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Review

Emergence of Small Interfering RNA-Based Gene Drugs for Various Diseases

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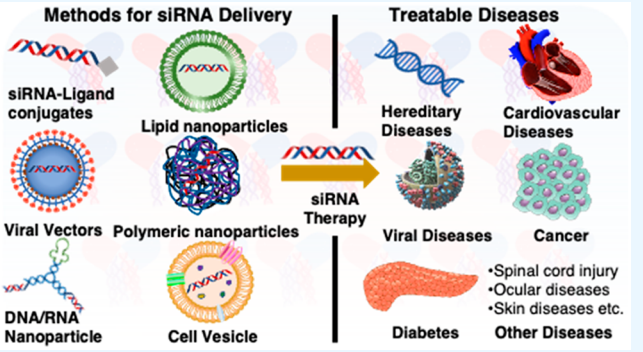
ABSTRACT: Small molecule, peptide, and protein-based drugs have been developed over decades to treat various diseases. The importance of gene therapy as an alternative to traditional drugs has increased after the discovery of gene-based drugs such as Gendicine for cancer and Neovasculgen for peripheral artery disease. Since then, the pharma sector is focusing on developing gene-based drugs for various diseases. After the discovery of the RNA interference (RNAi) mechanism, the development of siRNA-based gene therapy has been accelerated immensely. siRNA-based treatment for hereditary transthyretin-mediated amyloidosis (hATTR) using Onpattro and acute hepatic porphyria (AHP) by Givlaari and three more FDA-approved siRNA drugs has set up a milestone and further improved the confidence for the development of gene therapeutics for a spectrum of diseases. siRNA-based gene drugs have more advantages over other gene therapies and are under study to treat different types of diseases such as viral infections, cardiovascular diseases, cancer, and many more. However, there are a few bottlenecks to realizing the full potential of siRNA-based gene therapy. They include chemical instability, nontargeted biodistribution, undesirable innate immune responses, and off-target effects. This review provides a comprehensive view of siRNA-based gene drugs: challenges associated with siRNA delivery, their potential, and future prospects.



1. INTRODUCTION

Gene therapy is one of the most advanced biotechnologies in medicine.[1] Gene therapy is defined as the correction of defective/disease-causing gene/s by transferring genetic medicine such as DNA/RNA to a patient's cells to deactivate, replace, or repair.[2,3] The first gene manipulation was attempted as early as 1989 when human DNA is modified by direct insertion of DNA into the nuclear genome.[4] Later on, several scientific groups developed various types of gene-based drugs.[5] The nucleic acid-based gene drugs broadly can be categorized into DNA, RNA, and artificial nucleotide (XNA)-based drugs. DNA-based drugs include plasmids, antisense oligonucleotides based on DNA, DNA aptamers, and DNAzymes.[6−8] Likewise, RNA-based drugs can be further categorized as RNAi (viz., siRNA and miRNA), shRNA, mRNA, RNA-based antisense oligonucleotides, ribozyme, and aptamer-based drugs.[7,9,10] XNA-based gene drugs mainly fall under antisense oligonucleotide-based drugs.[11] CRISPR/Cas9 system, chimeric antigen receptor T (CAR T) cell therapy for cancer, and stem cell therapy also come under gene therapy.[12] Gene therapy mainly works by three methods: (i) Introducing a healthy copy of a gene in place of a disease-causing gene, (ii) inactivation of a nonfunctioning disease-causing gene, and (iii) treating disease by introducing a new copy of a modified gene into the body.[13] The nucleic acid-based gene drugs are studied to treat several diseases including cancer, viral infections, heart diseases, and rare genetic diseases.[14−16] By 2021, more than 2600 gene therapy trials are going on worldwide.[17,18] For example, Luxturna is a DNA-based gene drug to treat inherited retinal disease by targeting retinal pigment epithelium-specific 65 (RPE65),[19] Spinraza is an antisense oligonucleotide to treat spinal muscular atrophy by correcting the Survival of motor neuron 2 (SMN2) gene,[20] Patisiran is a siRNA-based gene-drug to treat familial amyloidotic polyneuropathy by targeting Transthyretin (TTR) gene,[21] Givosiran is also a siRNA-based gene-drug to cure acute hepatic porphyria by targeting δ-aminolevulinate synthase 1 (ALAS1),[22] and Golodirsen is an antisense oligonucleotide-based gene-drug to treat Duchenne muscular dystrophy (DMD) by targeting the mutated dystrophin gene.[23] The single-stranded antisense oligos silences a gene (mRNA) by following an RNaseH-dependent mRNA cleavage mechanism.[24] Kynamro is an another

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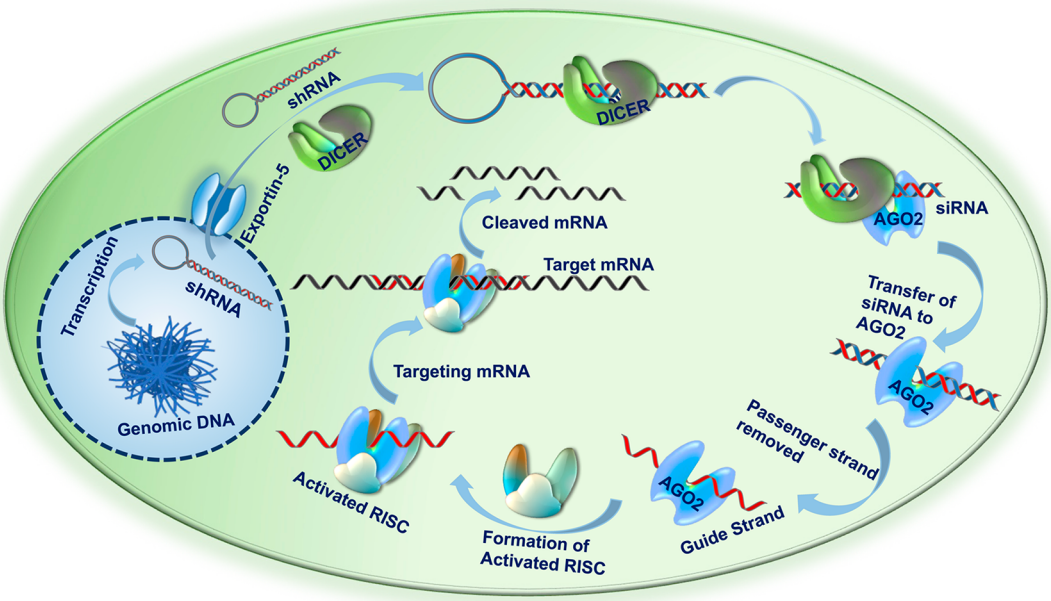

Figure 1. Schematic representation of the natural siRNA mechanism and target mRNA degradation.

antisense oligonucleotide-based gene-drug to treat homozygous familial hypercholesterolemia by deactivating the apolipoprotein B-100 (apoB-100) gene translation.[25] Besides, cell-based therapies are also considered as gene therapy, for example, Yescarta is a CAR-T-based gene therapy where T-cells are genetically engineered and are used to treat B-cell cancer.[26] These are a few examples of approved gene drugs. There are almost 39 gene drugs that have been approved by 2022 and many more are in clinical trials at different levels.[27] However, siRNA-based gene therapy got more attention due to the ease of siRNA production and characterization. siRNAs are smaller in size, hence easy to modify chemically to improve their nuclease stability, and can be produced in large quantities by synthetic means.[28] Besides, siRNA-based gene drugs are widely studied for various diseases; moreover, five siRNA-based drugs have already been approved by FDA for the treatment of various diseases like hereditary transthyretin amyloidosis (hATTR), acute hepatic porphyria (AHP), primary hyperoxaluria type 1 (PH1), primary hypercholesterolaemia, and amyloid transthyretin-mediated (ATTR) amyloidosis.[29−33] Thus, this review mainly focuses on siRNA-based gene therapy and its potential, challenges, and prospects.

2. RNAI MECHANISM

The discovery of the RNAi mechanism won the Noble Prize in Physiology in the year 2006. Most eukaryotic cells use the regulatory RNAi mechanism to defend themselves against viruses and transposons by developing small RNAs such as small interfering RNAs (siRNAs) and micro RNAs (miRNAs) naturally.[7] The siRNAs and miRNAs are central to the RNAi mechanism. Particularly, the siRNAs are produced from endonuclease cleavage of the double-stranded noncoding small hairpin (shRNAs) by Dicer (an RNase III endonuclease). Dicer is an important enzyme and is responsible for the processing and handover of the siRNAs to Argonaute-2 (Ago-2). Ago-2 unwinds and removes the "passenger" strand while leaving the "guide" strand on Ago-2 to form an activated RISC as shown in Figure 1, following the natural siRNA mechanism.[34] The breathing ends of the siRNAs are responsible for the selective loading of them into RISC. The less thermodynamically stable end of the passenger strand binds to the PIWI domain of Ago-2 and is cleaved by the Ago-2.[35] The activated RISC with a guide strand specifically binds to the target mRNA through Watson−Crick complementarity. The guide strand of siRNA binding to mRNAs results in mRNA cleavage by the RISC complex, thus deactivation of translation. Therefore, siRNA is a widely used research tool both *in vitro* and *in vivo* as the siRNAs introduced to cells can suppress the gene of interest with high selectivity and efficacy. It has become evident from various studies that siRNA-based therapy is a precise, efficient, and stable gene therapy to treat various diseases.[36] However, siRNA-based therapy has its challenges to translate as an entirely acceptable gene therapeutic.[37]

3. PHYSIOLOGICAL AND INTRACELLULAR CHALLENGES IN SIRNA DELIVERY

There are various physiological and intracellular barriers to overcome to realize the full potential of siRNA-based gene therapeutics. After systemic administration of the siRNA formulation, it must reach the target site by avoiding renal clearance, phagocyte uptake, interaction with serum proteins, and nuclease degradation. Various siRNA modifications are studied to overcome the problems.[38] Though, the siRNA modification whether they are naked siRNAs, nonviral vectors, or viral vectors exhibits high efficiency *in vitro* but face a chain of obstacles *in vivo* before they reach the target cells. The siRNAs can be administered topically or intravenously depending upon access to the site of disease.

3.1. Route of Administration. Topical administration: The siRNA-based drugs are initially administrated topically to treat ocular pain and dry eye disease. However, topical administration is possible only for external tissues such as the eye and epidermis.[39,40]

Intravenous administration: Administration of siRNA through an intravenous (IV) is one of the choices when

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topical administration is not possible. For example, a vast majority of tumors and diseases in humans are not accessible for topical administration where IV administration is only preferable.[41] siRNAs can be administered through the subcutaneous,[42] intrathecal (IT),[43] and intracerebroventricular (ICV) routes as well.[43]

3.2. Physiological Barriers. Most of the drugs need to overcome and cross all physiological barriers before reaching the site of action. There are several types of physiological barriers such as the dermal barrier, nasal barrier, and intestinal barriers, but when it comes to siRNA delivery, the main physiological barriers are nuclease stability in serum, renal clearance, and reticuloendothelial system (RES) as the siRNA administration is mostly intravenous.

3.2.1. Nuclease Cleavage. Unmodified siRNAs are unstable in biological fluids due to their degradation by exonucleases such as RNases. Nuclease degradation is one of the biological barriers the siRNA encounters in plasma and tissues. Exonuclease is one of the major nucleases present in plasma. The half-life of the naked siRNAs is about 5−10 min.[44]

3.2.2. Renal Clearance. Renal clearance is another problem that naked siRNAs face. Systemic delivery of siRNA of the naked siRNAs are very susceptible to renal clearance due to their smaller size, which reduces their half-life in the blood. Various biodistribution studies in animals showed that the majority of the naked siRNAs accumulated in the kidneys.[45]

3.2.3. Reticuloendothelial System (RES). Besides the aforementioned physiological barriers, the uptake of siRNA by RES is another major barrier to overcome for efficient siRNA delivery *in vivo*. The RES is made-up of phagocytic cells. The macrophages which are part of phagocytic cells are highly abundant in the liver and spleen and result in a high accumulation of siRNA in these organs.[46]

3.3. Intracellular Barriers. Even after crossing the physiological barriers, the siRNA also must overcome the intracellular barriers to reach the cytoplasm of the cells, thus to exert the RNAi mechanism and the knockdown of the target mRNA. The intracellular barriers are endosomal trapping, immune stimulation, and off-target effects.[38]

3.3.1. Endosomal Trapping. Naked siRNA is anionic and hydrophilic that cannot be readily taken up by cells due to repulsion by the anionic cell surface.[47] Though delivery vehicles such as nanoparticles are employed to deliver siRNAs to target cells, the nanoparticles remain trapped inside endosomes, leading to siRNA degradation. Therefore, nanoparticles must be accompanied by an endosomal escape strategy to deliver siRNA into the cell cytoplasm.[38]

3.3.2. Immune Stimulation. Immune stimulation is another challenge to address in order to realize the full potential of siRNA therapy. Innate immune stimulation is the body's first response against germs and foreign substances entering the body.[48] Therefore, innate immune stimulation is possible if too much siRNA is used, and it might be activated through the double-stranded RNA (dsRNA) sensor. GC-rich sequences in siRNA might activate nuclear factor kappa B (NF-κB), interferon regulatory factors, and toll-like receptors (TLR7, TLR8, and TLR9).[49]

3.3.3. Off-target Effects. As the siRNA sequences are short, there is a possibility for off-target binding. For example, a gene with shared strong homology to the target gene would potentially be knocked down and can lead to severe unintended side effects.[50]

4. METHODS OF SIRNA DELIVERY

The naked siRNA as such cannot be used as a drug candidate for clinical applications due to their physiological and intracellular barriers. Various groups worked on addressing the challenges and could overcome some of the hurdles associated with siRNA delivery. Different methods are developed for safe siRNA delivery for clinical use; they mainly include (i) lipid nanoparticle (LNP), (ii) siRNA-ligand conjugates, (iii) polymer-based nanoparticles, (iv) viral vectors, (v) cell vesicles, and (vi) nucleic acid-based nanoparticles.

4.1. Lipid Nanoparticles (LNP)-Based siRNA Delivery. Lipid nanoparticles are spherical in shape and are composed of a lipid bilayer with an aqueous core, and the liposomal membrane is either positively charged, negatively charged, or neutral. The commonly used lipids to synthesize the lipid nanoparticles are (i) *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA), (ii) 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), (iii) dioleoylphosphatidylethanolamine (DOPE), (iv) 1,2-dioleyl-*sn*-glycero-3-phosphocholine (DOPC), (v) 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), (vi) dimethyldioctadecylammonium bromide (DDAB), and (vii) cholesterol (Chol).[51] On the basis of the charge of the liposome surface, they are categorized into cationic, anionic, and neutral liposomes.[52] The lipid nanoparticles are used as a carrier to treat various diseases, including TTR-mediated amyloidosis, human immunodeficiency virus (HIV)-associated Kaposi's sarcoma, neoplastic meningitis, and various types of tumors.[53]

4.1.1. Cationic Liposomes. Cationic lipids usually stabilize the siRNAs through electrostatic interactions, thus facilitating siRNA delivery. Cationic liposomes are lipid-bilayered structures that carry an overall positive charge and can be used in the delivery of hydrophobic cargo.[54] Cationic liposomes are synthesized by utilizing positively charged lipids such as DOTAP, DSPE, and DDAB, along with neutral lipids like DOPE, DOPC, and PEG. Several research groups have explored the use of cationic liposomes for siRNA delivery both *in vitro* and *in vivo* to treat various cancers and diseases.[55,56] Sheng Yu and colleagues developed and utilized the cationic nanoparticles functionalized with AS1411 antinucleolin aptamer for targeted delivery of siRNA against the Polo-like kinase 1 (siPLK-1) gene and Paclitaxel (chemotherapeutic) and could inhibit the breast cancer progression due to the synergistic effect between siPLK-1 with Paclitaxel.[57] Another group tested the cationic liposomes to deliver a combination therapy of siRNA (siMcl-1) targeting myeloid cell leukemia 1 (MCL1) and gemcitabine (chemotherapeutic) against pancreatic cancer. The cationic liposomes carrying the combination therapy (siMcl-1 + Gemcitabine) could inhibit tumor progression *in vivo* with higher antitumor efficiency than liposomes delivering individual drugs (siMcl-1 or Gem).[58] Though the success rate for siRNA delivery using the cationic liposomes is high *in vitro*, it is too less *in vivo* due to their toxicity and pulmonary inflammation due to the positive charge present on the liposomes.[59] To avoid the toxicity arising from the cationic nature of the cationic lipids, the (polyethylene glycol) PEG polymer is usually mixed with the cationic lipids to synthesize hybrid PEG/lipid-based liposomes.[55] For example, Guan et al., developed a cationic liposome formulation with an outer layer composed of DSPE-PEG2000 (DSPE-(polyethylene glycol)-2000]) for siRNA delivery to overcome the toxicity arising from the cationic

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liposomes. The DSPE-PEG2000 coating on the liposome surface improved the circulation time and reduced the toxicity. The PEG-coated liposomes are loaded with a combination therapy of siRNA targeting the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and paclitaxel. The nanoparticle formulation reduced the tumor burden significantly in a mouse model (Hela) upon IV injection of the combinational therapy.[60] The cationic liposomes derived from the blending of PEG, positively charged DC-cholesterol, and DOPE (PEG/DC-Chol/DOPE) which are also used as carriers to deliver siRNA targeting kinesin spindle protein (KSP) to inhibit ovarian cancer growth in PDX-resistant mouse model.[61] Alnylam Pharmaceuticals used the lipid nanoparticle formulation to test two siRNAs (ALN-TTR01 and ALN-TTR02) against transthyretin (TTR) mRNA to treat TTR-mediated amyloidosis. Out of the two siRNA-based drugs, ALN-TTR02 (Patisiran) exhibited better prospects compared to ALN-TTR01 with fewer adverse reactions with more effectiveness and is approved by FDA.[62] LNP-formulated siRNA accumulates in the liver, and the siRNA degrades the TTR mRNA using the RNAi pathway and reduces the TTR protein production.[21,63−65] Though the PEG covering on the liposomal surface helped reduce the toxicity, the PEG limits the cellular uptake of lipid nanoparticles, thus lowering the transfection efficiency. Besides, PEG interferes with endosomal escape and leads to siRNA degradation.

4.1.2. Stable Nucleic Acid-Lipid Particle (SNALP). The SNALPs are composed of both cationic and neutral lipids. The cationic lipid enhances the cellular uptake while the neutral lipid promotes the endosomal escape of the siRNA drug.[66] The lipid bilayer is further protected from rapid systemic clearance by modifying it with a PEG-lipid conjugate.[67] Tekmira Pharmaceuticals first tried to utilize the SNALP to treat hypercholesterolemia by delivering TKM-ApoB siRNA, then the same company used siRNA-mediated treatment against the Ebola virus (TKM-100201).[68] The company also used a SNALP/PLK1-siRNA formulation to treat neuroendocrine tumors and adrenocortical carcinoma by targeting PLK1 (Polo-like kinase 1).[69] However, the trials do not yield good results though the formulation has good tolerance. The SNALP-siRNA nanoparticles are tested against the hepatitis-B virus (HBV), and they showed reduced serum HBV levels; however, the HBV levels reduction in serum is dose-dependent.[70] Alnylam Pharmaceuticals also utilized the SNALP formulation to deliver two siRNAs; siRNA targeting vascular endothelial growth factor (VEGF), and siRNA targeting KSP (the formulation is named as ALN-VSP02) have shown increased therapeutic effect in treatment of liver tumors. The phase-I clinical trials of the ALN-VSP02 from the initial 28 patients were demonstrated to be safe even at the highest dose of 1.25 mg/kg.[71]

4.1.3. Anionics Liposomes. Anionic liposomes are also used as one of the vectors for the delivery of siRNA-formulated drugs.[72] Similar to the cationic liposome, anionic liposomes is also a made of lipid bilayer and are negatively charged macromolecules that delivers DNA or RNA or oligonucleotides.[73] Anionic lipid nanoparticles for siRNA delivery are often developed by blending anionic lipids such as dioleoylphosphatidylethanolamine (DOPE), phosphatidylserine (PS), 1,2-dioleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DOPG), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), and caproylamine (CAP) with other cationic or neutral lipids. Anionic liposomes that are composed of anionic lipids, Ca^{2+} ions, and siRNA have been demonstrated to be secure and effective in siRNA delivery.[74−77] The anionic liposomes exhibit less immunogenicity, have more circulating time, and showed high tolerance in animal models.[72] The anionic liposomes composed of both anionic and cationic lipids results in lipid nanoparticles with a pH-responsive zeta potential profile which indeed helps in releasing the siRNA from endosomes through the proton-sponge effect.[78] Anionic liposomes have been explored for siRNA delivery to treat various diseases such as breast cancer, hepatocellular carcinoma, neuronal diseases, prion diseases, systemic disease, and protein misfolding diseases in CNS.[72,74,79−82] A study states that siRNA shows improved resistance, and it can play an effective role in gene knockdown and also states that anionic liposomes prepared using DOPG, DOPE, and glycerol show safe and effective means of nucleic acid-based drug delivery.[83] siRNA delivery through anionic liposomes (DOPG/DOPE/Ca2+/siRNA molar charge ratio) has been shown to be safe and efficient in breast cancer.[79] Anionic liposomes composed of DOPG and DOPE are used as carriers to deliver siRNA against the GFP gene and could silence the GFP expression with about 70% efficiency.[84] Though, the anionic liposomes show promising results in siRNA delivery; however, finding the right composition of anion lipids with other cationic or neutral lipids with just the right proportion is difficult. Moreover, the lipid nanoparticles formed are heterogeneous in size.

4.1.4. Neutral Liposomes. A lipid bilayer macromolecule that carries no charge can also be used for the delivery of siRNA. Neutral lipids such as DOPC and DOPE can encapsulate and deliver siRNA into tumor cells with high efficacy compared to naked siRNA.[85,86] For example, DOPC-encapsulated siRNA liposomes are studied against EPH Receptor A2 (EphA2), focal adhesion kinase (FAK), and neuropilin-2 genes, and demonstrated significant inhibition of tumor growth inhibition in both ovarian carcinomas as well as colorectal cancer xenografts.[87] Likewise, neutral liposomes loaded with mTOR siRNA inhibited the tumor growth of breast cancer and restored its morphological alterations. Furthermore, the same study found that the accumulation of siRNA in mammary cancer tissue has been enhanced to a great extent that promoted its distribution into the deep cytosolic tumor area, allowing apoptosis and aiding its antitumor capability.[88] Neutral liposomes have been explored for siRNA delivery to treat various diseases such as ovarian carcinoma therapy, DMBA-induced mammary carcinogenesis, and lung cancer.[88−91]

4.2. siRNA-Ligand Conjugates. FDA-approved siRNA-based drugs were developed by conjugating them with cell-specific ligands to sense strands of the siRNA, thus facilitating siRNA delivery to diseased/cancer cells.[38] The sense strand of the siRNA is chemically modified to stabilize the siRNA against nuclease degradation.[149] And, the ligands that are conjugated to the siRNA could be small molecules, peptides, or aptamers. For example, Vutrisiran is developed by conjugation of hepatocyte cell-specific tri-GalNAc ligands to the sense strand of the siRNA-targeting TTR gene and was approved in the year 2022.[33,92] Acute hepatic porphyrias, a hereditary disorder can be treated using Givosiran (ALN-AS1). Givosirna is a GalNAc-siRNA conjugate that targets hepatic 5-aminolevulinic acid synthase 1 (ALAS1).[93−95] Another GalNAc-siRNA conjugate drug Inclisiran (Leqvio; Novartis)

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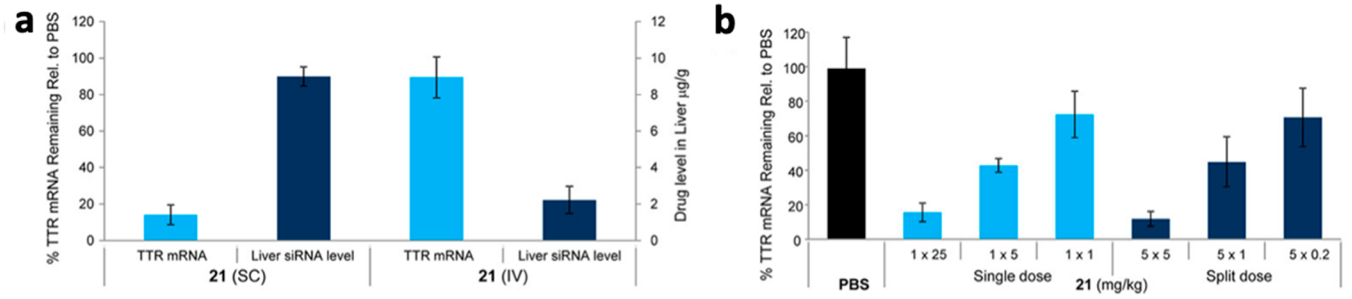

Figure 2. TTR gene silencing by conjugate 21 in mice. (a) % mTTR mRNA expression and tissue levels of conjugate 21 in liver (light blue is primary ordinate and dark blue is secondary ordinate). (b) % TTR mRNA expression comparison with control; (light blue) single dose treatments of 25, 5, and 1 mg/kg and (dark blue) multidose treatments of 5 × 5, 5 × 1, and 5 × 0.2 mg/kg. Adapted from ref 103. Copyright 2014 American Chemical Society.

is developed to treat homozygous familial hypercholesterolemia and elevated low-density lipoprotein cholesterol (LDL-C).[32,96,97] Recently, the FDA approved another siRNA base drug Lumasiran (ALN-GO1) and is also a GalNAc-siRNA conjugate used in the treatment of primary hyperoxaluria (PH) type 1 by targeting glycolate oxidase (GO).[98,7,99,100] Likewise, arginylglycylaspartic acid (RGD) peptides and their derivatives such as cyclopentapeptide (RGDfK) and octreotide (cyclo-octapeptide) are selective toward integrin receptors ($\alpha v\beta 3$) and somatostatin subtype-2 receptor (SSTR2), highly expressed in various tumor cells.[101] Aptamers are also widely used as targeting ligands that are specific to cell-surface receptors. Aptamers are specific to prostate-specific membrane antigen (PSMA) and are employed to home two siRNAs targeting EGFR and Survivin genes using a bivalent aptamer chimera to treat prostate cancer. The two siRNAs are sandwiched between two PSMA aptamers, and the resulting bivalent aptamer chimera could home the two siRNA to a prostate tumor and exhibit high tumor inhibition and angiogenesis with low immunogenicity and high tumor specificity.[102] Nair and co-workers demonstrated that the GalNAc-siRNA conjugate targeting the TTR gene enters into hepatocytes through ASGP receptor-mediated endocytosis, and results showed robust TTR gene silencing (Figure 2).[103] As mentioned earlier, the off-target effect is one of the biggest barriers for siRNA, and to mitigate the off-target effect, Schlegel et al. utilized the rotated nucleobase orientation ability of the glycol nucleic acids (GNAs) by placing them within the region of the guide strand of a siRNA and exhibited fewer off-target effects while retaining the siRNA activity both *in vitro* and *in vivo*.[7,104] Strand selection between sense and antisense by the RISC complex is another crucial step in mitigating off-target effects. The antisense strand selection over the sense strand by the RISC can be achieved by blocking the phosphorylation of the 5′-end of the sense strand. Manoharan et al. could solve the problem by placing a morpholino nucleotide at the end of the sense strand which not only improved antisense loading into the RISC but also increased its siRNA activity.[105]

4.3. Polymer-Based Nanoparticles and siRNA Delivery. Polymer-based nanoparticles are widely tested as a vehicle for siRNA delivery due to their favorable properties such as biocompatibility and diversity in modifiable structures.[106] The following are a few examples of the polymeric-nanoparticle studied for siRNA delivery, they include poly-L-lysine (PLL), polyethylenimine (PEI), PEG, cyclodextrin, chitosan, hyaluronic acid (HA), proteins, and dendrimers.

4.3.1. PEI-Derived Nanosystems. Polyethylenimine (PEI) polymers resemble PEG where the two oxygen atoms are replaced with two amine groups and are responsible for improved solubility.[107] Though, the PEI nanoparticles are responsible for effective intracellular delivery of siRNA; however, PEI often causes cytotoxicity.[108] Therefore, the PEI is usually blended with biocompatible polymers such as chitosan, cyclodextrin (CD), PEG, and hyaluronic acid (HA) to form nanoparticles with reduced toxicity and safe delivery of the siRNA to treat different cancer types and diseases.[109] For example, the HA is used as a targeting ligand as it is selective for CD44 receptors, and the receptors are abundantly expressed on the cancer cell surface.[109] Thus, the HA decorated HA-PEI/PEG nanoparticle formulation utilized to deliver two siRNAs: (i) siRNA targeting the Sjogren syndrome antigen B (SSB) gene (small RNA binding exonuclease protection factor La) and (ii) siRNA targeting PLK1 gene in mice model to treat lung cancer.[110] Likewise, nanoparticles composed of PEI and cyclodextrin are utilized for the delivery of siRNA and hydrophobic drugs. The amphiphilic nanoparticles could enhance the pharmacodynamics (PD) properties of the hydrophobic drugs; they usually diminished PK properties.[111] The cationic PEI binds to siRNA through electrostatic interactions, whereas hydrophobic drugs accumulated in the interior of the cyclodextrin making them ideal carriers for combinational therapeutics. Also, the CD-PEI-gold nanoparticles are used as a vehicle to deliver the hydrophobic chemotherapeutic drug docetaxel (DTX) alone with siRNA against the p65 protein. Inhibition of the p65 protein leads to an enhanced cytotoxic effect by DTX and thus inhibited tumor growth *in vivo*.[112] Likewise, polyplexes of PEI and siRNA specific to human epidermal growth factor receptor 2 (HER-2) receptor have led to gene knockdown and induced antitumor effects *in vivo*.[87] Lipid-linked PEI nanoparticles also improved the siRNA delivery; for example, a phase I trial (NCT00689065) of siRNA CALAA-01 against the ribonucleoside-diphosphate reductase subunit M2 (RRM2) resulted in the inhibition of tumor growth in humanized cancer-bearing xenograft of mice by downregulation of RRM2. Also, the siRNA is covered by adamantane PEG-modified cationic cyclodextrin nanoparticles and a human transferrin protein which serves as a targeting ligand to deliver the siRNA to cancer cells.[113]

4.3.2. PLL-Derived Nanosystems. Poly-L-lysine (PLL) is synthesized by polymerizing L-lysine and is studied for siRNA delivery by complexing with the siRNA through electrostatic interactions.[114,115] However, the cationic PLL/siRNA or PLL/

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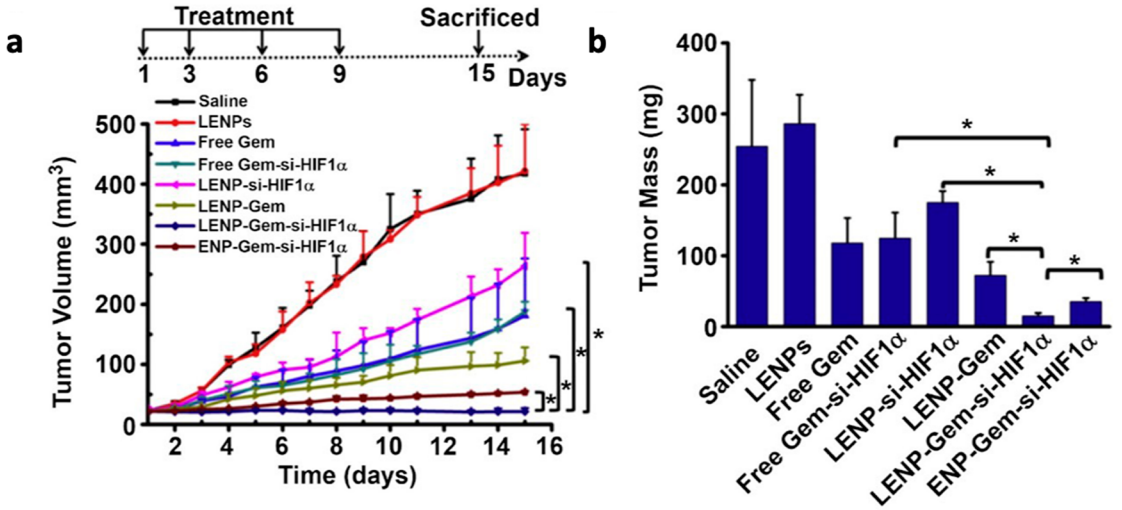

Figure 3. (a) Tumour growth curves of lipid nanoparticles formulation with siHIF1 and Gemicidine. (b) Tumour weights of the mice after the treatment. Adapted from ref 117. Copyright 2015 Elsevier.

PEG/siRNA complexes bind to serum proteins and thus reduces their ability to reach the target site.[116] Nevertheless, Zhao and his team created a nanoparticle formulation LENP-Gem-si-HIF1α by loading siRNA against hypoxia-inducible factor 1α (HIF1α) and Gemicidine into the lipid-polymer hybrid nanoparticles (LENPs) and evaluated them for their efficacy in both *in vitro* and *in vivo*, showing improved results (Figure 3).[117] Likewise, blending of PLL with poly(ε-caprolactone), PEG, and siRNA resulted in micelle formation and could overcome the aforementioned problem to some extent.[118] Likewise, the copolymerization of PLL-PEG-PCL carrying oxaliplatin, cisplatin, and the siBcl-2 (siRNA targeting B-cell lymphoma 2) gene showed Bcl-2 mRNA down-regulation *in vitro*.[119] On the other hand, melanin-conjugated PLL polymers are also used as a vehicle, particularly to overcome the problem of endosomal escape by generating heat after NIR irradiation.[120] The melanin/PLL polymer carrying siRNA against survivin could inhibit 4T1 cell proliferation *in vitro* and could reduce tumor burden *in vivo*.[120] A triblock copolymer developed by Sun et al. was evaluated for combinational delivery of DOX and siRNA against the Bcl-2 gene and could inhibit HepG2/adriamycin tumors *in vivo*.[121] Wang et al. developed PLL-SS-PEG nanoparticles by incorporating a disulfide bond (-SS-) between PLL and PEG to improve endosomal escape of the siRNA encapsulated into the nanoparticles. The PLL-SS-PEG nanoparticles could carry and deliver a siRNA against VEGF gene (siVEGF) to HepG2 tumor which resulted in the tumor inhibition in a mice model.[122] A study conducted by Kozielski et al. designed and studied a biodegradable poly(beta-amino ester) (PBAE) nanoparticle for its ability to deliver five different siRNAs (anti-GBM genes) to primary human GBM cells both *in vitro* and then *in vivo*. This study has shown effective knockdown of all five genes and reduced the cancer burden.[123]

4.3.3. Dendrimers for siRNA Delivery. Dendritic polymers with tunable size and terminal functional groups can encapsulate payload into a nanometer size.[124] Polymeric dendrimers are being extensively used in drug delivery for the treatment of cancers and infectious diseases.[125−127] Perez et al. tested the polyamidoamine (PAMAM) dendrimers for their ability to deliver siRNA against the Green Fluorescent Protein (siGFP) gene in both T98G-EGFP and J774-EGFP cells, and results showed that the GFP successfully down-regulated.[128] Surface-modified PAMAM-siRNA dendrimers were used for the delivery of siRNA targeting TWIST1 transcription factor in breast cancer cell metastasis. PAMAM-based delivery systems are also used for the delivery of siRNA against ApoB.[129] Patil et al. improved siRNA (siRNA against BCL-2 gene) stability by formulating it with a triblock nanocarrier and resulting in the downregulation of the BCL-2 gene in cell uptake studies on the ovarian cancer cell line.[130]

4.3.4. siRNA-Polymer Bioconjugates. Biocompatible conjugation to the sense strand of the siRNA is one of the widely used methods to deliver the siRNA double strands to overcome their physiological as well as intracellular barriers, thus improving efficient cellular uptake.[131] Polymers such as PEG and poly(lactic-*co*-glycolic acid) (PLGA) are often conjugated to sense the strand of the siRNA. The siRNA-PLGA conjugate usually self-assembles into nanoparticles, thus facilitating the delivery of siRNAs.[132] Saltzman et al. used the PLGA-spermidine nanoparticles for siRNA delivery against endogenous gene silencing in the vaginal lumen and uterine in a mice model.[133]

4.3.5. Polymeric Complexes. Polymers of positively charged tertiary amines are also tested for siRNA delivery as they can self-assemble with siRNA through electrostatic interactions.[134] Being positively charged the polymeric complex enables endosomal release due to proton sponge effects, thus improving the siRNA's cellular uptake.[135] In addition to positively charged functional groups that usually bind to siRNA, hydrophobic polymers are also efficacious for siRNA delivery.[136,137] The hydrophobic molecules such as cholesterol conjugation to siRNA with an optimal balance of pK_a between 6.0 and 6.5 are implicated in effective siRNA delivery.[134] For example, PEG-*b*-PLA/BHEM-Chol nanoparticles are a combination of both cationic lipid (N,N-bis(2-hydroxyethyl)-*N*-methyl-N-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide, BHEM-Chol) and an amphiphilic polymer that could deliver siRNA against the PLK1 gene and inhibit tumor growth in mice.[138] PLGA-based block copolymers are explored for siRNA delivery against prostate cancer mice models.[139] A combination of siRNA-based drugs and chemotherapy is also one of the cancer therapeutic methods. Local Drug EluteR (LODER), a biodegradable implant, is developed to deliver a

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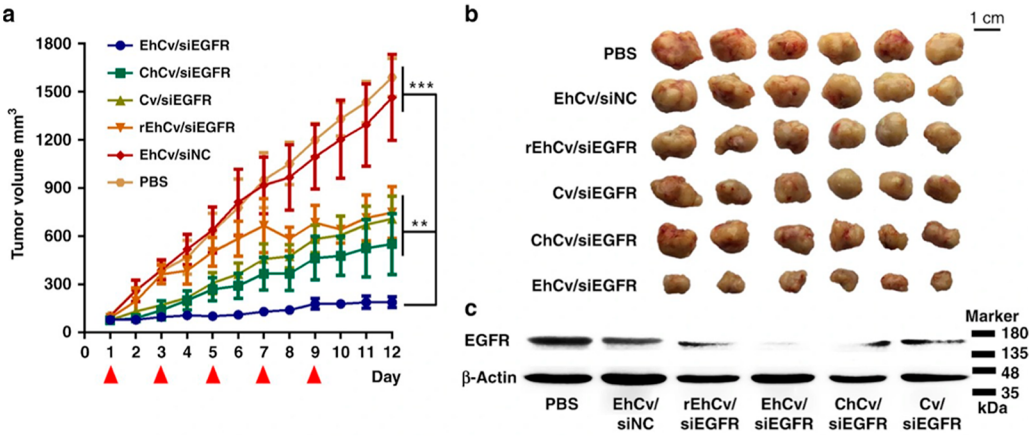

Figure 4. (a) *In vivo* tumor inhibition effects of siRNA-loaded nanoparticles (NPs). Tumour growth curves of nude mice after treated by siRNA-loaded NPs at a dose of 0.5 mg kg^{-1}. (b) Tumours harvested from mice after the treatment. (c) Western blot analysis after the treatment for EGFR gene regulation. Adapted from ref 162. Copyright 2019 Nature Portfolio.

combination therapy of siG12D (siRNA against mutated KRAS) and chemotherapy (gemcitabine or FOLFIRINOX) to treat locally advanced pancreatic cancer (LAPC) and has completed phase I trials and is in phase II clinical trials.[140,141]

4.3.6. Biopolymers. Biocompatible biopolymers such as cyclodextrin, chitosan, and proteins have been utilized for the siRNA delivery to diseases like melanoma, lung metastasis, and breast cancer.[142−144] CD47 immunoglobulin is highly expressed in many cancer models, as CD47 and signal-regulatory protein α (SIRPα) forms a signaling complex that helps in the escape of cancerous cells from macrophage-mediated phagocytosis. Thus, targeting the CD47 selectively can lead to the inhibition of tumor growth. Thus, silencing of CD47 using siRNA can inhibit tumor growth and is evident from various phase 1 clinical trials.[145] In another example, cyclodextrin is used as a carrier to deliver siRRM2 (siRNA targeting RRM2) and reduced the RRM2 mRNA and protein levels drastically in humans as well as in mice models.[146] Chitosan is another natural biopolymer often used for siRNA delivery.[147] Chitosan with higher molecular weight provides better complexation and stability with siRNA targeting CD47 while lower molecular weight chitosan needs to be mixed with specific degrees of protamine-hyaluronic acid (LPH) to achieve better silencing effects of the CD47 in tumor tissues.[86,143] Protein is a biocompatible material and is explored as a carrier for the safe delivery of siRNA. Particularly, the protamine (HIV-1 envelope antibody fusion protein) is explored to deliver siRNA to the cells which expresses the HIV-1 envelope.[148,149] For example, HER2-ScFv-protamine fusion protein expressed on the cell surface of various cancer models is used for targeted delivery of siRNA by complexing the siRNA with the HER2-ScFv-protamine fusion protein and forms a siRNA-protein complex.[150] Likewise, cell-penetrating peptides (CPPs) are another class of peptides proven to be effective in delivering siRNA to cells by passive cell membrane penetration.[151]

4.4. Viral Vectors based siRNA Delivery. The recombinant adeno-associated viruses (rAAV) are explored for siRNA delivery where the inverted terminal repeat (ITR) sequences needed for viral replication as well as packaging are retained while deleting all the viral protein genes.[152,153] Therapeutic transgenes (siRNA) along with regulatory elements are inserted in between the ITR sequences. The transgene capacity of rAAV can be increased by deleting the viral protein genes which in turn reduces the viral toxicity.[154] The rAAV vector also does not integrate into the host genome as it cannot express the Rep protein, thus reducing the immunogenicity and cytotoxicity.[154] And the rAAV vector is used for siRNA delivery in the treatment of many diseases such as myotnic dystrophy, tumor, hepatocellular carcinoma, and Huntington's disease.[155−158] Likewise, Lentiviruses are also used for siRNA delivery, for example, doxycycline (Dox)-inducible Lentiviral-mediated siRNA delivery against the Spinal microglial Toll-like receptor 4 (TLR4) gene in rats with chronic constriction injury (CCI) and reduced TLR4 expression in the spinal cord of CCI rats that resulted in reduced neuropathic pain.[159] Also, siRNA offers a great opportunity to treat untreatable neurodegenerative disorders. A recent study showed that siRNA delivery using rAAV against the mutant ataxin-1 gene rescues a phenotype in a spinocerebellar ataxia mouse model.[160] Likewise, administration of lentiviral vectors expressing β-secretase siRNA (siRNA against BACE1) to a transgenic model of Alzheimer's disease lowered the BACE1 levels and thus reduced amyloid production, indicating the potential therapeutic value of siRNAs in treating Alzheimer's.[161]

4.5. Cell Vesicle-Based siRNA Delivery. Cell vesicle-based nanoparticles are also used as one type of siRNA-based drug delivery for different varieties of cancer treatment.[162] Biomimetic drug delivery systems have been developed by modifying natural cell membranes of red blood cells (RBC), white blood cells (WBCs), platelets, cancer cells, macrophages, and mesenchymal cells into a cell-membrane-modified nanoparticle.[162] A deep study is going on cell membranes to form biomimetic nanoparticles to achieve functions like targeted drug delivery, immune escape, and immune modulation that can interact within *in vivo* environments.[163,162] Along with cell vesicle-based RNA delivery, there are also extracellular vesicles that are used for RNA delivery from cells to intercellular space to the target site.[164] Using ultrasonic dispersion, Qiu et al have developed an endoplasmic reticulum membrane (EM) decorated hybrid nanoparticle (EhCv/siRNA NPs), which has an improved version of the intracellular fate of siRNA. These EhCv/siRNA NPs after being injected peritumorally escape from the endosome/lysosome pathway. The NP is taken up by the coat protein complex I or II (COPI or COPII) vesicles for directional transportation, which is induced by the

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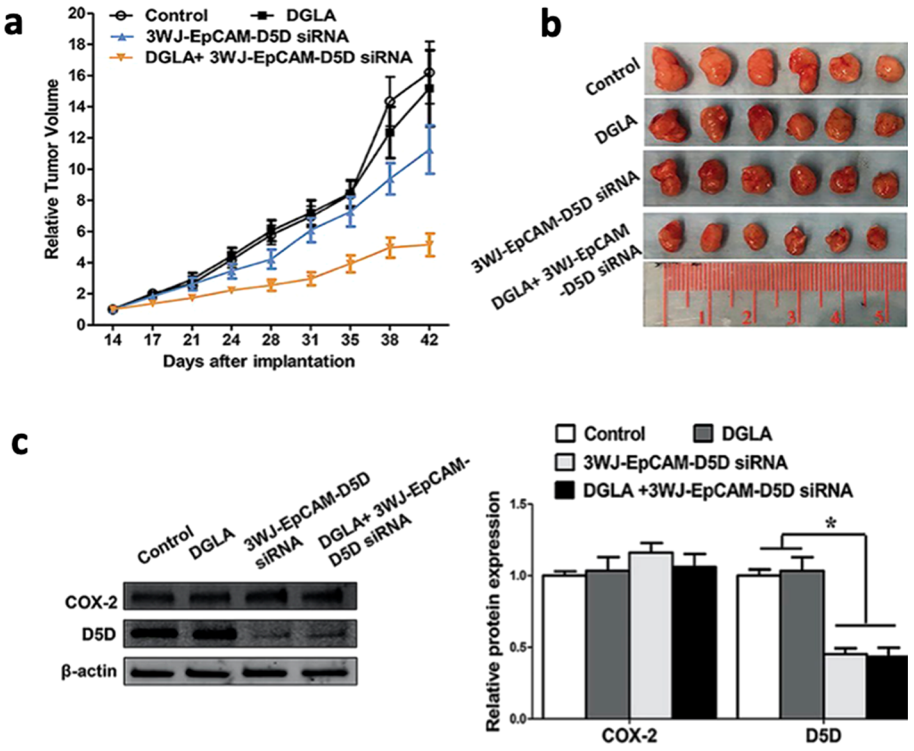

Figure 5. (a) Tumor Curves of mice treated with nanoparticles carrying D5D siRNA. (b) Images of tumors at the end of the treatment. (c) COX-2 and D5D expression in tumor tissues after treatment with nanoformulation carrying D5D siRNA. Adapted from ref 174. Copyright 2020 Elsevier.

several EM resident proteins present between the endoplasmic reticulum (ER) and Golgi complex. The EM-decorated EhCv/siRNA NPs have shown maximum tumor growth inhibition for EhCv/siEGFR formulation (Figure 4).[162]

Exosomes (Exos) are naturally derived biocompatible nanocarriers with a size range of 30−160 nm.[165] They are harvested from various cell types and biological fluids such as milk and blood. Exos are natural carriers and are explored to develop exosome-based drug delivery systems.[166] As the exosomes are biocompatible, they are versatile carriers of small RNAs, mRNAs, and proteins. Besides, the surface proteins of exosomes can be engineered to append cell-specific targeting ligands on the surface of exosomes for targeted siRNA delivery.[167] Also, the targeting ligands can be imparted on the exosome surface using RNA nanoparticles. For example, Guo et al. decorated the exosomes with folate using the engineered 3WJ RNA nanoparticles for targeted delivery of siRNA to various cancer types expressing folate receptors on the cell surface.[168] Plant-based ginger-derived exosome-like nanovesicles (GDENs) are also explored for siRNA delivery. The surface of GDENs are displayed with folic acid as a ligand using RNA nanotechnology and delivered the survivin siRNA to folate receptor expressing KB cancer xenograft models, and the results showed significant inhibition of tumor growth.[169] Non-small-cell lung cancer (NSCLC) poses considerable health risks and exosomes decorated with RNA aptamer-targeting EGFR (epidermal growth factor receptor) as a ligand could deliver survivin siRNA for NSCLC regression by silencing the antiapoptotic factor survivin in animal trials. And tumor regression is observed at an IC50 value of 20 nmol/kg siRNA *in vivo*.[170]

4.6. Nucleotide Nanoparticles-Based siRNA Delivery. Materials composed of DNA and RNA are extensively used for smart siRNA delivery. For example, DNA nanodevices are used for the targeted delivery combination therapy of siRNAs against Bcl-2 and P-glycoprotein genes and chemotherapeutic drug Doxorubicin to human breast adenocarcinoma (MCF-7R), while the transactivator of transcription (TAT) peptide was used as a targeting ligand. Both Bcl2 and P-glycoprotein genes are suppressed *in vitro* as well as *in vivo*.[171] Likewise, a 3D DNA nanogel is employed for siRNA delivery. The nanogel is assembled along with siRNA against EGFP having overhang RNA strands. The siRNA then evaluated the silencing effect on the EGFP gene, and the EGFP gene is suppressed significantly.[172] The naturally derived pRNA-3-way junction (pRNA-3WJ) derived from bacteriophage nanoparticles is explored and studied as a vehicle for the delivery of various small RNA to various cancer models. For example, a 3-WJ pRNA-HER2apt-siMED1 nanoparticle bearing human epidermal growth factor receptor 2 (HER2)-RNA aptamer as a targeting ligand and siRNA against MED1 gene is employed to target HER2-overexpressing human breast cancer. The RNA nanoparticles harboring siMED1 inhibit cancer proliferation and metastasis, by sensitizing the breast cancer cells to the chemotherapeutic drug tamoxifen.[173] Pang et al. created a combination of epithelial molecule (EpCAM) aptamer and Delta-5-Desaturase (D5D) siRNA into a 3WJ-EpCAM-D5D siRNA nanoparticle which specifically targets and accumulates in lung cancer cells and knockdown D5D gene. By targeting the D5D gene, the 3WJ-EpCAM-D5D siRNA nanoparticle could inhibit lung cancer growth (Figure 5).[174] The 3/4WJ RNA nanoparticles are explored to treat various cancer types and include ovarian cancer, breast cancer, and glioblastoma.[175−178]

5. TREATABLE DISEASES BY SIRNA

siRNA has been studied to treat various diseases, and some of them are already approved for treatment and many are in

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Table 1. Approved and Clinical Trials of siRNA-Based Drugs for Different Diseases

disease	target	vehicle	status	ref
hereditary transthyretin-mediated amyloidosis	transthyretin	siRNA-lipid nano particle (Patisiran)	approved	65, 192, and 208
acute hepatic porphyria	targets amino levulinate synthase I (ALAS-1)	siRNA-GalNAc conjugate (Givosiran)	approved	94, 95, and 209
primary hyperoxaluria type 1 (PH1)	hydroxy acid oxidase I	chemically stabilized siRNA (Lumasiran)	approved	210−212
transthyretin-mediated amyloidosis	TTR	siRNA-GalNAc conjugate (Vutrisiran)	approved	33 and 213
heterozygous familial hypercholesterolemia	PCSK9	siRNA-GalNAc conjugate (Inclisiran)	approved	97 and 213
primary hyperoxaluria	PHI	siRNA-GalNAc conjugate (Nedosiran)	phase III	211 and 214
hemophilia A and B	antithrombin (AT)	siRNA-GalNAc conjugate (fitusiran)	phase III	215 and 216
acute kidney injury	p53	chemically stabilized-siRNA (teprasian)	phase III	217
nonarteritic anterior ischemic optic neurotherapy and primary angle glaucoma	CASP2	naked siRNA (cosdorian)	phase III	211
ocular pain and dry eye disease	TRPV1	naked siRNA (tivanisiran)	phase III	211 and 218
multiple cancers	PLK1	lipid nanoparticle	phases II and III	192 and 219
solid tumor	KSP and VEGF	lipid nanoparticle	phases II and III	192 and 220
AMD/CNV	VEGFR	naked siRNA	phase II	192 and 221
AMD/DME	RTP801	naked siRNA	phase II	192 and 222
RSV virus infection	RSV nucleocapsid	naked siRNA	phase II	192 and 223
hypercholesterolemia	PCSK9	lipid nanoparticle	phase II	192 and 224
diabetic AMD	VEGF	naked siRNA	phase II	192 and 222
advanced solid tumors	PKN3	lipid nanoparticle	phase II	192 and 225
HCC, multiple melanoma	c-Myc	lipid nanoparticle	phase II	192 and 226
Ebola virus infection	VP24, VP35, Zaire Ebola Lpolymerase	lipid nanoparticle	phase II	192 and 227
glaucoma ocular hypertension	ADRB2	naked siRNA	phases I and II	192
delayed graft function kidney transplant	P53	naked siRNA	phases I and II	228
advanced cancers	EphA2	lipid nanoparticle	phase I	192 and 229
chronic optic nerve atrophy	caspase 2	naked siRNA	phase I	192 and 230
metastatic melanoma	LMP2, LMP7, MECL1	LODER polymer	phase I	140 and 192
prostate cancer treatment	polo-like kinase gene	siRNA-peptide based	clinical trials	231
targeted stem cell therapy	OCT4 transcript	dendrimer	pre-clinical trials	232 and 233
idiopathic pulmonary fibrosis (IPF)	Interleukin-11 (IL-11)	polymeric nanoparticle	clinical trials	234
chronic myeloid leukemia (CML)	BCR-ABL fusion oncogene	lipid nanoparticle	preclinical studies	235 and 236

clinical trials at different stages. Thus, siRNA gene therapy can be used for the treatment of cardiovascular diseases, diabetes, cancer, viral infections, rare genetic diseases, and many more.

5.1. Hereditary Genetic Diseases. A disease or disorder that is inheritable from one generation to another is called hereditary genetic disease. Huntington's disease, arthritis, cystic fibrosis, and multiple sclerosis are a few examples of hereditary genetic diseases. siRNA therapeutics are being developed to treat several hereditary genetic diseases such as hereditary transthyretin-mediated (hATTR) amyloidosis, Huntington's disease, hemophilia A or B, Primary hyperoxaluria (PH), and Duchenne muscular dystrophy.[36,179−181]

5.2. Cardiovascular Disease. Increased levels of low-density lipoproteins (LDL) and cholesterol in the blood are causative in atherosclerotic cardiovascular diseases.[182] Alnylam Pharmaceuticals are developing siRNA-based drugs for the treatment of cardiovascular diseases (CVD) that are in phase 3 clinical trials.[183] The SNALP system is also used to deliver siRNA to target the ApoB gene which is responsible for recurrent cardiac events. Suppression of the ApoB gene resulted in a reduction in ApoB and LDL levels.[184] Alnylam pharmaceuticals developed a GalNAc-siRNA conjugate (Inclisiran, ALB-PPCSSC) targeting the PCSK9 gene.[185]

5.3. Diabetes. Diabetes is a metabolic disorder caused by genetic or environmental factors.[186,187] Apart from conventional methods, diabetes also would be treated using siRNA, and the ongoing research is in clinical trials. Unregulated gluconeogenesis is common in Type 2 diabetes and causes fasting and postprandial hyperglycaemia. The downregulation of the phosphoenolpyruvate carboxykinase-1 (PCK-1) by siRNA against PCK1 improved glucose homeostasis.[188] Galectin-1 (Gal-1) is a highly expressed protein in diabetic mice kidneys due to Hyperglycaemia and causes renal fibrosis. And research showed that the inhibition of Gal-1 by siRNA-Gal-1 prevents the accumulation of Gal-1 in kidneys and would be a novel therapeutic method to treat renal fibrosis in diabetes.[189]

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5.4. Cancer. Research on cancer treatment using siRNA is actively going on both *in vitro* and *in vivo* to treat a variety of cancer types such as liver cancer, pancreatic cancer, lung cancer, colorectal cancer, breast cancer, ovarian cancer, lymphoma, glioblastoma, and many more.[190−197]

5.5. Viral Infection. The demand for antiviral vaccines and drugs using RNA is rapidly growing nowadays. For example, the siRNA gene therapy is extended to treat various viral infections by targeting the viral RNA or DNA, and they include influenza-A virus, coxsackievirus, SARS-CoV, food-and-mouth-disease virus (FMDV), human papillomavirus (HPV), hepatitis B virus (HBV), and Zaire ebolavirus (ZEBOV).[198,199]

5.6. Other Diseases. siRNA-based therapy is involved in a variety of disease treatments and is studied to treat various other diseases including acute lung injury/respiratory distress syndrome, spinal cord injury, neurological diseases, ocular diseases, inflammatory diseases, rheumatic diseases, and skin diseases.[43,200−207]

6. FUTURE PROSPECTIVES

The discovery of the RNAi mechanism leads to the advancement in biotechnology and medicine. The development of gene-based drugs using small RNA such as siRNA, miRNA, shRNA, Gapmers, and antisense oligos has gained immense attention due to the ease of their synthesis with modifications to improve their stability and specificity toward target genes.[10,237,238] Among them, siRNA-based gene therapy has provided treatments for various untreatable diseases and it shows great potential for the treatment of a spectrum of diseases as discussed in this review. The small RNA are easy to synthesize as they are usually about 21−27 nucleotides.[35] The siRNAs can perfectly search and bind to their target mRNA through perfect Watson−Crick complementarity. Moreover, the modification of the sense strand of the siRNA improves its stability, half-life, specificity, and efficacy.[239] The FDA approved and ongoing clinical trials show great promise for the future of this exciting expanding field (Table 1).[213] Nevertheless, the siRNAs can be excreted out through urine due to their small size.[240] And lack of targeting ability to the naked siRNAs often leads to off-target effects.[241] Therefore, various materials have been explored to home the siRNA to the site of action to overcome the aforementioned physiological as well as intracellular barriers. However, biocompatibility and endosomal escape of the nanomaterials are the two major concerns to address for safe siRNA delivery.[242] pH-sensitive polymers of arginine, lysine, aspartic acids, and glutamic acids can be conjugated to nanoparticles to induce the endosomal release of the siRNA through proton sponge effects. Endosomolytic peptides and fusogenic lipids also can be employed to improve endosomal escape.[243] Nevertheless, adding these pH-sensitive materials to the nanoparticles further complicates their pharmacodynamic and pharmacokinetic properties.[244] Thus, choosing the right nanoparticles for the delivery of siRNA is important while avoiding most of the aforementioned problems. The use of biocompatible nanoparticles such as RNA nanoparticles and exosomes might be used as carriers and would eliminate the need for extra material for endosomal escape as the nanoparticles themselves are pH-sensitive as they can be protonated at low pH and escape from the endosomal entrapment.[243] Targeted delivery is an important strategy to deliver nanoparticles to the disease site. RNA nanoparticles are perfectly suited for this purpose as they can be conjugated targeting ligands conveniently.[169] However, RNA strands with high GC content usually exert immune responses, therefore it is important to tailor RNA nanoparticles carefully to minimize innate immune activation.[49] On the other hand, exosomes are highly biocompatible and can release the payload through the back-fusion mechanism.[168] However, exosome production from various cell sources is expensive. Nevertheless, exosomes extracted from biological fluids such as bovine colostrum would be cheaper than other sources.[245] Besides, surface modulation of exosomes can also impart targeting ability to the exosomes.[92] Thus, the engineered exosomes derived from cheap biological fluids would be another option for siRNA delivery. Besides choosing the right carrier for the siRNA delivery, targeting multiple disease-causing genes would be ideal compared to a single gene suppression. Thus, the RNA nanoparticles and engineered exosomes might serve as carriers to deliver combinational siRNA-based gene drugs to treat various cancer or diseases.

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Notes

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