siRNA Delivery Technology for Cancer Therapy: Promise and Challenges

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Abstract- Small interfering RNAs (siRNA) technology has shown great promise as a new class of therapeutic interventions for the treatment of cancer and other diseases. It is a remarkable endogenous pathway that can regulate sequence-specific gene silencing. Despite the excitement about possible applications of this biological process for sequence-specific gene regulation, the major limitations against the use of siRNA-based therapeutics are their rapid degradation by serum nuclease, poor cellular uptake, and rapid renal clearance following systemic delivery, off-target effects and the induction of immune responses. Many researchers have tried to overcome these limitations by developing nuclease-resistant chemically-modified siRNAs and a variety of synthetic and natural biodegradable lipids and polymers to enhance the efficacy and safety profiles of siRNA delivery. Ideal siRNA-based delivery systems for cancer therapy must be clinically suitable, safe and effective. In this review, we introduce the greatest challenges in achieving efficient RNAi delivery and discuss design criteria and various delivery strategies for cancer therapy, including chemical modifications, lipid-based nano-vectors, polymer-mediated delivery systems, conjugate delivery systems, and others.

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Introduction

Cancer is a leading cause of death worldwide. Despite the significant progress made in our understanding of cancer biology, which has led to the development of better diagnostic and treatment methods, overall cancer mortality remains high. A major reason for this is the poor ability of current therapeutic agents to target cancerous cells selectively and without any adverse effects on healthy tissues. Surgical resection, radiation therapy, and chemotherapy are the current therapeutic strategies for cancer. Chemotherapy has many limitations, including difficult administration owing to the poor solubility of chemotherapeutic agents in aqueous solutions, its inability to target cancer cells selectively, its toxicity to healthy tissues, and cancer cell resistance, which hinder its effectiveness. The field of nanotechnology provides promising methods with which to address these challenges. RNA interference (RNAi) is an evolutionarily conserved mechanism in which double-stranded RNA (dsRNA) molecules silence the post-transcriptional expression of homologous target genes (1). The phenomenon of RNAi was first described by Fire *et al.*, in plants in the late 1980s, after which they discovered the ability of dsRNA to silence genes in *Caenorhabditis elegans* in 1998 (2). The emergence of new tools in the field of RNAi applications led to the demonstration of similar processes in mammalian cells in 2001 (3). Small interfering RNA (siRNA) molecules are dsRNAs that are 21 to 23 base pairs (bp) in length, which are mediators of RNAi, and silence the expression of target genes. When exogenous dsRNA enters a cell in a short form (21–23 bp) or in the form of long dsRNA molecules, they are processed by the endogenous RNAi machinery (Figure 1). First, long dsRNAs are cleaved into siRNAs by the cytosolic enzyme Dicer, leaving 2-3-nucleotide 3′ overhangs, and 5′ phosphate and 3′ hydroxyl groups (4,5). Double-stranded siRNA is split into sense (passenger) and antisense (guide) strands. The sense strand is degraded by an endonuclease of the AGO2–RISC enzyme complex, while the antisense strand guides the RISC towards the complementary sequence in the target

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messenger RNA (mRNA). siRNAs will bind to sequences with perfect or nearly perfect complementarity and induce the cleavage of targets by post-transcriptional gene silencing instead of translational suppression (6,7). Because they can efficiently silence target gene expression in a sequence-specific manner, siRNAs became indispensable tools for studying the function of single genes (6,8).

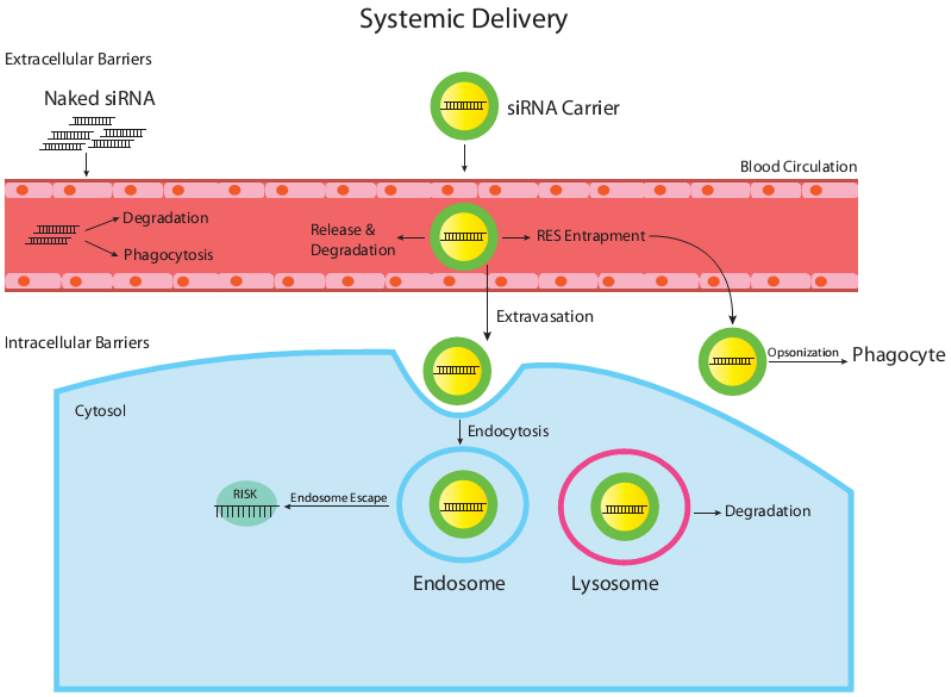

Figure 1. Barriers encountered by systemic siRNA delivery. Extracellular barriers to the distribution of siRNA and carriers targeting organs include enzymatic degradation, opsonization, and phagocytosis by the mononuclear phagocyte system (step 1) and entrapment in the reticuloendothelial system (RES) (step 2). Intracellular barriers include extravasation and penetration into the extracellular matrix, which is dependent on the physiological structure of the target tissue reticuloendothelial system (RES) and cellular internalization are dependent on the surface properties of siRNA and carriers (e.g., charge, size, PEGylation, and specific binding antigen). The crucial barriers for delivering siRNAs to its site of action are the endosomal entrapment and lysosomal degradation of siRNA and carriers (step 3)

Challenges with siRNA-based therapeutics

1- Off-target effects: Although siRNAs are designed to knockdown specific target genes, studies have shown that they may also silence an unknown number of non-target genes through partial sequence complementarity to their 3' UTRs; also, exogenous siRNA can saturate the endogenous RNAi machinery, causing widespread effects on miRNA processing and function (1).

2- Efficacy: siRNAs show different levels of efficacy in gene silencing. The selection of optimal mRNA target sequences requires the thorough mining of databases and pathways (9). Efficacy for different parts of the same mRNA sequence varies widely among siRNAs, and only a limited number of siRNAs have been shown to be functional in mammalian cells (10). Among the randomly selected siRNAs, 58–78% induce silencing with greater than 50% efficiency and only 11–18% induce 90–95% silencing (11).

3- Delivery: Systemic delivery of siRNA to target tissues is prevented by many barriers at different levels (12). Intracellular trafficking of siRNA starts in early endosomal vesicles after the injection of siRNA into the blood; it is readily degraded by endogenous nucleases, easily filtered from the glomerulus, rapidly excreted from the kidney, taken up by phagocytes or aggregated with serum protein (13). Susceptibility to degradation by endo- and exonucleases is the main problem, leading to a short half-life from several minutes to 1 h in the plasma, potentially limiting the use of siRNAs in systemic

delivery via blood (14,15). Physicochemical properties of siRNA, such as negative charges as well as their large molecular weight and size, hampers passive diffusion via cellular membranes, which makes endocytosis the major pathway for internalization (1). In addition to endocytosis, plasma nuclease degradation, and renal clearance, another major barrier to the systemic delivery of siRNA is uptake by the components of the reticuloendothelial system (RES). The RES is composed of phagocytic cells, such as circulating monocytes and tissue macrophages, which remove foreign pathogens, cellular debris and apoptotic cells (Figure 1) (16). Some chemical modifications can significantly protect siRNAs from nuclease degradation without interfering with the siRNA silencing efficiency and enhance the stability and uptake of naked siRNAs. Some modifications such as 2-o-methyl modifications have been shown to reduce susceptibility to endonuclease activity and to abrogate off-target effects (17). Further, linkage of phosphorothioate (PS) or hydrophobic ligands (e.g., cholesterol, polyethylene glycol [PEG]) increased protein binding and extended serum half-life (18,19). Besides these, nanocarriers are important tools, providing protection against both rapid renal clearance and nuclease degradation during the delivery of siRNAs to target tissues (20).

4- Immune response and toxicity: RNAi is a mechanism that is also involved in innate immunity, protecting cells from invasion by nucleic acids of pathogens such as viruses and bacteria. Several studies have demonstrated that some siRNAs can activate innate immune responses in cells in a sequence-specific manner by inducing interferon expression, even at low concentrations (21). siRNAs can also activate protein kinase receptor (PKR) and several toll-like receptors (TLR) signaling pathways in a sequence-independent manner. Some particular immune stimulatory sequence motifs in siRNA such as 5′-UGUGU-3′ (22) or 5′-GUCCUUCAA-3′ (23) as well as some secondary structures and uridine content of the sequence activate endosomal TLR7/8 sensors. Therefore, chemical modifications of siRNA such as 2′-O-methylation 2′-deoxy-2′-fluoro groups, locked or unlocked nucleic acids, or phosphorothioate linkages are required to prevent recognition by the innate immune system. Therefore, not only are chemical modifications of the siRNA needed, but additional delivery materials are also essential to eliminate other barriers in the body. Hence, the immunostimulatory effects of therapeutic siRNAs must be tested prior to clinical applications (12).

Delivery: local *vs*. systemic (delivery of siRNA therapeutics: barriers and carriers)

The site of action of siRNA therapeutics is the cytosol. The barriers to siRNA delivery are multiple and depend on the targeted organs and the route of administration. In general, the systemic delivery of siRNA poses greater barriers than local delivery. For example, intravitreal or intranasal routes of siRNA against the respiratory syncytial virus, either naked or encapsulated in polycationic liposomes, was almost equally effective in reducing the viral infection (24). Several excellent reviews have outlined the physical and immunological barriers to siRNA delivery to the eye, skin, lung, and brain (25-27). Figure 1 shows barriers to systemic siRNA traveling from the site of administration to the site of action. After delivery into the bloodstream, the siRNA undergoes an initial distribution to organs via the circulatory system. In the interior of an organ, siRNA extravagates the intravascular space towards the interstitial space. There, the siRNA is transported across the interstitial space to target cells. After reaching the target tissue, siRNA can be internalized within endocytic vesicles and then a part of the siRNA undergoes endosomal escape, releases from its carrier into the cytosol and load onto RISC (28).

Carriers

It is becoming clear that due to its instability and degradability, naked siRNA is rarely applied in systemic delivery accordingly; this section will deal primarily with siRNA-loaded carriers, such as nanospheres, nanocapsules, liposomes, micelles, microemulsions, conjugates, and other nanoparticles.

Owing to the similar physicochemical properties of DNA and siRNA, DNA carriers have also been applied to siRNAs. These vehicles for gene delivery can be divided into two categories: viral and non-viral (29). With regard to the importance of low toxicity in delivery systems, and also due to the unacceptable levels of toxicity caused by some viral vectors, several synthetic non-viral vectors have been developed offering alternatives to viral vectors for nucleic acid delivery applications (30). Non-viral vectors are classically biodegradable and positively charged (e.g., cationic cell-penetrating peptides, cationic polymers, dendrimers, cationic lipids, etc.). Conjugation of siRNA with a variety of small molecules (e.g., cholesterol, bile acids, and lipids), polymers, peptides, proteins (e.g., antibodies), as well as aptamers (e.g.,

RNAs), and encapsulating siRNA in nanoparticulate formulations improves the stability, cellular internalization, or cell-specific active targeted delivery (31). Several studies have revealed that modification of the RNA backbone improves the stability of the siRNA in serum without significantly affecting its RNAi efficiency. The selection of siRNA carrier systems depends on the siRNA properties, the type of target cells, and the delivery routes for *in vivo* application (29)

Peptide-based siRNA delivery system

Cationic cell penetrating peptides

Cationic cell penetrating peptides (CPP) have been successfully used for carrying different macromolecules that might vary in size and nature, including proteins (e.g., antibodies), peptides, antisense oligonucleotides, plasmid DNA and nanoparticles (32). CPP and siRNA form non-covalent complexes (non-covalent CPP–siRNA) via electrostatic and hydrophobic interactions between positively charged CPPs and anionic nucleic acids, leading to the formation of positively charged complexes with different sizes and stabilities (33). The main advantage of the non-covalent strategy is its simplicity, and the lower concentration of siRNA and CPP needed to elicit a biological response (34). The lower concentration of siRNA reduces any undesired side effects, like possible toxicity and off-target effects that will lead to sustainability of the siRNA, preserve the activity of the siRNA, protect it from digestion by nucleases both in extra- and intracellular milieu and markedly enhance its half-life (35).

Polymer-based siRNA delivery system

Linear or branched cationic polymers including peptides readily bind and condense DNA and have been used as efficient transfection reagents, delivering genes, oligonucleotides, and siRNA (36-38). The structural and chemical properties of these polymers are well recognized (39). The positively charged polymers, via electrostatic interactions with the negatively charged phosphates of DNA, form nanosized complexes called polyplexes (31). This process leads to DNA condensation and protects plasmids from nuclease degradation; facilitates their cellular uptake via endocytosis and results in prolonged half-life. In addition, complete encapsulation of siRNA inhibits off-target effects such as immune activation by a toll-like receptor-dependent mechanism (40). Other polymeric vehicles of siRNAs comprise micelles, nanoplexes, nanocapsules, and nanogels (41). The polyplex characteristic (e.g., size, surface charge, and structure) is associated with the ratio of positive charges of the cationic polymers to the number of phosphate groups in the siRNAs.

Polymers are classified into natural and synthetic polymers.

Natural: peptides, proteins, polysaccharides

Synthetic: Dendrimers, Polyethylenimine (PEI), Poly-L-lysine (PLL), Poly-D,L-lactide-co-glycolide (PLGA), Polymethacrylate (42)

a. Dendrimers

Dendrimers are known as spherical hyper-branched synthetic polymers. The unique structural properties such as flexible arrangement and molecular size, large number of accessible terminal functional groups, as well as capacity to encapsulate cargos will enhance their potential as drug vehicles (43).

Polycationic dendrimers like poly(amidoamine) (PAMAM) and poly(propylenimine) (PPI) dendrimers are considered attractive candidates for delivery of negatively charged siRNA are owing to their positive charge (44). PAMAM dendrimers have become the most commonly used dendrimer-based siRNA delivery vehicles due to their relatively simple synthesis and commercial availability. However, PAMAMs are known to be cytotoxic, mainly through inducing apoptosis mediated by mitochondrial dysfunction (45). A number of studies have shown, some modifications in PAMAMs can reduce their inherent cytotoxicity without compromising gene silencing efficiency (46). For example, modification by conjugating either lauroyl chains or polyethylene glycol (PEG) 2000 onto the surface of cationic PAMAM dendrimers decreases its cytotoxicity (47). It is reported that surface-modified and cationic PAMAM dendrimers including QPAMAM-OH, QPAMAM-NHAc and PAMAM-NH 2 can enter cancer cells in vitro while presenting very low cytotoxicity to normal cells, even at high concentrations (48). siRNA nanoparticles were first formulated with poly(propyleneimine) (PPI) dendrimers, and these nanoparticles showed efficient gene silencing (49).

b. Chitosan

Chitosan is a natural cationic polysaccharide that possesses several characteristics including efficient complexation and condensation of siRNA into nanoparticles (50,51), biodegradability, biocompatibility, high nuclease resistance and mucoadhesive properties which are crucial factors for in vivo administration (52,53). Besides, the possibility of simple chemical changes in the polymer structure causes the acquisition of new properties and an improvement in the uptake

efficiency (54,55). Altogether, Chitosan can be considered as a suitable means of siRNA transfer. However, Chitosan has shown low water solubility at pH values above 6.5 and poor colloidal stability in physiologically relevant media (56). Moreover, it is reported that the transfection efficiency and endosomal escape of Chitosan are limited owing to its relatively weak buffering capacity (57). Research has concluded that structural modification of Chitosan in the form of Polyethylene glycol (PEG)-grafted chitosan (C PEG), may enhance the solubility and stability of the colloidal nanoparticles (58). Moreover, the gene transfection efficiency of the nanoparticles was improved by including PEI in the C PEG (56), which is ultimately appropriate for *in vivo* administration.

The conjugation of Chitosan with arginine–glycine–aspartate (RGD) peptide or RGD peptidomimetic (RGDp) mimicking the RGD motif to the distal ends of the PEG chains has been investigated with regard to its affinity towards αVβ-3 integrin receptors. With regard to the high levels of integrin expression in tumor cells and in angiogenic endothelial cells compared to normal cells, RGD-grafted structures are attractive targets, which should be considered for the delivery of siRNA in cancer therapy (59). Using RGD peptidomimetic (RGDp) compared to RGD peptide in RGD-grafted systems results in a longer half-life and higher bioavailability of nanoparticles, which is associated with the high chemical stability of the peptidomimetics (60).

c. Polyethylenimines

Polyethylenimines (PEIs) are water soluble cationic synthetic polymers which have been widely investigated for siRNA delivery. PEIs are present at different lengths and with different molecular weights, such as branched (bPEI) or linear (lPEI) and low molecular weight (<1000 Da) or high molecular weight (>1000 kDa) (61). PEIs are considered a gold standard reagent for gene transferring purposes in vivo and in vitro because they have a high density of amine groups, leading to a protein sponge effect, followed by stopping the acidification of endosomal pH. PEI has the ability to cause the influx of chloride within the compartment, thereby increasing the osmotic pressure, resulting in the swelling and rupture of the endosomal membrane (42). These synthetic polymers may enhance intracellular delivery by facilitating endosomal escape and inducing lysosomal disruption, endosomal release, and siRNA protection from lysosomal degradation by way of buffering the endosomes (62).

d. Poly (l-Lysine) (PLL)

Poly (l-Lysine) (PLL) is one of first polymers explored for non-viral gene delivery. The primary ε-amine groups of lysine in PLL have positive charges that form electrostatic complexes with negatively charged siRNA and can improve the affinity to proteins and cells (63). PLL can be produced on a large scale and is physiologically stable and safe (64). Although PLL may protect siRNA from nucleases degradation, it is hampered by several barriers that restrict its clinical application. It lacks the ability to provide proton buffering and thus is not capable of increasing the lysosomal release of transported siRNA (65).

Li *et al*., synthesized a ternary copolymer mPEG-b-PLL-g(ss-IPEI) which was used for the siRNA delivery of SKOV-3 ovarian cancer treatment. The administration of the targeted polyplex to SKOV-3-implated Balb/c mice has had a great effect on tumor growth inhibition and prolonged animal survival times (66).

e. Poly-D, L-lactide-co-glycolide (PLGA)

PLGAs are biodegradable and biocompatible, enabling them to undergo hydrolytic degradation, yielding non-toxic and neutral pH degradation products, thereby providing sustained gene delivery. PLGA has been approved by the FDA as a pharmaceutical excipient (67). However, the efficiency of siRNA delivered by PLGA nanoparticles is generally poor compared to that observed for lipid-based carriers (68). Therefore, the incorporation of common cationic excipients such as PEI, DOTAP, or polyamine (69) into PLGA nanoparticles has been widely used as a strategy to improve their transfection capability (70). Cationic lipids, such as dioleoyltrimethylammoniumpropane (DOTAP), have been successfully combined with PLGA, by using different preparation procedures. This kind of modification results in the incorporation of siRNAs in lipid–polymer hybrid nanoparticles (LPNs) (71,72). Among these, LPNs prepared at a DOTAP: PLGA weight ratio of 15:85 by using a double emulsion solvent evaporation (DESE) method resulted in nano-sized carriers with enhanced siRNA loading efficiency, sustained release, and improved transfection efficiency in vitro. Also, these carriers present promising outcomes and therapeutic effects in vivo (73-75).

f. Polymethacrylate

Polymethacrylates is a cationic vinyl-based polymer which is able to condense polynucleotides into nanometer-sized particles. The use of Polymethacrylates for transfection is limited due to their low ability to interact with membranes (42).

Lipid-based carriers

Lipid-based siRNA carrier systems include liposomes, micelles, microemulsions, and solid lipid nanoparticles (76). Among the preferable non-viral vectors, liposomes are by far the most advantageous for siRNA delivery, as they have a high gene transfection efficiency, efficient interaction with lipidic cell membranes, efficient *in vivo* delivery, enhanced endosomal release and flexible and versatile physicochemical properties. Liposomes are globular vesicles composed of an aqueous core and phospholipid bilayer, with natural body constituents (e.g., lipids and sterols), and are biocompatible and biodegradable. Moreover, owing to their relative simplicity and well-known pharmaceutical properties, liposomes are popular siRNA vehicles. Lipid-based and liposomal delivery vehicles for siRNA molecules have shown their therapeutic potential by a fast entry in the market and their inclusion in many clinical trial programs (31). A great example is Stable Nucleic Acid Lipid Particles (SNALPs), designed as the most important liposomal-like formulation for siRNA delivery (12). Morrissey *et al*., have shown that HBV replication was inhibited via the delivery of a siRNA–SNAlP complex that targeted HBV RNA (77).

Various liposomes, such as neutral, anionic, and cationic liposomes, are used in siRNA delivery studies (78). Cationic liposomes for siRNA delivery can protect the siRNA against enzymatic degradation, facilitate crossing the cell membrane, promote escape from the endosomal compartment, and reach the target genes with good biocompatibility. However, cationic lipids can cause unwanted interactions with negatively charged serum proteins because of their high cationic charge density; also, they can induce potential unwanted effects by stimulating interferon responses (79). It is reported that the transfection efficiency of cationic lipids is linked to the length and structure of hydrocarbon chains of lipids (80).

Neutral lipids have been characterized with lower toxicity and lack of immune response, longer circulation time and limited interactions with proteins in the blood. However, neutral liposomes exhibit low transfection efficiency because of their absence of surface charges (81). The commonly used cationic monovalent lipids for siRNA delivery such as 1,2-dioleoyloxy-3-trimethylammonium propane (DOTAP) and 1,2-di-o-octadecenyl-2-trimethylammonium propane (DOTMA) have combined with neutral lipids including 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) and have been successful at improving transfection efficiency. In this combination, neutral lipids facilitate fusion to the host cell's membrane, and cationic lipids can facilitate electrostatic complexation with siRNA to form more stable formulations and enter cells more easily (82).

Divalent cations like calcium have been used to prepare anionic lipid-siRNA complexes. Positively charged calcium ions improved the complex formation between anionic liposomes and negatively charged siRNA (83).

The amphipathic nature of liposomes allows them to form a wide range of hydrophilic and hydrophobic drug incorporations. Hydrophilic molecules will display greater affinity for the hydrophilic head groups of phospholipid bilayers and the aqueous core of the liposomes also, while hydrophobic molecules tend to be intercalated into the fatty acyl chains of the lipid bilayer. Several liposomal-based anticancer drugs have shown good safety records in humans and one of them, named Doxil, has received FDA approval for human use (31).

Clinical studies with siRNA based therapeutics

Therapies based on siRNA are entering clinics, especially for diseases requiring locoregional treatments, including age-related macular degeneration, diabetic macular edema, respiratory virus infection, pachyonychia congenital, hepatitis, human immunodeficiency virus infection, and cancer (31,84).

Locally delivered siRNA-based therapeutics

Local delivery of siRNAs is beneficial for diseases, as tissues are externally accessible or locally restricted. To date, locally administered siRNAs have been used in clinical trials for topical diseases mostly including the eye such as age-related macular degeneration (AMD), diabetic macular edema (DME), and glaucoma, as well as in those for a small number of other diseases, involving respiratory syncytial virus (RSV) infections, pachyonychia congenita, and pancreatic ductal adenocarcinoma.

In 2004, the first clinical trial involving siRNA was carried out for the treatment of AMD and DME (85). In this study Nguyen *et al*., utilized bevasiranib, a siRNA targeting vascular endothelial growth factor (VEGF) to inhibit retinal neovascularization in patients with AMD and DME. They observed biological activity in both phases I and II clinical trials. However, the phase III trial was terminated early because of poor efficacy in reducing sight loss.

One of the local delivery examples of siRNA in cancer treatment is siG12D, which was encapsulated in a

biodegradable polymer Local Drug EluteR (LODER) to provide controlled and prolonged delivery for pancreatic ductal adenocarcinoma (86).

Systemically delivered siRNAs based therapeutics:

Currently, there are some examples of cancer clinical trials using nanoparticle-based systemic siRNA delivery (Table 1). The first clinical trial of the siRNA for human solid tumors was performed in 2008. They used ribonuclease reductase regulatory subunit M2 (RRM2) using a cyclodextrin-based polymer conjugated siRNA (87) Self-assembled cyclodextrin nanoparticles were pegylated and conjugated with the transferrin ligand (88). Dose-limiting toxicity was observed in several patients, and the trial was terminated (89).

Table 1. Examples of siRNA cancer therapeutics in clinical trials

Target gene	Intervention	Malignancy	Phase
EphA2	Neutral liposome (DOPC)	Advanced solid tumors	I
Fus1	Nanoparticle (DOTAP): Chol-fus1	Lung cancer	I/II
EGFR	Phosphorothioate ODN	Advanced head & neck squamous cell carcinoma	I/II
M2 subunit ribonucleotide reductase (RRM2)	Cyclodextrin nanoparticle, Transferrin, PEG	Solid tumors	I
Polo like kinase I (PLKI)	Lipid nanoparticle (SNALP)	Solid tumors	I
Bcl2 interacting killer (Blk)	BikDD Nanoparticles	Advanced pancreatic cancer	I
HIF-1α	LNA antisense oligonucleotide	Advanced solid tumors or lymphoma	I
Protein kinase N3 (PKN3)	Liposome (Lipoplex, a cationic lipid)	Advanced solid tumors	I
VEGF	Dendrimer type bio-reducible polymer (PAM-ABP)	human hepatocarcinoma (Huh-7), human lung adenocarcinoma (A549), human fibrosarcoma (HT1080) cells	-

Alnylam Pharmaceuticals developed ALN-VSP02, a nearly neutral lipid nanoparticle formulation, containing two distinct siRNAs targeting kinesin spindle protein (KSP) and VEGF for the use of SNALP as a carrier. In phase I, ALN-VSP02 was well tolerated, and an anti-VEGF effect was observed in patients with advanced solid tumors presenting with liver involvement (90).

Atu027 is a cationic lipoplex-based siRNA delivery system containing chemically stabilized siRNAs which target protein kinase N3 (PKN3) carried in AtuPLEX. (91). Atu027 is recently being assessed in a Phase II trial in combination with gemcitabine for patients with locally advanced or metastatic pancreatic adenocarcinoma (NCT01808638) (92).

Landen *et al*., have developed neutral 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC)-based nanoliposomes (93). DOPC-nanoliposomes incorporating siRNAs targeting either EphA2, FAK, neuropilin-2, TMRRS/ERG, IL-8, EF2K, or Bcl-2 were active in orthotropic and subcutaneous xenograft models of various tumors.

Ewe *et al*., explored polyethyleneimine-based lipopolyplexes comprising a low-molecular-weight PEI and the phospholipid DPPC for therapeutic siRNA use. Upon systemic administration in tumor-bearing mice, it was revealed that this complex does not cause blood serum parameter alterations, erythrocyte aggregation or immunostimulation, and also the good physical condition of animals and a stable body weight confirmed by the biocompatibility of the complex (94).

Conclusion and future prospects

siRNA-based therapeutics are highly effective pathways for the treatment of multiple cancers due to specific silencing of gene expression or selective regulation of the pathways involved in cancer progression. Although fundamental progress has been made in the field of *in vivo* siRNA delivery, there are a number of obstacles and concerns that should be overcome before RNAi will be used as a new therapeutic technique. The problem with off-target effects, immune responses, degradation by nucleases, competition with cellular RNAi components and *in vivo* delivery, is reaching target cells or tissues; this has been partially overcome through strategies that are used in the design of nano-particles and manipulating their biopharmaceutical properties. In conclusion, strategies for siRNA delivery based on chemical modifications of siRNA, targeting of siRNA by viral vectors, or non-viral delivery systems all are being developed and might be considered as optimistic strategies for the treatment of cancer or other diseases.

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