed fc-rPEI-Cdots were capable of targeted siRNA delivery and were biocompatible.

Iron-oxide nanoparticles modified with biodegradable polyester nanoparticles composed of the polymers poly(lactic-co-glycolic acid) (PLGA) and PEG were loaded with telomerase siRNA. Telomerase expression is responsible for inhibition of apoptosis and cancer mutations associated with lung cancer malignant cells. This study demonstrated that the self-assembly of magnetic diblock copolymers encapsulated telomerase siRNA and resulted in reduced telomerase expression when compared to that of naked siRNA. The reduction of telomerase gene expression leads to increased tumor cell apoptotic death in lung cancer cells treated with siRNA magnetic copolymers than compared to naked siRNA treated cells.[85]

Summary and Future Directions

Nanoparticles carrying siRNA molecules have revealed high transfection rates and targeting ability for lung carcinoma tumors through systemic intravenous or localized inhaled administration. Therapeutic efficiency of gene therapy can be improved by active targeting on specific lung cancer tumors or metastases through modification or conjugation of targeting agents on the surface of the nanoparticles. The use of polymer, lipid, metals, and carbon-based nanoparticle systems in the field of targeted siRNA delivery has grown tremendously and has demonstrated promising in vitro and in vivo therapeutic efficacy results. The challenges of delivering nanoparticle mediated siRNA therapy within the body, such as maintaining the nanoparticle stability and siRNA stability, controlling the biodistribution and pharmacokinetics, penetrating biological barriers and minimizing the potential toxicity of the nanoparticles needs to be considered and overcome before entering clinical trials. The field of nanomedicine will continue to expand in the areas of cell or tissue targeting ability, circulation longevity, improved aerosol pulmonary delivery, enhanced intracellular penetration, stimuli sensitivity, and carrier-mediated visualization through using different nanocarrier properties or surface functional moieties. To increase the application of nanoparticle systems in siRNA therapy to the clinic, standards in the examination of nanoparticle safety and evaluation of therapeutic efficacy should be established to guide the direction of research and development of siRNA loaded nanoparticle therapeutic interventions.

Conflict of Interest

None of the authors identify any conflict of interest.

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