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## Delivery Strategies and Potential Targets for siRNA in Major Cancer Types

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### Abstract

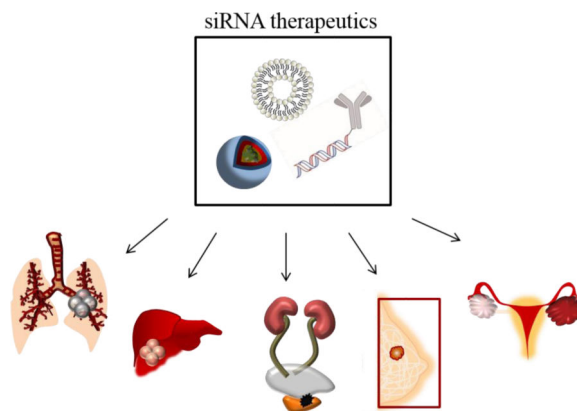
Small interfering RNA (siRNA) has gained attention as a potential therapeutic reagent due to its ability to inhibit specific genes in many genetic diseases. For many years, studies of siRNA have progressively advanced toward novel treatment strategies against cancer. Cancer is caused by various mutations in hundreds of genes including both proto-oncogenes and tumor suppressor genes. In order to develop siRNAs as therapeutic agents for cancer treatment, delivery strategies for siRNA must be carefully designed and potential gene targets carefully selected for optimal anti-cancer effects. In this review, various modifications and delivery strategies for siRNA delivery are discussed. In addition, we present current thinking on target gene selection in major tumor types.

### Graphical Abstract

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## Keywords

Small interfering RNA (siRNA); Delivery strategy of siRNAs; Cancer; siRNA therapeutics; Gene target

## 1. Introduction

The discovery of RNA interference (RNAi) has opened doors that might introduce a novel therapeutic tool to the clinical setting [1]. For many decades, small molecules have been developed and utilized in cancer therapy; however, critical problems, such as undesirable toxicity against normal tissues due to a lack of selectivity, still remain today. Using RNAi as a therapeutic tool will allow targeting previously unreachable targets with its potential to silence the function of any cancer causing gene [2]. This unique advantage is made possible by utilizing the biological functions of double-stranded RNA molecules (dsRNA). Endogenous dsRNA is recognized by a ribonuclease protein, termed dicer, and cleaved into small double stranded fragments of 21 to 23 base pairs in length with 2-nucleotide overhangs at the 3' ends. The cleaved products are referred to as small interfering RNAs (siRNAs). The siRNAs consist of a passenger strand and a guide strand, and are bound by an active protein complex called the RNA-induced silencing complex (RISC). After binding to RISC, the guide strand is directed to the target mRNA, which is cleaved between bases 10 and 11 relative to the 5' end of the siRNA guide strand, by the cleavage enzyme argonaute-2. Thus, the process of mRNA translation can be interrupted by siRNA [3-5].

The therapeutic application of siRNA has the potential to treat various diseases including cancer [6, 7]. Cancer is a genetic disease caused by the generation of mutated genes within tumor cells; multiple gene mutations both activate disease driving oncogenes and inactivate tumor suppressor genes in cancer [8-10]. Small interfering RNAs that can inactivate specific cancer driving genes have shown great potential as novel cancer therapeutics. Several anti-cancer siRNA based drugs have entered clinical trials, and many are actively sought after in preclinical research [11-13].

Even though the usage of siRNA as therapy has shown promise in the treatment of cancer, many obstacles that hinder the ultimate functionality of siRNAs in the clinic remain to be solved [14, 15]. In order to make this therapy effective, the first and most crucial step is to

ensure the delivery of siRNA to the tumor cells from the injection site. In practice, siRNAs face physiological and biological barriers that prevent their delivery to the active site when administered systemically [16-18]. These barriers include, but are not limited to, intravascular degradation, recognition by the immune system, renal clearance, impediments to tumor tissue penetration and uptake into tumor cells, endosomal escape once in tumor cells, and off-target effects [19-21]. Delivery formulations as well as chemical modification of siRNA are required to overcome these challenges and facilitate siRNAs in reaching their target cells [22]. Furthermore, selection of gene targets in cancer is also crucial in designing siRNA therapeutic strategies. Discoveries of mechanisms in cancer provide innovative targets for siRNA therapy that in many cases cannot be targeted with conventional drugs. However, the particular gene pool that drives cancer varies depending on the origins and types of the tumors. Thus, careful selection of gene targets according to their cancer type is essential in siRNA therapeutic strategies.

To summarize, target discovery in cancer leads to the selection of siRNA gene targets, followed by their incorporation of the siRNAs into suitable delivery systems that allow access to the desired sites. Once therapeutic effect is observed, further application in varying organs and tissues can be anticipated as shown in Figure 1. This review examines current thoughts on the therapeutic potential of siRNA delivery strategies and the optimal targets for siRNA in major cancer types.

## 2. Strategies for siRNA based Therapeutics

In order to activate the RNAi pathway, double stranded siRNA must travel through the bloodstream and gain access to the cytosol of target cells. The hydrophilic nature and large molecular weight of siRNAs prevent the molecules from diffusing across the cellular membrane into the cell; therefore, modifications to the nucleic acid and generation of clever delivery strategies are necessary for the creation of siRNA therapeutics.

### 2.1. Chemical Modifications

With current bioorganic techniques, oligonucleotides can be synthesized and modified as single strands, then annealed into the desired double stranded material. Customizable oligonucleotide synthesis incorporating artificial modifications enhances the potential of RNA therapeutics by overcoming problems associated with administration of naked siRNA. In particular, unmodified siRNA exposed in the bloodstream stimulates the innate the immune response and is readily degraded by serum nucleases. One of the methods to increase stability in serum and potency of gene silencing efficacy is to employ chemical modifications on the RNA-backbone of siRNA. A wide variety of chemical modifications, listed in Figure 2, have been proposed to overcome existing challenges of siRNA therapeutics.

One of the most common alterations of RNA is modification of the 2' position on the ribose backbone. These modifications include 2'-O-methyl, 2'-O-methoxyethyl, 2'-deoxy-2'-fluorouridine, locked nucleic acid (LNA), and many more [23-26]. These chemical modifications increase stability against nucleases and improve thermal stability. As a naturally occurring RNA variant, 2'-O-methyl RNA has shown reduced potency or even

inactivation in siRNA activity in the RNAi pathway upon heavy modification [27]. The 2'-fluoro modification is compatible with siRNA function and lends stability in presence of nucleases. Combined modification with 2'-fluoro pyrimidines and 2'-O-methyl purines results in highly stable RNA duplexes in serum and improved *in vivo* activity [28]. The 2'-O-methoxyethyl RNA modification has also shown significant nuclease resistance as well as increased thermal stability (T<sub>m</sub>). Nevertheless, this modification is not generally used as frequently as the 2'-O-methyl and 2'-Fluoro RNAs. LNA contains a methylene bridge that connects the 2'-O with the 4'-C positions of the ribose backbone. This causes the siRNA to have "locked" sugar that results in higher stability with increased T<sub>m</sub>. Though incorporation of LNA also interferes with the siRNA activity, limited modification retains the functionality [27].

In addition to the sugar modifications, variations in phosphate linkage of siRNA are also accepted as an alternative strategy to overcome functional limitations. The phosphorothioate (PS) linkage, perhaps the most commonly modified linkage in siRNA, often displays cytotoxicity when used extensively; however, PS incorporation does not appear to have a major effect on biodistribution of siRNA. [29]

Apart from modifications made on the backbone, chemical modifications are also made on other parts of siRNA to facilitate delivery to the target site. One of the hurdles in siRNA delivery is that weak negative charge and high molecular weight makes the nucleic acid more prone to serum degradation and capture by the reticuloendothelial system (RES). In order to form more stable delivery complexes, polymerized siRNA can be synthesized, resulting in greater electrostatic interactions and facilitating incorporation into nanoparticles. Lee *et al.* developed polymerized siRNA using a thiol group to form a stable complex with glycol chitosan via not only electrostatic interaction but also disulfide bond crosslinking. Polymerized siRNA synthesized with thiol groups was also shown to form stable complexes with PEI, albumin, transferrin, hyaluronic acid, and other nanoparticles [30-35]. This delivery reagent was shown to have an anti-tumor effect in xenograft cancer models when systemically injected.

Other chemical alterations of siRNA include base modification, change in overhangs and termini of the RNA duplexes, and varying tertiary structure of the siRNA. In an attempt to develop siRNA for use in clinical trials as drugs, various chemical modifications are being investigated to improve qualities such as serum stability, siRNA potency, low immunostimulation, off-target effects, and target organ/cell delivery [36].

## 2.2. Polymeric Nanoparticles

Incorporation of siRNAs into nanoparticles is widely used to overcome limitations of nucleic acid formulations. Biodegradable polymeric nanoparticles synthesized via a variety of methods have shown significant therapeutic potential allowing improved stability in serum, better delivery and controlled release. Nanoparticles used in siRNA delivery studies are divided into two categories—natural polymers and synthetic polymers. Cyclodextrin, chitosan, atelocollagen, albumin, gelatin and others are promising natural polymer candidates for siRNA delivery in cancer and other diseases [31, 33, 37]. Synthetic polymers

such as polyethylene glycol (PEG), polyethyleneimine (PEI), poly (d,l-lactide-co-glycolic acid) (PLGA), and others have also been extensively investigated as siRNA delivery agents.

Cyclodextrin polymer (CDP) was used as a raw material for first nanoparticles delivery system for siRNA used in clinical trials [38]. CDP is a polycationic oligosaccharide produced during bacterial digestion of cellulose and used by the pharmaceutical industry to deliver small molecules [39]. As a siRNA delivery formulation, self-assembled CPD with PEG and human transferrin (Tf), denoted as CALAA-01, has demonstrated improved targeting ability for cancer cells in preclinical tests. Subsequently, this system was tested in a clinical phase trial 1. Another polymeric nanoparticle class frequently used in nanomedicine is chitosan-based systems. Chitosan, a polysaccharide originally derived from chitin, is comprised of  $\beta$ -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The cationic nature of the resulting nanoparticle allows electrostatic interactions with negatively charged siRNAs facilitating formulation into the delivery system. Various modifications of chitosan, such as PEGylation and thiolation, have also shown potential in cancer models via a variety of routes of administration [30, 40-43]. Atelocollagen/siRNA complexes have also exhibited anti-cancer effects in several xenograft cancer models. Collagen is a fibrous structural protein in connective tissue. By pepsin treatment, the telopeptide of collagen is removed to reduce immunogenicity generating highly purified type I collagen, also known as atelocollagen. Atelocollagen particles have shown improved cellular uptake, nuclease resistance, and controlled release of nucleic acids [44]. Inhibition in tumor growth of an orthotopic xenograft cancer model has been seen when atelocollagen/siRNA complexes were intratumorally administered [45]. Several factors, such as low toxicity, biodegradability, reduction in immunostimulation, and facile condensation with nucleic acids, contribute to advantages of using natural polymers as delivery agents for siRNA.

Synthetic polymers have been extensively investigated in the drug delivery field because of their well-defined chemistries and the high degree of molecular diversity obtainable via chemical modifications [46]. PEI is considered the most potent in its ability to form stable complexes with nucleic acids due to its highly positive charged nature. Branched PEI (bPEI), which has been found to have lower toxicity than linear PEI, and its derivatives have demonstrated successful siRNA delivery to the tumor sites with resultant anti-cancer effects in preclinical studies. In addition to PEI/siRNA complexes, PEGylation is often incorporated into nanoparticles to increase stability in biological fluids and lend protection against nucleases. Several groups have demonstrated a PEI-PEG platform for effective siRNA delivery in various cancer models [32, 47-49]. Another widely studied synthetic polymer is PLGA. PLGA is a copolymer made from lactic acid and glycolic acid that is approved in therapeutic applications due to its high biodegradability and biocompatibility. With a sustained drug release profile due to slow hydrolytic degradation, PLGA was linked with siRNA through disulfide linkage to form spherical micelles which showed gene silencing effects in breast cancer cells [50].

Though other delivery strategies are gaining attention, particularly including lipid-based siRNA delivery, many investigators continue to use and seek to improve polymeric nanoparticles, based on their stability, flexibility of modification, and high targetability.

Nevertheless, deeper understanding into the toxicity and immunogenicity of these delivery methods is necessary to develop polymers as a siRNA delivery strategy for clinical use.

### 2.3. Lipid-based Delivery

Among various strategies to overcome challenges of siRNA therapeutics, lipid-based nanoparticles have great potential due to their biocompatibility and low toxicity in comparison to inorganic, viral and synthetic polymers. In particular, cationic lipids have emerged as attractive siRNA delivery vehicles owing to their electrostatic interaction with nucleic acids, high transfection efficiency into mammalian cells, and improved pharmacokinetic profiles. 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) is a type of cationic lipid that is commonly used in laboratories and is also commercially available. Kim *et al.* demonstrated a liposomal siRNA/DOTAP/Cholesterol platform for liver-targeting delivery of siRNAs against HBV [51]. Yagi *et al.* demonstrated a siRNA delivery complex utilizing cationic DOTAP attached to egg phosphatidylcholine (egg-PC) and PEG lipid in a weight ratio of 24:14.8. This complex has been shown to inhibit tumor growth in a xenograft cancer model via systemic injection [52].

For *in vivo* delivery studies, stable nucleic acid-lipid particles (SNALPs) have been formulated and tested in multiple disease models. SNALPs consist of a lipid bilayer of fusogenic and cationic lipids entrapping nucleic acids in the core. The surface of the SNALP is coated with PEG to provide enhanced hydrophilicity for improved stability in the serum. The half-life of a siRNA-SNALP complex is much longer compared to unformulated siRNA. An HBV targeted siRNA-SNALP has shown specific reduction in HBV mRNA when intravenously administered in a mouse model of HBV replication at a dosage of 3 mg/kg/day [28]. A siRNA-SNALP delivery complex was also tested against Ebola virus (EBOV) related genes in a guinea pig model [53]. Furthermore, an ApoB specific siRNA encapsulated in a SNALP has shown to have >90% maximal silencing effect of ApoB mRNA in liver upon a single systemic dosage of 2.5 mg/kg in cynomolgus monkeys [54]. Thus, RNAi-mediated gene silencing in non-human primates has clearly demonstrated the therapeutic potential of this new class of drug using SNALP technology.

Although cationic lipid-based siRNA delivery has demonstrated potential in therapy in various disease models, several hurdles remain to enter commercialization of this class of drugs. Toxicity and immediate immune responses elicited by lipid-based delivery designs must be further investigated, and it is likely that further thoughtful modifications will need to be devised.

### 2.4. Bioconjugated siRNAs

In addition to chemically modifying siRNA or incorporating it into nanoparticles, covalently conjugating biological agents to siRNA cargo is an alternative method to overcome barriers to siRNA efficacy *in vivo*. Such conjugated delivery systems currently include cholesterol, various peptides, antibodies, aptamers, and biopolymers with various physicochemical profiles, as summarized in Table 1.

Cholesterol conjugation of siRNA facilitates cellular import and improves intracellular activity of siRNA when injected systemically [22]. Cholesterol in circulation is transported



by lipoproteins in serum and taken up by hepatocytes through low density lipoprotein (LDL) receptor-mediated endocytosis. Apolipoprotein B (apoB) targeted siRNA conjugated with cholesterol showed enhanced stability in serum and greater suppression in apoB mRNA levels in the liver [55].

Biofunctional or cell-penetrating peptides can be covalently conjugated to siRNA for improved targeting of cancer cells. In a study by Choi *et al.*, siRNA conjugated with branched PEG was functionalized with a cell penetrating peptide, Hph1, as well as a cationic self-crosslinked fusogenic KALA peptide to form a polyelectrolyte complex micelle for gene silencing in MDA-MB-435 breast cancer cells [56]. In an attempt to reduce innate immune responses, siRNAs directed against the p38 MAP kinase conjugated to the HIV TAT cell penetrating peptide were intratracheally administered to in the lung [57].

For targeted delivery of siRNA to specific tissues or cell types, antibodies or aptamers are being conjugated directly to siRNAs. A considerable number of antibody based drugs including Trastuzumab, Pertuzumab, and Cetuximab are currently given to cancer patients with great success. Antibodies conjugated to chemotherapeutic small molecules have also shown successful therapeutic results, with TDM-1 serving as a prominent example of this class of antibody conjugate [58]. Recently, an anti-EGFR antibody conjugated to a siRNA targeted to KRAS has shown activity in vitro and in vivo in colon cancer resistant to EGFR inhibitors [59].

An alternative target agent that can be conjugated to siRNA is the nucleic acid based aptamer. These aptamers consist of synthetic short single-stranded RNA or DNA ligands that have been selected for target binding with high affinity and specificity. Ever since the generation of aptamers that target the extracellular domains of transmembrane receptors overexpressed in cancer cells, aptamers have gained extensive attention as active targeting moieties for cancer therapeutic agents including siRNA [60]. Meyerholz *et al.* developed an aptamer conjugated RNA-only approach for prostate cancer therapy. When siRNAs targeting the pro-survival genes, Plk1 and Bcl2, were conjugated with aptamers that specifically binds to prostate-specific membrane antigen (PSMA) and injected intratumorally in a xenograft cancer model, inhibition in tumor growth was observed [61]. Despite the high specificity and binding affinity of aptamers, aptamer-siRNA conjugation faces barriers arising from, among other causes, stability issues due to unprotected negative charge.

In addition to being used as for direct coupling to siRNAs both antibodies and aptamers can be used to target nanoparticles containing siRNAs. As a surface targeting moiety, different types of aptamer facilitate nanoparticle delivery to the specific tumor sites.

### 3. Current Targets for siRNA in Cancer

Cancer occurs as a result of a series of gene mutations in a cell. Generally, a combination of activating mutations in so-called oncogenes and the loss of tumor suppressor genes lead to uncontrolled cell growth and blockage of natural apoptotic processes [62, 63]. Because many key gene mutations involved in driving cancer, also known as driver genes, have been identified [64, 65], it is easy to see that siRNA therapeutics could be effective in cancer

treatment [66, 67]. A major advantage of using siRNA in cancer treatment is its ability to specifically inhibit any of the large set of cancer-associated genes without regard to the druggability of their protein products [67]. This allows us to potentially drug the undruggable. Furthermore, a diverse set of therapeutic siRNA molecules can be developed to target genes associated with the multiple signaling pathways which are aberrantly activated in tumors [68]. Table 1 summarizes the current status of siRNA targets for major cancers, which are discussed in more detail below.

### 3.1. Lung Cancer

Worldwide, lung cancer is the most frequently occurring tumor type with the highest incidence of cancer-related mortality. Among three main types of lung cancer, non-small cell lung cancer (NSCLC) is the most common, comprising approximately 85% of all lung cancers [69, 70]. Lung cancers often metastasize, leading to treatment failures. Despite the development of novel molecular therapies, aggressive surgery and radio-chemical therapy, prognosis for most of these patients is still very poor. It is possible to deliver siRNA based therapeutics to the lung either by systemically or via intrapulmonary administration [71, 72]. The latter route allows lower doses of siRNAs, reducing undesirable systemic side effects, and also improves the half-life of siRNAs in tumors. Hence, siRNA based therapeutics should be considered for lung cancer treatment [73].

Since cancer is a genetic disease it is always worth asking which genes are most frequently altered in a given tumor type. Epidermal growth factor receptor (EGFR) nucleotide variants are found in various types of cancer including lung cancer [74]. NSCLC in particular displays frequent EGFR mutations, which occurs on exons 18-21, encoding a portion of the EGFR kinase domain. The most common mutation class consists of exon 19 deletions, which activate the tyrosine kinase activity of EGFR, resulting in induction of downstream of pro-growth and survival signaling pathways [75]. Notably, it has been possible to construct allele specific siRNAs against certain oncogenic EGFR mutants, which have displayed significant therapeutic effects in lung tumor models with mutant EGFR alleles. The wet-weight of tumors treated with siRNAs targeting mutant EGFR were observed to be much lower than those of the control siRNA treated group. Furthermore, caspase-3 activity was upregulated in EGFR treated tumor tissue, indicating the induction of apoptosis. The allele specific EGFR siRNA treatment inhibited specific oncogenic EGFR alleles without affecting the normal EGFR allele, leading to a safe and effective treatment [76].

Activating mutations in KRAS frequently occur in lung tumors. Notably, in EGFR mutant lung tumors KRAS mutations can render them resistant to treatment with EGFR directed therapies. A seminal recent publication explored the anti-cancer therapeutic effect of targeting of both KRAS activation and loss of p53 function, another common lesion in many tumor types including lung tumors. The authors used siRNA and miRNA loaded polymer based nanoparticles in a genetically engineering mouse (GEM) model of lung cancer [77]. Nanoparticles loaded with siRNAs targeting KRAS and with a miRNA-34a mimic, which partially restores p53 downstream functions, were intravenously injected in a *Kras*/*p53* GEM model (*Kras*<sup>LSL-G12D/wt</sup>;*p53*<sup>flox/flox</sup>). Combination treatment with miR-34a and KRAS siRNA resulted in average lung tumor regression of 63% of its original volume and



increased apoptotic cells. This form of targeted multi-gene combination therapy is perfectly suited for siRNA strategies and allows personalized cancer therapy adaptable to various mutations identified in a particular patient cancer type. The mice which were treated both cisplatin and the nanoparticle formulation consisting of a combination of miR-34a and a KRAS siRNA survived significantly longer compared with mice treated with either single treatment. (Combination of miR-34a/siKRAS and cisplatin;  $159.5 \pm 19.5$  d, siLuc ;  $93.7 \pm 16.1$  d, cisplatin;  $127.4 \pm 9.0$  d, miR-34a/siKRAS;  $129.2 \pm 16.2$  d)

Human-ribophorin II (RPN2) is part of an N- oligosaccharyltransferase complex. Generally, this protein is known to facilitate human cancer resistance against chemotherapy drugs such as docetaxel. In addition, it has been reported RPN2 can serve as an anti-apoptotic protein that regulates tumor survival and cancer stem cell properties through the stabilization of mutant p53 [78]. To deliver naked RPN2 siRNA to the lung through inhalation, specifically astructured siRNAs termed, PnkRNATM and, nkRNATM were used [79]. The RPN2 targeting siRNAs inhibited the growth of a A549 luc-C8 lung xenograft and suppressed RPN2 expression.

The oncoprotein mouse double minute 2 (MDM2) is a negative regulator of the p53 tumor suppressor, which inhibits the transactivation activity of p53 via a direct interaction. A high level of MDM2 gene amplification has been detected in many human tumor types, including lung carcinomas [80, 81]. While inhibition of MDM2 function could obviously be beneficial in tumors with wild type p53, it has also been reported that MDM2 can play a significant role in tumors featuring mutant p53. Thus MDM2 could be a potential therapeutic target for treatment of human NSCLC with either wild type or mutant p53 [82]. In this regard it has been found that MDM2 siRNA modified with a pH-responsive diblock copolymer (termed MDM2 siRNA/PMPC-b-PDPA), showed significant downregulation of MDM2 expression in a xenograft of the p53 mutant H2009 cell line. MDM2 knockdown also resulted in impaired growth of H2009 tumors through cell cycle arrest and apoptosis. The tumor sizes of MDM2 siRNA/PMPC-b-PDPA treated H2009 tumor xenograft mice were significantly smaller than the tumors in control siRNA treated mice [83].

### 3.2. Liver Cancer

The total set of cancers in each organ type almost invariably constitutes a family of unique diseases. The major form of liver cancer is hepatocellular carcinoma (HCC), which is one of the most frequently occurring cancers worldwide. HCC is a complicated disease due to its broad epidemiology. Its causes include not only mutagenic environmental insults common to many tumors but also viral insults including those arising from hepatitis viruses. Hepatitis B and C infections are major risk factors for the development of HCC. Currently the treatment of HCC is limited. Surgical resection and transplantation are available to a minority of HCC patients who have an organ donor and are free of preexisting liver conditions [84, 85]. In the search for alternative modes of liver cancer treatment, many nucleotide based therapies, including siRNA therapeutics, that target signaling pathways specific to liver tumors, are now undergoing clinical trials [86].

HDACs, histone deacetylases, are crucial enzymes that regulate gene expression by deleting acetyl groups from histone substrates [87]. HDACs interact with key cancer-associated

transcription factors, such as  $\beta$ -catenin, Myc, p53, Stat3, NF-Kb, TFIIE, etc., to regulate the expression of numerous proteins implicated in tumor formation and development. Thus, altered expression and pathological activity of HDACs can lead to tumor onset and progression. HDAC2, which is overexpressed in solid tumors, promotes cell cycle progression and prevents apoptosis by inhibiting action of the tumor suppressor p53 [88]. HDAC2 targeting siRNAs have demonstrated a reduction of liver cancer cell proliferation *in vitro*. The therapeutic effect of HDAC siRNA/Lipid nanoparticles (LNP) was demonstrated in effectively reduced liver tumor growth in an orthotopic xenograft model [89].

Integrins are a superfamily of cell adhesion receptors that bind to and interact with the extracellular matrix (ECM). They are assembled from small families of  $\alpha$  and  $\beta$  glycoprotein subunits, generating 24 unique noncovalent  $\alpha/\beta$  heterodimers. The roles of integrins are both important and diverse; they take part in the regulation of cell motility, differentiation, survival, and proliferation. Among the integrin subunits, Itgb1 ( $\beta$ 1-integrin subunit) plays a critical role in liver formation and in the proliferation of liver tumor cells [90].

Overexpression of Itgb1 promotes survival and induces resistance to chemotherapy. It has been reported that activating mutations in Itgb1 affect tumorigenesis [91, 92]. Daniel G. Anderson's group reported that HCC progression can be delayed by targeting Itgb1 with siRNAs. In this study human MET and  $\delta$ N90- $\beta$ -catenin were delivered to mice hepatocytes to construct a mouse model of spontaneous HCC [93, 94]. After the introduction of the oncogenes, injection of Itgb1 siRNA/LNP inhibited HCC progression [95]. Liver weights of the Itgb1 siRNA/LNP treated group were significantly reduced compared to those of control groups. HCC tissues also showed a significant reduction in the size and number of tumor foci and enlarged hepatocytes in the residual tumor nodules.

Survivin is a protein inhibitor of apoptosis that is strongly expressed in hepatocellular carcinomas, but is not expressed in differentiated adult tissues [96]. Over-expression of survivin protein can inhibit caspase activation, thereby leading to inhibition of apoptosis and stimulation of HCC cell proliferation [97]. A tumor growth inhibition study using survivin targeting siRNA delivered by RGD-PEG-g-PEI-SPION nanoparticles was reported [98]. Mice bearing tumors arising from human HCC cell line, Bel-7402, were injected with survivin siRNA/RGD-PEG-g-PEI-SPION and exhibited delay in tumor growth. Inhibition of the survivin gene expression by siRNA resulted in an increase of cleaved caspase-3 expression.

Pokemon, a member of the POK family of transcriptional repressors, has been observed to have aberrant overexpression in multiple human cancers including liver tumors [99]. Pokemon plays a critical role in cellular transformation, as a central regulator of the ARF (Alternative reading frame)/p53 pathway and the Rb (retinoblastoma)/ E2F (Early- region -2 transcription factor) pathway [100]. A reconstituted high density lipoprotein (rHDL) based delivery system was used for Pokemon siRNAs in a tumor model with HepG2 cells overexpressing scavenger receptor class B type I (SR-BI). SR-BI, which is expressed in the liver and most malignant cells, interacts with the major component of HDL, apoprotein A-I, to maintain cellular cholesterol homeostasis. The relative expression levels of the Pokemon and Bcl-2 protein were markedly reduced in tumor tissues of the mice treated with

cholesterol conjugated Pokemon siRNA/ rHDL as compared to the control group resulting in tumor growth inhibition [101].

Human telomerase reverse transcriptase (hTERT) is an essential component of human telomerase, which is often required to maintain stable telomere length in cancer cells. In liver and breast cancer cells, hTERT is highly expressed, which correlates with telomerase activity. Treatment with a siRNA targeting hTERT conjugated with bioreducible polyethylenimine(SS-PEI) reduced telomerase activity levels and proliferation of HepG2 cells *in vitro*. Also, when hTERT siRNA/SS-PEI was injected into HepG2 tumor bearing mice by intratumoral (i.t.) injection, tumor sizes were significantly smaller compared to the control siRNA treated tumors [102].

The CSN5 protein is the catalytic center of the mammalian COP9 signalosome (CSN) complex and plays a crucial role in cell proliferation and senescence. In particular, CSN5 binds to transcription factors c-Jun and JunD. It also regulates the stability and controls the function of numerous intracellular regulators of cell proliferation and/or apoptotic signaling such as MYC, c-Jun, JunD, NFkB, and p53 [103]. Generally, high expression of CSN5 has been found in numerous human cancers, suggesting that knockdown of CSN5 could be an effective anti-cancer strategy [104, 105]. Therapeutic efficacy of CSN5 gene knockdown was reported as early as 2011 [106]. In orthotopic mouse model of hepatocarcinoma, Huh-luc cells, were treated with either  $\beta$ -galactosidase siRNA/SNALP or CSN5 siRNA/SNALP (2mg/kg) by intravenous injection. The CSN5 siRNA/SNALP effectively inhibited the hepatic tumor growth whereas the  $\beta$ -galactosidase siRNA/SNALP injected group showed partial liver parenchyma. Tumor growth inhibition effect arising from CSN5 knockdown was driven by induction of apoptotic cell death and delay of cell cycle progression.

### 3.3. Prostate Cancer

Prostate cancer is the most frequent malignant cancer in men. Several therapies are recommended for prostate cancers including prostatectomy, anti-androgenic hormone therapy, chemotherapy and radiotherapy. However, these therapies often permanently lower the quality of life or result in negative impact to healthy organs. A frequent genetic lesion in prostate cancer is the loss of the PTEN tumor suppressor gene, the protein product of which antagonizes the PI3K pathway. However in clinical trials, PI3K inhibitors have showed little effect as monotherapies. Thus the area is wide open for new targets and therapies directed toward them. Notably, oncogenes related to proliferation and metastasis of prostate cancer, have been identified as potential targets for RNAi based prostate cancer therapy [107].

Myc is a transcription factor associated with various biological processes, including replication, transcription, protein synthesis, cell division, and more [108]. Overexpression of Myc is observed frequently in primary and metastatic prostate cancers. A recent study by the Catapano lab provides insight into the efficacy of Myc inhibition by siRNA in prostate cancer-stem like cells using *in vitro* and *in vivo* models of human prostate cancer. Treatment with Myc targeting siRNAs resulted in a reduction of stem-like properties such as self-renewal and tumor-initiation, while further inducing cell senescence in monolayer cultures of human prostate cancer cells. Tumor masses of Myc siRNA/jet-PEI treated mice group

were almost completely suppressed, whereas the control group had a sharp increase in tumor growth [109].

Casein kinase II (CK2) is a serine/threonine kinase, which exists as tetramer of two  $\alpha$  catalytic subunits and two  $\beta$  regulatory subunits. CK2 is amplified in various cancer types and can activate and regulate the stability of several tumor suppressor proteins, oncogenes, and even key transcription factors like c-Myc, c-Jun, and NF $\kappa$ B [110, 111]. To test whether CK2 might be a suitable target for therapy, mice bearing human prostate cancer cell xenografts were treated with CK2 siRNA using nanocapsules. The treated mice resulted in about 50% less tumor mass and reduced tumor nodules compared to the vehicle treated animals [112].

As noted above prostate cancer is largely promoted by altered signaling in the androgen receptor (AR) and the PI3K/PKB/mTOR pathways. Notably, signaling in both pathways is coordinated by KLK4 (Kallikrein-related peptidase 4). Thus KLK4 has an important role in PCa progression via its role in regulating cell-cycle gene expression. Although KLK4 is expressed in the normal prostate gland, it is significantly more expressed in malignant prostate cancer. [113, 114]. Therefore, it is not surprising that LNCaP or VCaP prostate cancer cells treated with siRNA/liposome targeting KLK4 showed down-regulated AR signaling and KLK4 gene expression. Furthermore, the KLK4 siRNA treated mice showed a dramatic tumor regression of 90% [115].

Notch signaling is associated with multiple cellular processes including differentiation, proliferation, and apoptosis. Dysfunctional regulation of the Notch pathway has been demonstrated in a range of cancers. In prostate cancer cells, abundant Notch1 expression influences tumor invasion. Recent studies indicate that siRNA mediated Notch1 knockdown inhibited invasion and proliferation of prostate cancer cells [116]. Down-regulation of Notch1 expression by a Notch1 targeting siRNA/PSAM-protamine inhibited tumor growth and increased apoptosis in a LNCaP subcutaneous murine xenograft model [117].

### 3.4. Breast Cancer

Breast cancer is the most frequently diagnosed type of cancer and the leading cause of cancer related death in women [118]. Breast cancer can be divided into distinct subtypes by using either immunohistochemical (IHC) staining patterns for key receptors or, more recently, molecular profiling. The resulting subtypes display different clinical behaviors and responses to treatment [119]. There are many treatments for breast cancer including surgery, chemotherapy, and radiotherapy as well as the use of targeted therapies. Notably, inhibition of various genes that cause breast cancer through siRNA has been tested in animal models.

As one common way of subdividing breast cancers, tumors are classified by their expression of three receptor molecules: the estrogen receptor, the progesterone receptor, and the human epidermal growth factor receptor 2 (HER2). The estrogen receptor alpha (ER- $\alpha$ ), a ligand-activated transcription factor, is one of two types of estrogen receptor. It plays a crucial role in regulation of the cell cycle progression of mammary epithelial cells. Approximately 70% of breast cancer cases are observed to be ER- $\alpha$  positive often with overexpression of estrogen receptors [120]. To test siRNA targeting the estrogen receptor, MCF-7 cells, an ER-

$\alpha$  positive cell line, were used to create xenografts. Intravenous injection with ER- $\alpha$  targeted siRNA-encapsulated stealth nanocapsules composed of PEG-co-poly( $\epsilon$ -caprolactone-co-dodecyl  $\beta$ -malate) reduced tumor growth and downregulated the level of ER- $\alpha$  [121].

Approximately 15 % of breast tumors are known as triple-negative breast cancer (TNBC), because they lack IHC expression in HER2, estrogen receptor, and progesterone receptor [122, 123]. Since the growth of TNBC is not dependent on the hormones and/or HER2 receptors, common therapies such as anti-hormonal or HER2 targeting therapies are ineffective. Goga *et al.* provided a potential target for TNBC treatment by discovering that inhibition of cyclin-dependent kinase 1 (CDK1) induced synthetic lethality in TNBC overexpressing c-Myc. As a synthetic lethality-based TNBC treatment, CDK1 targeting siRNA were used with cationic lipid assisted poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PLA) nanoparticles (lipid NP) as the siRNA carrier. CDK siRNA/lipid NP delivered by systemic injection significantly reduced tumor growth in mice bearing c-Myc overexpressing SUM149 and BT549 xenografts without any systemic toxicity or innate immune response [124]. Obviously, siRNA directly targeting Myc described earlier could also be tried in this setting.

Another kinase target that has been examined in breast cancer is the ataxia-telangiectasia mutated (ATM) protein, a serine/threonine kinase that regulates DNA damage repair and cell cycle checkpoints and activates downstream signals including p53, CHK2, and BRCA following as DNA damage. For breast cancer therapy, an ATM targeting siRNA/porous silicon-based multistage vector (MSV) was administered in an orthotopic MDA-MB 231 mouse model. Biweekly treatment of ATM siRNA/MSV inhibited tumor growth and reduced ATM expression in IHC assay of tumor tissue [125].

Aberrant epigenetic regulation is frequently associated with the tumorigenic process. DNA methylation is a key epigenetic marker that serves as an important regulator of gene transcription. DNA methylation is organized and maintained by DNA methyltransferases (DNMTs). Aberrant patterns of DNA methylation have been identified in many types of human malignancies; for example, hypermethylation of tumor suppressor genes at CpG islands is associated with gene inactivation [126]. Jun Wang *et al.* reported that a DNMT targeted siRNA coupled with a fusion protein consisting of an anti-HER2 single-chain antibody fragment with a positively charged protamine carrier has successfully suppressed DNMTs in the HER2-expressing BT474 breast tumor model. Down regulation of DNMTs by siRNA induced re-expression of the RASSF1A tumor suppressor gene, which led to inhibition of tumor growth [127].

The osteopontin (OPN) protein binds to multiple cell surface receptors to induce cell adhesion and migration. OPN has been considered as a potential prognostic marker in breast cancer progression because elevated levels of OPN have been found in blood and plasma of patients with metastatic breast cancer. For this reason, suppression of OPN may be utilized as a therapeutic strategy. When mice bearing MDA-MB-231 xenografts were treated with an OPN targeting siRNA encapsulated in glycerol propoxylate triacrylate (GPT) and spermine (SPE) nanoparticles, significant inhibition of breast tumor growth with accompanying knockdown of OPN were observed [128].

### 3.5. Ovarian cancer

Ovarian cancer is one of the most common types of cancers in women and the leading cause of death in gynecologic cancers. Debulking surgery and chemotherapies with platinum-taxane drugs are generally used in the treatment of ovarian cancer. Despite treatment, the majority of ovarian cancer patients develop recurrent tumors that are resistant to chemotherapy. Consequently, there is an important need for alternative therapeutics for ovarian cancers [129, 130]. However, in contrast to the situation discussed above for TNBC, for ovarian cancers there is a relative dearth of genetically defined targets. One frequent genetic lesion is the loss of the tumor suppressor BRCA1, which has been attacked via PARP inhibitors. Genetic or epigenetic loss of expression of the tumor suppressor PTEN is also frequent, a lesion that can be attacked with PI3K inhibitors. However, there is a clear need for new targets and strategies in this disease.

Recently, Cheung *et al.* used genome-scale pooled shRNA screens in human cancer cell lines to define 54 overexpressed and essential genes in ovarian cancer that require further validation *in vivo* [131]. Among them, ID4, a helix-loop-helix (HLH) transcriptional regulator, is highly expressed in most primary ovarian cancers and is, moreover, overexpressed in 32% of high-grade serous ovarian cancers but not in normal tissues including ovary. In a flank xenograft tumor model using OVCAR-8 cells, both intravenous and intraperitoneal injection of ID4 siRNA in a tumor penetrating nanocomplex (TPN) lowered tumor burden compared to control groups. Histological analysis of tumor tissues from ID4 siRNA/TPN treated mice revealed suppression of ID4 expression levels and higher levels of apoptosis in tumor tissues [132].

Ephrin type-A receptor 2 (EphA2) is a tyrosine kinase receptor in the ephrin family that functions as an oncoprotein. EphA2 is highly expressed in many cancer types, but expressed at low levels in normal tissue in adults. This is particularly true for ovarian cancer, where approximately 70% of human ovarian tumors overexpress EphA2. Treatment of several orthotopic ovarian cancer models with paclitaxel and an EphA2 siRNA/DOPC liposome via intraperitoneal injection significantly reduced tumor growth by 81% in the case of a SKOV3ip1 model and by 48% in a HeyA8 model [133].

Enhancer of Zeste Homolog 2 (EZH2) functions as an epigenetic regulator of gene expression that works via histone methylation. Overexpression of EZH2 appears to be involved in cancer progression, functioning by silencing the expression of tumor suppressor genes via specific histone modifications [134]. Recently, EZH2 targeting siRNA were incorporated into chitosan nanoparticles and utilized along with docetaxel conjugated PLGA-PRINT nanoparticles as a combination treatment for ovarian cancer. The combination therapy resulted in 95% reduction in tumor weight and further reduced metastasis in orthotopic mice models of ovarian cancer [135].

CD44 is a cell-surface glycoprotein and a major receptor for hyaluronic acid. Hyaluronic acid is a main component of the peritoneum, where ovarian cancer metastases frequently occur. CD44 is involved in cancer progression and metastases but is not expressed in normal cells [136]. For ovarian cancer treatment, paclitaxel and CD44 targeting siRNA with a tumor specific targeting peptide conjugated dendrimer were intraperitoneally administered in mice



bearing human cancer cells directly isolated from malignant ascites of patients with advanced ovarian carcinoma. The combination treatment group showed almost complete tumor inhibition of tumor growth compared to tumors in mice treated with paclitaxel and a control siRNA [137].

C-Jun-NH2-kinases (JNKs) are serine/threonine kinases that bind and phosphorylate c-Jun. They are members of the mitogen-activated protein kinase family that regulates cell proliferation, apoptosis, and differentiation. Continuous activation of JNKs leads to cancer initiation and progression [138]. Of the two major JNK isoforms, JNK1 and JNK2, JNK1 specifically plays an important part in cell survival by controlling cell cycle arrest and apoptosis [139]. To determine whether JNK1 knockdown by siRNA is indeed capable of eliciting anti-ovarian tumor effects *in vivo*, JNK1 siRNA packaged in DOPC-liposomes was used to treat HeyA8-bearing and SKOV3ip1-bearing nude mice in combination with docetaxel, the current standard of care. Significant decreases in tumor weight and the number of metastatic nodules were observed in the combination treatment group in comparison to the groups treated with either JNK1 siRNA/DOPC or docetaxel alone [140].

X-linked inhibitor of apoptosis protein (XIAP) is an anti-apoptotic protein that prevents apoptotic cell death in tumors. XIAP binds to caspase proteases that are primarily responsible for cell death and stops the proteolytic activation of caspases. Because deregulation of XIAP can contribute to cancer, high level of XIAP expression has been identified as a tumor marker. In SKOV-3 tumor bearing mice, administration of XIAP-siRNA/HER2-PLI (HER2 targeting PEI and PLL based copolymer) via tail vein injection significantly delayed tumor growth and increased survival time compared to the control groups [141].

#### 4. Future prospects

Despite our ever-increasing knowledge of cancer, cancer is still the second leading cause of death, overall, and is predicted to become the leading killer as heart disease therapies improve. Thanks to a multitude of large scale sequencing efforts, numerous genetic alterations have been identified in tumors, opening the way for the generation of siRNA therapeutics targeting both the mutant genes and in lesions in cancer signaling pathways arising from these genetic defects. Small molecule or antibody drugs have proven quite effective for targeting certain cell surface and intracellular protein targets. Recently small molecules and antibodies for cancer immunotherapy have been shown to have strong antitumor effects [142, 143]. However, transcription factors and certain key oncoproteins such as Ras have proven difficult to access and block with conventional drug-based or antibody mediated approaches. In terms of “undruggable” disease targets, siRNA therapeutics have the potential to specifically target and silence almost any gene target [144]. In addition, their process of identifying and optimizing an siRNA for a target is relatively rapid and siRNAs are easy to synthesize [145]. These characteristics strongly support the need to develop siRNA therapeutics for cancer treatment.

Key challenges to siRNA usage in clinic lie both in selecting the best of this vast selection of possible targets and in optimizing delivery of the siRNA agents to individual tumors. Choice

of target and delivery route are very important for enhancing therapeutic efficacy in cancer, and should be done in a manner designed to minimize side effects in normal tissue. Since delivery and target selection are perhaps the ultimate limitations for siRNA based therapy we will conclude with a few thoughts on these final challenges.

#### 4.1. Delivery challenge

Problems with pharmacokinetics and delivery are known to limit the usefulness of many of the small molecule cancer therapeutics that have been developed in recent years. However, delivery of siRNA into target cells is even more challenging than delivery of conventional molecular drugs to deliver into target cells. Inherent characteristics of siRNAs, including negative charge, rigid structure, size, and stability, complicate their passive diffusion across the cell membrane; thus, endocytosis is the major mechanism for intracellular delivery of siRNA. Another challenge in siRNA therapy is the off-target silencing of unintended genes. While the design of an siRNA is meant to maximize knockdown of a specific gene target, nevertheless, marginal mismatch of the complementary sequence in mRNA is often tolerated, leading to non-specific knockdown of genes [146].

Exogenous siRNA triggers Toll-like receptor (TLR) mediated innate immune response in both sequence-dependent and independent manners. The TLR-3 pathway is activated by siRNA independent of sequence, whereas TLR-7 in dendritic cells and TLR-8 in monocytes are activated to produce proinflammatory cytokine in a sequence-specific manner [147-149]. Several modification strategies, including 2'-O-methyl chemical modification, have proved to avoid stimulation of the innate immune response; however, the mechanism behind the activation of the immune system still needs further explanation to help avoid side effects in clinical trials.

The pharmacokinetics and pharmacodynamics of siRNA based therapeutics *in vivo* are just beginning to be studied in the clinic. Optimal dosage levels, timing and duration are expected to vary depending on delivery and targeting strategy, choice of target genes, and disease. Thus our understanding of siRNA based therapies is in its infancy.

#### 4.2. Targeting challenge

As can be seen in the previous sections on specific diseases, cancer is a target rich disease. A major question is which targets deserve the most attention, in the target rich environment that we have described. Here perhaps we can rely on a combination of our knowledge of the genetic basis of cancer and common sense to help make these difficult choices. Cancer is a genetic disease and many successful small molecule therapeutics have directly targeted key genetic lesions. Thus, we should strongly consider potential genetic targets but perhaps concentrate our efforts on targets that are difficult to reach with small molecules. For example, oncoproteins like c-Myc or KRAS are presented in mutated form in about 50% of human cancer types [150]. However, no drugs targeting either c-Myc or KRAS directly have been approved, mainly because of their chemically intractable characteristics. Therefore, RNAi therapeutics against these genes justifiably rank high on target lists.

Further targets are being identified that will allow marshalling of the immune system against tumors and blocking cancer support systems such as the vasculature that facilitate tumor

growth. Immunotherapies targeting programmed cell death protein 1 (PD1, Nivolumab), programmed cell death ligand 1 (PDL1) and cytotoxic T lymphocyte antigen 4 (CTLA4, Ipilimumab) have shown exceptional success in melanoma, Hodgkin lymphoma, non-small-cell lung cancer (NSCLC) and bladder cancer, by blocking immune-inhibitory signals, thus enhancing the immune response against cancer [151]. SiRNA therapy is also suited for targeting immune cells because it can be modified to target a specific cell type and used for individual or multiple targets. Anti-tumor immunotherapies with siRNAs against immunosuppressive factors have been reported in dendritic cells [152-155], monocytes [156] and tumor associated macrophage [157] and have exhibited significant results in cancer treatment. In study by Dolina *et al.*, Pdl1 was effectively silenced in Kuffer Cells via in vivo administration of PD-L1 siRNA encapsulated in lipidoid nanoparticles (LNP) into mice. Silencing of Pdl1 effectively enhanced the NK cell and CD8+ T cell intrahepatic accumulation, viral clearance, and CD8+ T cell memory to hepatotropic viral infection. This study demonstrated that transient knockdown of PD-L1 using siRNA directly on the disease-causing cell type might even have benefits when compared to monoclonal antibody usage [158].

Alternatively, a high priority should be placed on investigating multi-targeted gene therapy utilizing a combination of siRNAs to block several pathways. This approach parallels efforts to generate combinations of small molecule therapies, but can be more easily achieved using siRNAs. As mentioned earlier, different types of small RNAs (e.g., siRNA and miRNA) can be partnered in combination therapy [77].

Combination therapy with siRNAs and chemotherapeutic drugs is a credible alternative method to combat tumor heterogeneity and chemo-resistant tumors [159-161]. The combination therapy can overcome multi-drug resistance and improve drug therapeutic response. Amiji group tested combination treatment with two anti-apoptotic genes, bcl-2 and survivin, targeting siRNA and cisplatin to overcome drug resistance in NSCLC [162]. Overexpressed bcl-2 in NSCLC is involved in tumorigenesis and drug resistance as an activator of anti-apoptotic cellular defenses. Using cisplatin-resistant tumor-bearing mice, combination therapy exhibited more effective tumor growth inhibition compared to single therapies as proved by following % inhibition: control siRNA + cisplatin 29%, bcl-2 siRNA + cisplatin 58%, and survivin siRNA + cisplatin 52%. Furthermore, the combination of bcl-2 and survivin siRNA and cisplatin treatment suppressed tumor growth by 62%.

Finally, a combination approach using siRNA with a variety of cancer therapies such as chemotherapy, immunotherapy, radiation therapy, or photodynamic therapy may dramatically improve the efficacy of cancer therapy. In this strategy, each form of therapy can be used on targets particularly suited to the therapy type, such as small molecules inhibitors for kinase targets and siRNAs for targets that are structurally unsuited to small molecule attack. Moreover since different therapeutic modalities may trigger different forms of resistance mechanisms such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRP1, MRP2) for small molecules drugs and other yet to be determined modes for siRNAs, such multimodal therapies may be harder for tumors to circumvent. It is our strong hope that by skillful delivery and careful target selection siRNA nanoparticles may take a

prominent place in the armamentarium that is being assembled to treat the many diseases that constitute cancer.

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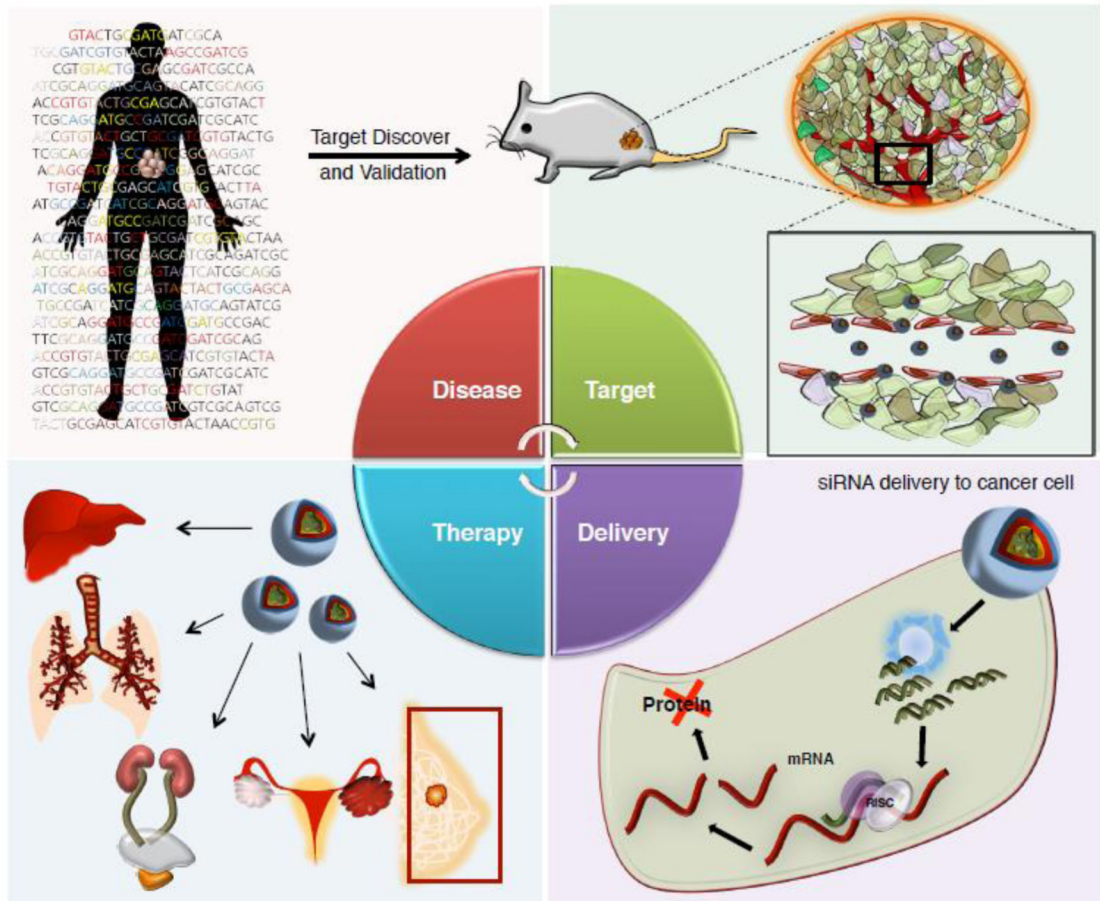
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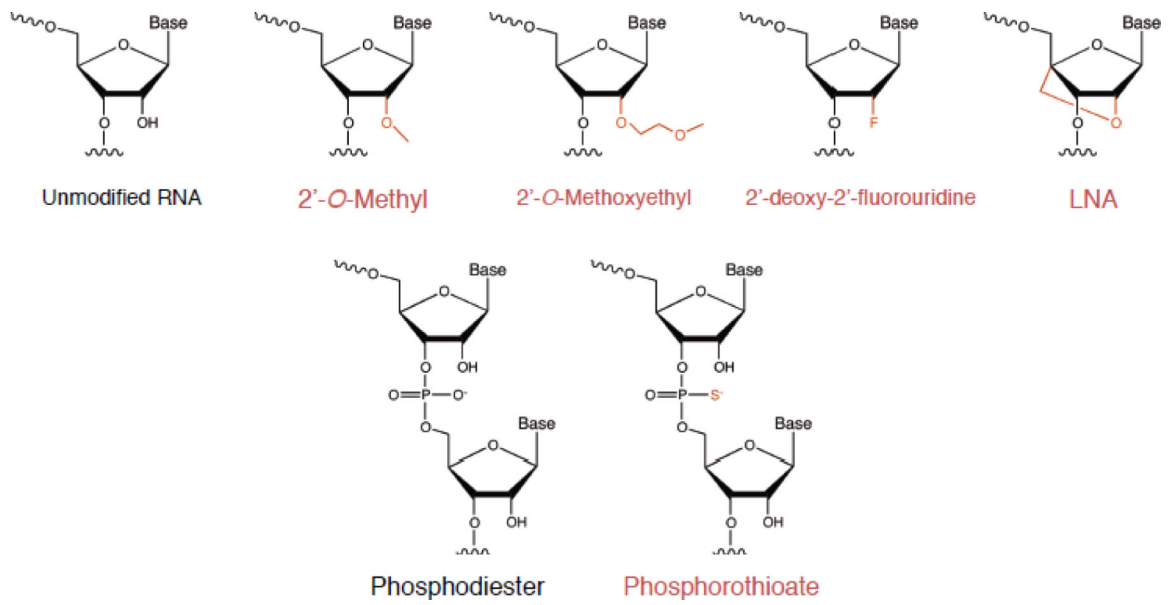
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**Figure 1.** Development process of siRNA therapeutics for cancer treatment





**Figure 2.**  
Chemical Modifications and siRNA

**Table 1**

## Types of Conjugated siRNA

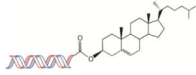
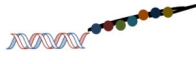
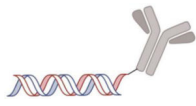
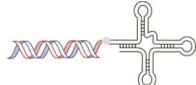
Conjugation	Structure	Function	Example
<b>Cholesterol</b>		Increases hydrophobicity for stability, free nucleases resistance of siRNA in serum, and mRNA suppression by polyplexes	Cholesterol (C <sub>27</sub> ) [55, 57, 163, 164] Cholesteryloxypropan-1-amine (COPA) and Cholesteryl-2-aminoethylcarbamate (CAEC) [165]
<b>Peptide</b>		Cell-penetration peptides (CPP) is able to cross the biological membrane for intracellular delivery	TAT [166, 167] MPG8 [168] Pep-3 [169] Penetratin [57] Transportan [170]
<b>Antibody</b>		Antibody increases the target ability by ligand binding to specific receptors of cancer	Trastuzumab anti-TENB2, anti-NaPi2b [171] F105-P[172] F5-P [173] HIRMAb, TfRMAb [174] Anti-EGFR [59]
<b>Aptamer</b>		Aptamer selectively deliver siRNA to affected tissue via specific binding with reduced side effects	PSMA aptamer [61, 175-177] BAFF-R aptamer [178] HER2 aptamer [179]

Table 2

siRNA Targets for Treatment of Major Cancers

Cancer type	siRNA/target gene	Route	Animal model	siRNA modification/Delivery system	Ref.
Lung	RPN2	inhalation	A549-luc-C8 cells via I.V.	naked PnkRNA and nKRNAS	[79]
	C7orf24	jet injection	EBC-1, S.C.	naked siRNA	[180]
	Mcl1	Intratracheal	B16F10 or LLC, metastatic lung cancer	liposome	[71]
	CD31	i.v.	LLC, metastatic lung cancer	liposome	[181]
	Bcl2	i.v.	B16, metastatic lung cancer	Protein, cationic bovine serum albumin (CBSA)	[182]
	NPT2b	inhalation	K-ras <sup>LA1</sup> model	polymer	[183]
	MDM2	i.v.	H2009, S.C.	Polymer	[83]
	Survivin/cyclin B1	i.v.	TSA-Luc, metastatic lung cancer	sticky siRNA/polymer	[184]
	HDM2/C-Myc/VEFG	i.v.	H460 or A546 NSCLC, S.C.	liposome	[185]
	Survivin/Bcl-2+Cisplatin	i.v.	A549 resistant, S.C.	Polymer	[162]
Liver	HDAC2	i.v.	Huh7-luc+, orthotopic liver tumor	2'-OMe modification / LNPs	[89]
	Polo-like kinases (PLKs),	i.v.	induction by Diethyl nitrosamine (DEN)	fusogenic liposome	[186]
	$\beta$ 1-integrin	i.v.	MET/DN90- $\beta$ -catenin-induced tumour	2'-OMe-modification/ LNPs	[95]
	RRM2, adriamycin	i.v.	HepG2 Orthotopic HCC	liposome	[187]
	various siRNA (KRAS, NHP2L1, BIRC5, CDCA1, PSMA2, Aurora B, etc)	i.v.	HuH7 liver tumors	liposome	[188]
	Survivin	i.v.	Bel-7402, S.C.	RGD-Polymer	[98]
	Pokemon	i.v.	HepG2, S.C.	Cholesterol conjugation/Liposome	[101]
	hTERT	i.t.	HepG2, S.C.	Cationic Polymer	[102]
	RhoA	i.v.	SMMC-7721, S.C.	anti-EGFR Fab'-liposomes	[189]
	CSN5	i.v.	Huh7, orthotopic transplantation	2'-Ome modifications / LNPs	[106]
	COPI	i.v.	HepG2, Huh7, orthotopic	2'-Ome modifications / LNPs	[190]
Prostate	HSP 27	i.t.	PC-3, S.C.	amphiphilic dendrimer	[191]
	survivin	i.t.	PC-3, S.C.	lipid+PEI hybrid nanocarrier (LPN)	[192]
	Bcl2	i.v.	PC-3, Orthotopic	cholesterol conjugation/ lipid nanoplatfrom,	[193]
	Myc	i.p.	PC-3, S.C.	<i>in vivo</i> -jetPEI	[109]
	plk-1	i.t.	PC-3 and LNCaP, S.C.	Aptamer	[175]
	YB- 1+ rapamycin	retro orbital	PC3, S.C.	trilayer polymeric micelle	[194]
	notch1	i.v.	LNCaP, S.C.	anti-PSMA scFv fusion proteins	[117]
	HIF-1 $\alpha$ + Dox	i.v.	PC3, S.C.	Micellar nanoparticle (MNP)	[195]
	VEGF	i.v.	PC3, S.C.	thiolated siRNA/chitosan based polymer	[30]
			PC3, S.C.	cyclodextrin modified polymer	[196]
TRAMP C1, S.C.			pH-triggered amphiphilic polymer	[197]	
AR	i.v.	LNCaP, S.C.	LNP	[198]	

Cancer type	siRNA/target gene	Route	Animal model	siRNA modification/Delivery system	Ref.
	Bcl-Xl+ cisplatin	i.v.	PC-3, S.C.	atelocollagen	[199]
	PKN3	i.v.	DU1-45, S.C. / PC-3, Orthotopic	Atu027	[200]
	AURKB, and EGFR	i.t.	PC3, S.C.	Hiperfect	[201]
	cyclin B1	i.v.	PC3, S.C.	Amphipathic peptide carrier MPG-8	[168]
	EZH2, and p110a plk-1+ paclitaxel Plk1	i.v.	PC-3M-luc-C6, heart injection/bone metastasis	atelocollagen	[202]
Breast	AURKB, and EGFR	i.v.	MDA-MB-435s, S.C.	polymer based Micelleplex	[203]
	cyclin B1	i.v.	MDA-MB-435s, S.C.	cationic lipid assisted PEG-PLA nanoparticles	[204]
	CDK1	i.v.	SUM149 or BT549, S.C.	cationic lipid assisted PEG-PLA nanoparticles	[124]
	Erc	i.v.	MCF-7, S.C.	polymer nanocapsule	[121]
	DNMTs	i.v.	BT474, S.C.	anti-Her2 ScFv-protamine (F5-P)	[127]
	RhoA	i.t.	MDA-MB-231, S.C.	Cytofectin™ Transfection Reagent	[205]
	ataxia-telangiectasia mutated (ATM)	i.v.	MDA-MB-231, orthotopic	porous silicon-based multistage vector (MSV)	[125]
	osteopontin (OPN)	i.t.	MDA-MB-231, S.C.	glycerol propoxylate triacrylate-spermine copolymer	[128]
	Mcl-1 +RPS6KA5	i.t.	MDA435_WT and MDA435_resistant (R), S.C.	lipid-substituted polymer	[206]
	P-gp +DOX	i.v.	MCF7/A, S.C.	RGD-Liposome	[207]
	MnSOD	-	MCF7-BK-TR cells co-transplanted with siRNA/NPs	PAMAM dendrimer based nanoparticles	[208]
	Raf-1	i.t.	MDA-MB-435, S.C.	histidine-lysine carrier	[209]
	VEGF	i.v.	MCF-7 and HT1080, S.C.	cholesterol conjugation/ high density lipoprotein	[210]
Ovary	EZH2+Docetaxel	i.v.	HeyA8 or SKOV3ip1, Orthotopic	PLGA-PRINT nanoparticles	[210]
	VEGF	i.t.	A2780, S.C.	arginine-grafted polymer-microbubble	[211]
	EphA2+paclitaxel	i.p.	HeyA8, orthotopic	neutral liposomes	[133]
	Akt+paclitaxel	i.t.	SKOV-3, S.C.	dendrimer	[212]
	XIAP	i.v.	SKOV-3, S.C.	ternary copolymer	[141]
	Vasohibin2	i.v.	DISS and SKOV-3, S.C.	atelocollagen	[213]
	CD44+paclitaxel	i.p.	human ascitic cells, S.C.	Dendrimer	[137]
	POSTN, FAK, and PLXDC1	i.v.	SKOV3ip1, HeyA8, and A2780, Orthotopic	RGD-Labeled Chitosan Nanoparticles	[214]
	EphA2	i.v.	SKOV3ip1 or HeyA8, Orthotopic	Mesoporous Silicon Particles	[215]
	c-Jun-NH2-kinases (JNK)+Docetaxel	i.p.	HeyA8 or SKOV3ip1, Orthotopic	liposomes	[140]
	KLF6-SV1+Cisplatin	i.p.	SKOV3-Luc, Orthotopic	Accell chemically synthesized siRNAs (Dharmacon)	[216]
	FAK+Docetaxel	i.p.	SKOV3ip1, A2780-CP20, and HeyA8MDR, Orthotopic	Neutral liposome	[217]