Review Article

Targeted Delivery of siRNA

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Therapeutic application of siRNA requires delivery to the correct intracellular location, to interact with the RNAi machinery within the target cell, within the target tissue responsible for the pathology. Each of these levels of targeting poses a significant barrier. To overcome these barriers several strategies have been developed, such as chemical modifications of siRNA, viral nucleic acid delivery systems, and nonviral nucleic acid delivery systems. Here, we discuss progress that has been made to improve targeted delivery of siRNA in vivo for each of these strategies.

INTRODUCTION

The functional mediators of RNA interference (RNAi) are small interfering RNAs (siRNA) [1, 2]. These double-stranded RNA molecules are typically 19 to 23 nucleotides in length, and consequently have a molecular weight of approximately 13 to 15 kd and 38 to 46 negative charges. As a consequence, passive transport over the lipophilic cell membrane is poor [3–5]. At the same time, intracellular entry and translocation into the cytoplasm (and/or nucleus), where the RNAi machinery is located, is a prerequisite, for gene silencing activity [6–9]. More importantly, for in vivo applications, intracellular entry into the target cell within the diseased tissue is required and should lead to appearance in the cytoplasm to silence the mRNA of interest (Figure 1). Ideally, siRNA should therefore be targeted to three levels: to the target tissue, the target cell type, and the subcellular compartment.

Primary obstacles for achieving this in vivo include competitive uptake by nontarget cells, excretion in urine, degradation by nucleases, and endosomal trapping.

Some literature reports claim entry of siRNA in the target cells of the target tissue after intravenous injection [10]. The observations have been attributed to translocation of siRNA over the cell membrane by a dsRNA-receptor, referred to in *Caenorhabditis elegans* as SID-1, which is responsible in this organism for systemic spreading of the silencing effects [11]. Indeed, overexpression of the mammalian homologue increases the intracellular uptake of siRNA [12]. In contrast, "naked" siRNA is used by many researchers as a negative control which fails to produce silencing effects after injection in vivo and even after prolonged incubation of cells with high siRNA concentrations in vitro. This lack of activity of "naked" siRNA indicates that not all cell types express (enough of) the SID-1 homologue, to observe silencing effects. In addition, the rapid removal of "naked" siRNA after intravenous administration from the circulation, with more than 99% of the injected dose renally excreted and taken up by liver Kupffer cells within minutes, makes a very small percentage of the administered dose available for the target tissue. This small percentage is additionally subject to nuclease degradation. Therefore, intravenous injection of naked siRNA relying on passive targeting of the diseased tissue, and SID-1 homologue-mediated target cell uptake seem to be inefficient and as yet unpredictable.

Local injection at the site of pathology avoids many of the difficulties encountered after intravenous administration, most notably the rapid elimination, and is therefore a popular approach to increase target tissue concentrations of siRNA. With this approach chances of obtaining sufficient intracellular levels of siRNA for therapeutic effects are increased [13, 14]. Furthermore, helper molecules (like cationic lipids or polymers) or physical methods (like electroporation, sonoporation, or hydrodynamic pressure) can be employed to facilitate intracellular entrance of siRNA [13, 15–19]. In addition, local production of siRNA by genes encoding for short hairpin RNA (shRNA) can ensure prolonged levels of the dsRNA intracellularly [20, 21]. The encoding genes can be delivered by viral vectors or one of the aforementioned nonviral methods.

Unfortunately, local administration is not always feasible because the target tissue cannot be reached, or covers an area that is too large to be feasible for a local injection protocol. In addition, using local injection (possibly supplemented with helper molecules or physical stimuli) selectivity in delivery to nontarget and target cell types has usually not been taken into account. This is an important aspect when considering the nonspecific effects that can be induced by dsRNA. Over the past few years, it has become clear that cells can respond strongly to siRNA by different proinflammatory reactions depending on cell type, siRNA sequence, and intracellular location [22–24]. These effects can be intensified by employing cationic helper molecules [25], an effect probably mediated by a change in the intracellular trafficking of the dsRNA [23]. As a consequence, nontarget cells may also take up siRNA and respond with induction of proinflammatory pathways in addition to the therapeutic RNAi effects within the target cells at the diseased site.

In this review we will focus on strategies for targeted siRNA delivery that are designed to improve accumulation of siRNA at three in vivo levels of delivery: at the target tissue, the target cell, and at the intracellular target site of action. We will concentrate on delivery approaches for systemic administration as such systems have broadest applicability. Three approaches will be discussed:

(1) chemical modifications of siRNA,
(2) viral nucleic acid delivery systems,
(3) nonviral nucleic acid delivery systems.

Most attention will be given to in vivo delivery strategies, as in vitro targeting studies often do not represent a fair evaluation of the many barriers that exist in vivo, possibly impeding efficient and site-specific delivery (eg, phagocyte uptake, uptake by competing cell types, excretion, intracellular processing, and siRNA (delivery system) stability).

CHEMICAL MODIFICATIONS

The nuclease sensitivity and poor tissue distribution/membrane permeation qualities of siRNA provide a reason to investigate possible chemical modifications that would improve these characteristics which would not interfere with the silencing efficiency of the molecules. Several strategies have been proposed to improve nuclease resistance and target cell uptake.

Increasing nuclease resistance

Chemical modifications in the nucleobases, sugars, and the phosphate ester backbone of siRNA can reduce siRNA sensitivity to nucleases [26–28]. Initial studies centered on the tolerance of the RNAi system for modifications in the two RNA strands [29]. A number of chemical modifications have been proposed to increase nuclease resistance, like boranophosphates [30], 4′-thioribonucleosides, phosphorothioates, 2′deoxy-2′ fluorouridine, 2′-O-methyl, 2′-O-(2-methoxyethyl), and locked nucleotides [31–36]. All of these chemically modified siRNAs were still able to induce
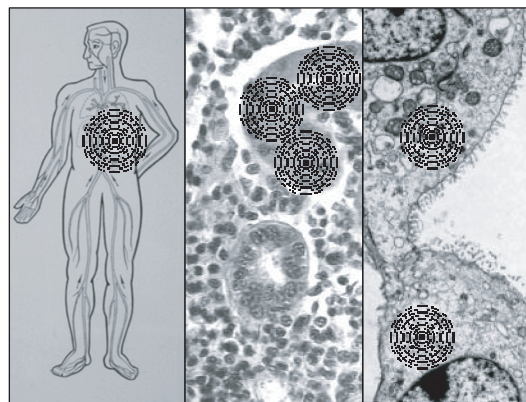

FIGURE 1: Three levels of targeting: preferably, siRNA should be targeted to the diseased tissue (I). Within this tissue it should be delivered to the correct cell type for silencing the mRNA of interest (II). Following entry of the target cell, siRNA should be delivered to the cytoplasm (and/or nucleus) to interact with the components of the RNAi machinery (III).

siRNA-mediating gene silencing provided that the modifications were absent in specific regions of the siRNA and included to a limited extent. These specific restrictions regarding position and degree of modifications were dependent on the characteristics of the incorporated modification. Although increased nuclease resistance of siRNA would be expected to increase in vivo silencing effects, Layzer et al showed that this is not necessarily the case. They studied silencing effects in the liver after hydrodynamic injection, and showed that unmodified siRNA had a similar potency as the stabilized version [36].

In contrast, chemical modifications were shown to enhance therapeutic effects in a mouse model of hepatitis B virus infection. Chemically modified siRNA designed against a conserved region of the hepatitis B virus was shown to decrease viral DNA, hepatitis B surface antigen levels in serum, as well as viral RNA levels in liver over 1000-fold as compared to chemically modified control siRNA and buffer-treated animals in a hydrodynamic injection protocol. The benefit of chemically modified siRNA was supported by the fact that indicators of viral infection were 30-fold higher in animals treated with unmodified siRNA [37].

Increasing intracellular uptake

Several approaches have been developed where chemical modifications have been introduced to enhance intracellular uptake of siRNA. Liao and Wang developed poly-2′-O-(2,4-dinitrophenyl) modified siRNA. The enhanced lipophilicity of this siRNA allows passive diffusion over the cell membrane, while at the same time enhancing nuclease resistance. This approach has only been investigated in vitro and shows that chemical modification can enhance siRNA potency at both fronts. As a result the silencing effects of the chemically modified siRNA specific for insulin-like growth factor receptor were strongly enhanced as compared to unmodified siRNA [38].

In a similar setup, membrane permeant peptides (penetratin and transportin) were coupled to siRNA to facilitate their intracellular uptake. By coupling the peptide via a reducible disulfide linker, the bulky peptides are expected to be cleaved-off liberating the siRNA in the cytoplasm. The approach was validated in vitro, thus supporting wide application of the basic technology. Nevertheless, cell specificity is lacking [39].

Probably the furthest developed work on chemically modified siRNAs has been reported by Soutcheck et al [40]. They have developed siRNAs with partial phosphorothioate backbone modifications and 2′-O-methyl sugar variations on the sense and antisense strands to promote nuclease resistance, while at the same time cholesterol was conjugated to the 3′ end of the sense strand using a pyrrolidine linker to change tissue distribution. The cholesterol-modified siRNA silenced reporter gene expression in vitro in the absence of transfection agents, something not observed for unmodified siRNAs. Probably the interaction of the cholesterol with serum components in the culture medium improved siRNA translocation over the cellular membrane. The cholesterol modification particularly enhanced binding of siRNA to serum albumin, probably explaining the prolonged circulation half-life measured after intravenous injection as compared to unmodified siRNA. In addition to a prolonged presence in the circulation, cholesterol-modified siRNAs were detected in liver and jejunum at concentrations of 100–200 ng per gram tissue 24 hours after the last injection of 50 mg/kg doses. These levels were sufficient to reduce the levels of the apolipoprotein apoB-100 in plasma by 31–68%. This reduction was paralleled by a 37% reduction in overall cholesterol levels, and reduction in high-density lipoprotein, low- density lipoprotein, and chylomicron levels. Despite these impressive results using relatively simple modifications, the doses of chemically modified siRNAs needed are relatively high and seem to underline that changing tissue distribution of siRNA in favor of target cell uptake by conjugation with comparatively small chemical groups is difficult to achieve. At the same time, small molecular weight modifications seem to be needed to preserve correct interaction with the RNAi enzymes. Cleavable linkers for coupling of bulkier modifications may be an approach to avoid these problems. In conclusion, chemical modifications do promise important advances regarding nuclease resistance and reduced induction of the stress response. Invitrogen has developed a second-generation siRNA, known as Stealth RNAi, in which chemical modifications are designed to increase the specificity of RNAi effects by allowing only the antisense strand to efficiently enter the RNAi pathway and eliminating induction of interferon-related pathways. Others have also demonstrated that sequence and modifications can strongly influence intensity of silencing efficiency and inflammatory reactions, providing tools to optimize these [41, 42].

Taken together, chemical modifications can markedly increase nuclease resistance of siRNA improving cellular persistence and conjugation with translocating/hydrophobic functional groups can increase membrane permeation. Strategies to affect tissue distribution profiles of siRNA with chemical modifications seem more difficult.

VIRAL NUCLEIC ACID DELIVERY

Viruses are at present the most efficient gene delivery vectors. After cell binding they are capable of delivering their nucleic acid payload intracellularly in a proficient way along with nuclear localization. Although virus-mediated delivery methods are usually based on delivery of genes encoding shRNA, few approaches used viruses to deliver chemically synthesized siRNA in vivo [43, 44].

Delivery of chemically synthesized siRNA

In this approach, reconstituted viral envelopes derived from influenza virus are used to encapsulate and deliver siRNAs. The reconstituted membrane vesicles contain the influenza virus spike protein hemagglutinin and additionally added cationic lipids. This protein is responsible for binding to and fusion with cellular membranes. The siRNA-loaded vesicles are taken up by receptor-mediated endocytosis, and are able to escape endosomal degradation by fusion with the endosomal membrane. Functional siRNA delivery was demonstrated in vitro, while in vivo uptake by macrophages in the peritoneal cavity was demonstrated after intraperitoneal injection. A similar approach, described siRNA delivery by simian virus SV40-based particles in vitro in lymphoblastoid cells [44]. As with many viral approaches, drawbacks of the systems are the difficulties of repeated administration and limited control over transduced cell type.

Delivery of DNA encoding siRNA/shRNA

A number of studies investigated the use of DNA encoding for shRNA delivered by viruses for gene silencing in vivo. Intravenous injection of 5×10^9 plaque forming units (pfus) recombinant adenovirus expressing shRNA targeting hepatitis B virus transcripts in mice with active replication of the hepatitis B virus, showed almost complete inhibition of viral protein production [45]. This in turn led to arrest of viral replication at day 17 after viral infection. The inhibitory effect persisted for at least 10 days. Interestingly, there appeared to be a fraction of viral protein that was not susceptible to RNAi-mediated silencing, which is suggested to be attributable to protection through binding of their mRNA to specific proteins. The exact nature of this protection and its possible involvement in RNAi resistance remains to be determined.

Uchida et al used expression of two separate siRNA strands against survivin by adenoviral transduction to inhibit tumor growth. Survivin is a protein that inhibits cancer cell apoptosis. Mice bearing subcutaneous U251 glioma tumors were treated with intratumoral injections of 10^{10} viral particles on three consecutive days every twenty days, ultimately leading to four-fold smaller tumors at day 48 after start of treatment as compared to empty adenoviral vector and adenoviral vector expressing irrelevant siRNA [20].

These studies demonstrate the possibilities for single intravenous or multiple local injections of virally delivered DNA encoding si/shRNA for gene silencing. This strategy has been further confirmed in a number of different in vivo models and with a number of different vectors, like intracranial delivery of lentivirus-produced shRNA for inhibition of reporter gene expression in cortical neurons [46], intraperitoneal delivery of lentivirus-produced shRNA for inhibition of viral cyclin to prevent primary effusion lymphoma in mice [47], intramuscular or intraspinal delivery of lentivirus-produced shRNA for inhibition of mutant SOD1 in amyotrophic lateral sclerosis [48, 49], and ex vivo delivery of lentivirus-produced shRNA for inhibition of CC-chemokine receptor 2 in hematopoietic cells in mice [50].

Taken together, the viral DNA-based sh/siRNA delivery process is very efficient: binding to the target cell surface and subsequent transduction, carrier stability, and protection against nucleases appear satisfactory [51–54]. However, as the discussed approaches illustrate, viruses usually lack selectivity for the target cell type. To improve specificity, the natural tropism of viruses for certain cell types may be used. Currently, much attention is focused on redirecting the natural preferred cell type of viruses towards therapeutically interesting receptors on the surface of target cells. Examples include the retargeting of murine coronavirus to the human epidermal growth factor receptor [55], directing adenovirus via fibroblast growth factor ligand towards its associated receptor (FGFR1) for delivery to glioma, or adenoviral delivery to angiogenic endothelium via RGD-peptides binding alpha v-integrins [56]. However, such approaches have not been tried as yet in combination with RNAi-mediated gene silencing in vivo.

The strength of the viral delivery approach is the efficient transduction of cells. Challenges that remain are the control over transduced cell type, especially after systemic administration. In addition, inflammatory reactions, immunogenicity, and oncogenic transformations continue to be important safety considerations for viral vectors that need to be addressed [57, 58].

Nonviral nucleic acid delivery

Whereas viral vectors possess many of the desired characteristics for efficient nucleic acid delivery, nonviral vectors possess several advantages. Important benefits of synthetic vector systems are the safety (related to their lack of immunogenicity and low frequency of integration) and ease of large-scale production. In addition, they can accommodate a wide variety of nucleic acid sizes and they allow easy modification. On the downside, transfection efficiency can be a limiting factor.

To face this weakness, many functional groups need to be incorporated into nonviral nucleic acid delivery systems. A cationic functional group is usually required to bind and condense the nucleic acid, thereby protecting it against nucleases and (important for siRNA) increasing the apparent molecular weight above the renal clearance cut-off. In addition, some cationic compounds are being used as endosomal escape enhancers. Due to the resulting positive charge, complexes tend to form aggregates by binding in the blood stream to negatively charged biomolecules. As a result, their clearance is usually rapid. Moreover, such cationic complexes possess a propensity to interact with virtually any cell type they encounter, creating a need to insulate the interactive surface of the particle to promote specificity. For that purpose, shielding groups can be added to enhance colloidal stability and reduce surface charge thereby avoiding nonspecific cell uptake. To restore cell interaction in a target-specific manner targeting ligands can be coupled to induce site-specific binding and uptake. In the case of delivery of DNA encoding for shRNA by non-viral delivery systems, nuclear translocation of the DNA is often inadequate. As such, the cytoplasmic site of activity of chemically synthesized siRNA provides an important advantage.

Delivery system based on RNA

A system consisting completely of RNA was proposed by Guo et al [59]. Their system is based on the packaging RNA of the DNA-packaging motor of bacteriophage phi29, which can spontaneously form dimers via interlocking right- and left-hand loops. By attaching the siRNA to one loop and an RNA aptamer to CD4 to the other, a cancer cell targeted system was created that could silence survivin gene expression in vitro. Alternatively, the system could also be targeted by folate.

Cationic delivery systems

Unshielded, untargeted complexes of siRNA with cationic polymers or lipids, can provide local or systemic transfection of a sufficient number of target cells for therapeutic effects. Several studies employed cationic lipids to complex siRNA to silence, amongst others, TNF-alpha in intraperitoneal macrophages after intraperitoneal administration [60], delta opioid receptor in spinal cord and dorsal root ganglia after intrathecal administration [61], polo-like kinase-1 in bladder cancer after intravesical administration, and c-raf-1 in prostate cancer cells after intravenous administration [62]. Although, a sufficient number of cells must have been reached as silencing is observed, it is fair to assume that a large part of the dose will arrive in nontarget cells. In view of the nonspecific effects that can be induced by cationic lipids themselves and in particular in combination with dsRNA, this may severely hamper therapeutic application [25, 63].

A variety of other cationic compounds have also been investigated for siRNA-delivery purposes. A linear low molecular weight form of the cationic polymer poly(ethylene imine) (PEI) has been used for treatment of (subcutaneously implanted) ovarian carcinoma in mice [64]. After intraperitoneal administration complexed siRNA was primarily recovered from muscle, liver, kidney, and tumor. Interestingly, the major organ where PEI nucleic acid-complexes are usually recovered, the lung, was largely avoided. Importantly, silencing of Her-2 with these polyplexes inhibited ovarian carcinoma growth in vivo.

Atelocollagen (a highly purified type-I collagen of calf dermis digested by pepsin), was shown to be a suitable vehicle for local delivery of siRNA [17, 65]. In addition, when administered intravenously, atelocollagen-siRNA was able to localize at sites of tumor mestastases and inhibit metastasis outgrowth [66]. More specifically, tumor levels increased ~ 6-fold as compared to levels after "naked" siRNA administration (from 0.7 to 4.3 ng/mg after injection of 25 μg siRNA). This effect was, albeit less pronounced, also seen in the other organs investigated (ie, liver, lungs, kidneys, and spleen) demonstrating that the enhanced tissue uptake is not exclusively tumor-specific. Nevertheless, delivery of these levels of siRNA silencing EZH2 (enhancer of zest homologue-2, a gene overexpressed in hormone-refractory metastatic prostate cancer) or p110-α (a phosphatidylinositol 3-kinase regulating cell survival, proliferation, and migration) resulted in strong inhibition of growth of bone metastases of prostate cancer cells. Importantly, siRNA-atelocollagen complexes failed to induce nonspecific proinflammatory responses like secretion of IFN-α and IL-12.

Targeted cationic delivery systems

A targeted amino-acid-based system was based on the cationic peptide protamine [67]. To the system's protamine-block the C-terminus of the heavy chain Fab fragment of an HIV-1 envelope antibody was coupled to form a protein construct known as F105-P. This system was highly efficient in binding to and transfection of cells expressing HIV-envelope protein, although it is unclear why the HIV-envelope protein would be internalized. Importantly, expression of interferon-β, 2′, 5′-oligoadenylate synthetase, and Stat-1, as indicators of nonspecific effects, were not increased upon siRNA transfection of HIV-envelope-expressing melanoma cells. In addition, when these cells formed subcutaneous tumors in vivo, 30% of cells took up fluorescent siRNA when delivered by F105-P after intravenous administration. Naked siRNA was not taken up, nor was F105-P-siRNA delivered to cells that were envelope-protein negative. Delivery of a combination of siRNAs against c-myc, MDM2, VEGF strongly inhibited tumor growth in vivo when delivered using the F105-P system. This combination of siRNAs attacking the tumor at multiple fronts is an important advantage of the siRNA technology as it allows simultaneous interference with a number of different pathways, while the delivery problem for each individual drug molecule (siRNA) remains the same. The versatility of this targeted system was demonstrated by exchanging the HIV-envelope antibody for an ErbB2-antibody changing the specificity of the system to ErbB2-positive breast carcinoma cells.

In a cationic lipid-based approach, Pirollo et al coupled a transferrin receptor single chain Fv region antibody fragment to the surface of cationic DOTAP: DOPE complexes containing siRNA [68]. They evaluated the targeting potential of these systems in different murine tumor models: an orthothopically implanted pancreatic carcinoma (that produced spontaneous metastases), an orthotopically implanted prostate carcinoma, and intravenously administered breast carcinoma cells giving rise to metastases in the lung. In all these models, specific accumulation of fluorescently labeled siRNA complexed to the targeted cationic lipid particles at the site of the malignancy could be demonstrated as compared to surrounding normal tissue and liver. The question whether targeted delivery resulted in gene silencing was not addressed.

Shielded targeted cationic delivery systems

Targeted cationic systems have the important advantage that they possess a recognition signal for specific interaction with the target cell type. However, the cationic surface may also be able to interact with biomolecules or nontarget cells. As such, shielding of the cationic surface may further enhance target cell specificity by reducing nontarget tissue uptake and may additionally increase colloidal stability of the siRNA complexes.

In our studies we focused on the cationic polymer PEI coupled to PEG as shielding polymer. To the distal end of the PEG-chain a cyclic RGD-peptide was coupled. This peptide is a high-affinity ligand for alpha v-integrins that are overexpressed on angiogenic endothelial surfaces [69]. Tissue distribution studies in vivo of fluorescently labeled siRNA in subcutaneous neuroblastoma-bearing mice showed that injection of "naked" siRNA did not produce appreciable tumor levels, but rather rapid clearance into the urine. PEI-siRNA complexes also lacked the production of high fluorescence in the tumor, but did increase liver and especially lung levels. The fluorescence appeared punctuate in both latter tissues, probably reflecting formation of aggregates in the circulation.

When the PEG-shielded, targeted nanoparticles were used, a higher level of specificity for the tumor and lower levels of fluorescence in the lung and liver were observed. In a therapeutic setting, siRNA against murine VEGF receptor-2 was used, since the receptor is one of the driving factors of tumor angiogenesis. Delivery to host tumor endothelium is required to inhibit tumor proliferation. Efficacy studies with VEGFR2-specific siRNA complexed in RGD-PEG-PEI nanoparticles resulted in strong inhibition of sc neuroblastoma growth rate, which was sequence-specific. These experiments suggest that the targeted shielded nanoparticles indeed deliver the siRNA to the angiogenic endothelial cells. In line with these findings, the reduced tumor growth rate was paralleled by a reduction in blood vessels in the periphery of the tumor and changes in vascular morphology of remaining vessels, supporting an antiangiogenic mechanism of action. These results were supported by studies in a model of pathological angiogenesis in the eye [70], again demonstrating vasculature-specific delivery and inhibition of angiogenesis leading to therapeutic effects. Importantly, the studies in the eye also showed that combining siRNAs against different driving factors in the VEGF-pathway in the same delivery system improved therapeutic effects. Attacking the various receptors and growth factors simultaneously seems to offer advantages. Especially in multifactorial diseases, where functional redundancy is likely, this cocktail approach seems to offer important benefits.

Chemical modifications

(a)

Viral nucleic acid delivery

(b)

Nonviral nucleic acid delivery

(c)

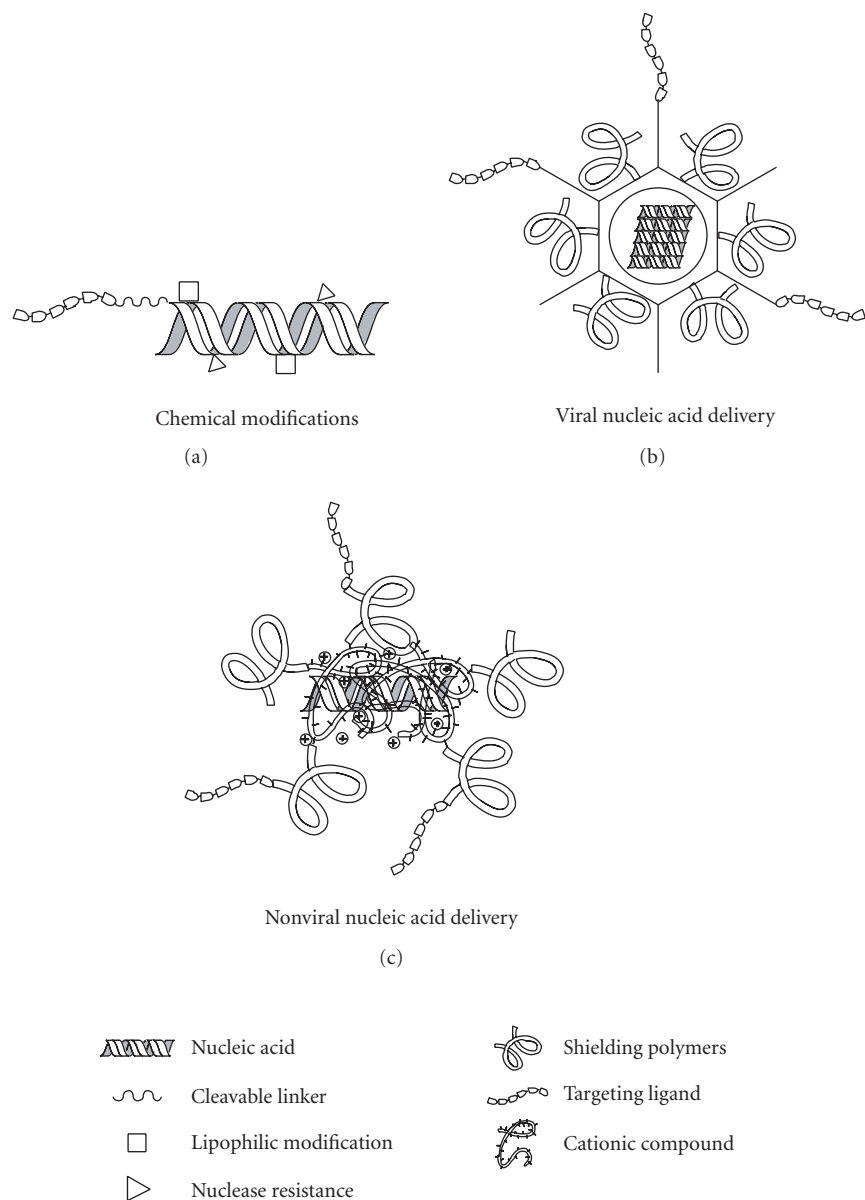| AWAW | Nucleic acid | | Shielding polymers |
~~	Cleavable linker		Targeting ligand
□	Lipophilic modification		Cationic compound
▷	Nuclease resistance		

FIGURE 2: Strategies for siRNA delivery. Strategies are based on (a) chemical modifications of siRNA, (b) targeting of siRNA using viral vectors, or (c) nonviral delivery systems.

Synthetic nonviral delivery systems are a diverse class of molecules used in different nucleic acid delivery strategies that range from relatively simple cationic complexation for local administration to targeted shielded systems for intravenous injection. Their adaptability to specific targeting requirements is an important advantage, although optimization of delivery efficiency continues to remain important.

FINAL REMARKS

Over the last decades, research on the promises of nucleic acids for therapeutic intervention and the difficulties encountered in turning these promises into clinical reality has provided a clearer picture of the development steps that are needed to transform nucleic acids into actual drug molecules. As a result siRNA has been able to make a remarkable rapid progress from initial discovery as functional mediator of RNA interference in mammalian cells in 2001 to three clinical trials at the end of 2005: two in age-related macular degeneration, the other in respiratory syncytial virus infection [71]. Nevertheless, the choice of the diseases also reflects the delivery difficulties encountered for this class of nucleic acids. These diseases were selected partly because the target cell delivery problems are relatively low as these pathologies are confined to specific and accessible sites. To further improve target specificity, also in view of possible adverse effects occurring when siRNA is processed by nontarget cells, and to allow application of siRNA for systemic treatment several strategies can be proposed (Figure 2). Taken together they serve to increase nuclease resistance, to reduce renal

excretion/specific cell uptake, to promote uptake by the target cells, and to ensure correct intracellular trafficking to the site of action. As the first preclinical proofs of principle have been delivered showing therapeutic effects of locally and systemically delivered siRNAs, it is expected that these strategies will soon translate into viable clinical development programs.

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