siRNA Versus miRNA as Therapeutics for Gene Silencing

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Discovered a little over two decades ago, small interfering RNAs (siRNAs) and microRNAs (miRNAs) are noncoding RNAs with important roles in gene regulation. They have recently been investigated as novel classes of therapeutic agents for the treatment of a wide range of disorders including cancers and infections. Clinical trials of siRNA- and miRNA-based drugs have already been initiated. siRNAs and miRNAs share many similarities, both are short duplex RNA molecules that exert gene silencing effects at the post-transcriptional level by targeting messenger RNA (mRNA), yet their mechanisms of action and clinical applications are distinct. The major difference between siRNAs and miRNAs is that the former are highly specific with only one mRNA target, whereas the latter have multiple targets. The therapeutic approaches of siRNAs and miRNAs are therefore very different. Hence, this review provides a comparison between therapeutic siRNAs and miRNAs in terms of their mechanisms of action, physicochemical properties, delivery, and clinical applications. Moreover, the challenges in developing both classes of RNA as therapeutics are also discussed.

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The term “non-coding RNA” is commonly employed for RNA that does not encode a protein.1 Although the current understanding of these RNA molecules represents perhaps only the tip of the iceberg, with the rapid development of molecular biotechnology, noncoding RNAs are increasingly found to have far more important functions than previously recognized and many new classes of noncoding RNA have been identified. Among them, small interfering RNAs (siRNAs) and microRNAs (miRNAs) have attracted considerable attention because their role in gene regulation makes them likely targets for drug discovery and development. Indeed, the therapeutic potential of siRNAs and miRNAs has been demonstrated in the treatment of many different diseases including cancers2–4 and infections.5–7 Compared with conventional small therapeutic molecules, siRNAs and miRNAs offer the advantages of being highly potent and able to act on “non-druggable” targets (for example, proteins which lack an enzymatic function or have a conformation that is inaccessible to traditional drug molecules),8 as they can be designed to affect virtually any gene of interest.

Therapeutic approaches based on siRNA involve the introduction of a synthetic siRNA into the target cells to elicit RNA interference (RNAi), thereby inhibiting the expression of a specific messenger RNA (mRNA) to produce a gene silencing effect.9 By contrast, miRNA-based therapeutics comprise two approaches: miRNA inhibition and miRNA replacement. The former approach resembles antisense therapy,10 with synthetic single stranded RNAs acting as miRNA antagonists (also known as antagonors or anti-miRs) to inhibit the action of the endogenous miRNAs. In the replacement approach, synthetic miRNAs (also known as miRNA mimics) are used to mimic the function of the endogenous miRNAs.11 It thus leads to mRNA degradation/inhibition, and produces a gene silencing effect. This review focuses on the therapeutic approach achieved by gene silencing, and so only the miRNA replacement approach is discussed and compared with siRNA. The therapeutic potentials and applications of the miRNA inhibition approach have been reviewed previously.12–14 siRNAs and miRNAs have similar physicochemical properties but distinct functions (Table 1). Both are short RNA duplexes that target mRNA(s) to produce a gene silencing effect, yet their mechanisms of action are distinct. As a result, the requirements for sequence design and therapeutic applications of siRNAs and miRNAs are different. On the other hand, for clinical development, the two types of small RNA molecules face a similar set of barriers: poor stability in vivo, delivery challenges and off-target effects15; and so, the same strategies can be employed to improve their in vivo efficacy.

Gene silencing mechanism of siRNA and miRNA

RNA interference and siRNA

RNAi is a natural cellular process that silences gene expression by promoting the degradation of mRNA. It plays an important role in gene regulation and innate defense against invading viruses.16 RNAi was first described by Fire and Mello based on their Nobel prize winning study investigating the mechanisms for effective gene inhibition by exogenous RNA in *C. elegans*.17 According to their observations, long double-stranded RNA (dsRNA) mediates potent and specific silencing of homologous genes. It appeared later that a similar process also occurs in mammals.18 After years of investigation, the mechanism underlying RNAi is better understood (Figure 1). In general, the dsRNA (either transcribed from cellular genes or infecting pathogens, or artificially introduced into the cells) is processed by a specialized ribonuclease

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(RNase) III-like enzyme named Dicer in the cytoplasm into a smaller dsRNA molecule. This short dsRNA molecule is known as the siRNA, which has 21–23 nucleotides with 3′ two-nucleotide overhangs. The siRNA interacts with and activates the RNA-induced silencing complex (RISC). The endonuclease argonaute 2 (AGO2) component of the RISC cleaves the passenger strand (sense strand) of the siRNA while the guide strand (antisense strand) remains associated with the RISC. Subsequently, the guide strand guides the active RISC to its target mRNA for cleavage by AGO2. As the guide strand only binds to mRNA that is fully complementary to it, siRNA causes specific gene silencing.16,19

Since the discovery of RNAi, dsRNAs have been used as research tools to study the gene functions of different cell types. However, in mammalian cells, the delivery of exogenous, long dsRNAs (over 30 nucleotides) is associated with the activation of the interferon (IFN) pathway,20 which is part of the defense mechanism against viral infection. The long dsRNAs bind and activate protein kinase R (PKR), which in turn stimulate a plethora of genes belonging to the IFN pathway, resulting in nonspecific mRNA degradation and apoptosis.21 An in vitro study in mammalian cells, including human cell cultures, showed that the direct introduction of synthetic siRNAs, instead of the long dsRNAs (thus skipping the step of Dicer processing), leads to effective RNAi without the complication of activating the IFN response.18 In view of this finding, siRNAs have become useful tools to inactivate target gene expression. However, later studies suggest that synthetic siRNAs may also induce partial IFN response and innate immune responses.22,23 As this effect can be either sequence-dependent or -independent, special care must be taken when designing siRNA therapeutics. Alternatively, short hairpin RNAs (shRNAs) can be used to achieve a specific gene silencing effect via the RNAi mechanism.24 shRNAs are stem-loop RNAs, which are expressed in the nucleus, typically through the delivery of viral vectors. Once expressed, they are transported to the cytoplasm for further processing, and subsequently loaded into the RISC for specific gene silencing activity in the same manner as synthetic siRNAs. However, the requirement of viral vectors for shRNA expression poses safety concerns in therapeutic applications, which are discussed in section “Viral vectors”.

Table 1  Comparison of general properties between siRNA and miRNA

|                      | siRNA                                      | miRNA                                      |
|----------------------|--------------------------------------------|--------------------------------------------|
| Prior to Dicer processing | Double-stranded RNA that contains 30 to over 100 nucleotides | Precursor miRNA (pre-miRNA) that contains 70–100 nucleotides with interspersed mismatches and hairpin structure |
| Structure            | 21–23 nucleotide RNA duplex with 2 nucleotides 3′ overhang | 19–25 nucleotide RNA duplex with 2 nucleotides 3′ overhang |
| Complementary        | Fully complementary to mRNA                | Partially complementary to mRNA            |
| mRNA target          | One                                        | Multiple (could be over 100 at the same time) |
| Mechanism of gene regulation | Endonucleolytic cleavage of mRNA | Translational repression                    |
| Clinical applications | Therapeutic agent                          | Drug target                                |

Gene silencing mediated by miRNA
Similarly to siRNAs, miRNAs also inhibit gene expression in a post-transcriptional manner. Although the gene silencing effects of siRNAs and miRNAs are distinct, the distinction has been obscured because they are associated with common enzymes (e.g., Dicer and RISC) and their functions overlap with each other to a certain extent. The major difference between siRNAs and miRNAs is that the former inhibit the expression of one specific target mRNA while the latter regulate the expression of multiple miRNAs. A considerable body of literature now classifies miRNAs as RNAi molecules.15,25–27

The first miRNA was discovered in 1993 in a study examining developmental regulatory genes in C. elegans.28 Soon after its discovery, miRNA was quickly found to be a class of small RNA molecules that negatively regulate gene expression (Figure 1). miRNA gene transcription is carried out by RNA polymerase II in the nucleus to give primary miRNA (pri-miRNA), which is 5′ capped, 3′ polyadenylated RNA with double-stranded stem-loop structure. The pri-miRNA is then cleaved by a microprocessor complex (comprising Drosha and microprocessor complex subunit DCGR8) to form precursor miRNA (pre-miRNA), which is a duplex that contains 70–100 nucleotides with interspersed mismatches and adopts a loop structure. The pre-miRNA is subsequently transported by Exportin 5 from the nucleus to the cytoplasm, where it is further processed by Dicer into a miRNA duplex of 18–25 nucleotides. The miRNA duplex then associates with the RISC forming a complex called miRISC. The miRNA duplex is unwound, releasing and discarding the passenger strand (sense strand)—unlike in the processing of siRNA, in which the AGO2 of the RISC causes the cleavage of the passenger strand of siRNA. The mature single-stranded miRNA guides the miRISC to the target miRNAs. The miRNA bonds to the target miRNAs through partial complementary base pairing with the consequence that the target gene silencing occurs via translational repression, degradation, and/or cleavage.25,29

Recognition of mRNA targets by siRNA and miRNA
To elicit RNAi, the siRNA must be fully complementary to its target mRNA (Figure 2). The complementary binding activates the AGO2, which then cleaves the phosphodiester
backbone of the mRNA between bases 10 and 11 relative to the 5′ end of the guide strand. The mRNA fragments generated are subsequently degraded by different exonucleases. By contrast, the target recognition of miRNA is more complex, as different binding sites and different degree of complementarity between the miRNA and the target RNA exist. This is a consequence of imperfect base pairing; miRNA only needs to be partially complementary to its target mRNA. The complementary pairing between mRNA and the mature miRNA typically occurs at the 3′ untranslated region (UTR) of the former and the seed region (nucleotides 2–7 from the 5′ end) of the latter (Figure 2). Other miRNA binding sites, such as the centered sites, 3′ supplementary sites and bulged sites, are considered to be atypical. Since miRNA-mRNA
recognition does not require perfect pairing, one miRNA strand can recognize an array of mRNAs, and hence miRNA has the characteristic of having multiple targets. For example, a microarray analysis showed that miRNA-124, which is preferentially expressed in brain tissues, can downregulate 174 annotated genes. Due to the partially complementary base pairing between miRNA and mRNA, AGO2 of the miRISC is not activated. Instead, the silencing of the mRNA targets of miRNA occurs through translation repression, or degradation by deadenylation, decapping or exonuclease action. In rare cases, high level of complementary between miRNA and miRNA leads to the endonucleolytic cleavage of miRNA by AGO protein, a mechanism that is similar to siRNA-mediated gene silencing.

siRNA and miRNA as therapeutic agents

The specific gene silencing effect of siRNAs makes them useful tools for target identification and validation in drug discovery and development. Since miRNAs have multiple mRNA targets and the disruption of their functions contributes to the development of many diseases including cancers, neurodegenerative disorders and cardiovascular diseases, their clinical use as biomarkers and in diagnostics is rapidly developing. Furthermore, both siRNAs and miRNAs have huge potential as therapeutic agents. They can overcome the major limitation of traditional small drug molecules, which can only target certain classes of proteins. Even for protein-based drugs including monoclonal antibodies that are highly specific, their targets are mainly limited to cell-surface receptors or circulating proteins. By contrast, siRNAs and miRNAs can downregulate the expression of virtually all genes and their mRNA transcripts. Since many diseases result from the expression of undesired or mutated genes, or from overexpression of certain normal genes, the discovery of siRNA and miRNA opens up a whole new therapeutic approach for the treatment of diseases by targeting genes that are involved causally in the pathological process. A comparison between conventional small molecules, protein-based therapeutic agents and siRNA/miRNA-based drugs is summarized in Table 2. Although the therapeutic potential of siRNAs and miRNAs is promising, different sets of hurdles retard their development into clinical use. Some of these challenges, such as problems regarding stability and poor efficiency of delivery, are similar for both RNA molecules.

Design of therapeutic siRNA

The first essential step for successful siRNA therapy is the design of a siRNA sequence that is potent and specific to the intended mRNA to minimize any off-target effect. A conventional siRNA consists of 19–21 nucleotides with two nucleotide overhangs at the 3' end, usually TT and UU, which are important for recognition by the RNAi machinery. Increasing the length of the dsRNA may enhance its potency, as demonstrated by an in vitro study that dsRNAs with 27 nucleotides were up to 100 times more potent than the conventional siRNAs with 21 nucleotides. The long dsRNAs require processing by Dicer into the shorter siRNAs (hence they are termed as “Dicer-ready” or “Dicer-substrate” siRNAs), which are more efficiently loaded into the RISC, thus facilitating the subsequent gene silencing mechanism. On the other hand, dsRNAs longer than 30 nucleotides can activate the IFN pathway and should be avoided for therapeutic applications.

The gene silencing efficiency of the siRNA varies greatly, depending on the region of the mRNA to which they are complementary. An understanding of this relationship can permit the design of a siRNA sequence with optimal efficacy, and hence the rational design of effective siRNA sequences has been a focus of research. While many siRNA design algorithms have emerged in recent years to predict efficacy, it is nevertheless essential to validate the gene silencing efficiency of siRNA experimentally. Some commonly used
strategies for the design of therapeutic siRNAs are summarized in Table 3.

**Strand selection.** To ensure effective gene silencing, the siRNA must be correctly orientated and loaded into the AGO of the RISC in order for the passenger strand to be cleaved and discarded, so that the guide strand that is complementary to the target mRNA remains bound to the active RISC and directs it to the target mRNA. The guide strand of the RNA duplex is determined during the AGO loading step. However, both strands in the RNA duplex could potentially be loaded into the AGO as the guide strand. An incorrect loading orientation results in the intended guide strand being discarded and off-target effects are produced as the remaining strand (the intended passenger strand) base-pairs to the nonintended mRNA. Since this phenomenon can occur with both siRNA and miRNA, the RNA duplex needs to be carefully designed to warrant correct guide strand selection by the RISC. Two major sequence parameters are known to determine the guide strand selection: (i) the asymmetry rule and (ii) 5’ nucleotide preference; both of which can be applied to siRNA as well as miRNA design.

The *asymmetry rule* is based on the finding that the relative thermodynamic stability of the two ends of the duplex contributes to the selection of the strand to be loaded into AGO. The strand with a relatively unstable 5’ end (i.e., higher A/U content) is selected as guide strand while the strand with a more stable 5’ end is discarded as the passenger strand. For this reason, RNA duplexes should always be designed with the intended guide strand having the less stable 5’ end. In addition to the asymmetry rule, the *5’ nucleotide preference* is also important in correct AGO loading. AGO proteins appear to have a preference for the strand with a U, or less favorably, an A at position one at the 5’ end as the guide strand. Therefore, the guide strand should ideally contain a U or A at the 5’ end, whereas the passenger strand should always contain C and G at the 5’ end to minimize the risk of being incorrectly selected as a guide strand.

**Efficiency affected by G/C content.** The overall G/C content of the siRNA influences the siRNA activity, although the importance of this influence is still debated. The G/C content affects the overall duplex thermodynamic stability as well as target site accessibility; siRNAs with very high G/C content appear to be less functional. Some studies suggest that the optimal G/C content of siRNA is around 30–50%, while others show that siRNAs with G/C contents of about 60% are highly efficient. As a general guideline, the G/C content of siRNA is ideally between 30 and 64%. Furthermore, sequences with G/C stretches of nine or more nucleotides should be avoided as this may reduce the gene silencing efficiency of siRNA.

**Minimizing off-target effect.** Although one of the distinctive features that differentiate siRNA from miRNA is that siRNA is designed to silence the expression of a specific target mRNA, siRNA may lead to the downregulation of unintended, unpredicted targets, resulting in off-target effects. Indeed, one of the major challenges of siRNA therapy is to reduce off-target effects, as these compromise the therapeutic effect, specificity and can even lead to cell death.

The most common type of off-target effect of siRNA is the miRNA-like effect. This occurs when the 5’ end of the guide strand of siRNA is complementary to the 3'UTR of the mRNA (reminiscent of the target recognition by the seed region of miRNA). In some situations, this off-target effect occurs simply due to the poor design of the siRNA, as siRNA can tolerate several mismatches at the mRNA (imperfect complementarity) without losing gene silencing ability. Under these circumstances, siRNA behaves like a miRNA molecule; it enters the natural miRNA pathway leading to the inhibition or degradation of multiple mRNAs. In certain cases, this type of off-target effect is nearly as efficient as the on-target effect in reducing the protein levels. Another type of off-target effect is not sequence-dependent, but due to the saturation of the RNAi machinery. When synthetic siRNAs (or miRNAs) are introduced into the cells, they compete with the endogenous miRNAs for common proteins such as RISC and other factors. As a result, gene regulation by endogenous miRNAs is perturbed, leading to unpredictable off-target effects.

### Table 3 A summary of commonly employed strategies to enhance the efficacy and specificity of siRNAs, and to reduce the off-target effects

| siRNA feature       | Strategy                          | Description                                                                 |
|---------------------|----------------------------------|-----------------------------------------------------------------------------|
| Strand selection    | Apply asymmetry rule             | Strand with a relatively unstable 5’ end is selected as guide strand       |
|                     | Utilize 5’ nucleotide preference | Strand with U or A at position one at the 5’ end is preferentially selected as guide strand |
| Activity            | Manipulate G/C content           | G/C content is ideally between 30–64%                                      |
|                     | G/C stretches of ≥9 nucleotides  | Should be avoided                                                           |
| Off-target          | Reduce siRNA concentration       | Lowest possible siRNA concentration to achieve a therapeutic effect is used |
|                     | Use multiple siRNAs              | siRNAs with different sequences for targeting the same mRNA are pooled for therapeutic effect |
| miRNA-like effect   | Avoid sequences similar to miRNA | Avoid seed sequences of miRNA that have already been identified             |
| Immune stimulation  | Avoid immune stimulatory motifs  | Avoid U-rich sequences and motifs that contain: GUCCUUCAA, UGUGU, UGU, UGGGC |

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Reduction of siRNA off-target effects is one of the research priorities in siRNA therapeutics development. Several strategies have been proposed to mitigate such off-target effects. One approach is to use the lowest possible siRNA concentrations, as the off-target effects due to the miRNA-like effect and RNAi machinery saturation are concentration-dependent. Pooling of multiple siRNAs targeting the same mRNA is another strategy, which allows the gene silencing effect to be achieved at low concentrations of each siRNA in the pool. As each siRNA has a unique off-target signature, the off-target effects can be selectively reduced. However, there is also a risk that siRNA pool may cause more off-target effects than the beneficial effects from on-target activity.

To avoid miRNA-like off-target effect, the logical approach is to reduce complementarity between the seed region (2-7 nucleotides of 5’ end) of siRNA and the 3’UTR of mRNA. Clearly, the seed sequences of miRNA (identified with miRNA databases) should be avoided in siRNA design. In addition, the siRNA should have a low thermodynamic stability of the duplex between the seed region of the guide strand of siRNA and its target mRNA, since a low seed-target duplex stability reduces the capability of siRNA to induce seed-dependent off-target effects.

Although the seed region of siRNA is implicated in the miRNA-like off-target effect, only some mRNAs, with this type of sequence complementarity, are silenced by siRNA. By analogy, miRNAs regulate the expression of multiple mRNAs through binding of the seed region to the target 3’UTR, but not all mRNAs with the same degree of sequence complementarity are the targets of a given miRNA. Therefore, it is speculated that several characteristics, other than sequence complementarity of the mRNA, are involved in defining the mRNA as a siRNA and/or miRNA target. However, these characteristics are still poorly understood and the identification of these features could contribute to the design of siRNA with minimal off-target effects. Another way to avoid the occurrence of off-target effects is by chemical modification, which is discussed in more details in section “Chemical modification”.

Avoidance of immune response. Initial studies suggested that long dsRNAs (over 30 nucleotides) could trigger an immune response by activating the IFN pathway. This led to the development of synthetic siRNAs (with smaller number of nucleotides), in the hope to generate therapeutic gene silencing without immunogenic adverse effect. However, it was soon discovered that siRNAs could also activate innate immunity; this complication creates another major hurdle to the development of siRNAs as therapeutic agents.

Indeed, siRNAs can cause immune responses in a sequence-independent and sequence-dependent manner. The former involves the PKR and toll-like receptor (TLR) 3 signaling pathways, although they may play only minor roles. The latter is mediated by TLR 7 and TLR 8 on dendritic cells and monocytes, respectively. These receptors are transmembrane receptors present in the endosomes of immune cells. Several immune-stimulatory sequence motifs have been reported. They include (5’ to 3’) “GUCCUUCAA”, “UGUGU”, “UGU”, and “UGGC”. Moreover, the presence of U-rich sequences correlates with TLR 7/8 activation. While avoiding these immune-stimulatory sequence motifs could reduce the siRNA immunogenicity, it may be impractical to exclude U from the primary siRNA sequence. Alternatively, stimulation of the TLR 7/8 mediated-immune response could be minimized by the use of delivery agents that exclude siRNA endosomal delivery (e.g., electroporation) or by chemical modification of the immune-stimulatory sequences to render them unrecognizable by TLR. At present, the rules of sequence-dependent immune activation are still poorly understood. Therefore, all therapeutic siRNAs must be carefully tested for any possible immunostimulatory adverse effects.

Design of therapeutic miRNA

Compared with siRNAs, miRNAs have a broader therapeutic application. Over 2,500 human miRNAs have been recorded in the miRBase (version 20 accessed June 2015), a searchable online miRNA database. Since more than 60% of the human protein-coding genes contain at least one conserved miRNA-binding site, together with the presence of numerous nonconserved sites, the majority of protein-coding genes are under the control of miRNAs. The extensive involvement of miRNAs across many human diseases makes them attractive targets for therapeutic strategies, as well as prognostic and predictive biomarkers.

The goal of miRNA replacement therapy using synthetic miRNAs (or miRNA mimics) is to achieve the same biological functions as the endogenous miRNAs. Therefore the synthetic miRNAs should possess the ability to be loaded to RISC and silence the target mRNAs through the natural miRNA signaling pathway. In theory, a single-stranded RNA molecule containing the sequence that is identical to the guide strand of the mature miRNA could be functional as miRNA mimic. However, the double stranded miRNA containing both guide and passenger strands was found to be 100 to 1,000 times more potent than the single stranded one. The double stranded structure can facilitate the proper loading of the RNA molecule into the RISC, thereby enhancing the gene silencing effect. Therefore, designing miRNA mimics with a duplex structure has become the direction of therapeutic development. Synthetic miRNA precursors with longer sequences (from a few extra nucleotides to a full-length pri-miRNA) have also been proposed as therapeutic agents. Since pri-miRNAs require processing in the nucleus, whereas pre-miRNAs and miRNAs do not, different strategies are required for the delivery of different types of miRNA mimics to their cellular targets. Similarly to shRNAs, viral vectors can be used to express miRNAs inside the cells. This review only discusses exogenously delivered, synthetic miRNAs.

The design of therapeutic miRNA is more straightforward than that of siRNA, as the sequence of the former should be almost, if not entirely, identical to the endogenous miRNA of interest. Nevertheless, the development of miRNA therapeutics faces similar hurdles that are encountered by siRNAs. In vivo administration of miRNAs can activate the innate immune system via TLR, leading to significant undesirable effects. As the sequence variation for therapeutic miRNA is limited, chemical modification is the major approach to tackle this problem. Furthermore, therapeutic miRNAs also face the barriers of poor stability and inefficient delivery. The
strategies to overcome these barriers are discussed in the following sections.

**Chemical modification**

RNAs are extremely vulnerable to serum nucleases. Although double-stranded RNA is more resistant to nuclease degradation than single-stranded RNA, naked RNAs in their unmodified forms are degraded rapidly following administration by the abundant nuclease present in the bloodstream, which contributes to a short half-life in vivo. Poor stability is one of the major obstacles toward the successful application of siRNAs and miRNAs as therapeutic agents. Chemical modifications of RNA were developed initially to address this issue. In addition, chemical modification of the RNA duplexes can minimize immunogenicity and reduce off-target effects.

A wide variety of RNA modification approaches have been investigated since the development of antisense therapies in the 1980s. The techniques have matured over the years and some are also applicable to siRNAs and miRNAs. Successful chemical modification should not compromise the gene silencing efficiency of these RNA molecules. In order for the modified siRNAs or miRNAs to be compatible with the endogenous silencing pathways and be loaded into the RISC in the correct orientation, a number of factors, including the position and the type of modification, and its effect on the charge of the RNA duplex, needs to be considered. Since the 5′ phosphate, the 5′ proximal part, and the central positions of the guide strand mirror the important areas of the RNA duplex for the interaction with and the action of the RISC and AGO proteins, RNA duplexes are less tolerant to chemical modifications at these sites. By contrast, chemical modifications at the entire passenger strand and the 3′ proximal part and 3′ overhang of the guide strand would have the least influence on the specificity and/or function of the RNA.

The major types of chemical modification that are commonly investigated in siRNA and miRNA design include: (i) ribose 2′-OH group modification; (ii) locked and unlocked nucleic acids; and (iii) phosphorothioate (PS) modification (Figure 3). Different RNA modification approach may be employed to serve different functions. Combinations of different modification strategies are also commonly used.

**Ribose 2′-OH group modification.** Modification of the ribose 2′-OH group is the most diverse and also the most popular type of modification in RNA duplex design, as the gene silencing activity of siRNAs or miRNAs does not depend on this group. This strategy involves the substitution of the ribose 2′-OH group with other chemical groups, including 2′-O-methyl (2′-O-Me), 2′-fluoro (2′-F) and 2′-methylthioethyl (2′-O-MOE) (Figure 3), and can effectively enhance the stability of the RNA duplex in serum. In particular, substitutions with 2′-O-Me and 2′-F are the two most extensively studied modifications in siRNA. Although these modifications are generally well-tolerated at most siRNA positions, extensive or full modification may lead to significant loss of silencing efficiency. By alternating 2′-O-Me and 2′-F substitutions in a fully substituted siRNA, nuclease-resistant and highly potent modified siRNA can be produced. Bulky substitution such as 2′-O-MOE may enhance nuclease resistance, but is poorly tolerated in terms of activity.

Apart from enhancing nuclease stability, ribose 2′-OH modifications also reduce immune activation of the RNA duplex. The substitution of the 2′-OH at only the U sites with either 2′-O-Me or 2′-F can abrogate immune responses without affecting siRNA potency. Such modification is believed to render the RNA duplex unrecognizable by TLR 7/8, which is responsible for siRNA-mediated immune response, as U-rich RNA sequences are associated with TLR 7/8 activation. Another approach proposed to reduce TLR 7 activation is to alternate 2′-O-Me modification of the passenger strand thereby preserving the gene silencing potency of the guide strand of the siRNA.

**LNA and UNA modifications.** Locked nucleic acid (LNA) is a type of chemically modified nucleic acid containing a methylene bridge between the 2′-O and the 4′-C of the sugar to create a stable “locked” ring conformation (Figure 3). This modification improves RNA duplex stability by increasing its resistance against nuclease degradation. However, multiple LNA modifications may lead to decreased efficacy in vitro and in vivo. In order to function properly, siRNA or miRNA must be designed in a way that favors the selection of the intended guide strand by the RISC to minimize off-target effects resulting from the passenger strand being wrongly selected. LNA modification can avoid this type of off-target effect as the modification of the passenger strand at the 5′ end precludes its incorporation into the RISC. Moreover, LNA modification in general can reduce RNA duplex-induced immunogenicity by preventing the immunogenic sequence-motifs from being recognized by TLR 7/8, without affecting its silencing activity.

In recent years, unlocked nucleic acid (UNA) modification has been introduced to siRNA. UNA monomers are acyclic derivatives of RNA, lacking the C2′ and C3′-bond of the RNA ribose ring, but structurally similar to unmodified RNA upon incorporation into RNA duplexes. Single UNA modifications are well-tolerated at most tested positions in the passenger and guide strands, exhibiting efficient gene silencing and improved performance and stability both in vitro and in vivo. However, additional UNA modification, especially in the guide strand, results in reduced silencing efficiency, possibly by destabilizing the siRNA duplex or by interactions with the target mRNA. Furthermore, UNA modification in the seed region of the guide strand can prevent miRNA-like off-target effect without compromising siRNA activity. Overall, results from various studies suggested that UNA modification represent an important modification with potential for future therapeutic RNA design.

**Backbone modification.** Backbone modifications are commonly used to improve the stability of nucleic acids against nuclease resistance by substituting the phosphodiester backbone linkages with other types of linkage. Among those, phosphorothioate (PS) modification is the most widely used strategy, in which one of the nonbridging phosphate oxygen atoms is replaced with a sulfur atom. This approach was first described in the development of antisense oligonucleotides, and is very efficient in increasing exonuclease resistance following parenteral administration. The PS modification also promotes plasma protein binding, thereby
reducing clearance by glomerular filtration and urinary excretion, and hence improving the pharmacokinetic profile of nucleotides.\textsuperscript{103} This technique was successfully employed in antisense PS drug fomivirsen, which was approved by the Food and Drug Administration in the late 90s.\textsuperscript{104}

When this approach was applied to siRNAs, the stability of the modified siRNA was successfully enhanced \textit{in vivo}.\textsuperscript{105,106} However, increased toxicity and reduction of gene silencing was also observed.\textsuperscript{86,89,105,106} This is probably because siRNAs, unlike antisense oligonucleotides, tolerate only limited modifications to remain RISC-compatible. It has been suggested that PS modification at the center of the duplex, especially at the scissile phosphate position, impairs the activity of the RISC.\textsuperscript{49} Therefore, partial PS modification was recommended, together with other types of modifications to enhance exonuclease resistance of siRNAs.\textsuperscript{107} This strategy has not been popular due to the limitations mentioned above. However, by carefully controlling the stereochemistry of phosphorothioate siRNA during synthesis, nuclease resistance could be improved without compromising biological activity.\textsuperscript{108} Alternatively, boranophosphate modification, which involves the replacement of nonbridging phosphate oxygen atoms with an isoelectronic borane (BH\textsubscript{3}) moiety (Figure 3),\textsuperscript{109} is more nuclease-resistant and less toxic compared to its PS counterparts. Whether or not this modification can retain the biological activity of siRNAs remains to be determined, and the application of this modification in miRNA therapeutics is yet to be studied.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of chemically modified RNA. (i) In the ribose 2′–OH group modification, the 2′–OH group is modified with 2′–O-methyl (2′ O-Me), 2′–fluoro (2′–F) or 2′–methoxyethyl (3′–O-MOE). (ii) In locked nucleic acid (LNA) modification, the ribose is locked in a C3′ endo conformation by introducing a 2′-O and 4′-C methylene bridge; In unlocked nucleic acid (UNA), the ribose ring is cleaved between 2′-C and 3′-C. (iii) In backbone modification, the phosphodiester backbone linkage is being substituted. The nonbridging phosphate atom is replaced with a sulfur atom to give a phosphorothioate modification, or replaced with a borane (BH\textsubscript{3}) moiety to give a boranophosphate modification.}
\end{figure}
Delivery of siRNA and miRNA Therapeutics

While chemical modification can improve the stability and reduce off-target effects of siRNAs and miRNAs, poor delivery is still a major challenge in translating therapeutic siRNAs and miRNAs into the clinic. Both types of RNA molecules have an intracellular site of action, but their intrinsic properties, including hydrophilic nature, negative charge and high molecular weight (~14–15 kDa), render them poorly permeable across biological membranes. The primary role of a delivery system is to facilitate the cellular uptake of siRNAs or miRNAs to their target sites. A delivery system can also protect the nucleic acids from premature nuclease degradation, thereby reducing the need for chemically modifications, which may affect the specificity and functionality of the RNA molecules. Since siRNAs and miRNAs have similar physicochemical properties (double-stranded RNAs with 21–23 nucleotides) and the same intracellular site of actions (both require enzymatic functions of the RISC to be active against the target mRNAs), similar delivery technologies can be applied to both types of RNA molecules.

Viral vectors

Viral vectors encoding shRNAs or miRNAs have been used to trigger RNAi and gene silencing effects. Viruses that are commonly employed for this purpose include lentiviruses, adenoviruses, and adeno-associated viruses (AAVs). They are extremely efficient in transferring the RNA-encoding vectors into the nucleus of mammalian cells to ensure high expression of RNA. Almost 70% of all gene therapy clinical trials have involved the use of viral vectors to deliver nucleic acids because of their high transduction efficiency. Viruses that are used to carry therapeutic RNA are genetically engineered to remove their virulence, and their tropism can be altered by genetic manipulation of the viral capsid for targeting to specific cell types. In addition, long-term expression can be achieved by using viruses, such as lentiviruses, that can integrate into the host genome. However, there are serious safety concerns associated with the use of viral vectors, including high immunogenicity (especially in adenoviruses) and the risk of insertional mutagenesis (especially in lentiviruses). In addition, low packaging capacity (especially in AAVs) and high production cost have also limited their clinical applications.

Therefore, despite their inferior transfection efficiency, nonviral vectors have become attractive alternatives in delivering synthetic siRNAs and miRNAs due to their better safety profile and lower production cost.

Nonviral vectors

Most of the nonviral vectors that have been investigated for RNA delivery are also used to deliver other types of nucleic acids including plasmid DNA and antisense oligonucleotides. Since the development of nonviral vectors has been extensively reviewed, readers who are interested in the design and structure of different types of nonviral delivery systems are referred to a number of recent articles. Polymer-based and lipid-based systems are the two main categories of RNA delivery systems. Apart from the advantages mentioned above (i.e., relatively good safety profile and low production cost), nonviral vectors are highly versatile. They can be easily modified to improve their delivery efficiency, e.g., to achieve site-specific delivery by incorporating targeting ligands, or to improve serum stability and extend the circulation time by PEGylation (attachment of polyethylene glycol (PEG) polymer chains). Despite the effort to develop suitable RNA delivery systems for clinical use, a lack of correlation between in vitro and in vivo efficacies is observed. It is often reported that a delivery system worked efficiently in vitro but failed in vivo either due to toxicity problems, poor pharmacokinetic profiles, nonspecific uptake or immune responses. The success of therapeutic siRNAs and miRNAs is highly dependent on the availability of a safe and efficient delivery system. Selected examples of nonviral delivery systems that have been investigated to deliver therapeutic siRNAs and miRNAs in animal and preclinical studies are summarized in Table 4.

Polymer-based delivery systems. Cationic polymers can form polyplexes with the negatively charged RNA through electrostatic interactions. The preparation of polyplexes is simple, and the nanosized polyplexes can facilitate cellular uptake through endocytosis. In addition, polymers that exhibit high proton buffering capacity can promote endosomal escape, thereby avoiding endosomal-lysosomal RNA degradation. Synthetic polyethylenimine (PEI), which has an extensive pH buffering capacity, is one of the early generation polymers studied for nucleic acid delivery. It is the most widely investigated polymer for siRNA and miRNA delivery in vivo. Because of its high transfection efficiency, PEI is regarded as the gold standard among the nonviral vectors. Apart from PEI, dendrimers, which are highly branched synthetic polymers with well-defined molecular architecture, are also frequently studied for nucleic acid delivery. Polyplexes that are formed between dendrimers and nucleic acids are also known as dendriplexes. Poly(amidoamine) (PAMAM) and polypropyleneimine are cationic dendrimers that have been evaluated for delivering RNA in vivo. However, because of their high charge density, cationic PEI and dendrimers are often associated with high toxicity which has limited their clinical use. Therefore, modified versions of PEI or dendrimers are developed to address this issue and to further improve their delivery efficiency. Alternatively, natural cationic polymers such as chitosan, which is derived from chitin (commonly found in the exoskeleton of crustaceans), and atelocollagen, which is highly purified protein derived from calf dermis, are considered to be safer options for RNA delivery.

Cyclodextrins, the cyclic oligomers of glucose, have been used in pharmaceutical formulations and their long-term effects in humans are well-established. Due to their low toxicity, high stability and lack of immune stimulation, cyclodextrin-based nanoparticles were investigated as a carrier of siRNA. Poly(lactic-co-glycolic acid) (PLGA) is a Food and Drug Administration-approved synthetic biodegradable polymer that is widely studied for delivering various types of therapeutic molecules including RNA due to its low toxicity and good safety profile. The rate of drug release can be controlled by the molecular weight and composition of PLGA. Since PLGA is a neutral polymer, it does not form polyplexes with nucleic acids. Instead, RNA can be loaded in PLGA nanoparticles or microparticles. Due to the hydrophilic nature of RNA and the hydrophobic nature of...
| Delivery system | Disease | miRNA/siRNA | Animal model | Route of administration | Reference |
|-----------------|---------|-------------|--------------|-------------------------|-----------|
| **Unmodified PEI** | | | | | |
| PEI | Asthma | siRNA targeting IL-13 | Mouse sensitized and challenged with ovalbumin | Intravenous | 202 |
| | Sepsis | siRNA targeting IL-6 and TNFα | Mouse with polymicrobial sepsis induced by cecal ligation and puncture | Intravenous; intraperitoneal | 203 |
| | Colon cancer | miRNA-145; miRNA-33a | Mouse xenograft tumor | Intratumoral; intraperitoneal | 204 |
| **Modified PEI** | | | | | |
| SA-PEI-CNT | Melanoma | siRNA targeting Braf | In situ mouse melanoma model | Topical | 138 |
| PU-PEI | Lung cancer | miRNA-145 | Mouse xenograft tumor | Intratumoral | 139 |
| | Glioblastoma | miRNA-145 | Mouse xenograft tumor | Intratumoral | 140 |
| **Dendrimers** | | | | | |
| PAMAM | Ovarian cancer | siRNA targeting Akt | Mouse xenograft tumor | Intratumoral | 134 |
| | Drug-resistant prostate cancer | siRNA targeting Hsp27 | Mouse xenograft tumor | Intratumoral | 135 |
| PAMAM-folic acid | Glioma | miRNA-7 | Mouse xenograft tumor | Intratumoral; intravenous | 136 |
| | Ovarian cancer | siRNA targeting CD44 | Mouse xenograft tumor | Intraperitoneal | 137 |
| **Natural polymers** | | | | | |
| Glycol chitosan | Drug-resistant breast cancer | siRNA targeting P-glycoprotein | Mouse xenograft tumor | Intravenous | 141 |
| Hyaluronic acid-chitosan | Breast cancer | miRNA-34a | Mouse xenograft tumor | Intravenous | 142 |
| Atelocollagen | Prostate cancer | siRNA targeting Bcl-xL | Mouse xenograft tumor | Intravenous | 143 |
| | Muscular dystrophy | siRNA targeting Mst | Genetically modified mouse with limb-girdle muscular dystrophy | Intramuscular | 144 |
| | Metastatic prostate cancer | miRNA-16 | Mouse xenograft tumor | Intravenous | 145 |
| **PLGA** | | | | | |
| PLGA microspheres with PEI | Sarcoma | siRNA targeting VEGF | Mouse xenograft tumor | Intratumoral | 149 |
| | Joint inflammation | siRNA targeting FcyRIII | Rat with temporomandibular inflammation induced by Complete Freund’s Adjuvant injection | Intra-articular | 150 |
| PLGA nanoparticles with PEI | Lung cancer | siRNA targeting STAT3 | Mouse with lung cancer induced by carcinogen | Intraperitoneal | 151 |
| **Other nanoparticles** | | | | | |
| TF-targeted nanoparticles of CDP | Subcutaneous tumor | siRNA targeting RRM2 | Mouse with subcutaneous tumor of murine neuroblastoma cells | Intravenous | 147 |
| Mesoporous silica nanoparticles with pDMAEMA | Cervical cancer | siRNA targeting PLK1 | Mouse xenograft tumor | Intravenous | 205 |
| Mesoporous silica nanoparticles with KALA peptide-PEG-PEI | Ovarian cancer | siRNA targeting VEGF | Mouse xenograft tumor | Intravenous | 154 |
| Porous silica nanoparticles with GD2 antibody | Neuroblastoma | miRNA-34a | Mouse xenograft tumor | Intravenous | 155 |
| **Lipoplexes** | | | | | |
| Cationic liposomes | Melanoma with lung metastasis | siRNA targeting Mcl1 | Mouse with lung cancer induced by intravenous injection of murine melanoma or lung carcinoma cells | Intrapulmonary | 206 |
| PEG-cationic liposomes | Prostate and pancreatic cancer | siRNA targeting PKN3 | Mouse xenograft tumor | Intravenous | 190 |
| | Drug-resistant renal cancer | siRNA targeting PLK1 | Mouse xenograft tumor | Intravenous | 207 |
| RGD peptide-PEG-cationic liposomes | Melanoma with lung metastasis | siRNA targeting c-Myc, MDM2 and VEGF | Mouse with lung cancer induced by intravenous injection of murine melanoma cells | Intravenous | 162 |
| Peptides-modified PEG-cationic liposomes | Glioma | siRNA targeting VEGF | Mouse xenograft tumor | Intratumoral; intravenous | 163 |

Table 4 Continued on next page
PLGA, it is always a challenge to obtain a high loading efficiency. Moreover, the neutral PLGA particles do not promote cellular uptake as effectively as cationic polypelexes. Incorporating small amount of cationic polymers such as PEI into the PLGA nanoparticles can enhance the encapsulation and transfection efficiency. This can also lower the toxicity when compared to the use of cationic polymers alone. Incorporating of PEG, but also promotes cellular uptake. To improve specificity, targeting moieties such as antibodies and small peptides can be employed.

Lipid-based delivery systems. Similarly to cationic polymers, cationic lipids and liposomes can form lipoplexes with RNA through electrostatic interactions. In general, lipids used for lipid-based delivery systems is not satisfactory due to toxicity, nonspecific uptake, and unwanted inflammatory and immune responses. Incorporation of PEG is a commonly employed strategy to reduce immunogenicity as well as prolong circulation following systemic delivery, but PEGylation may lead to reduction of cellular uptake. The incorporation of D-α-tocopheryl polyethylene glycol succinate into the delivery system has been reported to overcome this problem. Many of the commercial available transfection reagents such as Lipofectamine, Oligofectamine, DharmaFECT, siPORT, and TransIT-TKO are lipid-based systems and have been frequently used for RNA delivery. Despite their high transfection efficiency in vitro, the in vivo performance of most lipid-based delivery systems is not satisfactory due to toxicity, nonspecific uptake, and unwanted inflammatory and immune responses. Incorporation of PEG is a commonly employed strategy to reduce immunogenicity as well as prolong circulation following systemic delivery, but PEGylation may lead to reduction of cellular uptake. The incorporation of D-α-tocopheryl polyethylene glycol succinate into the delivery system has been reported to overcome this problem. D-α-tocopheryl polyethylene glycol succinate is a water-soluble derivative of natural vitamin E, which is formed by esterification of vitamin E succinate with PEG. It has the advantages of PEG, but also promotes cellular uptake. To improve specificity, targeting moieties such as antibodies and small peptides can be employed.

Lipid-based nanoparticles with low toxicity and high efficiency are developed to produce a more sophisticated delivery system for RNA. For example, “stabilized nucleic acid lipid particles” in which RNA is encapsulated inside the highly PEGylated liposomes prepared by ethanol dilution method have been proposed. In some studies, RNA is loaded into the cationic solid lipid nanoparticles for...
| Name | Indications | siRNA target | Phase | Delivery system | Route of administration | Trial ID (reference) |
|------|-------------|--------------|-------|-----------------|-------------------------|---------------------|
| **Cancer** | | | | | | |
| ALN-VSP02 | Advanced solid tumors with liver involvement | KSP and VEGF | 1, completed | Lipid nanoparticles | Intravenous | NCT01158079; NCT00821806 |
| Atu027 | Advanced solid tumor | PKN3 | 1, ongoing | Liposomal particles (AtuPLEX®) | Intravenous | NCT00938574 |
| CALAA-01 | Solid tumor | RRM2 | 1, terminated | Polymer-based targeted nanoparticles | Intravenous | NCT00689065 |
| DCR-MYC (Dicer-substrate siRNA) | Solid tumor, multiple myeloma, non-Hodgkin's lymphomas | MYC oncogene | 1, ongoing | Lipid nanoparticles (EnCore) | Intravenous | NCT02110563 |
| siG12D LODER | Hepatocellular carcinoma | mutated KRAS oncogene | 1/2, ongoing | Biodegradable polymer-based scaffold | Local implantation | NCT02314052 |
| siRNA-EphA2-DOPC | Advanced pancreatic cancer | EphA2 | 1, ongoing | Neutral liposomes | Intravenous | NCT01591356 |
| TKM-080301 (TKM-PLK1) | Primary or secondary liver cancer | PLK1 | 1, completed | Lipid nanoparticles | Intravenous | NCT01437007 |
| **Infectious Diseases** | | | | | | |
| ALN-RSV01 | RSV infection | RSV nucleocapsid | 2, completed | Naked | Intranasal | NCT00496821; NCT00658086; NCT01065935 |
| ARC-520 | Chronic HBV infection | conserved regions of HBV | 1, completed; 2, ongoing | DPC (membrane lytic peptides with cholesterol conjugated siRNA) | Intravenous | NCT01872065; NCT02065336; NCT02349126 |
| TKM-100201 | Ebola virus infection | Ebola L polymerase, VP24 and VP35 | 1, terminated | Lipid nanoparticles | Intravenous | NCT01518881 |
| TKM-100802 | | | 1, ongoing | | | NCT02041715 |
| **Ocular Conditions** | | | | | | |
| AGN211745 (Sirna-027) | CNV, AMD | VEGF receptor 1 | 1/2, completed; II, terminated | Naked | Intravitreal | NCT00396714; NCT00399507 |
| Bamosiran (SYL040012) | Ocular hypertension, glaucoma | ADRB2 | 1, completed | Naked | Topical ocular | NCT00939743; NCT01227291 |
| Wet AMD, open-angle glaucoma | Ocular hypertension, open-angle glaucoma | | | | | NCT01739244; NCT02250612 |
| Bevasiranib (Cand5) | Wet AMD | VEGF | 2, completed | Naked | Intravitreal | NCT00722384; NCT00529753 |
| Diabetic macular edema | Diabetic macular edema VEGF | | | | | NCT00306904 |
| Wet AMD | | | 3, terminated | | | NCT00499590 |
| PF-04523655 (PF-555) | AMD | RTP801 (hypoxia-inducible factor 1 responsive gene) | 1, 2 completed | Naked | Intravitreal | NCT00725686; NCT00713518; NCT01445899 |
| | CNV, diabetic retinopathy, diabetic macular edema | | | | | NCT00701181 |
| | Diabetic retinopathy, diabetic complications | | | | | NCT00701181 |
| QPI-1007 | Optic atrophy, nonarteritic anterior ischemic optic neuropathy | CASP2 | 1, completed | Naked | Intravitreal | NCT01064505 |
| SYL1001 | Ocular pain, dry eye syndrome | TRPV1 | 1, completed; 1/2, completed | Naked | Topical ocular | NCT01438281; NCT01776656 |
| **Cardiovascular and metabolic diseases** | | | | | | |
| ALN-PCS02 | Hypercholesterolemia | PCSK9 | 1, complete | Lipid nanoparticles | Intravenous | NCT01437059 |
| ALN-PCS8c | | | 1, ongoing | GalNAc-siRNA conjugation | Subcutaneous | NCT02314442 |
| PRO-040201 (TKM-ApoB) | Hypercholesterolemia | ApoB | 1, terminated | Lipid nanoparticles | Intravenous | NCT00927459 |

Table 5 Continued on next page
Modification of PEI with stearic acid led to stearic acid being incorporated into the backbone of PEI for better transfection efficiency in vivo.171 PEI. Similarly, cholesterol and deoxycholic acid-modified PEI also being developed for RNA delivery.167,168

In recent years, lipopolyplexes have emerged as a new generation delivery system for nucleic acids. Typically, such a system is composed of both polymers and lipids in an attempt to address the limitations of polymer-based and lipid-based delivery systems by combining the advantageous characteristics of both.178 This approach has been employed for the delivery of RNA in vivo. For example, stearic acid was incorporated into the backbone of PEI for siRNA delivery.171 Modification of PEI with stearic acid led to better transfection efficiency in vivo compared to unmodified PEI. Similarly, cholesterol and deoxycholic acid-modified PEI improved the transfection efficiency of siRNA in vivo.172–174

sustained release.165,166 The low toxic liposome-polycation (or protamine)-hyaluronic acid nanoparticles, in which the negatively charged RNA and hyaluronic acid are complexed with the cationic protamine to form the core of the liposomes, are also being developed for RNA delivery.167,168

Lipopolyplexes. In recent years, lipopolyplexes have emerged as a new generation delivery system for nucleic acids. Typically, such a system is composed of both polymers and lipids in an attempt to address the limitations of polymer-based and lipid-based delivery systems by combining the advantageous characteristics of both.178 This approach has been employed for the delivery of RNA in vivo. For example, stearic acid was incorporated into the backbone of PEI for siRNA delivery.171 Modification of PEI with stearic acid led to better transfection efficiency in vivo compared to unmodified PEI. Similarly, cholesterol and deoxycholic acid-modified PEI improved the transfection efficiency of siRNA in vivo.172–174

siRNA AND miRNA therapeutics in clinical studies

The first clinical trial of siRNA therapeutics was initiated in 2004,178 merely 6 years after the discovery of RNAi. The rapid progress of siRNA advancing into clinical trials is perhaps due to the experience gained during the development of antisense and other nucleic acid-based therapies. To date, around 30 siRNA candidates have reached various stages of clinical trials for the treatment of different diseases (Table 5). In comparison, the clinical development of miRNA as therapeutics is lagging behind, with only two miRNA therapeutics, both of which are indicated for the treatment of cancers, being registered in clinical trial to date (Table 6). The first miRNA therapeutic trial began in 2013 with the second one starting in early 2015. Although siRNAs share many similarities with miRNAs, the relatively slow progress of miRNA therapeutics could be due to their uncertain mechanism of action and specificity. The diverse potential applications of miRNAs (e.g., as drug target and biomarkers) may also have distracted from their development as therapeutic agents. However, miRNAs have an advantage over siRNAs as the therapeutics for complex multigenic diseases such as cancers and neurodegenerative disorders, which require modulation of multiple pathways for effective treatment. With the ability to inhibit the expression of a number of target genes, which often work together as a network within the same cellular pathway, a whole disease phenotype can potentially be changed by a single miRNA sequence.176,177 By contrast, the therapeutic potential of siRNAs is limited by its ability to target only one specific gene. It will be challenging to use siRNAs to modulate complex diseases, although the strategy of employing multiple siRNA sequences in a single formulation has been reported in clinical studies for the treatment of cancers and viral infections.178 On the other hand, siRNAs are extremely useful for targeting single gene disorders such as PNH, paroxysmal nocturnal hemoglobinuria; RRM2, ribonucleotide reductase subunit M2; RSV, respiratory syncytial virus; TRPV1, transient receptor potential cation channel subfamily V member 1; TTR, Transthyretin; VEGF, vascular endothelial growth factor; VP, viral protein.
Molecular Therapy—Nucleic Acids

Figure 4 Therapeutic indications of siRNA and miRNA therapeutics.

Cancer
Cancer is a leading cause of death worldwide. Almost one-third of the siRNA and miRNA based therapeutics in clinical trials are targeted at cancer. Both siRNAs and miRNAs aim to silence cancer-related gene(s) in order to inhibit tumor cell growth, angiogenesis, metastasis and/or drug resistance. Oncogenes, mutated tumor suppressor genes and other genes that contribute to tumor progression are potential targets for gene silencing. The specificity of siRNAs makes them possible to serve as a platform for personalized medicine in cancer therapy.8 On the other hand, since miRNA therapeutics can target multiple genes, typically in the context of a network, they are efficient in regulating distinct biological cell processes relevant to malignant cell biology. This characteristic makes them particularly attractive in cancer treatment,77 and may explain why the two miRNA clinical trials to date aim at cancer therapy.

By targeting the mRNA of cell-cycle proteins, tumor cell growth can be inhibited. An overexpression of polo-like kinase 1 (PLK1), a cell-cycle protein that is important in mitosis and cytokinesis, is observed in many human tumors, and the inhibition of its activity induces apoptosis and tumor cell death.181,182 A lipid nanoparticle-based delivery system containing therapeutic siRNA targeting PLK1, TKM-080301, has been developed. It is currently in phase 1/2 clinical trial for the treatment of neuroendocrine and adrenocortical cancers. Ribonucleotide reductase is an enzyme involved in DNA replication. The M2 subunit of ribonucleotide reductase (RRM2) is an established anticancer target.183,184 Inhibition of RRM2 by siRNAs reduces the growth potential of cancer cells in vitro and in vivo.184 CALAA-01 is a siRNA therapeutic targeting RRM2 for the treatment of solid tumors. Adopting a transferrin-receptor targeting cyclodextrin nanoparticle delivery system, CALAA-01 prevents the proliferation of transferrin receptor-expressing tumor cells. A phase 1 study showed that CALAA-01, following systemic administration, silenced the cancer-associated gene by RNAi mechanism in targeted tumor cells.185 However two patients experienced dose-limiting toxic events in a later trial, possibly due to the formulation problems (mis-folded, aggregated or degraded transferrin); this outcome thus underlines the significance of quality control assays of protein-targeted nanoparticle-based therapeutics.186

Other siRNA therapeutics currently undergoing clinical trials include siG12D LODER, which targets the mutated KRAS oncogene for the treatment of locally advanced pancreatic cancer and is delivered by a biodegradable polymer matrix for sustained release; siRNA-EphA2-DOPC, which targets cancer-related EphA2 gene using liposomal delivery for the treatment of advanced solid tumors; and DCR-MYC, a Dicer-substrate siRNA that targets the MYC oncogene carried by lipid nanoparticles for the treatment of various types of cancer.

Angiogenesis is a key process that promotes tumor growth and survival. One of the major regulators of this process is vascular endothelial growth factor (VEGF), which thus is an attractive target for inhibiting tumor angiogenesis.187 ALN-VSP02 is a dual targeted siRNA therapeutics carried by lipid nanoparticles that suppresses not only the cell-cycle protein kinesin spindle protein (KSP) to promote cell-cycle arrest and eventually cell death,186 but also VEGF.2 It is indicated for advanced solid tumors with liver metastasis, and the initial data from a completed phase I trial showed that ALN-VSP02 possessed antitumor activity while being well-tolerated by patients.2 The siRNA was detected in tumor biopsies following intravenous administration of loaded lipid nanoparticles. Moreover, siRNA-mediated mRNA cleavage in the liver and complete regression of liver metastases was observed in patients with endometrial cancer. Protein kinase N3 (PKN3), a downstream effector of phosphoinositide 3-kinase (PI3K) signaling, is another validated target in cancer. PI3K is only transiently activated after growth factor stimulation in normal cells. Excessive and/or chronic activation of this pathway occurs in many cancer types, and is believed to be involved in the process of metastasis.189 Inhibition of PKN3 resulted in the significant inhibition of tumor growth and the reduction of lymph node metastases in vivo.190,191 Atu027, a liposomal siRNA targeting PKN3, is currently undergoing a phase 1/2 clinical trial for the treatment of advanced or metastatic cancer.

### Table 6 A summary of miRNA therapeutics in clinical trials (registered with clinicaltrials.gov, last accessed 13 June 2015)

| Name       | Indications                                      | miRNA Phase | Delivery system                  | Route of administration | Trial ID        |
|------------|-------------------------------------------------|-------------|----------------------------------|-------------------------|----------------|
| MRX34      | Primary liver cancer or liver metastasis from other cancers | miRNA-34a   | 1, ongoing                       | Liposomes (SMARTICLES)  | Intravenous    | NCT01829971   |
| TargomiRs  | Malignant pleural mesothelioma; non–small-cell lung cancer | miRNA-16    | 1, ongoing                       | Nanoparticles (nonliving bacterial minicells) | Intravenous     | NCT02369198   |

![Figure 4](image-url)
pancreatic tumor. Early results showed that Atu027 was safe in patients with advanced solid tumors, with 41% of patients showed no further progression of tumors after 8 weeks of treatment. The role of miRNA in cancer has been a focus of research in recent years, evidenced by the number of registered clinical trials evaluating the use of miRNA as biomarkers for patient diagnosis, prognosis, and response to treatment. 194

Several miRNAs are upregulated or downregulated in various tumors. They can act either as oncogenes (also known as oncomiRs) or tumor suppressors (tumor suppressor miRNAs). 195 The latter are employed in miRNA replacement therapy for the treatment of cancer.

MRX34 is a first-in-class cancer therapy and the first synthetic miRNA to enter clinical trials. 196 MRX34 was designed to deliver miRNA-34 mimic by liposomal formulation. Indeed, miRNA-34 is a well-characterized, naturally occurring regulator of tumor suppression, 197 and it is downregulated in many cancers. It inhibits a series of signaling molecules that contribute to cancer processes including cell proliferation, anti-apoptosis, metastasis, and chemoresistance. 198 Currently, in phase 1 study, MRX34 is indicated for primary liver cancer or liver metastasis from other solid tumors, and the study is expected to be completed in late 2015. The other miRNA therapeutic that has also reached the clinical trial stage is TargomiRs, which is indicated for malignant pleural mesothelioma and non–small-cell lung cancer. TargomiRs consists of three components, namely a miRNA-16 mimic, a nanoparticle drug delivery system using nonliving bacterial minicells, and an antiproliferative growth factor receptor antibody as a targeting moiety. miRNA-16 is another tumor suppressor. 199 Restoration of miRNA-16 leads to inhibition of tumor-promoting gene transcription and hence tumor growth, as well as sensitzation of tumor cells to certain chemotherapeutic agents. The phase 1 clinical study of TargomiRs is expected to be completed in mid-2016.

Although only two miRNA replacement therapies have reached clinical trials, many tumor suppressor miRNAs, such as miRNA-7, miRNA-126, miRNA-143/145, miRNA-200, miRNA-355, and the members of the let-7 families, 200,201 have been identified with the ability to downregulate oncogenes. Some of these tumor suppressor miRNAs are currently in the preclinical stage and ready to enter phase 1 clinical trials very soon. In terms of safety profile, clinical trials have demonstrated that siRNA therapeutics are generally well-tolerated by the patients. By contrast, the clinical study of miRNA therapeutics is still in its infancy. The potential for any adverse effects may only become apparent after more clinical trials have been carried out. Since a single miRNA can affect multiple target genes, it is also difficult to predict its long-term systemic effect.

Conclusions and future prospects

Synthetic siRNAs and miRNAs hold great promises as new classes of therapeutic agents by silencing the gene(s) of interest. They have been studied for the treatment of various human diseases including cancers, viral infections, ocular conditions, genetic disorders, and cardiovascular diseases. The most attractive aspect of siRNA and miRNA therapeutics is their ability to target virtually any gene(s), which may not be possible with small molecules or protein-based drugs. While the therapeutic efficacy of siRNAs and miRNAs has been successfully demonstrated in vivo, several technical barriers still need to be overcome in order for these RNA molecules to be used clinically. The experience from antisense and gene therapy has contributed to the rapid progress of siRNAs and miRNAs into clinical studies. In particular, the technologies of chemical modification and delivery of nucleic
acids developed previously can be applied to both siRNAs and miRNAs. While the former possesses a high specificity by targeting one single gene, the latter can target multiple related genes, often in the same cellular pathway or process, to generate pronounced therapeutic effect. Currently, the development of siRNAs is advancing ahead of miRNAs, with a larger number of candidates already entered clinical trials, possibly due to the uncertainties of the complex roles of miRNAs during the early years of their discovery. With the recent surge in intensive research concerning miRNAs, it can be expected that significant advance will be made for their future role in therapeutics.

For proper therapeutic use, the sequences of RNA must be carefully designed to avoid any specific or nonspecific unwanted effects and immune responses. The transition from bench to bedside of RNA-based therapy also depends heavily on the availability of a safe, clinically relevant delivery system that can facilitate cellular uptake of the RNA into target tissues/cells and offer protection against nuclease degradation. The use of nonviral vectors including polymer-based or lipid-based delivery systems offers the advantages of better safety profile and lower production cost over viral vectors despite the inferior delivery efficiency.

Among the various diseases being investigated, cancer is currently the major target of siRNA and miRNA therapeutics. While a large number of cancer-related genes have been identified with therapeutic potential, the duration of silencing effect has not been properly investigated or reported, and it may affect the dose interval and length of treatment. The duration of silencing effect after a single dose of siRNAs or miRNAs depends on a number of factors. These include the stability of the RNA molecules, the rate of RNA release from the delivery system, the type of target tissues, as well as the half-life and turnover rate of the target proteins. A good understanding of this area can contribute to the rational design of treatment strategy to improve clinical outcome of siRNA and miRNA therapeutics. PEGylated nanoparticles incorporated with targeting ligands are frequently employed to prolong circulation time and achieve specific targeting to tumor sites following systemic administration. However, the potential toxicity and immunogenicity effects associated with the delivery agents need to be carefully monitored.

Efficient gene silencing can also be achieved with naked RNA, but this may be limited to local administration to organs such as the eyes and the lungs. Nevertheless, chemical modification of the RNA molecule may be required to improve its stability against nuclease activity in the vitreous humor of the eye or in the airway fluid of the lungs. It is expected that by overcoming the delivery barrier, and better understanding of the effects and the duration of gene silencing, siRNAs and miRNAs will become practical therapeutics in the clinic in the near future.

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35. Grimm, D, Streetz, KL, Jocelyn, CL, Storm, TA, Pandey, K, Davis, CR et al. (2006). Fatality in mice due to oversaturation of cellular microRNA/shorn hairpin RNA pathways. Nature 441: 537–541.

36. Ketter, R, Surendranath, V, Heinsius, AK, Slabicki, M, Theis, M, Putz, G et al. (2007). Genome-wide resources of endoribonuclease-prepared short interfering RNAs for loss-of-function studies of natural la. Methods 4: 337–344.

37. Kozomara, A and Griffiths-Jones, S (2011). miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 39(Database issue): D152–D157.

38. Hao, J and Xie, K (2013). Designing functional siRNA with reduced off-target effects. Methods Mol Biol 942: 57–104.

39. Judge, AD, Sood, V, Shaw, JR, Fang, D, McLintock, K and MacLachlan, I (2005). Sequence-dependent silencing of the mammalian innate immune response by siRNA. Nat Biotechnol 23: 457–482.

40. Robbins, M, Judge, A and MacLachlan, I (2009). siRNA and innate immunity. Oligonucleotides 19: 135–152.

41. Kleinman, ME, Yamada, K, Taieba, A, Chandrasakeran, V, Nozaki, M, Battf, JZ et al. (2008). Sequence- and target-independent agonism suppression by siRNA via TLR3. Nature 452: 501–507.

42. Kim, DH, Behlke, MA, Birmingham, A, Reynolds, A, Karpilow, J, Robinson, K et al. (2005). Synthetic Pre-miRNA-Based shRNA as Ligands of Toll-like receptors. RNA 11: 179–191.

43. Leary, TM, McCall, AP, Tanner, AK, Huang, Z, Kay MA and Sullenger, BA (2004). RNA interference: a chemical modification analysis. Nat Biotechnol 22: 458–46.

44. Li, W, Wang, Y, Guo, T and He, J (2009). Synaptic transmission and synaptic plasticity. J Neurosci 29: 131579.

45. Li, W, Wang, Y and Zhong, R (2008). Increased RNAi screens. Cold Spring Harb Symp Quant Biol 73: 486–493.

46. Li, W and Zhong, R (2006). Positional effect of chemical modifications on short interference RNA activity in mammalian cells. RNA 12: 1188–1196.

47. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA mediating potent gene silencing in vivo. J Med Chem 53: 589–595.

48. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

49. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

50. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

51. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

52. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

53. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

54. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

55. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

56. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

57. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

58. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

59. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.

60. Li, W, Pan, F, Huang, H, Fesik, SW et al. (2010). Synthetic siRNA against mTOR. J Med Chem 53: 596–602.
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acids, 2'-O-methyl RNA, phosphorothioates and small interfering RNA, Nucleic Acids Res 31:3185–3193.

99. Hornung, V, Geurtten-Biller, M, Bourquin, C, Ablasser, A, Schier, M, Uematsu, S et al. (2005). Sequence-specific potent induction of RIG-I alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat Med 11:263–270.

100. Laursen, MB, Pakula, MM, Gao, S, Fluit, K, Mook, OR, Baas, F et al. (2010). Utilization of unlocked nucleic acid (UNA) to enhance siRNA performance in vitro and in vivo. Mol Biotechnol 46: 862–870.

101. Vaish, N, Kim, YJ, Kim, S, Park, HO and Choi, YC (2006). Chemical modification of siRNAs to improve serum stability without loss of efficacy. Biochem Biophys Res Commun 342: 819–827.

102. Souchet, J, Aikac, B, Bramlage, B, Carius, K, Constein, R, Donoghue, M et al. (2004). Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 432: 173–178.

103. Jahns, H, Roos, M, Imig, J, Baumann, F, Wang, Y, Gilmour, R et al. (2015). Stereochemical bias introduced during RNA synthesis modulates the activity of phosphorothioate siRNAs. Nat Met 12: 631–637.

104. Hall, AH, Wan, J, Shaughnessy, EE, Ramsay Shaw, B and Alexander, KA (2004). RNA interference using boranophosphate siRNAs: structure-activity relationships. Nucleic Acids Res 32: 6991–6000.

105. Ters, J, Lam, S, Stojkovic, P, Ren, G, Oz, OK, Mason, RP et al. (2004). Biodistribution of phosphodiester and phosphorothioate siRNA. Bioorg Med Chem Lett 14: 1139–1143.

106. Choung, S, Kim, YJ, Kim, S, Park, HO and Choi, YC (2006). Sequence-specific potent induction of IFN-alpha by short interfering RNA in vivo. Mol Biotechnol 32: 862–870.

107. Höbel, S and Aigner, A (2013). Polyethyleneimines for siRNA and miRNA delivery in vivo. Wiley Interdiscip Rev Nanomed Nanobiotechnol 6: 484–501.

108. Urban-Klein, B, Werth, S, Aburabdeh, S, Cuboysko, F and Aigner, A (2006). RNAi-mediated gene-targeting through systemic application of polyethyleneimine (PEI)-complexed siRNA in vivo. Gene Ther 12: 461–466.

109. Dufes, C, Uchegbu, IF and Schätzlein, AG (2005). Nucleic Acid Therapeutics: towards the match between fundamental science and engineered systems. Adv Drug Deliv Rev 57: 975–985.

110. Lu, J, Li, R, Su, Z, Jiang, Z, Chen, L, Wang, J and Jie, J (2013). Polyamide amine is an ideal carrier of miR-7 for enhancing gene silencing effects on the EGFR pathway in U251 glioma cells. Oncol Rep 29: 1387–1394.

111. Shah, V, Taratula, O, Garbuzaenko, OB, Taratula, OR, Rodriguez-Rodriguez, L and Mirko, T (2013). Targeted nanomedicine for suppression of CD44 and simultaneous cell death induction in ovarian cancer: an optimal delivery of siRNA and anticancer drug. Curr Cancer Res Clin Pract 19: 6193–6204.

112. Liu, S, Ko, D, Zheng, X, Zhang, X, Johnston, N, Liu, Y et al. (2014). Non-covalently functionalized single-walled carbon nanotube for topical siRNA delivery into melanoma. Biomaterials 35: 3435–3442.

113. Chiou, YF, Cheng, YF, Hsu, YJ, Wang, ML, Tsai, CM, Lu, KH et al. (2012). Cationic polyethyleneimine-short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. J Control Release 159: 240–250.

114. Yang, YF, Chien, YF, Chiu, YC, Cheng, YF, Wang, ML, Lo, W et al. (2012). Injection of cationic stem cell-like protein (sc-Prp) nanoparticles for glioblastoma using microRNA145 with cationic polyethyleneimine short branch PEI. Biomaterials 33: 1462–1478.

115. Yhee, JY, Song, J, Lee, S, Park, SG, Kim, KS, Kim, MG et al. (2015). Cancer-targeted MDR-1 siRNA delivery using self-cross-linked glycol chitosan nanoparticles to overcome drug resistance. J Control Release 198: 1–9.

116. Deng, X, Cao, M, Zhang, J, Yu, K, Yin, Z et al. (2014). Hydroacid-achitosan nanoparticles for co-delivery of MR-34a and doxorubicin in therapy against triple negative breast cancer. Biomaterials 35: 4335–4344.

117. Yuan, Y, Makita, N, Cao, D, Mihara, K, Kadomatsu, K and Take, Y (2015). Ateclocollagen-mediated intravenous siRNA delivery specific to tumors surgically xenografted in proxates of nude mice and in a pancreatic cancer model. Nucleic Acid Ther 25: 85–94.

118. Kawakami, E, Kawai, N, Kinouchi, N, Mori, H, Ohashi, Y, Ishimaru, N et al. (2013). Local applications of myostatin-siRNA with ateloecollagen increase skeletal muscle mass and rescue of muscle function. PLoS One 8: e67419.

119. Takeishi, F, Patrawala, L, Osaki, M, Takahashi, RY, Yamamoto, Y, Kosaka, N et al. (2010). Systemic delivery of synthetic microRNA-18 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. Mol Ther 18: 181–187.

120. Davis, ME and Brewster, ME (2004). Cyclodextrin-based pharmaceuticals: past, present and future. Nat Rev Drug Discov 3: 1023–1033.

121. Bartlett, DW and Davis, ME (2008). Impact of tumor-specific targeting and dosing schedule on tumor growth inhibition after intravenous administration of siRNA-containing nanoparticles. Biotechnol Bioeng, 99: 975–985.

122. Hu-Leskov, S, Heidel, JD, Bartlett, DW, Davis, ME and Trinch, TJ (2005). 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 65: 8964–8972.

123. Murata, N, Takashima, Y, Toshiyoshi, K, Yamamoto, M and Okada, H (2008). Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice. Hum Gene Ther 19: 975–985.

124. Hu-Leskov, S and Heidel, JD (2012). Polyethyleneimine short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. J Control Release 159: 240–250.

125. Brandt, MP, Kirsch, AG, Pozutto, T, Schubert, S, Kandolf, R and Fecher, H et al. (2013). Adenovirus vector-mediated RNA interference for the inhibition of human papovavirus B19 replication. Virus Res 175: 156–169.

126. Esquela-Kerscher, A and Slack, FJ (2006). Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 6: 255–260.

127. Liou, W, Chen, O, Ma, L, Liu, J, Yang, Z, Shen, J et al. (2013). Oncolytic adenovirus co-expressing miRNA-34a and BAG4 inhibits superior tumor immunity in experimental tumor model. J Mol Med (Berl) 91: 715–725.

128. Kore, F, Kay, MA and Mueller, C (2014). Recombinant AAV as a platform for translating the therapeutic potential of RNA interference, Mol Ther 22: 692–701.

129. Koto, J, Chivukula, RR, O'Donnell, KA, Wenzelt, EA, Montgomery, CL, Hwang, HW et al. (2008). Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 137: 1005–1017.

130. Stanek, LM, Sardi, SP, Mastis, B, Richards, AR, Treileaven, CM, Takac, T et al. (2014). Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Hum Gene Ther 25: 461–474.

131. Hendrickx, R, Stichting, N, Koelen, J, Kuryk, L, Lipiec, A and Greber, UF (2014). In vivo delivery of nucleic acid therapeuticss. Nanomedicine 11: 1575–1584.

132. Vilela, M, Arranja, A, Rafael, D and Gaspar, R (2014). Preclinical development of siRNA therapeutics: towards the match between fundamental science and engineered systems. Nanomedicine 10: 985–992.
154. Chen, Y, Wang, L, Xu, L, Zhang, D, Wang, Y, Gu, H, et al. (2015). Highly effective antiangiogenic escape pathways for non-viral nucleic acid delivery systems. In Cersa B (ed.), Molecular Regulation of Endocytosis, Intech, 2012, pp. 429–456.

155. Tivnan, A, Orr, WS, Gubala, V, Nooney, R, Williams, DE, McDonagh, C, et al. (2012). Lipidic systems for in vivo siRNA delivery. AAPS J 33: 639–652.

156. Liang, W and Lam, JKW. Endosomal escape pathways for non-viral nucleic acid delivery systems. In Cersa B (ed.), Molecular Regulation of Endocytosis, Intech, 2012, pp. 429–456.

157. Wu, Y, Crawford, M, Tao, Y, Lee, RJ, Davis, IC, Elton, TS et al. (2013). Therapeutic Delivery of MicroRNA-218 by Cationic Lipoplexes for Lung Cancer. Mol Ther Nucleic Acids 2: e64.

158. Zhang, Z, Yan, Y, Feng, G, Gao, QM and ETPGS as a molecular tool for biodegradable drug delivery. Biomaterials 33: 4898–4906.

159. Kenjo, E, Asai, T, Onenaga, N, Ando, H, Ishii, T, Hatanaka, K, et al. (2013). Systemic delivery of small interfering RNA by use of targeted polycation liposomes for cancer therapy. Bio Pharm Bull 36: 287–291.

160. Chen, Y, Wang, X, Liu, T, Zhang, DS, Wang, Y, Gu, H. (2014). Antiangiogenesis via magnetic mesoporous silica-based siRNA vehicle targeting the VEGF inhibitor. Mol Pharm 11: 11448–11454.

161. Sakurai, Y, Hatakeyama, H, Akita, H and Harashima, H (2014). Improvement of doxorubicin delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung cancer growth and metastasis by systemic delivery of siRNA via targeted nanoparticles. Nature 464: 1067–1070.

162. Tivnan, A, Orr, WS, Gubala, V, Nooney, R, Williams, DE, McDonagh, C, et al. (2012). Lipidic systems for in vivo siRNA delivery. AAPS J 33: 639–652.

163. Lobovkina, T, Jacobson, GB, Gonzalez-Gonzalez, E, Hickerson, RP, Leake, D, Kaspar, RL, et al. (2011). Intrapulmonary delivery of anticancer siRNA for lung cancer therapy using cationic poly(2-dimethylaminoethyl methacrylate) functionalized mesoporous silica nanoparticles. Nat Nanotechnol 6: 339–352.

164. Liu, X and Erikson, RL (2003). Polo-like kinase (Plk)1 depletion induces apoptosis in human neuroblastoma. Proc Natl Acad Sci USA 100: 5878–5894.

165. Shao, J, Zhou, B, Chu, B and Yen, Y (2008). Bromocriptine reductase inhibitors and future drug development. Curr Drug Targets 9: 409–431.
213. Nguyen, QD, Schachar, RA, Nduaka, CI, Sperling, M, Basile, AS, Klamerus, KJ et al.; PF-04523655 Study Group. (2012). Phase 1 dose-escalation study of a siRNA targeting the RTP801 gene in age-related macular degeneration patients. Eye (Lond) 26: 1099–1105.
214. Nguyen, QD, Schachar, RA, Nduaka, CI, Sperling, M, Klamerus, KJ, Chi-Burris, K et al.; MONET Clinical Study Group. (2012). Evaluation of the siRNA PF-04523655 versus ranibizumab for the treatment of neovascular age-related macular degeneration (MONET Study). Ophthalmology 119: 1867–1873.
215. Nguyen, QD, Schachar, RA, Nduaka, CI, Sperling, M, Basile, AS, Klamerus, KJ et al.; DEGAS Clinical Study Group. (2012). Dose-ranging evaluation of intravitreal siRNA PF-04523655 for diabetic macular edema (the DEGAS study). Invest Ophthalmol Vis Sci 53: 7666–7674.
216. Fitzgerald, K, Frank-Kamenetsky, M, Shulga-Morskaya, S, Liebow, A, Bettencourt, BR, Sutherland, JE et al. (2014). Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. Lancet 383: 60–68.
217. Coelho, T, Adams, D, Silva, A, Lozenon, P, Hawkins, PN, Mant, T et al. (2013). Safety and efficacy of RNAi therapy for transthyretin amyloidosis. N Engl J Med 369: 819–829.
218. Leachman, SA, Hickerson, RP, Schwartz, ME, Bullough, EE, Hutcherson, SL, Boucher, KM et al. (2010). First-in-human mutation-targeted siRNA phase Ib trial of an inherited skin disorder. Mol Ther 18: 442–446.

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