MINI-REVIEW

Nanoparticle-mediated siRNA delivery systems for cancer therapy

Han Gao¹ | Ruoyu Cheng¹ | Hélder A. Santos¹,²

¹ Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
² Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland

Correspondence
Hélder A. Santos, Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki FI-00014, Finland.
Email: helder.santos@helsinki.fi

Abstract
The small interfering RNA (siRNA)-based therapeutics have raised great attention since the first RNA interference (RNAi)-derived drug, patisiran, was approved by the US Food and Drug Administration, which represented a landmark in the field of gene therapy. Given the properties of interfering disease-associated gene expression, RNAi machinery is regarded as an essential factor for preparing precise medicine. However, over the past few years, siRNA drugs are undergoing a period of clinical translation, in which the major hurdle is the limited efficient delivery strategies. Therefore, this mini-review mainly focuses on describing the state-of-the-art of the nanoscale platforms for delivering siRNA payloads, also addressing their applications in cancer therapy. Finally, the status of siRNA drugs under clinical trials is discussed, providing a comprehensive understanding on the field of oligonucleotide-mediated therapeutics.

KEYWORDS

cancer therapy, nanomedicine, nanoparticles, small interfering RNA

1 INTRODUCTION

In the past few decades, gene therapy has drawn great attention due to the possibility of molecule-manipulation efficacy, for instance, the RNA interference (RNAi)-based therapeutics.¹ Compared with conventional clinical therapies, gene therapy has higher sensitivity and specificity in terms of triggering the expression of tailored genes, which exhibit extraordinary alterations in the progress of tumorigenesis, metastasis, and immune responses.² Moreover, given that the side effects caused by extracorporeal therapy, such as the disappointed outcomes occurred on adjacent tissues or organs of patients with chemotherapy,³,⁴ RNAi therapeutics is prospected to overcome these barriers, thereby reducing disease burden and improving treatment efficiency.⁵ Nevertheless, the use of RNAi for therapeutics has to confront various challenges based on its stability and property. Despite forced efforts from several companies and researchers applying the small interfering RNAs (siRNAs) in biological research, the first clinical trials using naked siRNAs have generated unfavorable results due to the inflammatory toxicities and off-target effects.¹ siRNAs without any chemical modifications might encounter several barriers involving their easily degradation in serum, difficulties to across the cell membranes, or short half-life.⁶ Therefore, effective delivery systems are urgently needed to attenuate these drawbacks of siRNAs. In recent
years, owing to the feasible design and rational modifications of delivery strategies, substantial improvements have been created, especially the siRNA drug (patisiran) approved by the US Food and Drug Administration (FDA) in 2018, representing a milestone for clinical siRNA therapies.

The patisiran (Onpattro) story symbolizes the clinical translation of nanomedicines, which play a significant role on the treatment of multiple diseases. To date, drug delivery systems based on nanotechnology have made prominent progress, for instance, more than 10 lipid nanoparticle (LNP) systems to carry drugs into targeted tissues or organs have been approved by the FDA. The natural characteristics of nanoparticles, including low toxicity profiles, well-controlled and favorable biocompatibility, endow them great interest for delivering siRNA payloads. Given the challenges that RNAi-based drugs would encounter, using nanoparticles as vectors is likely to reduce these obstacles, followed by enhancing cellular uptake of siRNAs and optimizing silence efficacy.

Therefore, this mini-review aims to briefly describe the fundamental mechanisms of RNAi machinery and provide insights on the current status on the use of nanoparticles for siRNA delivery, as well as to discuss the applications of siRNA in cancer research and clinical therapy.

2 OVERVIEW OF RNAI

2.1 Brief description of the mechanisms of RNAi machinery

Until recently, RNAi-based therapeutics, such as siRNA, have been regarded as accelerating the process of precision treatment of human diseases. The RNAi mechanism hypothesis was formally introduced and characterized in the nematode worm by a finding published in 1998, in which researchers demonstrated that compared with single-stranded molecules, double-stranded RNA (dsRNA) aroused more generous interference effects. The key point was how dsRNA triggered RNAi to induce gene silencing. The clues found in plants and animals indicated that dsRNA could act as a guide for the identification of substrates for RNAi. Therefore, under the function of RNA helicase A (RHA), dsRNA could steer short anti-sense RNA (guide strand) to interact with Ago2 protein, whereas the passenger strand was cleaved, leading to the formulation of RNA-induced silencing complex (RISC) complex. After this, the single-stranded siRNA would be guided by RISC at the post-transcriptional point, inducing its complementary combination with endogenous mRNA based on base-pair binding. Then, the downstream gene was unable to express proteins and cleaved in the end.

Therefore, RNAi is viewed as an editable transcriptional machinery that combines technology of genomic editing and ribonucleic acid together. In this context, it is promising to conduct siRNAs for performing gene silencing work, especially targeting tumor-associated mRNAs. Unfortunately, one crucial question still remains that how to directly transport siRNA drugs into cytoplasm without inducing its degradation.

2.2 Limitations of siRNA technology

During the process of delivering siRNA payload in vivo, two major barriers are encountered: the extracellular and intracellular barriers, which mainly induced by characteristics of siRNAs (strong anionic charged, hydrophilicity) and the complex circumstances of innate systems. During the process of delivering siRNA payload in vivo, two major barriers are encountered: the extracellular and intracellular barriers, which mainly induced by characteristics of siRNAs (strong anionic charged, hydrophilicity) and the complex circumstances of innate systems. First, considering the natural properties and short length of siRNAs (21–23 nucleotides), they are easily to be recognized by endogenous enzymes in serum and filtered by the kidneys, inducing a temporary half-life. Furthermore, siRNAs have to encounter the hurdles to cross plasma membranes, given the negatively charged property of the cellular walls, which can repel siRNAs that are also anionic in nature. Lastly, under the systematic administration, the blood–brain barrier and immune recognition system also create challenges in order to get the siRNAs inside the unhealthy cells. The physiological impact of the reticuloendothelial system, also known as the mononuclear phagocytic system, cannot be negligible, which is responsible for conducting the process of endocytosis. Similar with other molecules, siRNAs cannot escape from the endosomal system, resulting in further challenging to arrive at the cytosol of the cells.

The aforementioned drawbacks on siRNAs delivery should be considered in order to enhance the way siRNAs can be delivered intracellularly. Therefore, it is important to explore practical platforms that are capable of overcoming these challenges and enhancing RNA transfection efficiency.

3 NANOPARTICLES FOR SiRNA DELIVERY

Since the limited properties of siRNA hamper its ability for gene silencing, many efforts have been undertaken to develop effective transportation tools for siRNA therapeutic. To date, siRNA carrier systems are divided into two parts: viral and nonviral. Virus-based systems, such as the lentiviral vectors, have shown high transfection efficiency for gene transfer. However, high risks of immunogenicity and off-target problems hindered its...
FIGURE 1 Extracellular barriers to RNAi therapy. (A) Endonucleases degrade siRNA in circulation; (B) the mononuclear phagocytic system, in particular the macrophages of the major clearance organs (e.g., liver, lungs, and spleen), removes siRNA from circulation; (C) tissue penetration of siRNA is hindered by charge repulsion between the anionic siRNA and the plasma membrane of endothelial cells, as well as tight junctions in selective regions (e.g., the blood–brain barrier) that require transcellular or paracellular transport to reach the target tissue. Reprinted with permission from Ref. 13; Copyright © WILEY publications

further clinical translation. In contrast, nonviral systems have been shown promising siRNA delivery, especially nanoparticle-based platforms. Researchers have focused on nontoxic systems with the aim of maximizing the unique features of nanoparticles, referred to biocompatibility and low cytotoxicity. Currently, nanoparticles like lipids, polymers, and hybrid ones represent the frequently used types for siRNA loading. For example, certain amounts of lipid-based formulations have been approved for drug delivery by the FDA, especially the LNP-based siRNA (Patisiran) for polyneuropathy therapy. Such advanced progress has opened up a new avenue for establishing siRNA trafficable methods, while the potential for clinical translation remains to be explored.

Therefore, in this section, we will focus on the latest nanoparticles applied for siRNA delivery with effective formulations both in vitro and in vivo.

3.1 Lipid nanoparticles

So far, the first siRNA drug (trade name Onpattro) approved by the FDA was successfully translated in vivo and was shown to induce considerable gene silencing efficiency with an LNP formulation. The essential properties of this LNP system are the low surface charge, size of a diameter of 100 nm or less, and high encapsulation efficacy under intravenous administration. In parallel, the LNP system is ionizable and neutral under physiological pH, compared with conventional lipids, which are likely to retain positive charge regardless of different pH conditions. In general, PEGylated cationic lipids and cholesterol are essential components of LNPs, in which the ionizable amino lipids are critical for constructing stable nucleic acid lipid particles. Typically, these lipid particles are consist of ionizable lipids, including amine head parts, hydrophobic tails, and the parts linked together head-to-tails. Different chemical structures of ionizable lipids performed diverse potency for nucleic acids delivery. In addition, another hurdle preventing the clinical use of LNPs is the liver clearance, as the predominant targeted cell, hepatocytes are responsible for clustering LNPs via different pathways. These mainly involve the robust apolipoprotein E-mediated low-density lipoprotein receptor pathway, as well as the more gradual N-acetyl-D-galactosamine (GalNAc)/asialoglycoprotein receptor pathway. Therefore, enhancing the bioavailability and reducing clearance ratio are crucial factors that should be taken into account for the design of LNPs to deliver RNAs.

To this end, a recent work demonstrated that certain numbers of ionizable amine lipids by varying formulations
based on the linker moieties, gaining insight on how to build new structures of LNP s, administered beyond liver. Since the variation in the linker region could efficiently influence the efficiency of the lipids’ delivery, three commonly amino molecules are used for surface modification: hydrazine, hydroxylamine, and ethanolamine.28 The LNPs were synthesized by using a microfluidic mixing device, and the system contained a cationic lipid, cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and PEGylated lipids in the molar ratios of 50:38:5:10:1.5, respectively.28 Among the established 14 lipids with different chemical structures, lipid-10 performed more effectively than the other groups. The composition of lipid-10 from head-to-tail regions was piperazine and linoleic fatty acid chains, respectively. These formulations contributed to enhance the transfection potency and endosomal escape. Additionally, lipid-10-based LNP encapsulated with Cy5-labeled siRNA (siCy5) showed gene silencing effects without triggering immune responses. These results were attributed to the linker effect, which are more prominent in the case of ethanolamine and hydroxylamine linkers, with pKa values of 6.2–6.5.28 All in all, this work showed the transport of siRNA payloads by sung LNPs with novel structures established for efficient biodistribution and less immune-activity.

3.1.1 Lipid-formulated platforms for imaging

In the past few years, efforts on visualizing the release of siRNA from endosomes have been conducted to comprehensively understand the events happening during endocytosis. In addition, as the most advanced tools for drug delivery, ionizable LNPs are capable of fusion with the membranes of the endosomes.27 However, conventional microscopy assays could not directly image release events or functional mechanism of siRNA, endosomal escape remains an obstacle hampered the efficacy of siRNA drugs.27 Recently, a specific compartment sharing early and late endosomal characteristics was monitored for analyzing the intracellular fate of siRNA payloads.27,31 In this approach, the majority of siRNA released events were happening in a very narrow stage, termed as “window of opportunity,” same as the time endosomes reached maturation.20 Meanwhile, the lysosome-associated membrane protein 1-mediated, macropinocytosis regulators (Rab5 and Rab7)-participated process was the dominant pathway in terms of LNP-based siRNA cytosolic release.27 Rb7, rather than Rb5, was more responsible for enhanced RNAi efficiency due to its importance in early endosomal maturation. In addition, it was demonstrated that siRNA release mainly occurred in a moderately acidic chamber.31 All these results proposed an innovative perspective on characterizing siRNA escaped events, provided fundamental knowledge on improving the end fate of cytosolic siRNA release. Future studies focused on at least two parts: (1) whether the alteration of the specific stage had an impact on the siRNA release and gene knockdown efficiency; and (2) explore other systems to characterize endosomal entrapment,20,32 for example, polymers and chemically stabilized siRNA molecules.

3.2 Polymeric nanoparticles

In addition to lipids, polymers are also commonly used for delivery siRNA payloads. Under systematic design of polymeric structures, up to now, there are certain widely recognized components of polymers applied for siRNA delivery, including chitosan (CS), poly(lactic-co-glycolic acid) (PLGA), poly-L-arginine, and polyethylenimine (PEI).28,33 Cationic polymers can interact with siRNAs that are robustly negative charged via electrostatic associations, binding between the amino groups and phosphate groups of siRNA, which are beneficial for avoiding the chemical modification of siRNAs.33 However, previous studies demonstrated that some parameters should be considered to optimize the polymer/siRNA formulation, for example, the relative proportion (charged groups and concentration) of the polymer-based nanovector.34 In addition, the complexes composed of polymeric compositions are viable formulations that could combine the advantages of each component.

CS is a natural cationic polysaccharide with favorable biocompatibility and low toxicity, endowing its capacity to bind with siRNA via charge-to-charge connections.35 Nevertheless, the low solubility of CS is a major obstacle for its application in the synthesis of nanomedicines. To circumvent this problem, previous study36 designed a core-shell platform for siRNA delivery, in this system, PLGA (lactide:glycolide, 75:25) served as a solid shell to prevent the degradation of siRNA and protect the innate core. This nanoparticle presented a size of 110±5.7 nm in diameter with negatively charged surface (−22.4±2.1 mV) at pH 7.0.36 In particular, as the reagent bound with siRNA, the CS (1 kDa) was modified by guanidine group (chitosan–guanidinate [CG]). The purpose of this formulation was to enhance the endo/lysosomal escape in a pH-dependent manner.36,37 CG could react with CO2 reversibly, which means that certain amounts of CO2 were released under acidic pH, such as the endo/lysosomal interior, working like a “nano-bomb.” This pH-responsive nanoparticle showed low cytotoxicity and good biocompatibility across different cell lines, and the siRNA/NPs targeted
RNA Polymerase II Subunit A (POLR2A) significantly reduced the gene expression and inhibited POLR2A loss tumor growth by ~80% in vivo.36 Subsequent research built a CS (20 kDa) based platform coated with cancer cell membrane (CCM), obtained from DOX-resistant HeLa/DOX cells, aiming to increase the tumor-homing capacity of this delivery system.37 The mass ratio of CCM/CS/siRNA was 7.5, in which point the particle size was 122.39±4.69 nm, with a zeta-potential of −27.76±3.12 mV. Further results showed this nanoparticle with good stability and high transfection efficiency (~60% at 72 h).37 Of note, as a result of the CCM modification, the tumor volume was reduced by 328±48 mm in vivo anticancer studies. In addition, other functional groups, such as the PEI,38 have been adopted to optimize the formulations by modifying the backbone of CS. In brief, CS was modified with PEI by a reductive amination reaction, and then a pH-sensitive linker 4-hydrazinovenzoic (HBA) was attached to fabricate this nanoparticle.38 As a result, this CS-based vector showed high siRNA encapsulation potency (~81.3% at 10 h) and considerable antitumor effects (~88.0%) in vivo.

3.3 Hybrid nanoparticles

Albeit the properties of polymers and lipid-based nanoparticles, many of them are still under test for further clinical use, with the consideration of the existent drawbacks, for example, excessive charge interactions and limited cytosolic siRNA released.39,40 Considering the latter aspect, an integrated fabrication of the nanosystems has given a unique insight into nanomedicines, holding the advantages of combining together characteristics of different nanovectors. An innovative hybrid system specifically targeted macrophages in atherosclerotic lesions was introduced by the Shi et al.41 First, a cationic G0-C14 incorporated with siRNA was surrounded with PLGA to fabricate a stable inner core. Then, a S2P peptide (CRTLTVRKC) was used for the purpose of specific targeting macrophages in a ligand-receptor binding mode. The ratio of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)]-S2P (DSPE-PEG-S2P/DSPE-PEG) was considered and adjusted in order to optimize the nanoparticle formulations.41 Results suggested that the proportion of S2P should be kept at no more than 50%, in case of overlaying PEG-layer and weakening stability of the whole platform. Based on this delivery strategy, the expression level of a plaque-destabilizing gene was significantly decreased by ~60%, resulting in less necrotic core area thereby enhancing plaque stability, and little toxic effect in mice after intravenously injection (1 nmol siRNA per mouse).41 Another study utilized similar components to formulate a pH-responsive nano-vehicle, encapsulating with mitoxantrone (MTO)-based prodrug.40 In this case, the therapeutic siRNA and MTO prodrug were incorporated into the tumor microenvironment (TME) polymer, coated with a PEG shell (N/P molar ratio: 15, particle size ~100 nm). Such kind of structure increased siRNA encapsulation efficiency (EE) by ~50%, prolonged blood circulation, and enhanced siRNA released potency across the tumor tissues, resulting in seven-fold higher accumulation compared with naked siRNA (at a dose of 1 nmol siRNA per mouse).40

Furthermore, the liposomal-based system is another commonly used platform for delivering siRNA due to its similar compositions with endogenous cell membranes. However, original liposomes suffer from low cellular internalization and short circulation time during transportation, leading to transit stay and inefficient knockdown.13 Consequently, modifications on the surface of liposomes are necessary to address these barriers, such as PEG, approved by the FDA and recognized as gold standard for modifying with the ability of increasing hydrophilicity and stability.42 Other options are to dope traditional liposomal formulation with gold nanoparticles, termed as “auroliposomes.”43 In this case, the liposomes are constructed with certain types of components, where PE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]) is used for extending circulation time, DOTAP (1,2-dioleoyl-3-trimethylammonium-propane), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) for charge modulation, and the low-level Tween 20 for promoting cellular uptake.43 The siRNA EE increased to ~90% due to the incorporation of a 20-nm gold nanoparticle, compared with the commercial transfection reagents and traditional cationic nanoparticles. Such finding demonstrated the potential benefits of hybrid nanoparticles not only conquer the shortcomings of small molecules drugs, but also extend attractive effects of different kinds of nanoparticles combined.

Other hybrid nanoparticles have been prepared by a particular kind of biomimetic engineering-based siRNA delivery system. In this context, porous metal-organic framework (MOF) nanoparticles were coated by cell-derived platelet membrane.44 The formulation of this P-MOF-siRNA nanoparticles incorporated 500 nm siRNA loading.45,46 As an integrated nanosystem, hybrid nanoparticles are holding promising potential for further translation and application into the clinic.
3.4 Porous silicon nanoparticles

Porous silicon nanoparticles (pSiNPs) are relatively new nanoscale materials as the siRNA host. However, the ease of surface functionalization and tunable pore sizes of pSiNPs, together with high biocompatibility and degradability, make them feasible as delivery platform for siRNA. For example, The Voelcker’s group has focused on modifying the surface chemistry of pSiNPs by several amine-groups, including diethylenetriamine, tris(2-aminoethyl) amine, poly(ethylene glycol) diamine, and polyamidoamine (PAMAM) dendrimers generations 1–4 (G1-G4). The loading capacity of PAMAM(G4)-pSiNPs (413 μg siRNA/mg pSiNPs) was ∼73.8%, which was maximum value among groups in this work, and also higher compared with previous works. Additionally, the release rate of siRNA payloads was 27% in 72 h, suggesting the protective role of PAMAM(G4)-pSiNPs. Based on this formulation, a knockdown efficiency at 93% was observed in protein expression by loading with siRNA agents. Another work of this group has fabricated pSiNPs/siRNA coated with PEI, aiming to improve the colloidal stability and control the release. This PEI-capped pSiNPs showed extended EE at 70 ± 9%, with a dosage of 23 μg siRNA/250 μg PEI-pSiNPs. Multidrug resistance-associated protein 1 (MRP1) gene silencing effect was ∼65% with MRP1 siRNA in vitro, compared with the scrambled group. In parallel, in vivo studies (dose of 31.25 mg/kg of NPs per mouse) showed that the mRNA and protein expression level of MRP1 were reduced by 90% and 65%, respectively.

Recently, it has been of great interest to include pSiNPs and other kind of nanoparticles, such as a fusogenic (FNP) lipid. Briefly, the pSiNPs/siRNA complexes were coated with an FNP, which was synthesized with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), DSPE-PEG, and DOTAP at molar ratios of 76.2:3.3:20. The FNP coating has the ability to prevent siRNA release (20% in 24 h) and hinder pSi dissolution. Furthermore, since the important role of FNP lipid on endosomal escape, authors also fabricated a pSiNPs/siRNA/Lyp-1 peptide (CGNKRTRGC)-conjugated FNP nanoplatform, which specifically homed to tumor-associated macrophages (TAMs). In this way, siRNA payloads against PI3k, a mediator of immunosuppression during tumorigenesis, showed ~85% knockdown efficiency in vitro. In line with its in vivo therapeutic effect was 81% in 24 h after intraperitoneal (IP) injection (at 65 μg/kg of siRNA dose).

4 SIRNA THERAPEUTICS STRATEGY APPLIED IN THE TREATMENT OF CANCER

Usually, a solid tumor has always a complicated microenvironment, which consists of not only cancer cells, but also immune cells, such as macrophages, regulatory T cell, fibroblast, endothelial cells, and so on. Besides cancer cells, these cells also contribute to tumor growth, metastasis, and recurrence. Thus, in terms of cancer treatments, the function of siRNA is not limited to the elimination of cancer cells. Thus, in this section, we will discuss about the interaction between siRNA and different cells in the process of cancer therapy.

4.1 Macrophages

In human immune systems, macrophages take part in both innate immune response and adaptive immune response by detecting, engulfing, and destroying the related pathogens and apoptotic cells. As an antigen-presenting cell, macrophages can also present a protein attached to a major histocompatibility complex (MHC) class II molecule, which can give a signal to other white blood cells, such as helper T cells and cytotoxic T cells. In this way, macrophages are involved in diverse immune responses in the human immune systems, such as inflammation, regeneration, and cancer. As one of the danger signals, cancer cells are the targeted cells for the macrophages. However, these cancer cells develop different strategies to avoid elimination. For example, cancer cells can present CD47 a “don’t eat me” signal that interacts with signal regulatory protein alpha on macrophages to prevent phagocytosis. Some researchers indicated that blocking the CD47 cannot only enhance the phagocytosis of cancer cells by macrophages, but also boost the anti-tumor T cells immunity. Moreover, cancer cells secrete stimulating factors polarizing the TAMs from an anti-tumor M1 phenotype to a tumorigenic M2 phenotype. These M2 macrophages cannot only limit the cytotoxic effects of chemotherapy, but also secrete cytokines to promote the metastasis of cancer cells. They also contribute to the tumor suppressive microenvironment, tumor metastasis, and recurrence. Therefore, targeting and manipulating these tumor-associated M2 macrophages (TAMs) are promising strategies for cancer immunotherapy treatment.

One of the strategies would involve targeting the TAMs with siRNA to silence certain genes that take part in the
M2 function. Since the major challenge for siRNA-based therapeutics is their delivery, Shobakietal. prepared LPNs by mimicking the envelope-type viruses that followed a novel packaging strategy. The LPNs were composed of CL4H6 lipid (a pH-sensitive cationic lipid), in addition to cholesterol (cholesterol) and PEGylated-lipids, which led to a high EE of siRNA (> 90%). Furthermore, the intravenously injection of siRNA-loaded LPNs into the institute of cancer research mice, the LPNs modified with 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG 2000) at a level of 1 mol-% of total lipid resulted in minimal siRNA leakage and higher integrity and stability of the LNP in the blood circulation. Additionally, DSG-PEG 2000 modified LPNs slightly decreased the siRNA accumulation in the liver and increased the siRNA accumulation in the spleen and tumor area. Particularly, various cell populations in the TME were analyzed, CL4H6-LNPs were extensively taken-up by leukocytes (CD45+ cells) in general and by TAMs (CD45+, CD11b+, and F4/80+ cells). In addition, CL4H6-LNPs induced ~70% gene silencing of the model target gene (CD45) at the protein level, and a significant silencing (~80%) of hypoxia-inducible factor 1-alpha, as well as signal transducer and activator of transcription 3 (STAT3). Furthermore, Mp infiltration in the TME was increased, the protumorous functions of TAMs, such as angiogenesis and tumor cell activation/invasiveness, were inhibited, which finally resulted in the significant antitumor therapeutic response.

Besides silencing the certain functional genes in the TAMs, another strategy is reprogramming the immunosuppressive and profibrotic M2 phenotype to an immune stimulating and antifibrotic M1 phenotype through siRNA. Leber etal. prepared α-mannosyl-functionalized cationic nanohydrogel particles loading siRNA by electrostatic interactions (ManNP), aiming to target M2 macrophages and reprogram it. The in vitro study, comparing with untargeted nanoparticles (NonNP), ManNP exhibited lower accumulation in the nonspecific cells, such as hepatocytes (HepG2), endothelial cells (SVEC4-10), human macrophages (THP-1), and murine macrophages (RAW 264.7); in addition, ManNP showed more accumulation in M2 macrophages than M1 and M0 macrophages. Furthermore, significant knockdown was observed in M2 polarized primary macrophages rather than the (low CD206 expressing) M1 phenotype, which provide a method for the further investigations in reprogramming the M2 to M1 phenotype macrophages.

4.2 | Fibroblasts

Usually, fibroblasts are quiescent and become activated in the wound healing process, and they are identified by the expression of α-smooth muscle actin (αSMA; also known as ACTA2), a cytoskeletal protein associated with smooth muscle cells. The activated fibroblasts may have the extracellular matrix (ECM) remodeling ability, robust autocrine activation, and dynamic immunomodulatory signaling function. This process is associated with persisting and unabated injurious stimulation, such as cancer development and organs fibrosis. Tumor stroma usually consists of immune cells, capillaries, basement membrane, activated fibroblasts, ECM, and cancer cells. As one of the dominant components, tumor-associated fibroblasts (TAFs) were proved to take part in cancer progression and metastasis, which indicates that these activated TAFs contribute to a wide range of fibrotic stromal programs of many different tumors. Tumor stroma in the advanced stage of cancer includes increased amounts of various types of collagens, laminins, fibronectins, proteoglycans, peristin, and tenascin C, among others. Studies have shown that the activated fibroblasts regulate cancer progression via their active secretome, which includes growth factors and ECM. Thus, regulating the activated TAFs to be inactivated could be a strategy for the cancer treatment. Other studies indicated that eliminating TAFs could enhance the efficacy of chemotherapy on prostate tumors. However, some studies demonstrated that depletion of α-SMA fibroblasts increased the risk of tumor invasion or migration. Therefore, Lang etal. decided to reverse the function of TAFs instead of depleting TAFs. C–X–C motif chemokine ligand 12 (CXCL12) is a multiple function proinflammatory mediator that is mainly secreted by activated TAFs in tumors. In addition, CXCL12 directly activates a variety of responses through CXCL12/CXCR4 pathways in the TME, such as cell migration, survival, and proliferation. Authors prepared cell-penetrating peptide (CPP, nine-arginine (R9))-based self-assembly nanoparticles loaded with a CXCL12 silencing siRNA (siCXCL12), and further modified the nanoparticles surface with fibroblast activation protein-α monoclonal antibodies (anti-FAP-α mAb). They found that this nanosystem can specifically deliver siRNA into TAFs via targeting the FAP-α on the cell membrane of TAFs, thereby knocking down the expression of CXCL12 genes in TAFs, which could further inactivate TAFs and reshape the TAF-related malignant TME, as shown in Figure 2. As a result, tumor cell migration, invasion, and tumor angiogenesis were significantly inhibited, and therefore, the metastasis of orthotopic prostate tumor was suppressed.

Moreover, some studies also found that tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Specifically, authors cultured four liver cancer cell lines (CSQT-2 and HCC-LM3 were high-metastatic cancer cells vs. HepG2 and MHCC-97L) with different migration and
FIGURE 2  Activity and secretion profile changes of CAFs: (A) Confocal microscopy images of immunofluorescence. (B) Quantitative analysis of the normalized α-SMA expression from IF results in panel A. (C) Cell cycle analysis of CAFs in different formulation treatment groups. (D) Percentage of cells arrested at G0/G1, S, and G2/M. (E) Differential expression of cytokines from CAFs after treatment detected by the proteome array chip. (F) Differential expression of angiogenesis-related factors from CAFs after treatment detected by an angiogenesis proteome array chip. Reprinted with permission from Ref. 69; Copyright © ACS Publications
invasion abilities with MRC5, a kind of human embryonic lung fibroblasts. They proved that high-metastatic cancer cell-derived exosomes could improve the fibroblasts migration ability remarkably, leading to the high expression of proinflammatory genes, such as IL-1β, IL-6, IL-8, TGF-β, CXCL12, Collagen type I (COL1A1), Collagen type III (COL3A1), and Collagen type IV (COL4A1). These cytokines can modulate the inflammation microenvironment and promote carcinoma development. Furthermore, they identified the MiR-1247-3p as the trouble marker from abundant exosomes MiRNAs, which can lead to the fibroblast’s activation through B4GALT3-β1-integrin-NF-κB axis (B4GALT3 is the targeted gene of MiR-1247-3p in fibroblasts). Priming by the MiR-1247-3p, fibroblast can promote tumor stemness, epithelial-mesenchymal transition, chemoresistance, and tumorigenicity in liver cancer. Additionally, they observed that hepatocellular carcinoma patients suffering from lung metastasis showed higher levels of miR-1247-3p expression in serum exosomes than in patients without lung metastasis, with high miR-1247-3p expression correlated with increased alpha-fetoprotein level, liver cirrhosis, tumor thrombus, and distant metastasis. This study provides a solid foundation for the further siRNA therapy by interfering the expression of MiR-1247-3p.

4.3 | Dendritic cells

In addition to macrophages, dendritic cells (DCs) are another type of antigens presenting cells. They can express the costimulatory molecules and inflammatory cytokines, and induce the activation of T cells.71 In this way, they serve a protective role in antitumor immunity. Additionally, DCs are often called “nature’s adjuvants,” and thus have become the natural targets for antigen delivery. DCs also promote immunosuppression by secreting anti-inflammatory cytokines or expressing negative immunological checkpoint molecules that inhibit T cell activation.72 For example, the activation of signal transducer and activator of transcription-3 (STAT3) in DCs influences the DCs’ response to the toll-like receptor stimulation leading to the failure of DC maturation, which means that the activation of STAT3 shows a positive correlation with a suppression and immune tolerance.73 In order to stimulate the expression of tumor antigens and suppress of immunosuppressive proteins in DCs, Lee et al. incorporated extra RNAi sequence at the 3’end of poly A tails in the mRNA.74 After that, they introduced additional DNA thymine sequence to the 3’end of the complementary RNAi strand to form DNA/RNA hybrid between the double-stranded siRNA and the in vitro transcribed mRNA (IVT). This designed DNA/RNA hybrid can be readily cleaved by cytosolic RNase H after intracellular delivery to the DCs. After separating from each other, both IVT mRNA and the siRNA can function normally, which was proved by the successful antigen presentation and DC maturation in both in vivo and in vitro studies.

In the immunosuppressive state of the TME, the interaction between DCs and T cells is also influenced. For example, the inhibitory immune checkpoint molecules, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), is one of the main barriers in priming T cells by DCs. Through the ionic gelation, Esmaily et al. prepared siRNA-loaded CS-lactate to suppress the expression of CTLA-4 molecule on tumor-infiltrating T cells. In addition, they also fabricated the tumor-lysate-loaded DC vaccine to facilitate priming antitumor T cells.75 The administration of anti-CTLA-4 siRNA-loaded nanoparticles into CT26 and 4T1 tumor-bearing mice led to the downregulation of CTLA-4 on tumor-infiltrating T cells, which was associated with tumor regression and increased mouse survival. Moreover, the administration of DC vaccines in tumor-bearing mice caused mild therapeutic outcomes, and the combination of DC vaccines and siRNA-loaded nanoparticles showed synergistic antitumor effects attributed to the reduction of immunosuppressive cells, improved cytotoxicity of T lymphocytes, decreased inhibitory, and increased inflammatory cytokines, as well as reduced angiogenesis and metastasis processes.

5 | CLINICAL APPLICATIONS OF SIRNA THERAPEUTIC STRATEGY

As a therapeutic method, siRNA has its unique advantages comparing to the small molecular therapeutics and monoclonal antibody drugs.76 Additionally, siRNA can target to any interested genes as long as the right nucleotide sequence along the targeting mRNA is selected, which means the siRNA therapeutics could have shorter research and development span, and wider therapeutic area. Encouraging by the first FDA-approved ONPATTRO® (patisiran, ALN-TTR02), the first commercial RNAi-based therapeutic for the treatment of hereditary amyloidogenic transthyretin (hATTR) amyloidosis with polyneuropathy in adults in 2018, recently more and more researchers treat siRNA therapeutics as a next generation of novel therapeutic method.77

5.1 | Transthyretin amyloidosis

Transthyretin amyloidosis is a slowly progressive condition featured by the formation of abnormal deposits of a protein called amyloid (amyloidosis) in the body’s
organs and tissues. These deposited proteins usually appear in the peripheral nervous system, which is made up of nerves connecting the brain and spinal cord to muscles and sensory cells that detect sensations, such as touch, pain, heat, and sound. Deposited proteins in these nerves lead to a loss of sensation in the extremities. Currently, APOLLO-B (from Alnylam Pharmaceuticals) are under phase 3 study to evaluate patisiran in participants with transthyretin amyloidosis with cardiomyopathy. Patisiran safety was consistent, regardless of the concomitant or prior transthyretin stabilizers. In the phase II open-label extension (n = 225), patisiran-treated groups showed stabilization or improvements in neurological function (modified Neuropathy Impairment Score +7) and quality of life (Norfolk Quality of Life Diabetic Neuropathy questionnaire) at 18 months, regardless of the concomitant or prior transthyretin stabilizers. In APOLLO (n = 225), patisiran-treated groups showed stabilization or improvements in the hepatic clearance. 

5.2 | Porphyria

Porphyria is a group of disorders caused by abnormalities in the chemical steps leading to heme production. According to the genetic cause and their signs and symptoms, researchers have identified several types of porphyria, such as cutaneous porphyrias, acute porphyrias, and hereditary coproporphyria and variegate porphyria. A clinical trial (a study to evaluate the efficacy and safety of Givosiran [ALN-AS1] in patients with acute hepatic porphyrias) has been finished by the Alnylam Pharmaceuticals. Givosiran was rapidly absorbed from the subcutaneously injection site with peak plasma concentrations achieved within 0.5–5 h followed by elimination with a short half-life of 4–10 h. Plasma exposures of AS(N−1)3′ givosiran, an active metabolite with equal potency as givosiran, was 35–75%. Givosiran treatment resulted in a rapid and dose-dependent reduction in urinary aminolevulinic acid and porphobilinogen toward the upper limit of normal in acute hepatic porphyria patients.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

Overall, the approval of patisiran has taken RNAi therapeutics one-step further than ever before. Despite the limited number of efficient delivery strategies, there are still certain NP-based platforms under clinical trials, holding great potential on achieving siRNA therapeutics. Moreover, this review focused on discussing the advanced developments on nanocarriers for siRNA delivery, with multiple options on optimizing structural formulations. It may allow us working on materials to grasp design elements during formulation, considering the problems encountered and the requirements needed for the nanoformulations. For instance, some problems like endocytosis could be avoided by incorporating an FNP lipid into nanocomplexes, or alternatively conjugate a peptide to also tackle such problems. In the future work, a closer collaboration between chemists and biologists might be beneficial for developing functional systems on the field of RNAi therapy. In addition, in order to generate rational nanoplatforms, the following aspects should be taken into consideration: (1) biosafety and biodegradability of the materials used in the nanoformulations; (2) optimization of the formulations to achieve efficient endo/lysosomal escape; and (3) enhanced specific targetability of the nanosystems to target the desired cells/tissues, avoiding the hepatic clearance.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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ORCID
Han Gao https://orcid.org/0000-0002-2457-8152
Ruoyu Cheng https://orcid.org/0000-0002-7258-9489
Hélder A. Santos https://orcid.org/0000-0001-7850-6309

REFERENCES
1. R. L. Setten, J. J. Rossi, S.-P. Han, Nat. Rev. Drug Discov. 2019, 18, 421.
2. Y. Xin, M. Huang, W. W. Guo, Q. Huang, L. Z. Zhang, G. Jiang, Mol Cancer. 2017, 16, 134.
3. J. H. Lorch, O. Goloubeva, R. I. Haddad, K. Cullen, N. Sarlis, R. Tishler, M. Tan, J. Fasciano, D. E. Sammartino, M. R. Posner, Lancet Oncol. 2011, 12, 153.
4. A. Rühle, P. E. Huber, R. Safrich, R. Lopez Perez, N. H. Nicolay, Int. J. Cancer. 2018, 143, 2628.
5. C. Denkert, C. Liedtke, A. Tutt, G. von Minckwitz, Lancet. 2017, 389, 2430.
6. B. Hu, L. Zhong, Y. Weng, L. Peng, Y. Huang, Y. Zhao, X.-J. Liang, Signal Trans Target Ther. 2020, 5, 101.
7. A. Akinc, M. A. Maier, M. Manoharan, K. Fitzgerald, M. Jayaraman, S. Barros, S. Ansell, X. Du, M. I. Hope, T. D. Madden, B. L. Mui, S. C. Semple, Y. K. Tam, M. Ciufolini, D. Witzigmann, J. A. Kulkarni, R. van der Meel, P. R. Cullis, Nat. Nanotechnol. 2019, 14, 1084.
8. T. M. Allen, P. R. Cullis, Adv. Drug. Deliv. Rev. 2013, 65, 36.
9. M. K. Nguyen, C. T. Huynh, A. Gilewski, S. E. Wilner, K. E. Maier, N. Kwong, M. Levy, E. Alsberg, Sci. Adv. 2019, 5, eaax0801.
59. N. Shobaki, Y. Sato, Y. Suzuki, N. Okabe, H. Harashima, J. Control. Release. 2020; 325, 235.
60. N. Leber, L. Kaps, A. Yang, M. Aslam, M. Giardino, A. Klefenz, N. Choteschovsky, S. Rosigkkeit, A. Mostafa, L. Nuhn, Macromol. Biosci. 2019, 19, 1900162.
61. R. Kalluri, Nat. Rev. Cancer. 2016, 16, 582.
62. E. Sahai, I. Astsaturov, E. Cukierman, D. G. DeNardo, M. Egeblad, R. M. Evans, D. Fearon, F. R. Greten, S. R. Hingorani, T. Hunter, Nat. Rev. Cancer. 2020, 20, 174.
63. R. Kalluri, M. Zeisberg, Nat. Rev. Cancer. 2006, 6, 392.
64. B. Rybinski, J. Franco-Barraza, E. Cukierman, Physiol. Genomics. 2014, 46, 223.
65. A. Kudo, I. Kii, J. Cell. Commun. Signal. 2018, 12, 301.
66. B. Erdogan, D. J. Webb, Biochem. Soc. Trans. 2017, 45, 229.
67. L. Miao, Y. Wang, C. M. Lin, Y. Xiong, N. Chen, L. Zhang, W. Y. Kim, L. Huang, J. Control. Release. 2015, 217, 27.
68. B. C. Özdemir, T. Pentcheva-Hoang, J. L. Carstens, X. Zheng, C. C. Wu, T. R. Simpson, H. Laklai, H. Sugimoto, C. Kahler, S. V. Novitskiy, Cancer Cell. 2014, 25, 719.
69. J. Lang, X. Zhao, Y. Qi, Y. Zhang, X. Han, Y. Ding, J. Guan, T. Ji, Y. Zhao, G. Nie, ACS Nano. 2019, 13, 12357.
70. T. Fang, H. Lv, G. Lv, T. Li, C. Wang, Q. Han, L. Yu, B. Su, L. Guo, S. Huang, Nat. Commun. 2018, 9, 1.
71. Y. Wang, Y. Xiang, V. W. Xin, X.-W. Wang, X.-C. Peng, X.-Q. Liu, D. Wang, N. Li, J.-T. Cheng, Y.-N. Lyv, J. Hematol. Oncol. 2020, 13, 1.
72. T. F. Gajewski, H. Schreiber, Y.-X. Fu, Nat. Immunol. 2013, 14, 1014.
73. M. T. Brady, A. Miller, S. N. Sait, L. A. Ford, H. Minderman, E. S. Wang, K. P. Lee, H. Baumann, M. Wetzler, Leuk. Res. 2013, 37, 822.
74. K. Lee, T.-S. Kim, Y. Seo, S. Y. Kim, H. Lee, J. Control. Release. 2020, 327, 225.
75. M. Esmaily, A. Masjedi, S. Hallaj, M. N. Afjadi, F. Malakotikhah, S. Ghani, A. Ahmadi, M. Sojoodi, H. Hassannia, F. Atyabi, J. Control. Release. 2020, 326, 63.
76. G. Ozcan, B. Ozpolat, R. L. Coleman, A. K. Sood, G. Lopez-Berestein, Adv. Drug Deliv. Rev. 2015, 87, 108.
77. B. Hu, Y. Weng, X. H. Xia, X. j. Liang, Y. Huang, J Gene Med. 2019, 21, e0097.
78. T. Coelho, D. Adams, A. Silva, P. Loizzer, P. N. Hawkins, T. Mant, J. Perez, J. Chiesa, S. Warrington, E. Tranter, N. Engl. J. Med. 2013, 369, 819.
79. H. Lin, M. Merkel, C. Hale, J. L. Marantz, Neurodegener. Dis. Manag. 2020, 10, 289.
80. H. Thadani, A. Deacon, T. Peters, BMJ. 2000, 320, 1647.
81. S. Agarwal, A. R. Simon, V. Goel, B. A. Habtemariam, V. A. Clausen, J. B. Kim, G. J. Robbie, Clin. Pharmacol. Ther. 2020, 108, 63.

**AUTHOR BIOGRAPHIES**

Han Gao received her B.M. in preventative medicine from the North China University of Science and Technology (2017), and M.D. in health science from the Southeast University (2020). Currently, she is a Ph.D. candidate working under the supervision of Prof. Hélder A. Santos at the University of Helsinki. Her research focuses on developing nanomedicine for gene therapy.

Ruoyu Cheng got her MD master’s degree from The First Affiliated Hospital of Soochow University (China, 2018). Her previous work was mainly focusing on biomaterials for drug delivery and biomedical engineering. Currently, she is a PhD student in pharmacy under the supervision of Prof. Hélder A. Santos at the University of Helsinki, and her major is developing nanoparticles for the cancer immunotherapy.

Hélder A. Santos obtained his Doctor of Science in Technology (Chemical Engineering) in 2007 from the Helsinki University of Technology. Currently, he is a Full Professor at the Faculty of Pharmacy, University of Helsinki, and Head of the Nanomedicines and Biomedical Engineering Lab. His scientific expertise lies in the development of nanoparticles/nanomedicines for biomedical applications, particularly porous silicon and polymeric-based nanomaterials for simultaneous controlled drug delivery, diagnostic, and therapy for cancer, diabetes, and cardiovascular diseases.

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