

Expert Opinion

1. Introduction
2. siRNA for cancer treatment
3. Non-viral vectors for siRNA delivery
4. Non-viral targeted siRNA delivery to tumor
5. Expert opinion

informa
healthcare

Tumor-targeted delivery of siRNA by non-viral vector: safe and effective cancer therapy

Yunching Chen & Leaf Huang[†]

University of North Carolina at Chapel Hill, Eshelman School of Pharmacy,

Division of Molecular Pharmaceutics, Campus Box 7360 Kerr Hall, Chapel Hill, NC 27599, USA

RNA interference technology has been developed as a potential therapeutic agent for many indications, including cancer. Silencing a specific oncogene in tumor cells brings about cell death both *in vitro* and *in vivo*. However, there is a great need for powerful delivery strategies to enhance the therapeutic effect of small interfering RNA (siRNA). This review summarizes different signaling pathways inhibited by siRNA and the advantages of targeted siRNA as a delivery system.

Keywords: cancer therapy, gene delivery, liposome, nanoparticles, non-viral, polymer, siRNA, targeting ligand

Expert Opin. Drug Deliv. (2008) 5(12):1301-1311

1. Introduction

RNA interference (RNAi) was first discovered in plants in the late 1980s [1]. RNAi, serving as an antiviral mechanism [2], is a unique regulatory system that uses small double-stranded RNA (dsRNA) molecules to degrade the target mRNA in a homology-dependent manner [3]. Small interfering RNA (siRNA) of 21 – 23 bp in length is produced from longer dsRNA which is cleaved by Dicer, a dsRNA-specific endonuclease [4-6]. Dicer – siRNA interacts with TAR RNA-binding protein (TRBP) and is shuttled into the RNA-induced silencing complex (RISC). RISC further interacts with argonaute 2 (Ago2), an effector nuclease which cleaves the target mRNA between bases 10 and 11 relative to the 5' end of the antisense strand. The cleaved mRNA fragments are released from RISC and degraded.

RNAi technology has recently been developed as a potential therapeutic agent. Compared with antisense oligonucleotides, siRNAs are more resistant to nuclease degradation and show a more prolonged therapeutic effect [6,7]. RNAi works in both cell lines and various primary cells [8], if it is properly delivered. It is broadly, yet specifically, applicable to any target gene with which the sequence is known. The promise that siRNA can specifically downregulate 'undruggable' gene products brings hope to the 'incurable' diseases and ushers in a new era of pharmaceutical science [9]. This review summarizes different signaling pathways inhibited by siRNA and the advantages in the usage of targeted siRNA delivery systems.

2. siRNA for cancer treatment

2.1 Inhibition of angiogenesis

Angiogenesis is a main factor that regulates tumor growth, invasion and metastasis. The vascular endothelial growth factor (VEGF) family has been reported as a key mediator of these processes. VEGF, a glycoprotein, can activate the signaling pathway to enhance endothelial cell growth, differentiation and migration and

protect the pre-existing vasculature from death. As shown in Figure 1, activation of VEGFR-1 or VEGFR-2 can lead to cell survival, proliferation or migration via several different pathways. First, Ras pathway is activated to stimulate cell proliferation and survival via the mitogen-activated protein-kinase (MAPK) cascade. Secondly, VEGF can also phosphorylate phospholipase C (PLC- γ), which activates protein kinase C and triggers the MAPK pathway [10]. Thirdly, the AKT pathway is phosphorylated through VEGFR activation in a PI3K-dependent manner. Finally, focal adhesion kinase (FAK) activation is mediated via the C-terminal tail of VEGFR-2 and is required for cell migration [11,12].

siRNA, which downregulates VEGF expression, inhibits tumor growth in HeLa cells, ovarian carcinoma or melanoma cells *in vitro*, as well as in a PtdCho-3 xenograft model [13]. Blocking VEGF receptor expression is another way to inhibit angiogenesis. It has been shown in endothelial cells of different tumor models that siRNAs downregulating VEGFR-1 and VEGFR-2 could block angiogenesis. The combination of anti-VEGF and anti-VEGFR siRNA improves therapeutic effect [14-16].

2.2 Inhibition of tumor survival and induction of apoptosis

siRNAs targeting oncogenes that are involved in survival or antiapoptosis show great therapeutic potential for cancer therapy [17]. The Wnt pathway plays an important role in carcinogenesis. Wnt signaling is transduced through Wnt receptors to the beta-catenin-Tcf pathway, the c-Jun-N-terminal kinase (JNK) pathway or the Ca(2+)-releasing pathway. Wnt/beta-catenin signaling pathway is highly activated and results in beta-catenin accumulation in a variety of human cancers. Anti-Wnt-1 siRNA induced apoptosis in MCF-7 breast cancer cell line [18]. siRNAs blocking Wnt pathway have also been used to target cancerous stem cells in non-small cell lung cancer (NSCLC), which has limited response to single-agent chemotherapy [19]. These results suggest that siRNA inhibiting Wnt pathway may serve as a potential anticancer agent.

NF- κ B, a transcription factor which regulates various genes involved in different pathological states, has been related to oncogenesis and plays an important role in cell survival and antiapoptosis in response to chemotherapy. siRNA is currently used to inhibit the function of NF- κ B pathway. The combination of p53 siRNA and CPT-11, a topoisomerase I inhibitor, significantly delayed tumor growth and reduced tumor size in HCT116 xenograft models [20]. These studies demonstrate that siRNA targeting NF- κ B cannot only enhance apoptosis, but also increase sensitivity to radiation or chemotherapy in tumor cells [20,21].

siRNA targeting heparin binding-epidermal growth factor-like growth factor (HB-EGF), a ligand of epidermal growth factor receptor (EGFR), can inhibit every step involved in peritoneal dissemination in human ovarian cancer *in vitro*

and *in vivo* [22]. EGFR, which is overexpressed in a variety of tumors, is related to tumor proliferation, anti-apoptosis, enhanced metastasis and drug resistance [23,24]. As shown in Figure 1, activation of EGFR results in homo/hetero-dimerization of the receptor and phosphorylation of specific tyrosine kinases, which trigger several signal pathways. For example, PLC- γ binds directly to the receptor and activates PKC. The transcription factor, STAT, enters into the nucleus to activate the expression of target genes. Ras/Raf/MAPK pathway and PI3K/AKT pathway are both triggered by EGFR signaling and lead to transcription activation of target genes related to cell proliferation, antiapoptosis, invasion and metastasis [25]. siRNA against EGFR could trigger cell death and inhibit tumor growth in NCI-H460 xenograft tumor, and the complete tumor growth inhibition lasted for 1 week when combined with cisplatin treatment [26].

Many cancer cells overexpress *HER-2/neu*, which inhibits apoptosis and promotes cell growth. *HER-2/neu* overexpression activates Ras/MAPK pathway, that helps cell survival and growth [27]. Zhou *et al.* [28] demonstrated that activation of *HER-2/neu* activated AKT, which phosphorylates MDM2, could enhance MDM2-mediated ubiquitylation and degradation of p53. Loss of p53, a key molecule in regulating cell growth and apoptosis, makes the cancer cells resistant to DNA-damaging agents. Her-2 siRNA formulated in nanoparticles significantly inhibited Her-2 protein expression and suppressed tumor growth *in vitro* and *in vivo* [29].

Telomerase, an enzyme maintaining cancer cell immortality and promoting malignant transformation, is abundant in most malignant cells but is expressed only at low levels in normal somatic cells. Wang *et al.* [30] evaluated the ability of siRNA targeting the human telomerase reverse transcriptase component (hTERT) to inhibit telomerase activity in human cancer cells. In their research, tumor-specific siRNA expression system targeting hTERT driven by the survivin promoter could inhibit the growth and increase the radiosensitivity of human cervical carcinoma cells (HeLa). Shen *et al.* [31] demonstrated that siRNA targeting hTERT inhibited telomerase activity, HCT116 cell growth *in vitro* and tumorigenicity *in vivo*. They further showed the depletion of hTERT also repressed cell adhesion, migration and invasion by downregulating the expression of adhesion- and motility-related proteins such as c-Met and integrins. Taken together, hTERT siRNA may offer a new potential gene therapy strategy for cancer.

p53, a transcription factor, maintains the normal cell cycle and keeps the genome integrity through apoptosis induction in response to DNA damage. p53 is negatively regulated by MDM2. Downregulation of MDM2 by siRNA results in increasing p53 which is able to regulate its target genes [32]. Loss-of-function p53 gene mutations results in a decreased ability of the cancer cells to undergo apoptosis. Martinez *et al.* [33] showed that highly sequence-specific siRNA could suppress the expression of mutated p53 and restored the wild-type p53 in cells expressing both forms,

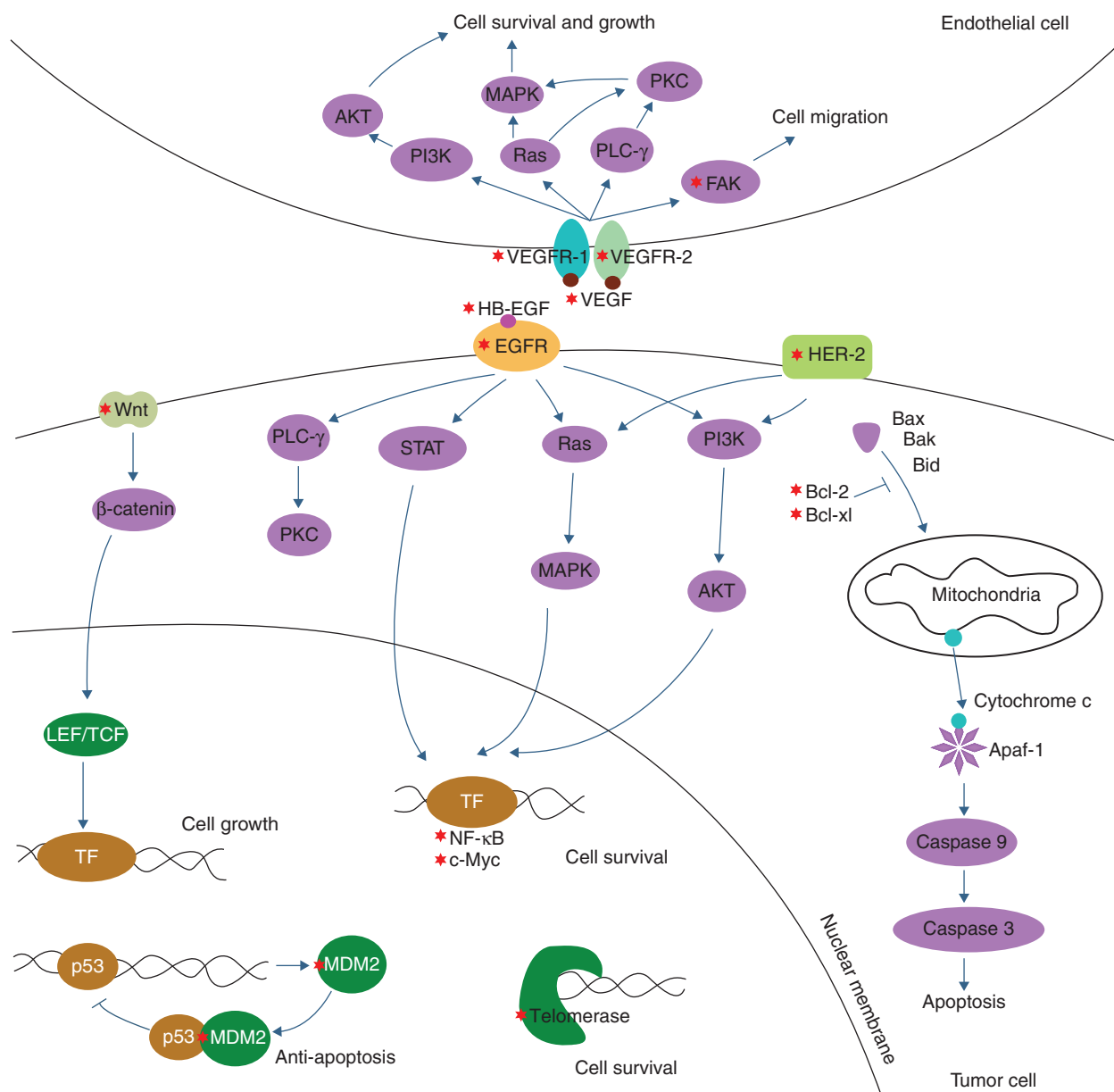


Figure 1. Cellular signaling in tumor and endothelial cells. Target genes to which siRNA has been used for downregulation are identified with a red star.

thereby promoting apoptosis of the treated tumor cells, reducing the cancer formation and inhibiting the development of malignant process.

Bcl-2, which is overexpressed in many cancer cells such as gastric cancer, has a strong antiapoptotic effect. Both Bcl-2 and Bcl-XL, which belong to the antiapoptotic Bcl-2 family, counteract the activity of pro-apoptotic molecules such as Bid, Bax and Bak and thus can suppress pro-apoptotic signaling in the mitochondria. When apoptotic signaling is triggered in the mitochondria, cytochrome c escapes from the mitochondrial inter-membrane space to the cytosol and forms apoptosomes with Apaf-1 and dATP.

After apoptosome formation, caspase-9 cleaves pro-caspase-3 into the active form caspase-3 and leads to cell death [34]. Hao *et al.* [35] reported that siRNAs inhibiting Bcl-2 expression decreased telomerase activity (by about 80%), promoted apoptosis and delayed the growth of human gastric cancer cells. Therefore, knockdown of Bcl-2, an important factor in carcinogenesis, may provide an efficient therapeutic approach for treating cancer.

2.3 Enhancing the chemo-sensitivity

Drug resistance of tumor cells is one of the major limitations of chemotherapy. Subpopulations of tumor cells resistant to

a given chemotherapeutic drug allow for continued tumor growth. Multiple drug resistance (MDR1) gene is directly related to the drug resistance to vinca alkaloids (vinblastine, vincristine), anthracyclins (adriamycin, daunorubicin), etoposide and paclitaxel. For enhancing the therapeutic effect of these chemotherapeutic agents, siRNAs were designed to inhibit MDR1 expression and convert the tumor back to the drug-sensitive state [36].

Furthermore, it has been shown that a combination of siRNAs targeting various oncogenes and angiogenic factors mediated a greater antitumor effect. In order to achieve this approach, Chen *et al.* [37] have constructed multiple siRNA expression vectors that simultaneously targeted VEGF, hTERT and Bcl-xl. The reduction in VEGF, hTERT and Bcl-xl expression significantly repressed tumor growth in human laryngeal squamous carcinoma (Hep-2) *in vivo*. Li *et al.* [38] showed that selective delivery of siRNA targeting c-myc, MDM2 and VEGF by LPD (liposome-polycation-DNA) nanoparticles significantly reduced the lung metastasis of B16F10 melanoma cells *in vivo*. These studies suggest that siRNA targeting multiple genes in human cancers could prove to be valuable in preventing cancer cell proliferation and metastasis and should be considered as a novel approach for cancer therapy.

In summary, siRNA shows great potential in many different anticancer strategies. However, there are serious obstacles that need to be overcome before it becomes a powerful new class of drug: difficulties with delivery, bio-stability, pharmacokinetics and the off-target effect, to name just a few. The half-life of the naked siRNA is less than an hour in human plasma, and the circulating siRNA is rapidly cleared by the kidneys because of their relatively small size. Recently, adverse off-target activity due to crossreactivity between RNAi pathways was identified as potential cause of toxicity [39]. It is unwise to systemically administer naked siRNA as a therapeutic agent. Thus, the use of a delivery system and/or chemical modification is developed to protect siRNA from degradation and to enhance its stability in serum.

3. Non-viral vectors for siRNA delivery

Effective strategies to deliver siRNA systematically into solid or metastatic tumor should fulfill at least five requirements: protection of siRNA from degradation and rapid clearance; prolonged circulation time after administration; efficient uptake of siRNA by the tumor; cellular uptake of siRNA; and endosomal release of siRNA into the cytoplasm [40]. Some viral vectors can effectively deliver their genomes into the tumor cells and express short hairpin RNA (shRNA) for gene silencing [41]. But none of the viral vectors can efficiently accumulate in the tumor after systemic administration. Their strong immunogenicity and other safety issues are also concerns [42].

It is of great importance to prevent instability and inactivation of siRNA in the human blood and to avoid side

effects such as the off-target effects of siRNA, the interferon response, or the activation of Toll-like receptors. This might be accomplished by several different strategies – chemical modification of siRNA, inhibition of RNase family enzymes that degrade siRNA in the blood circulation and use of siRNA carriers such as cationic liposomes and polymers [43,44]. Below are non-viral vectors that are commonly used.

3.1 Polycationic polymer based polyplexes

Systemic delivery of siRNA to tumor cells continues to be a major hurdle. Several synthetic cationic polymers and oligopeptides have been designed as systemic, non-viral delivery vectors for polynucleotides. The drawback of the carrier system is that the positively charged complex non-specifically interacts with anionic plasma proteins or other blood components, such as heparan sulfate and hyaluronic acid, resulting in the inactivation of the vector and undesired toxicity.

Therefore, using a water-soluble polymer, such as poly(ethylene glycol) (PEG) and polysaccharides, to modify the polymer/siRNA complex has been a major strategy to decrease such non-specific interactions, and thereby prolong the blood circulation time. Sato *et al.* [45] studied a cationic comb-type copolymer (CCC) consisting of a polycation backbone and side chains of water-soluble polymer and found that the dense brush of the water-soluble side-chain polymer enhanced inter-polyelectrolyte complex between the polycation backbone and siRNA. Furthermore, the CCC/siRNA complex exhibited a protective effect against nuclease activity and produced prolonged circulation time of siRNA in mice.

Bartlett and Davis [46] developed cyclodextrin-containing polycations (CDP) nanoparticles for siRNA delivery. Inclusion complex formed between adamantane (AD)-containing molecule and the β -cyclodextrin allowed for the attachment of poly(ethylene glycol) (AD-PEG) for steric stabilization and a targeting ligand (AD-PEG-transferrin) for target specific delivery of the siRNA. The nanoparticles protected siRNA from nuclease degradation, prevented aggregation at physiological salt concentrations and avoided complement fixation.

For developing a safe and serum stable carrier system that can rapidly release siRNA from endosome, calcium phosphate (CaP) is incorporated into the formulation. The siRNA-entrapped CaP nanoparticles made with poly(ethylene glycol)-block-poly(methacrylic acid) (PEG-PMA) was designed by Kakizawa *et al.* [47]. PMA nanoparticles undergo a conformational transition at pH 4 – 6, which is similar to the endosomal pH. The formulation can protect the entrapped siRNA from degradation until it arrives at the acidic endosome, where the siRNA escapes the nanoparticles and enters into the cytoplasm. They demonstrated highly efficient transfection activity of siRNA using these nano-sized calcium phosphate crystals with appreciable serum stability.

Polyethyleneimine (PEI) is a synthetic polymer which contains many cationic charges and a protonable amino

group in every third position [48]. PEI condenses DNA and delivers it into mammalian cells *in vitro* and *in vivo*. It is also known to exhibit a 'proton sponge effect' due to its strong buffering capacity at mildly acidic pH in the endosome and lysosome to facilitate the escape of DNA into the cytoplasm [49]. More recently, PEI was used to deliver siRNA but the siRNA has to be polymerized into greater lengths to increase its interaction with PEI [50]. Werth *et al.* [51] also demonstrated that the non-covalent complexation of siRNA and a commercially available polymer, Jet-PEI, led to enhanced siRNA stabilization and delivery efficacy. They also showed that lyophilized PEI/siRNA complexes retained the activity and the stability to serve as a ready-to-use reagent for specific and efficient silencing of genes.

Chitosan, a cationic polymer, has also been used to deliver siRNA. Anderson *et al.* developed the easy-to-use freeze-dried chitosan/siRNA complex capable of efficient knockdown of target gene *in vitro* with an extended storage period. These systems provide the advantage for RNAi based high-throughput screening, surface-mediated siRNA delivery for implants, and storage of siRNA therapeutics [52].

The Dynamic Polyconjugate technology has been recently developed by Rozema *et al.* [53]. A membrane-active polymer is designed to reversibly mask its activity until it reaches the acidic environment of the endosome. It also possesses the features of prolonged circulation time, reduced toxicity and targeted delivery to the hepatocytes *in vivo* after i.v. administration. They demonstrated that siRNA formulated in the dynamic polyconjugate could effectively silence either apolipoprotein B (apoB) or peroxisome proliferator-activated receptor alpha (PPARα) in the mouse liver.

3.2 Lipid-based lipoplex and liposomes

Lipid-based lipoplex and liposomes have been applied for the delivery of siRNA to provide an improved pharmacokinetic property and a decreased toxicity profile. Liposomes are composed of a single or multiple lipid bilayers and an aqueous core. Usually, a cargo is entrapped in the aqueous core of the liposomes. In contrast, a typical feature of lipoplex is a heterogeneous association of cationic lipid and nucleic acid [54]. Generally, liposomes are more stable than lipoplex in biological fluid.

Zimmermann *et al.* [55] developed a liposomal formulation, i.e., stable nucleic acid lipid particles (SNALP), for systemic delivery of siRNA in non-rodent species. siRNA against apoB formulated in SNALP could silence the disease target apoB in the liver 48 h after administration in cynomolgus monkeys. Twenty-four hours after the treatment, apoB protein, serum cholesterol and low-density lipoprotein levels were significantly reduced and the therapeutic effect lasted for 11 days. Their studies demonstrated that liposomal formulation of siRNA could be an efficient strategy for silencing hepatocyte genes.

To avoid the drawbacks of cationic lipids such as immunogenicity and instability in the serum, neutral liposomal

delivery systems have also been developed for siRNA delivery. Halder *et al.* [56] successfully delivered siRNA against FAK into human ovarian tumor in nude mice by neutral liposome 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC). The siRNA formulation inhibited FAK expression for up to 4 days in tumor tissue and reduced mean tumor weight by 44 – 72% in three different human ovarian cell lines (HeyA8, A2780-CP20 and SKOV3ip1). This group also reported recently that siRNA targeting IL-8 incorporated into neutral liposomes (siRNA-DOPCs) reduced the mean tumor weight by 32 and 52% in the HeyA8 and SKOV3ip1 mouse models and also decreased the microvessel density of these tumors [57]. Since the liposomes did not contain charges, it is unclear how siRNA could be efficiently encapsulated in the liposomes.

To prolong the circulation time of liposomes, Morrissey *et al.* [58] reported a sustained circulation in the blood of a formulation of chemically modified siRNA employing PEG-modified liposomes. They demonstrated that siRNA against hepatitis B virus (HBV) encapsulated by the modified liposomes could be delivered to the mouse liver and reduced the HBV DNA titer *in vivo*. Ueno *et al.* [59] have developed the LPD (liposome-polycation-DNA complex) which showed enhanced stability and increased transfection efficiency. Li and Huang [60] modified the LPD formulation for siRNA delivery. The tumor-targeted nanoparticles LPD-PEG-anisamide (LPD-PEG-AA) increased the tumor uptake of siRNA and the associated gene-silencing effect, resulting in an inhibition of tumor growth *in vitro* and *in vivo*.

Efficient endosomal release of siRNA into the cytoplasm can significantly improve siRNA delivery. pH-dependent (PD) liposomes have been designed for endosomal release of siRNA [61]. A polycationic block, either poly [2-(dimethylamino) ethyl methacrylate] (31 or 62 DMA repeat units) or polylysine (21 K repeat units), serves as an anchor for PEG. 1,2-dioleoyl-3-dimethylammonium-propane (DAP), a titratable lipid, was added into the liposomes to enhance the net cationic character at the acidic condition, resulting in polymer release and membrane fusion in the endosomes. Auguste *et al.* demonstrated that the polymer release from PD liposomes increased the siRNA-mediated gene silencing effect [61].

4. Non-viral targeted siRNA delivery to tumor

For cancer therapy, an effective delivery system is designed to specifically deliver functional siRNA into the target tumor cells and reach an effective intracellular concentration. A suitable targeting ligand is usually added into the carrier to achieve tumor-specific siRNA delivery. Below is a review of different targeting ligands used for delivery. A summary of the tumor-specific ligands for siRNA delivery is listed in Table 1.

4.1 Peptides

The RGD peptide has been used to target siRNA to integrins overexpressed in the tumor neovasculature.

Table 1. List of strategies for non-viral targeted siRNA delivery to tumor.

Targeting ligand	Method of delivery	Target gene	Tumor model	Ref.
RGD peptide	PEGylated PEI	VEGFR	Murine neuroblastoma N2A xenograft tumor	[62]
	Cationic liposome (DOTAP/DOPE) and PEG-PEI	Luciferase	Murine neuroblastoma N2A xenograft tumor	[63]
	Highly branched HK peptides	Luciferase and beta-galactosidase	SVR-bag4 and MDA-MB-435 cells	[64]
Tumor-homing peptide (F3)	PEGylated QD	EGFP	EGFP-transfected HeLa cells	[67]
Folate	Nanoparticles composed of cholesteryl-3-beta-carboxyamidoethylene-N-hydroxyethylamine (OH-Chol), Tween 80 and PEG(2000)-DSPE	Her-2	Human nasopharyngeal KB xenograft tumor	[29]
Sulfatide	Liposome	TN-C	Human glioma cells	[68]
AA	LPD	EGFR	NCI-H460 xenograft tumor	[26,60]
	LPD	MDM2, c-myc and VEGF	B16-F10 melanoma lung metastasis model	[38]
Anti-TfR antibody	Mono-biotinylated siRNA conjugated with the antibody-streptavidin	Luciferase	Intra-cranial C6 rat glioma or RG2 rat glioma	[69]
Anti TfR single-chain antibody fragment	Nanoimmunoliposome	HER-2	MDA-MB-435 xenograft tumor	[70]
Transferrin	OEI-HD	Ras-related nuclear protein Ran	Murine neuroblastoma N2A xenograft tumors	[71]
Aptamer against PSMA	Aptamer-siRNA chimera	Polo-like kinase and BCL2	LNCaP xenograft tumor	[74]

Schiffelers *et al.* [62] attached siRNA against VEGF receptor to PEGylated PEI with an RGD peptide as a targeting ligand. They showed suppression of angiogenesis, reduction of tumor growth in the murine neuroblastoma N2A xenograft tumor. De Wolf [63] further designed nanoparticles assembled upon complexation of siRNA with cationic liposome (DOTAP/DOPE) and RGD-PEG-PEI, a PEGylated polymer that carries RGD. They showed that both the circulation kinetics and the overall tumor accumulation of the siRNA complex were similar to non-complexed siRNA. However, the intratumoral distribution of siRNA was improved by the carriers. The benefits from using the targeted carrier were attributed to the specific transport towards the tumor mediated by the RGD ligand.

Peptide carriers have been developed that have proved effective for siRNA delivery. Leng *et al.* [64] demonstrated that the highly branched polymers composed of histidine and lysine were effective carriers of siRNA. Furthermore, RGD containing peptide carriers showed more siRNA silencing activity in an endothelial cell line (SVR-bag4) than the carriers without RGD peptide. Thus, RGD peptide can be used as a targeting ligand for siRNA delivery into the tumor neovasculature and enhance the therapeutic effect.

A tumor-homing peptide (F3) was found to target the cell-surface nucleolin [65]. It binds to the surface of the

tumor cells and is internalized by the tumor cells when administered systemically as a free peptide [66]. Derfus *et al.* [67] used a PEGylated quantum dot (QD) core as a scaffold conjugated with both siRNA and tumor-homing peptide (F3) on the particle surface. siRNA attached to the particle by a disulfide crosslinker showed a greater silencing effect than that attached by a non-reducible thioether linkage. Delivery of the enhanced green fluorescence protein (EGFP) siRNA by F3/siRNA-QD complex to EGFP-transfected HeLa cells led to significant knockdown of the EGFP signal. By replacing EGFP siRNA with other therapeutic siRNAs, the targeted complex may be useful to treat cancer.

4.2 Small molecular weight ligands

Yoshizawa *et al.* [29] developed a folate-linked nanoparticle (NP-F) for tumor-targeted siRNA delivery. NP-F was composed of cholesteryl-3-beta-carboxyamidoethylene-N-hydroxyethylamine (OH-Chol), Tween 80 and folate-poly(ethylene glycol)-distearoylphosphatidylethanolamine conjugate (f-PEG(2000)-DSPE). NP-F could deliver higher amounts of siRNA into the cytoplasm than the non-targeted nanoparticles in human nasopharyngeal KB cells, which overexpressed the folate receptor (FR). Her-2 siRNA formulated by NP-F significantly and selectively suppressed Her-2 protein expression and inhibited tumor growth *in vitro* and

in vivo. These results provide a reason for optimism regarding tumor-targeted siRNA therapy.

Tenascin-C is an extracellular matrix glycoprotein highly expressed in a range of tumors but not in normal tissues. Shao *et al.* [68] developed a liposomal carrier system using sulfatide as a targeting ligand that binds with tenascin-C. The targeted liposomes bound specifically with the tenascin-C expressing glioma cells. After binding to the extracellular matrix, the sulfatide-containing liposomes were internalized via both caveolae/lipid raft- and clathrin-dependent pathways, and the cargoes in the liposomes were released into the cytoplasm. Such targeted, lipid-based intracellular delivery shows promise for effective siRNA mediated cancer therapy in the future.

Li and Huang [60] have developed a tumor-targeted LPD formulation for siRNA delivery. This formulation included anisamide (AA), which binds with the sigma receptor overexpressed in NCI-H460 lung cancer cells. The tumor-targeted nanoparticles LPD-PEG-anisamide (LPD-PEG-AA) increased the siRNA delivery efficiency and the gene-silencing effect *in vitro*. siRNA against survivin formulated by LPD-PEG-AA induced 90% of apoptosis and sensitized the cells to cisplatin *in vitro*. Four hours after i.v. injection of LPD-PEG-AA into a xenograft model, 70 – 80% of the injected siRNA/g accumulated in the tumor, approximately 10%/g was detected in the liver and approximately 20%/g recovered in the lung [26]. siRNA against EGFR delivered by LPD-PEG-AA significantly silenced EGFR in the tumor, induced approximately 15% tumor cell apoptosis and completely inhibited tumor growth for 1 week when combined with cisplatin. They also selectively delivered a mixture of siRNA against MDM2, c-myc and VEGF co-formulated in LPD-PEG-AA into a lung metastasis model of B16F10, sigma receptor – expressing murine melanoma cells [38]. siRNAs delivered by targeted nanoparticle caused simultaneous silencing of each of the oncogenes in the metastatic nodules. Two consecutive i.v. injections of siRNA formulated in the LPD-PEG-AA significantly reduced the lung metastasis (~ 70 – 80%) and significantly prolonged the mean survival time of the animals by 30% as compared to the untreated controls. These studies indicate that surface-modified LPD may serve as a potent vector for RNAi-based tumor therapy.

4.3 Antibodies and proteins

The recently developed systemically administered, tumor-specific immunoliposome nanocomplex with high transfection efficiency could serve as a carrier for siRNA delivery and be utilized as effective anti-cancer clinical modalities when formulated with a therapeutic siRNA.

A receptor-specific monoclonal antibody delivery system and avidin – biotin technology was used to deliver siRNA into a brain tumor across the blood – brain barrier [69]. The siRNA was mono-biotinylated on either terminus of the sense strand, in parallel with the production of a conjugate

of the targeting antibody and streptavidin. Intravenous administration of the transferrin receptor antibody attached siRNA caused 69 – 81% suppression in luciferase gene expression in the intracranial brain cancer (C6 or RG-2) model *in vivo*. This study showed a therapeutic potential for brain cancer gene therapy.

Pirollo *et al.* [70] have developed an antitransferrin receptor (TfR) single-chain antibody fragment-directed nanoimmunoliposome to deliver siRNA to both primary tumor and metastatic disease. A pH-sensitive histidine – lysine peptide and a modified hybrid (DNA-RNA) anti-HER-2 siRNA molecule were used to enhance the efficiency of this complex. The nanoimmunoliposome anti-HER-2 siRNA complex could silence the target gene and its downstream pathway components *in vivo*, sensitize the tumor cells to chemotherapeutic agents and inhibit tumor growth in a pancreatic cancer model. Triche *et al.* have designed a non-viral delivery system using a cyclodextrin-containing polycation to bind and protect siRNA and transferrin. The targeted non-viral delivery system which systematically delivers siRNA against the EWS-FLI1 gene can inhibit tumor growth in a murine model of metastatic Ewing's sarcoma [71].

A polymer, OEI-HD (beta-propionamide-crosslinked oligoethylenimine) conjugated with transferrin, was used for siRNA delivery into the mouse Neuro2a neuroblastoma cells *in vitro* and *in vivo* [72]. siRNA against the Ras-related nuclear protein Ran delivered by transferrin-conjugated OEI-HD (three i.v. applications at 3-day interval) resulted in > 80% reduced Ran protein expression, increased apoptosis and a reduced tumor growth in a xenograft Neuro2A tumor models without unspecific toxicity. Bartlett *et al.* [73] used positron emission tomography (PET) and bioluminescence imaging to quantify the *in vivo* biodistribution and function of siRNA formulated in cyclodextrin-containing polycation nanoparticles. Both non-targeted and transferrin-targeted siRNA nanoparticles showed similar biodistribution and tumor localization through the enhanced permeability and retention (EPR) effect. However, the transferrin-targeted siRNA nanoparticles decreased luciferase activity in the tumor more than the non-targeted nanoparticles. Their results demonstrated that the function of the targeting ligand is to enhance the cellular uptake in tumor cells rather than tumor localization. Li *et al.* [26,60] reached the same conclusion in their work using the anisamide ligand.

4.4 Aptamers

Aptamer is a nucleic acid molecule selected for high affinity binding with a protein target [74]. McNamara *et al.* [75] have developed an aptamer-siRNA chimeric RNA capable of specific binding and delivery of therapeutic siRNAs into the target cells. The aptamer portion of the chimera had the ability to bind with PSMA, a cell-surface receptor overexpressed in prostate cancer cells and tumor vascular endothelium, but not the normal cells. siRNA delivered by aptamer – siRNA chimera were internalized and processed

by Dicer, resulting in repression of the target protein and cell death. siRNA against a survival gene delivered by aptamer – siRNA chimera also specifically inhibited tumor growth and mediated tumor regression in a xenograft model of the prostate cancer. The formulation did not contain an endosome release mechanism, so how the chimera could escape the endosomes is not clear.

5. Expert opinion

The various targeted siRNA delivery systems described above offer promising approaches for the development of safer and effective therapeutics for cancer. However, the following issues must be dealt with before a full-scale development effort can be embarked upon.

5.1 Safety

One of the important features of a good delivery system is its lack of non-specific immune stimulation. For example, transferrin-conjugated OEI-HD did not induce acute toxicity or significant changes in the host body weight, hematology parameters, or liver enzymes (AST, ALT, or AP). siRNA formulated by LPD-PEG-AA caused a low level of toxicity based on the serum level of liver enzymes and bodyweight monitoring during the treatment. The carrier itself only showed a little immunotoxicity (IMT). To improve the safety of siRNA delivery, Chono *et al.* [76] have developed a nanoparticle formulation (liposome – protamine – hyaluronic acid nanoparticles [LPH-NP]) for systemically delivering siRNA into the tumor. siRNA formulated in the targeted LPH-NP showed a similar gene silencing effect as LPD-PEG-AA, but the targeted LPH-NP showed very little immunotoxicity in a wide dose range (0.15 – 1.2 mg siRNA/kg) compared with LPD – NP (liposome-protamine-DNA nanoparticles), which had a much narrower therapeutic window (0.15 – 0.45 mg/kg).

5.2 Efficacy

As an effective therapeutic for oncology applications, siRNA formulated in nanoparticles can accumulate in the tumor through the EPR effect independent of the targeting ligand. However, targeting ligand attached to the nanoparticles can enhance the cellular uptake of siRNA and lead to enhanced potency compared to the non-targeted formulation. Receptor-mediated endocytosis of the targeted nanoparticles is considered a key feature for effective siRNA delivery. An ideal receptor targeted by the nanoparticles should have several properties, such as overexpression on the tumor cells rather than the normal cells, homogeneous distribution on all tumor cells, accessibility from the blood circulation and rapid internalization of carried cargo after binding to the target cells.

Internalization often occurs through receptor-mediated endocytosis. For example, when folate-targeted nanoparticles bind with the folate receptor, the nanoparticles internalize into the endosomes. As the pH in the endosome decreases, the formulated siRNA is released from the endosome into the cytoplasm. At the same time, the folate receptor released from the endosome returns to the cell membrane and starts a second round of internalization through binding with new folate-targeted nanoparticles [77]. A RGD – oligolysine containing cationic liposome showed the ability to deliver plasmid DNA through endocytosis. The vector complex internalizes into the early endosomes within 5 min, and then enters into the late endosomes and the lysosomes [78]. Since the vector internalization and trafficking is important for siRNA delivery, a better understanding of these processes should help design improved carriers.

Gene therapy is a potential method for the treatment of cancer. Major limitations of siRNA therapy such as low stability and poor cellular uptake need to be overcome by using a suitable vector. An ideal carrier system for tumor-targeted siRNA delivery should form neutrally charged and nano-scale particles to achieve a high EPR effect. Additional components such as PEG should be added into the nanoparticles to maintain prolonged circulation. A suitable targeting ligand facilitates the binding to the tumor cells and the internalization of the nanoparticles into the endosomes. The ability to release the cargo from the endosome to the cytoplasm such as the proton sponge effect is another key feature for improving siRNA delivery. Fortunately, siRNA does not need to penetrate into the nucleus, which is a significant barrier for plasmid delivery. In conclusion, siRNA has excellent potential to become a class of novel cancer therapeutics. A suitable carrier that can deliver siRNA into the tumor tissue and achieve the therapeutic effect is the most important issue facing the field. In particular, targeted nanoparticles offer the potential to improve the pharmacokinetics of siRNA, while providing clinical applications for diagnostic imaging and cancer therapy.

Acknowledgements

The authors would like to thank Wei-Yun Sheng and Qi Yang for their help in preparing this manuscript. The original work from this laboratory has been supported by NIH grant CA129825.

Declaration of interest

The authors state no conflicts of interest and have received no payment in the preparation of this manuscript.

Bibliography

1. Napoli C, Lemieux C, Jorgensen R. Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans. *Plant Cell* 1990;2(4):279-89
2. Ketzinil-Gilad M, Shaul Y, Galun E. RNA interference for antiviral therapy. *J Gene Med* 2006;8(8):933-50
3. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;391(6669):806-811
4. Zamore PD, Tuschl T, Sharp PA, et al. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* 2000;101(1):25-33
5. Elbashir SM, Harborth J, Lendeckel W, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001;411(6836):494-8
6. Kim K, Lee YS, Harris D, et al. The RNAi pathway initiated by Dicer-2 in *Drosophila*. *Cold Spring Harb Symp Quant Biol* 2006;71:39-44
7. Li CX, Parker A, Menocal E, et al. Delivery of RNA interference. *Cell Cycle* 2006;5(18):2103-9
8. Dave RS, Pomerantz RJ. RNA interference: on the road to an alternate therapeutic strategy! *Rev Med Virol* 2003;13(6):373-85
9. Hoerter JA, Walter NG. Chemical modification resolves the asymmetry of siRNA strand degradation in human blood serum. *RNA* 2007;13(11):1887-93
10. Butowski N, Chang SM. Small molecule and monoclonal antibody therapies in neurooncology. *Cancer Control* 2005;12(2):116-24
11. Qi JH, Claesson-Welsh L. VEGF-induced activation of phosphoinositide 3-kinase is dependent on focal adhesion kinase. *Exp Cell Res* 2001;263(1):173-82
12. Hadj-Slimane R, Lepelletier Y, Lopez N, et al. Short interfering RNA (siRNA), a novel therapeutic tool acting on angiogenesis. *Biochimie* 2007;89(10):1234-44
13. Xie FY, Woodle MC, Lu PY. Harnessing in vivo siRNA delivery for drug discovery and therapeutic development. *Drug Discov Today* 2006;11(1-2):67-73
14. Das B, Yeager H, Tsuchida R, et al. A hypoxia-driven vascular endothelial growth factor/Flt1 autocrine loop interacts with hypoxia-inducible factor-1alpha through mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 pathway in neuroblastoma. *Cancer Res* 2005;65(16):7267-75
15. List AF, Glimsman-Gibson B, Stadheim C, et al. Vascular endothelial growth factor receptor-1 and receptor-2 initiate a phosphatidylinositol 3-kinase-dependent clonogenic response in acute myeloid leukemia cells. *Exp Hematol* 2004;32(6):526-35
16. Kou R, SenBanerjee S, Jain MK, et al. Differential regulation of vascular endothelial growth factor receptors (VEGFR) revealed by RNA interference: interactions of VEGFR-1 and VEGFR-2 in endothelial cell signaling. *Biochemistry* 2005;44(45):15064-73
17. Huang C, Li M, Chen C, et al. Small interfering RNA therapy in cancer: mechanism, potential targets, and clinical applications. *Expert Opin Ther Targets* 2008;12(5):637-45
18. Wiczorek M, Paczkowska A, Guzenda P, et al. Silencing of Wnt-1 by siRNA induces apoptosis of MCF-7 human breast cancer cells. *Cancer Biol Ther* 2008;7(2):268-74
19. Rosell R, Cecere F, Cognetti F, et al. Future directions in the second-line treatment of non-small cell lung cancer. *Semin Oncol* 2006;33(1 Suppl 1):S45-51
20. Guo J, Verma UN, Gaynor RB, et al. Enhanced chemosensitivity to irinotecan by RNA interference-mediated down-regulation of the nuclear factor-kappaB p65 subunit. *Clin Cancer Res* 2004;10(10):3333-41
21. Guo J, Fu YC, Becerra CR. Dissecting role of regulatory factors in NF-kappaB pathway with siRNA. *Acta Pharmacol Sin* 2005;26(7):780-8
22. Miyamoto S, Yagi H, Yotsumoto F, et al. New approach to cancer therapy: heparin binding-epidermal growth factor-like growth factor as a novel targeting molecule. *Anticancer Res* 2007;27(6A):3713-21
23. Jones HE, Gee JM, Hutcheson IR, et al. Growth factor receptor interplay and resistance in cancer. *Endocr Relat Cancer* 2006;13(Suppl 1):S45-51
24. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;37(Suppl 4):S9-15
25. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. *Clin Cancer Res* 2006;12(18):5268-72
26. Li SD, Chen YC, Hackett MJ, et al. Tumor-targeted delivery of siRNA by self-assembled nanoparticles. *Mol Ther* 2008;16(1):163-9
27. Diehl KM, Grewal N, Ethier SP, et al. p38MAPK-activated AKT in HER-2 overexpressing human breast cancer cells acts as an EGF-independent survival signal. *J Surg Res* 2007;142(1):162-9
28. Zhou BP, Liao Y, Xia W, et al. HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nat Cell Biol* 2001;3(11):973-82
29. Yoshizawa T, Hattori Y, Hakoshima M, et al. Folate-linked lipid-based nanoparticles for synthetic siRNA delivery in KB tumor xenografts. *Eur J Pharm Biopharm* 2008
30. Wang R, Lin F, Wang X, et al. The therapeutic potential of survivin promoter-driven siRNA on suppressing tumor growth and enhancing radiosensitivity of human cervical carcinoma cells via downregulating hTERT gene expression. *Cancer Biol Ther* 2007;6(8):1295-301
31. Shen Y, Zhang YW, Zhang ZX, et al. hTERT-targeted RNA interference inhibits tumorigenicity and motility of HCT116 cells. *Cancer Biol Ther* 2008;7(2):228-36
32. Wang H, Nan L, Yu D, et al. Antisense anti-MDM2 oligonucleotides as a novel therapeutic approach to human breast cancer: in vitro and in vivo activities and mechanisms. *Clin Cancer Res* 2001;7(11):3613-24
33. Martinez LA, Naguibneva I, Lehmann H, et al. Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. *Proc Natl Acad Sci USA* 2002;99(23):14849-54
34. Lawen A. Apoptosis – an introduction. *Bioessays* 2003;25(9):888-96
35. Hao JH, Gu QL, Liu BY, et al. Inhibition of the proliferation of human gastric cancer cells SGC-7901 in vitro and in vivo using Bcl-2 siRNA. *Chin Med J (Engl)* 2007;120(23):2105-11
36. Nieth C, Pribsch A, Stege A, et al. Modulation of the classical multidrug resistance (MDR) phenotype by RNA

- interference (RNAi). *FEBS Lett* 2003;545(2-3):144-50
37. Chen SM, Wang Y, Xiao BK, et al. Effect of blocking VEGF, hTERT and Bcl-xl by multiple shRNA expression vectors on the human laryngeal squamous carcinoma xenograft in nude mice. *Cancer Biol Ther* 2007;7(5)
38. Li SD, Chono S, Huang L. Efficient oncogene silencing and metastasis inhibition via systemic delivery of siRNA. *Mol Ther* 2008;16(5):942-6
39. Scacheri PC, Rozenblatt-Rosen O, Caplen NJ, et al. Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells. *Proc Natl Acad Sci USA* 2004;101(7):1892-7
40. Pirollo KF, Chang EH. Targeted delivery of small interfering RNA: approaching effective cancer therapies. *Cancer Res* 2008;68(5):1247-50
41. Grimm D, Pandey K, Kay MA. Adeno-associated virus vectors for short hairpin RNA expression. *Methods Enzymol* 2005;392:381-405
42. Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther* 2004;15(12):1157-66
43. Meyer M, Wagner E. Recent developments in the application of plasmid DNA-based vectors and small interfering RNA therapeutics for cancer. *Hum Gene Ther* 2006;17(11):1062-76
44. De Paula D, Bentley MV, Mahato RI. Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. *RNA* 2007;13(4):431-56
45. Sato A, Choi SW, Hirai M, et al. Polymer brush-stabilized polyplex for a siRNA carrier with long circulatory half-life. *J Control Release* 2007;122(3):209-16
46. Bartlett DW, Davis ME. Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles. *Bioconjug Chem* 2007;18(2):456-68
47. Kakizawa Y, Furukawa S, Ishii A, et al. Organic-inorganic hybrid-nanocarrier of siRNA constructing through the self-assembly of calcium phosphate and PEG-based block anionomer. *J Control Release* 2006;111(3):368-70
48. Boussif O, Lezoualc'h F, Zanta MA, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci USA* 1995;92(16):7297-301
49. Vasir JK, Labhasetwar V. Polymeric nanoparticles for gene delivery. *Expert Opin Drug Deliv* 2006;3(3):325-44
50. Bolcato-Bellemin AL, Bonnet ME, Creusat G, et al. Sticky overhangs enhance siRNA-mediated gene silencing. *Proc Natl Acad Sci USA* 2007;104(41):16050-5
51. Werth S, Urban-Klein B, Dai L, et al. A low molecular weight fraction of polyethylenimine (PEI) displays increased transfection efficiency of DNA and siRNA in fresh or lyophilized complexes. *J Control Rel* 2006;112(2):257-70
52. Andersen MO, Howard KA, Paludan SR, et al. Delivery of siRNA from lyophilized polymeric surfaces. *Biomaterials* 2008;29(4):506-12
53. Rozema DB, Lewis DL, Wakefield DH, et al. Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. *Proc Natl Acad Sci USA* 2007;104(32):12982-7
54. Novobrantseva TI, Akinc A, Borodovsky A, et al. Delivering silence: advancements in developing siRNA therapeutics. *Curr Opin Drug Discov Devel* 2008;11(2):217-24
55. Zimmermann TS, Lee AC, Akinc A, et al. RNAi-mediated gene silencing in non-human primates. *Nature* 2006;441(7089):111-4
56. Halder J, Kamat AA, Landen CN Jr, et al. Focal adhesion kinase targeting using in vivo short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. *Clin Cancer Res* 2006;12(16):4916-24
57. Merritt WM, Lin YG, Spannuth WA, et al. Effect of interleukin-8 gene silencing with liposome-encapsulated small interfering RNA on ovarian cancer cell growth. *J Natl Cancer Inst* 2008;100(5):359-72
58. Morrissey DV, Lockridge JA, Shaw L, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nat Biotechnol* 2005;23(8):1002-7
59. Ueno NT, Bartholomeusz C, Xia W, et al. Systemic gene therapy in human xenograft tumor models by liposomal delivery of the E1A gene. *Cancer Res* 2002;62(22):6712-6
60. Li SD, Huang L. Surface-modified LPD nanoparticles for tumor targeting. *Ann NY Acad Sci* 2006;1082:1-8
61. Auguste DT, Furman K, Wong A, et al. Triggered release of siRNA from poly(ethylene glycol)-protected, pH-dependent liposomes. *J Control Release* 2008;
62. Schifferers RM, Ansari A, Xu J, et al. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res* 2004;32(19):e149
63. de Wolf HK, Snel CJ, Verbaan FJ, et al. Effect of cationic carriers on the pharmacokinetics and tumor localization of nucleic acids after intravenous administration. *Int J Pharm* 2007;331(2):167-175
64. Leng Q, Scaria P, Zhu J, et al. Highly branched HK peptides are effective carriers of siRNA. *J Gene Med* 2005;7(7):977-86
65. Christian S, Pilch J, Akerman ME, et al. Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels. *J Cell Biol* 2003;163(4):871-78
66. Porkka K, Laakkonen P, Hoffman JA, et al. A fragment of the HMGN2 protein homes to the nuclei of tumor cells and tumor endothelial cells in vivo *Proc Natl Acad Sci USA* 2002;99(11):7444-9
67. Derfus AM, Chen AA, Min DH, et al. Targeted quantum dot conjugates for siRNA delivery. *Bioconjug Chem* 2007;18(5):1391-6
68. Shao K, Hou Q, Go ML, et al. Sulfatide-tenascin interaction mediates binding to the extracellular matrix and endocytic uptake of liposomes in glioma cells. *Cell Mol Life Sci* 2007;64(4):506-15
69. Xia CF, Zhang Y, Boado RJ, et al. Intravenous siRNA of brain cancer with receptor targeting and avidin-biotin technology. *Pharm Res* 2007;24(12):2309-16
70. Pirollo KF, Rait A, Zhou Q, et al. Materializing the potential of small interfering RNA via a tumor-targeting nanodelivery system. *Cancer Res* 2007;67(7):2938-43
71. Hu-Lieskova S, Heidel JD, Bartlett DW, et al. Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. *Cancer Res* 2005;65(19):8984-92
72. Tietze N, Pelisek J, Philipp A, et al. Induction of Apoptosis in Murine

- Neuroblastoma by Systemic Delivery of Transferrin-Shielded siRNA Polyplexes for Downregulation of Ran. *Oligonucleotides* 2008;18(2):161-74
73. Bartlett DW, Su H, Hildebrandt IJ, et al. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *Proc Natl Acad Sci USA* 2007;104(39):15549-54
 74. Cerchia L, Hamm J, Libri D, et al. Nucleic acid aptamers in cancer medicine. *FEBS Lett* 2002;528(1-3):12-6
 75. McNamara JO 2nd, Andrechek ER, Wang Y, et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. *Nat Biotechnol* 2006;24(8):1005-15
 76. Chono S, Li S-D, Huang L. An efficient and low immunostimulatory nanoparticle formulation for systemic siRNA delivery to the tumor. *Journal of Controlled Release* 2008;
 77. Cho K, Wang X, Nie S, et al. Therapeutic nanoparticles for drug delivery in cancer. *Clin Cancer Res* 2008;14(5):1310-6
 78. Colin M, Maurice M, Trugnan G, et al. Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells. *Gene Ther* 2000;7(2):139-52.

Affiliation

Yunching Chen & Leaf Huang[†]

[†]Author for correspondence

University of North Carolina at Chapel Hill,

Eshelman School of Pharmacy,

Division of Molecular Pharmaceutics,

Campus Box 7360 Kerr Hall,

Chapel Hill, NC 27599, USA

Tel: +1 919 843 0736; Fax: +1 919 966 0197;

E-mail: leafh@unc.edu