REVIEW

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MicroRNAs as novel endogenous targets for regulation and therapeutic treatments

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MicroRNAs (miRNAs) are small non-coding RNAs that have been identified as key endogenous biomolecules that are able to regulate gene expression at the post-transcriptional level. The abnormal expression or function of miRNAs has been demonstrated to be closely related to the occurrence or development of various human diseases, including cancers. Regulation of these abnormal miRNAs thus holds great promise for therapeutic treatments. In this review, we summarize exogenous molecules that are able to regulate endogenous miRNAs, including small molecule regulators of miRNAs and synthetic oligonucleotides. Strategies for screening small molecule regulators of miRNAs and recently reported small molecules are introduced and summarized. Synthetic oligonucleotides including antisense miRNA oligonucleotides and miRNA mimics, as well as delivery systems for these synthetic oligonucleotides to enter cells, that regulate endogenous miRNAs are also summarized. In addition, we discuss recent applications of these small molecules and synthetic oligonucleotides in therapeutic treatments. Overall, this review aims to provide a brief synopsis of recent achievements of using both small molecule regulators and synthetic oligonucleotides to regulate endogenous miRNAs and achieve therapeutic outcomes. We envision that these regulators of endogenous miRNAs will ultimately contribute to the development of new therapies in the future.

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Introduction

MicroRNAs (miRNAs) are a novel class of endogenous small non-coding RNAs that are usually 19–23 nucleotides in length and function by regulating gene expression at the post-transcriptional level.^{1,2} Since the first discovery of *lin-4* in 1993,^{3,4} thousands of human miRNAs have been discovered and identified. Meanwhile, miRNAs have been shown to simultaneously regulate multiple human genes, and over 30% of human genes have been predicted to be miRNA targets.⁵ miRNAs are therefore extensively involved in various cellular processes, including proliferation, differentiation, and apoptosis.⁶ Recent evidence also points to the involvement of miRNAs not only in normal physiologies but also in pathologies. The abnormal expression or function of miRNAs is

closely associated with the occurrence and development of diverse human diseases, including cancers.^{7,8} miRNAs are therefore emerging as novel endogenous targets for therapeutic treatments.⁹

The biological roles of certain miRNAs in disease development have been elucidated in considerable detail. For example, miR-10b has been identified as an oncogenic miRNA in breast cancer that targets *Hoxd10*.¹⁰ miR-34a has been shown to be a tumor-suppressing miRNA in prostate cancer that targets CD44.¹¹ The active involvement of miRNAs in different human diseases makes them promising targets for therapeutic treatments. It is therefore possible to develop novel methods to treat diseases by regulating these disease-related miRNAs. Exogenous molecules, such as small molecules and synthetic oligonucleotides that are able to regulate abnormal miRNAs, may serve as powerful tools to treat related diseases by restoring miRNA-involved regulatory pathways inside cells.^{12–16} It is worth noting that several miRNA mimics or antisense miRNA oligonucleotides (AMOs) have even entered clinic trials,⁹ highlighting the potential of miRNA regulators in therapeutic treatments.

In this review paper, we summarize current strategies for identification of small molecule regulators of miRNAs and recently reported small molecules that are able to regulate miRNA expression or function. Synthetic oligonucleotides, including AMOs and miRNA mimics, as well as strategies for

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the delivery of these synthetic oligonucleotides into cells to regulate endogenous miRNAs are also summarized. Finally, typical biomedical applications of these regulators in therapeutic treatments are discussed.

Biogenesis and function of microRNA

As shown in Fig. 1, the biogenesis of endogenous miRNA starts with the transcription of a miRNA gene into primary miRNA (pri-miRNA), which occurs in the nucleus *via* RNA polymerase II.¹⁷ Pri-miRNA is cleaved by Drosha into precursor miRNA (pre-miRNA),¹⁸ which is further transported from the nucleus into the cytoplasm through Exportin 5.¹⁹ In the cytoplasm, maturation of pre-miRNA occurs through cleavage by Dicer into a miRNA duplex.²⁰ After degradation of the complementary strand, single-stranded miRNA is incorporated into RNA-induced silencing complexes (RISCs) and binds to the 3'-untranslated region (3'-UTR) of target messenger RNA (mRNA) through sequence complementarity, leading to mRNA degradation or repression of translation.² Several proteins in RISCs have now been identified, including Argonaute 2 (AGO2) and TAR RNA-binding protein (TRBP), with AGO2 as the key protein that binds to miRNA and exerts a gene silencing function.^{21,22}

Since the biogenesis and function of miRNA involve many other biomolecules and are multi-step processes, changes at any step may cause changes in miRNA expression or function. Small molecules that regulate these processes and synthetic oligonucleotides that modulate the expression level of miRNAs are thus potential regulators of miRNA and have attracted significant research interest in recent years.

Regulation of microRNAs with small molecules

Small molecules that can modulate the expression or function of miRNAs hold great potential in restoring abnormal miRNAs and corresponding miRNA-involved regulatory pathways. Differing from synthetic oligonucleotides that are di-

rectly synthesized according to the sequences of target endogenous miRNAs, small molecule regulators of miRNAs are usually identified *via* high-throughput screening.²³ In this section, we summarize current strategies for identifying small molecule regulators of miRNAs and recently reported small molecules that can regulate miRNAs.

Strategies for the identification of small molecule regulators of miRNAs

As shown in Table 1 and Fig. 2, strategies targeting different steps in the biogenesis and function of miRNAs have been developed and employed to discover potential small molecule regulators of miRNAs.

As shown in Fig. 1, miRNA maturation and function are multi-step processes, suggesting that regulation by small molecules at any step could lead to changes in miRNA expression or function. To develop unbiased strategies for screening small molecule regulators, cellular reporter systems were constructed (Fig. 2A).^{24,25} Using green fluorescent protein (GFP) or luciferase as reporter genes, complementary sequences of miRNAs were first inserted into the 3'-UTR of reporter genes. After transfection of these engineered reporter genes into cells expressing high levels of target miRNAs, the endogenous miRNAs were able to repress expression of the reporter genes. Small molecules that can regulate the biogenesis or function of target miRNAs could thus be identified by measuring the luciferase or GFP signals. Small molecules inhibiting endogenous miRNAs could lead to up-regulation of reporter signals while small molecules activating endogenous miRNAs could cause down-regulation of reporter signals. To avoid variations or false positives, internal controls and empty vectors are often used in combination. Since exogenous small-interfering RNAs (siRNAs) share the same pathway with endogenous miRNAs after introduction into cells,¹ cellular reporter systems could also be constructed by using reporter genes engineered with complementary sequences of designed siRNA.^{24,29} Small molecules that are identified through siRNA-based reporter systems usually can also regulate miRNAs.

On the other hand, some *in vitro* assays have also been developed to target miRNA maturation or function. Dicer, which can cleave a pre-miRNA substrate (Fig. 1), has been selected as a target for the construction of screening systems for identifying small molecules that can regulate miRNA maturation. A molecular beacon, a dual-labelled pre-miRNA with a fluorophore and a quencher at each end, was designed and constructed by Maiti *et al.*²⁶ Its fluorescence was initially quenched due to the fluorescence resonance energy transfer (FRET) between the fluorophore and quencher. Upon cleavage by Dicer and the subsequent dissociation of the fluorophore from the quencher, the fluorescence of the molecular beacon was recovered, indicating the maturation of miRNA. Small molecules that are able to inhibit the maturation of miRNA could be identified by measuring the fluorescence signals (Fig. 2B). By using this method, several small molecules

Fig. 1 Schematic illustration of the biogenesis and function of miRNA. The pri-miRNA transcript is cleaved by Drosha into pre-miRNA in the nucleus. The pre-miRNA is transported into the cytoplasm by Exportin 5 and is cleaved by Dicer into miRNA. After assembly into RISCs, the miRNA subsequently regulates its target mRNA through mRNA degradation or repression of translation.

Table 1 Current strategies for the identification of small molecule regulators of miRNAs

Strategies	Principles	Advantages	Disadvantages
Cellular assays	GFP reporter assay ²⁴ Luciferase reporter assay ²⁵	Reporter cells transfected with engineered GFP or luciferase genes for the read-out of changes in expression or function of endogenous miRNA	An unbiased assay that can identify small molecules targeting both miRNA biogenesis and function
<i>In vitro</i> assays	Dicer cleavage assay ²⁶ AGO2-binding assay ²⁷	A FRET probe based on pre-miRNA for measuring the activity of Dicer Fluorescence polarization assay using fluorophore-labelled miRNA for measuring the binding between miRNA and AGO2	Specific to the Dicer-mediated miRNA maturation process Specific to the AGO2-mediated miRNA function process
Bioinformatics	Inforna ²⁸	A prediction database about known structure relationships between small molecules and RNA motifs	Need further experimental validation in cells

that inhibit miRNA maturation were discovered.²⁶ Moreover, AGO2, the key protein of RISCs, and small molecules that can inhibit miRNA loading onto AGO2 have potential for inhibiting miRNA function. A fluorescence polarization method based on the binding of fluorophore-labelled miRNA and AGO2 was developed by Kiriakidou *et al.*²⁷ Upon binding of miRNA to AGO2, high polarization values were measured, while inhibition of binding by small molecules resulted in low polarization values (Fig. 2C). By measuring the polarization values, several small molecules that inhibit the loading of miRNA onto AGO2 were discovered as inhibitors of miRNA function.²⁷ Although the small molecules identified by these methods show activity *in vitro*, further validation in live cells is still needed since Dicer and AGO2 function with other chaperone proteins inside the cell.

Inforna, a bioinformatic method, was developed by Disney *et al.* to predict potential small molecule regulators that can inhibit the maturation of miRNAs (Fig. 2D).²⁸ This method involves building a database of structure relationships between small molecules and RNA motifs that can interact with each other. The RNA motifs are structural motifs, such as bulges, hairpins, and internal loops, that are present in the precursors of miRNAs. By collecting data from experimental screening of direct interactions between small molecules from a compound library and synthesized RNAs with different RNA motifs, a database was constructed, allowing small molecules that can potentially bind to miRNA precursors with specific RNA motifs to be predicted computationally. Using this method, several small molecules that regulate oncogenic miRNAs were successfully discovered.^{30,31}

With these developed strategies for the identification of small molecule regulators of endogenous miRNAs, several active small molecules were discovered to regulate miRNAs (Table 2). The regulatory mechanisms for some small molecules have also been elucidated in detail. Next, we introduce recently reported small molecule regulators of miRNAs.

Fig. 2 Strategies for the identification of small molecule regulators of miRNAs. (A) Cellular reporter systems based on engineered reporter genes. Luciferase or GFP genes were engineered with complementary sequences of miRNAs to report the changes in miRNA expression or function upon treatment with small molecules. (B) *In vitro* assays targeting miRNA maturation. A FRET probe that is pre-miRNA labelled with a fluorophore and corresponding quencher was used to identify whether small molecules could inhibit Dicer-mediated cleavage of pre-miRNA. (C) *In vitro* assays targeting miRNA function. A probe that is miRNA labelled with a fluorophore was used to measure its interaction with protein AGO2. (D) The Inforna approach is a bioinformatic method that was developed based on a database containing information on structure relationships between small molecules and different RNA motifs that interact with each other. Adapted from ref. 28 with permission.

Table 2 Summary of the reported small molecule regulators of miRNAs

Small molecule regulators	Activation/inhibition	Target miRNAs	EC ₅₀ /IC ₅₀ value
1 (ref. 24)	Activation	—	30 μM
2 (ref. 32)	Activation	—	—
3 (ref. 33)	Activation	—	—
4 (ref. 29)	Inhibition	—	—
5 (ref. 29)	Inhibition	—	—
6 (ref. 27)	Inhibition	—	0.47 μM
7 (ref. 34)	Inhibition	miR-21	2 μM
8 (ref. 35)	Inhibition	miR-21	—
9 (ref. 26)	Inhibition	miR-27a	—
10 (ref. 36)	Inhibition	miR-122	3 μM
11 (ref. 36)	Inhibition	miR-122	0.6 μM
12 (ref. 36)	Activation	miR-122	3 μM
13 (ref. 37)	Inhibition	myomiRs	2.5 μM (miR-1) 5 μM (miR-206)
14 (ref. 38)	Inhibition	miR-96	—
15 (ref. 39)	Inhibition	miR-96	50 nM
16 (ref. 40)	Inhibition	miR-544	—
17 (ref. 41)	Inhibition	miR-210	200 nM

Small molecule regulators

The first small molecule regulator of miRNAs, enoxacin (1, Fig. 3), was identified by Jin *et al.* in 2008.²⁴ They constructed a cellular reporter system that used HEK293 cells that stably expressed GFP reporter genes for siRNA, which was used to screen a collection of ~2000 US Food and Drug Administration (FDA)-approved small molecules. Compound 1 was found to increase siRNA-mediated down-regulation of GFP signals in a dose-dependent manner, and its half maximal effective concentration (EC_{50}) value was ~30 μ M. They then examined the effect of compound 1 on endogenous miRNAs. One-hundred-fifty-seven miRNAs were tested, showing that compound 1 could up-regulate the expression levels of 13 miRNAs (by ~2-fold) and down-regulate the expression levels of 2 miRNAs (by ~2-fold). Further investigations revealed that compound 1 down-regulated the expression levels of pri-miRNAs and pre-miRNAs when up-regulating the expression levels of miRNAs, suggesting that it functioned by promoting miRNA processing. Through *in vitro* processing assays mediated by Dicer or TRBP, TRBP was found to be the target protein for compound 1 regulation of endogenous miRNAs. Meanwhile, another small molecule, 2,2'-dipyridyl (2, Fig. 3), was also found to increase siRNA-mediated down-regulation of GFP signals.³² Compound 2 (10 μ M) led to an ~50% reduction in GFP signals. The effect of compound 2 on 369 miRNAs was then tested, showing that compound 2 caused an ~2 fold increase in the expression levels of 56 miRNAs. By measuring the expression levels of pri-miRNAs and pre-miRNAs, compound 2 was also found to activate miRNA by promoting miRNA processing. Since compound 2 is an iron chelator, they hypothesized that cytosolic iron might be involved in miRNA biogenesis. The effect of iron on modulating GFP signals was further tested using the same reporter system, showing that iron inhibited RNAi activity. Meanwhile, previous reports had already revealed that poly(C)-binding protein 2 (PCBP2) facilitates the loading of cytosolic iron into ferritin, which is an intracellular protein that can store and release iron. They examined whether PCBP2 or ferritin was involved in miRNA biogenesis. Further investigations revealed that PCBP2 associated with Dicer and was thus involved in miRNA biogenesis. Similarly, by construction of cel-

lular reporter systems consisting of cells transiently transfected with luciferase reporter genes bearing complementary sequences of different miRNAs, Zhang *et al.* identified another activator, a photocycloaddition product (3, Fig. 3), of miRNAs by screening their own compound library.³³ Compound 3 (10 μ M) was able to simultaneously down-regulate luciferase signals (~30–60%) for several miRNAs. When compound 3 was used as a probe, it was found to decrease the expression levels of several pre-miRNAs (~50–70%) and increase the expression levels of these miRNAs (~1.5–2-fold), indicating that it can promote miRNA maturation. Although the expression levels of the TRBP protein were up-regulated by compound 3, a detailed mechanism for the activation of miRNAs by compound 3 has not been elucidated.

In addition to these general activators, two general inhibitors of miRNAs were discovered by Jeang *et al.*²⁹ They screened 530 compounds using cellular reporter systems constructed from 293T cells that stably expressed luciferase reporter genes for siRNA, resulting in the discovery of poly-L-lysine (4, Fig. 3) and tryptaflavine (5, Fig. 3) (5 μ M), which were able to down-regulate luciferase signals (~50–60%). They tested the effect of compounds 4 and 5 on several endogenous miRNAs, showing that they inhibited expression of these miRNAs but in two different ways. Compound 4 caused down-regulation in the total expression levels of the miRNAs. By measuring Dicer activity, they found that compound 4 functioned by impairing Dicer activity. By measuring the ratios between the expression levels of AGO2-associated miRNA and the total miRNA levels, compound 5 was found to mainly inhibit the loading of miRNA onto AGO2.

In addition to these general regulators of miRNAs identified from cellular reporter systems, several small molecules that can inhibit miRNAs have also been found using *in vitro* assays. A fluorescence polarization assay based on tetramethylrhodamine (TAMRA)-labelled miR-21 and AGO2 was developed to screen ~1280 compounds from the Library of Pharmacologically Active Compounds (LOPAC). This approach led to the discovery of three small molecule regulators, including aurintricarboxylic acid (6, Fig. 3), that were able to inhibit miRNA loading onto AGO2.²⁷ The half maximal inhibitory concentration (IC_{50}) value for compound 6 was determined to be 0.47 μ M. When applying compound 6 (6.25–50 μ M) to cellular reporter systems of 293T cells transfected with luciferase reporter genes for siRNA, a dose-dependent increase in the luciferase signals was detected. Meanwhile, the protein expression levels of the endogenous target RNA helicase A (RHA) for siRNA were also up-regulated by compound 6.

In addition to small molecule regulators that can regulate multiple miRNAs, small molecule regulators of certain miRNAs have been identified.

miR-21, a well-known oncogenic miRNA, is significantly up-regulated in various cancers.⁴² Small molecules with inhibitory activity toward miR-21 thus hold great promise in therapeutic treatments with miR-21 as the target. Through

Fig. 3 General inhibitors or activators of endogenous miRNAs.

construction of a cellular reporter system of HeLa cells stably transfected with a luciferase reporter gene for miR-21, Deiters *et al.* screened over 1000 compounds from their compound collection and LOPAC.³⁴ A diazobenzene compound (**7**, Fig. 4) (10 μ M) was found to increase the luciferase signals ($\sim$ 5-fold). By measuring the miRNA expression levels, an $\sim$ 80% reduction in expression levels of both mature miR-21 and pri-miR-21 was induced by treating the HeLa cells with 10 μ M compound **7**. However, compound **7** did not affect the expression levels of miR-30 and miR-93 in the same cell line. The simultaneous decrease in the expression levels of miR-21 and pri-miR-21 indicated that the inhibitory activity of compound **7** was due to inhibition of miR-21 transcription. The detailed mechanism of miR-21 inhibition by compound **7** remains unclear. Aminoglycosides are known to bind to secondary structures of RNA, indicating that they are potential regulators of miRNAs. Maiti *et al.* constructed a cellular reporter system of MCF-7 cells transfected with a luciferase reporter gene engineered with the 3'-UTR of the miR-21 target, which they used to screen 15 aminoglycosides.³⁵ Streptomycin (**8**, Fig. 4) was discovered to be an inhibitor of miR-21, which induced an $\sim$ 1.4-fold increase of the luciferase signals after treating the MCF-7 cells with 10 μ M compound **8**. Meanwhile, an $\sim$ 80% reduction in the mature miR-21 level was detected. Further *in vitro* assays revealed that compound **8** may directly interact with pre-miR-21 to prevent its cleavage by Dicer.

miR-27 is another oncogenic miRNA that is overexpressed in a number of different cancers.⁴³ Using a molecular beacon of pre-miR-27a that was dual-labelled with 5'-FAM and 3'-BHQ1, 14 FDA-approved aminoglycoside small molecules were screened to assess their ability to inhibit Dicer-mediated cleavage of the pre-miRNA probe. Five small molecules, including neomycin (**9**, Fig. 4), were found to inhibit miRNA maturation.²⁶ By using cellular reporter systems of MCF-7 cells transfected with luciferase reporter genes for miR-27a, they found that 20 μ M compound **9** led to an $\sim$ 1.2–1.4-fold

increase in the luciferase signals. Meanwhile, 20 μ M compound **9** was able to inhibit the expression of mature miR-27a ($\sim$ 70%) and up-regulate the protein expression levels of prohibitin, the endogenous target of miR-27a. Using *in vitro* assays, compound **9** was showed to directly bind to pre-miR-27a.

miR-122 is a liver-specific miRNA that is actively involved in liver diseases, including liver cancer and hepatitis C.^{44–46} Using a cellular reporter system of Huh7 cells stably transfected with luciferase reporter genes for miR-122, Deiters *et al.* screened 1364 compounds from the National Cancer Institute (NCI) Developmental Therapeutic Program.³⁶ Compound **10** (Fig. 4) (10 μ M) and compound **11** (Fig. 4) (10 μ M) were found to induce $\sim$ 7.7-fold and $\sim$ 12.5-fold increases in the luciferase signals, respectively. The IC₅₀ values for compounds **10** and **11** were 3 μ M and 0.6 μ M, respectively. Compound **12** (Fig. 4) (10 μ M) caused an $\sim$ 90% reduction in the luciferase signals. The EC₅₀ value of compound **12** was determined to be 3 μ M. An $\sim$ 50–90% reduction in the expression levels of both mature miR-122 and pri-miR-122 was induced by compounds **10** (10 μ M) and **11** (10 μ M), while compound **12** (10 μ M) led to a 4-fold increase in the expression levels of both mature miR-122 and pri-miR-122. Meanwhile, compounds **10–12** did not affect the expression levels of miR-21 in the same cell line. Similar to compound **7**, compounds **10–12** regulated miR-122 by down-regulating or up-regulating miR-122 transcription. However, the detailed mechanism for the regulation of miR-122 by these compounds remains unclear.

Myogenic miRNAs (myomiRs), including miR-133a, miR-1, and miR-206, are highly expressed in cardiac and skeletal muscles, which are also involved in muscle and heart development or diseases.⁴⁷ Using a cellular reporter system of cells transiently transfected with a luciferase reporter gene for endogenous miRNAs, Zhang *et al.* screened potential small molecule regulators of myomiRs from their compound library.^{37,48} A photocycloaddition product, compound **13** (Fig. 4) (10 μ M), was found to up-regulate the luciferase signals ($\sim$ 6–10-folds) corresponding to myomiRs, but not other miRNAs, such as miR-150 or miR-25 in other cell lines. The IC₅₀ values of compound **13** for miR-1 and miR-206 were $\sim$ 2.5 μ M and $\sim$ 5 μ M, respectively. By measuring the expression levels of primary myomiRs, they found that compound **13** inhibited transcription of myomiRs. An $\sim$ 70–90% reduction in the expression levels of both mature myomiRs and pri-myomiRs was induced after treating muscle cells with 10 μ M compound **13**. Through further studies that investigated the expression levels of myogenic differentiation protein (myoD), the transcription factor of myomiRs, they found that compound **13** reduced only the protein levels but not the mRNA levels of myoD, suggesting that there is another unknown miRNA that regulates myoD. Through a bioinformatics prediction and further experimental validation, they demonstrated that myoD is the direct target of miR-221/222. This revealed a novel signaling pathway in muscle cells that involves miR-221/222 regulation of myomiRs by targeting myoD

Fig. 4 Inhibitors or activators of specific miRNAs. Compounds **7** and **8** are inhibitors of miR-21; compound **9** is an inhibitor of miR-27a; compounds **10** and **11** are inhibitors of miR-122; compound **12** is an activator of miR-122; compound **13** is an inhibitor of myomiRs.

since miR-221/222 regulates myoD and myoD regulates transcription of myomiRs.

In addition to these examples of miRNA regulators that were identified through experimental screening, Informa, which predicts potential small molecules from known information about small molecules binding to specific RNA motifs, has also been successfully used to find inhibitors of oncogenic miRNAs.²⁸ Since small molecules predicted using Informa tend to bind to Drosha or Dicer sites in the precursors of target miRNAs, they usually inhibit miRNA by blocking Drosha or Dicer-mediated miRNA maturation. For example, compound 14 (Fig. 5) was identified as an inhibitor of miR-96 biogenesis through an Informa design of small molecules to target pri-miR-96.³⁸ In MCF-7 cells, compound 14 reduced the expression levels of miR-96 in a dose-dependent manner, and an ~90% decrease in miR-96 expression was induced by 40 μ M compound 14. By profiling the expression levels of 149 abundant miRNAs in MCF-7 cells after treatment with 40 μ M compound 14, only miR-96 was shown to be affected. Recently, they found a more potent inhibitor of miR-96, Targaprimir-96 (15, Fig. 5), which is an optimal dimeric compound designed using Informa and synthesized by conjugating compound 14 with a *bis*-benzimidazole.³⁹ In MDA-MB-231 cells, compound 15 reduced mature miR-96 levels in a dose-dependent manner, with an IC₅₀ value of ~50 nM. By labeling compound 15 with a cross-linking module and biotin, compound 15 was developed into a probe, and intracellular targets of this probe were isolated through streptavidin purification. An ~5-fold enrichment of pri-miR-96 was detected in the pull-down samples, demonstrating that compound 15 directly interacts with pri-miR-96. Similarly, with miR-544 as the target miRNA, compound 16 (Fig. 5) was identified as an inhibitor of miR-544 biogenesis by using Informa to design small molecules to target pre-miR-

544.⁴⁰ In MCF-10A cells, an ~2-fold increase in the pre-miR-544 levels and ~90% reduction in the mature miR-544 levels were induced by treatment with 20 nM compound 16. Recently, with miR-210 as a target, the authors identified an inhibitor of miR-210 (Targapremir-210, 17, Fig. 5) by using Informa to design small molecules to target pre-miR-210.⁴¹ In MDA-MB-231 cells, compound 17 decreased the mature miR-210 levels in a dose-dependent manner, with an IC₅₀ value of ~200 nM. Meanwhile, treatment with 200 nM compound 17 led to a ~3-fold increase in pri-miR-210 levels and a ~2.6-fold increase in pre-miR-210 levels. Similarly to compound 15, after labeling compound 17 with a cross-linking module and biotin, the direct targets of compound 17 were isolated and analyzed. An ~20-fold enrichment of pre-miR-210 was detected in the pull-down sample, suggesting that compound 17 directly binds to pre-miR-210.

Regulation of microRNAs using synthetic oligonucleotides

In contrast to small molecule regulators of miRNAs, which can regulate not only miRNA biogenesis but also miRNA function, synthetic oligonucleotides are directly designed to modulate the expression levels of miRNAs.¹⁵ As a result, they are more specific in their ability to regulate miRNAs compared to small molecules. AMOs, also known as miRNA inhibitors (antimiRs), are able to bind endogenous miRNAs and serve as specific silencers that block the function of endogenous miRNAs (Fig. 6).⁹ miRNA mimics are able to replace endogenous miRNAs, and the elevation of endogenous miRNA levels through introduction of miRNA mimics has been achieved both *in vitro* and *in vivo*.¹⁵ In this section, we summarize the use of sequence-specific oligonucleotides to regulate miRNAs.

Fig. 5 Inhibitors of oncogenic miRNAs identified using Informa. Compounds 14 and 15 are inhibitors of miR-96; compound 16 is an inhibitor of miR-544; compound 17 is an inhibitor of miR-210.

Fig. 6 Schematic illustration of the use of AMOs and miRNA mimics to regulate endogenous miRNAs. miRNA mimics are able to replace endogenous miRNAs and AMOs can bind to endogenous miRNAs to block their function.

AMOs

Generally, AMOs bind to miRNA with high affinity, leading to changes in downstream gene expressions. Recently, different chemical modifications have been made to synthetic oligonucleotides to improve the specificity and potency of AMOs (Fig. 7).¹⁵ In comparison with the native structure, 2'-O-methyl (2'-OMe) modification can lead to a higher binding affinity for RNA and higher resistance to nuclease degradation.⁴⁹ To further improve nuclease resistance, phosphorothioate (PS) modification, which substitutes the non-bridging oxygen in phosphate with a sulfur atom, was developed. The PS modification further blocks the ability of nucleases to degrade oligonucleotides.⁵⁰ To realize both nuclease resistance and high binding affinities, locked nucleic acids (LNAs), which bridge the 2'-oxygen with a 4'-carbon, were developed.^{51,52} LNAs exhibit resistances to many endonucleases and significantly improved their ability to bind RNA. It is worth noting that LNA AMOs targeting miR-122 have entered clinic trials for the treatment of HCV.⁵³ Moreover, chemical modifications of the backbone of AMOs have also been made using non-natural backbones, including peptide nucleic acid (PNA)⁵⁴ and phosphorodiamidate morpholino oligonucleotide (PMO).⁵⁵ These backbone modifications also showed good potency as antisense oligonucleotides for inhibition of miRNAs. For example, PNAs⁵⁶ and PMOs⁵⁷ that target miR-155 were used to inhibit endogenous miR-155 and even showed effective inhibition of miR-155 in living animals.

miRNA mimics

Even though plasmid vectors can be designed and engineered to express miRNAs after introduction into cells,¹⁵ synthetic miRNA mimics are still the most often used agents to up-regulate the expression levels of miRNAs through replacement. Synthetic miRNA mimics are designed to have the same sequence as natural endogenous miRNAs and are expected to replace endogenous miRNAs as well as regulate the same target genes that are under regulation of endogenous miRNAs. The use of miRNA mimics to restore the function of tumor-suppressing miRNAs that are usually down-regulated in tumor cells has become a potential therapeutic

strategy. For example, miR-34a mimics have been employed in clinics for cancer treatment.⁵⁸ To ensure the stability and activity of miRNA mimics, double-stranded miRNA mimics are often used instead of single-stranded ones.

Although AMOs and miRNA mimics have shown promise in regulating endogenous miRNAs, their poor cellular permeability and stability are the major obstacles to further applications.¹⁶ To overcome these limitations, carriers that can protect and deliver these oligonucleotides can be used.

Delivery of synthetic oligonucleotides for the regulation of miRNAs

In contrast to small molecule regulators of miRNAs, which permeate into cells, synthetic oligonucleotides, including AMOs and miRNA mimics, are not able to enter cells by themselves due to their negative charges. Meanwhile, synthetic oligonucleotides can easily be degraded by RNase. To address these issues, additional carriers have been developed for the encapsulation and intracellular delivery of AMOs and miRNA mimics.^{15,16} Below, we briefly summarize currently used carriers, including lipids, polymers, nanoparticles, peptides and hydrogels, for the intracellular delivery of synthetic oligonucleotides to regulate endogenous miRNAs (Table 3).

Cationic lipid-based delivery systems are now the most commonly used vectors for the intracellular delivery of synthetic oligonucleotides. Many of them are now commercially available.^{59,60} Cationic lipids are able to encapsulate synthetic oligonucleotides and release them after endocytosis into the cell.⁵⁹ Lee *et al.* developed cationic lipoplexes as carriers to deliver tumor-suppressing pre-miR-133b into lung tissues and compared the delivery efficiency of lipoplexes with that of the commercial transfection agent NeoF. ⁶¹ Pre-miR-133b (100 nM) was complexed with lipoplexes or NeoF and used to treat non-small lung cancer A549 cells, leading to ~20 000-fold and ~8000-fold increases in the mature miR-133b levels using the lipoplexes and NeoF complexes, respectively. Meanwhile, an ~60% reduction in the protein expression levels of MCL-1, the target of miR-133b, was induced by the lipoplexes and only an ~30% reduction was caused by the NeoF complexes. When using the lipoplexes (1.5 mg kg⁻¹) to treat mice through tail-vein injection, an ~52-fold increase in the mature miR-133b levels in comparison with the levels in untreated mice was achieved, and ~30% accumulation of lipoplexes was observed in lung tissues. Similarly, solid lipid nanoparticles (SLNs) were developed by Sun *et al.* to encapsulate and deliver tumor-suppressing miR-34a into lung tissues.⁶² miR-34a was complexed with SLNs or the commercial transfection agent Lipofectamine and further administrated into mice through tail-vein injection (0.5 mg kg⁻¹ of miR-34a). Significant accumulation of miR-34a in lung tissues was induced by SLNs, but not by Lipofectamine. Moreover, SLNs led to an ~5.9-fold increase in the mature miR-34a levels in lung tissues compared to Lipofectamine. Although lipids have great potential for the delivery of synthetic oligonucleotides, one major concern when using lipids as carriers is

Fig. 7 Chemical modifications of units for synthetic oligonucleotides.

Table 3 Summary of typical delivery systems for the delivery of AMOs and miRNA mimics

Carriers	Target miRNA	Oligonucleotides	Function
Lipoplexes ⁶¹	miR-133b	Pre-miR-133b mimics	miRNA replacement
Solid lipid nanoparticles ⁶²	miR-34a	miR-34a mimics	miRNA replacement
Polyethyleneimine ⁶³	miR-145	miR-145 mimics	miRNA replacement
	miR-33a	miR-33a mimics	
Poly(lactide-co-glycolide) ⁵⁷	miR-155	Anti-miR-155 (PMOs and PNAs)	miRNA inhibition
Nanographene oxides ⁶⁴	miR-21	Anti-miR-21	miRNA inhibition
Nanographene oxides ⁶⁵	<i>let-7g</i>	<i>let-7g</i> mimics	miRNA replacement
Low molecular weight protamine ⁶⁶	miR-29b	miR-29b mimics	miRNA replacement
pH-induced transmembrane structure peptide ⁵⁶	miR-155	Anti-miR-155 (PNAs)	miRNA inhibition
Polymeric hydrogels ⁶⁷	miR-205	miR-205 mimics	miRNA replacement and inhibition
	miR-221	Anti-miR-221	
Supramolecular hydrogels ^{68–70}	miR-122	miR-122 mimics	miRNA replacement
	miR-34a	miR-34a mimics	

their biocompatibility, which requires further refinements for future use.

Polymers, such as polyethyleneimine (PEI), are biocompatible materials that complex with negatively charged synthetic oligonucleotides through electrostatic interactions for subsequent intracellular delivery. For example, Aigner *et al.* used PEI to complex tumor-suppressing miR-145 and miR-33a.⁶³ Upon administration in subcutaneous colon tumor-bearing mouse models, the complexes successfully delivered miR-145 and miR-33a to tumor tissues to down-regulate the expression levels of their targets. In comparison with PEI, poly(lactide-co-glycolides) (PLGAs) have excellent biocompatibility and biodegradability, making them attractive and promising carriers for the delivery of synthetic oligonucleotides. Saltzman *et al.* used PLGAs to encapsulate PMOs or PNAs, which are miR-155 inhibitors, and modified the formed nanocomplexes with nona-arginine to facilitate their delivery into cells.⁵⁷ Anti-miR-155 (1 μ M) was complexed with PLGA and delivered into KB cells, leading to an $\sim$ 50% reduction in mature miR-155 levels and an $\sim$ 5-fold increase in the expression levels of miR-155 targets.

Nanoparticles, which have defined shapes and sizes, have been widely used in biomedical applications.⁷¹ The easy modification or loading of nucleic acids onto nanoparticles through electrostatic interactions as well as their ability to penetrate into cells make them attractive carriers for the intracellular delivery of synthetic oligonucleotides to regulate endogenous miRNAs. For example, Tian *et al.* developed poly-amidoamine (PAMAM) functionalized nanographene oxides (PAMAM-NGOs) to encapsulate anti-miR-21.⁶⁴ In comparison with the commercial transfection agent Lipofectamine 2000, PAMAM-NGOs showed lower cytotoxicity and a higher transfection efficiency. When A549 cells were treated with 100 nM anti-miR-21 that was encapsulated with PAMAM-NGOs or Lipofectamine 2000, an $\sim$ 4.5-fold increase in the protein expression levels of PTEN, a target of miR-21, was induced by PAMAM-NGOs, while only an $\sim$ 3-fold increase was induced by Lipofectamine 2000. Moreover, the easy integration of other functionalities, including contrast agents or targeting ligands, into nanoparticles further allows the delivery process and targeted delivery of synthetic oligonucleotides to be

tracked. For example, PAMAM coated NGOs were further functionalized with gadolinium and used for intracellular delivery of *let-7g*, a miRNA transcribed from the *lethal-7* gene, into U87 cells.⁶⁵ A significant decrease in the protein expression levels of Pan-Ras, a target of *let-7g*, was induced by NGO-delivered *let-7g* (9.4 nM) with an efficiency that was even higher than that of Lipofectamine 2000. Meanwhile, the presence of gadolinium allowed the bio-distribution of the nanocarriers to be monitored through magnetic resonance imaging after systemic delivery into living animals, showing efficient accumulation in tumor tissues.

Peptides are biocompatible molecules that have been widely used in biomedical applications, including in the delivery of small non-coding RNAs.⁷² Based on the ability of cationic peptides to self-assemble with negatively charged nucleic acids through electrostatic and hydrophobic interactions, Park *et al.* used low molecular weight protamine (LMWP) to complex with miR-29b for delivery into osteogenic stem cells to modulate the expression levels of endogenous miR-29b.⁶⁶ Upon delivery of 50 nM miR-29b using LMWP or commercial Lipofectamine, LMWP induced an $\sim$ 600-fold increase in the miR-29b levels, while only an $\sim$ 100-fold increase was induced by Lipofectamine, indicating the high delivery efficiency of LMWP. Meanwhile, LMWP-delivered miR-29b led to an $\sim$ 40–60% reduction in the protein expression levels of miR-29b targets. Additionally, it is worth noting a recent example reported by Slack *et al.*, which used a special peptide to deliver PNAs for miR-155.⁵⁶ The pH (low) insertion peptide (pHLIP)⁷³ was sensitive to low pH and could insert into the cellular membrane at low pH. It is therefore specific toward a tumor microenvironment (low pH). By using a disulfide bond as the linkage between pHLIP and anti-miR-155, anti-miR-155 was directly delivered into the cytoplasm to inhibit miR-155 following the insertion of pHLIP into the membrane of tumor cells and cleavage of the disulfide bond by reducing molecules inside tumor cells. This peptide shows great promise for the delivery of oligonucleotides with high efficiency and tumor-specificity.

Hydrogels are self-assembled biomaterials that can mimic the extracellular environment and support cell culture.⁷⁴ Through co-encapsulation of nucleic acids and cells inside

hydrogels, nucleic acids can adhere to nanofibers inside hydrogels. Nanofibers have been shown to mediate the transportation of nucleic acids into cells. As reported by Artzi *et al.*, by using polymeric hydrogels to encapsulate miR-205 and anti-miR-221, these oligonucleotides are able to be delivered into tumor cells after local injection into tumor tissues.⁶⁷ After introduction of 1 μM miR-205 and anti-miR-221, a significant increase in miR-205 levels and decrease in miR-221 levels were detected in tumor tissues, demonstrating the successful delivery of these oligonucleotides into tumor cells. Meanwhile, the expression levels of the target genes for miR-205 and miR-221 were also correspondingly modulated. With low-molecular weight supramolecular hydrogels as carriers, Zhang *et al.* demonstrated that simultaneous encapsulation of miR-122 (80 pmol) and cells inside hydrogels led to a significant increase in the endogenous miR-122 levels (~ 10 -fold) and subsequent down-regulation in the expression levels of its targets ($\sim 80\%$).⁶⁸ Moreover, by using photosensitive molecules and targeting ligands as hydrogelator components, light-controlled and targeted delivery of miR-122 or miR-34a was also achieved.^{69,70}

Regulation of endogenous microRNAs for therapeutic treatments

With the identification of small molecule regulators of miRNAs and development of strategies for the intracellular delivery of miRNA mimics or AMOs, it is possible to realize therapeutic treatments by using these exogenous molecules to regulate aberrantly expressed miRNAs. In this section, we introduce recent applications of miRNA-targeted therapeutic treatments using small molecules or synthetic oligonucleotides.

Therapeutic applications of small molecule regulators of miRNAs

Oncogenic miR-21 is able to promote the growth of cancer cells through down-regulation of programmed cell death 4 (PDCD4), which is an apoptosis inducer.⁷⁵ Maiti *et al.* used an inhibitor of miR-21 (compound 8) to treat MCF-7 breast cancer cells.³⁵ In comparison with an untreated control, treatment with 5 μM compound 8 led to an ~ 2 -fold increase in the protein levels of PDCD4 and an ~ 2 -fold increase in the apoptotic rate of MCF-7 cells. Liver-specific miR-122 is able to enhance HCV replication and promote apoptosis of liver cancer cells.^{76,77} Based on the discovery of inhibitors and activators of miR-122, Deiters *et al.* used compounds 10 and 11 to treat HCV infected liver cells, showing an $\sim 50\%$ reduction in HCV RNA levels due to treatment with 10 μM compounds 10 and 11.³⁶ When treating HepG2 liver cancer cells with compound 12 (10 μM), an $\sim 80\%$ reduction in cellular viability was achieved. Hypoxia is essential and critical to the metastasis and invasion of cancer cells and has also been found to be regulated by miRNAs. Oncogenic miR-544 is able to silence mammalian target of rapamycin (mTOR) and increase hypoxia-related transcription factor HIF-1 α , reversing the re-

sponse of cancer cells to hypoxia. This suggests that miR-544 is a potential target for therapeutic treatments.⁴⁰ Using an inhibitor of miR-544 (compound 16) to treat MDA-MB-231 triple negative breast cancer cells, Phinney *et al.* found that compound 16 (20 nM) was able to induce an $\sim 60\%$ increase in apoptotic rates.⁴⁰ When using 4 nmol of compound 16 to treat MDA-MB-231 tumor-bearing mice through i.p. injection, tumor growth was also successfully inhibited. Similarly, miR-210 is able to up-regulate HIF-1 α by targeting the glycerol-3-phosphate dehydrogenase 1-like enzyme (GPD1L). Disney *et al.* used an inhibitor of miR-210 (compound 17, 20 pmol) to treat MDA-MB-231 tumor-bearing mice, leading to significant inhibition of miR-210 ($\sim 90\%$) and HIF-1 α ($\sim 75\%$) with up-regulation of GPD1L (~ 5 -fold) (Fig. 8).⁴¹ Meanwhile, tumor growth was also successfully inhibited. It is worth noting that the therapeutic efficiency of compound 17 was even comparable to that of anti-miR-210 (50 pmol).

Additionally, activators of miRNAs have also been employed in therapeutic treatments. Compound 1 was found to have a cancer-specific growth inhibition activity without killing normal cells, and an EC₅₀ value of 124 μM .⁷⁸ Importantly, the inhibitory activity of compound 1 was dependent on TRBP, indicating that the effect of compound 1 on miRNA activation was the major factor contributing to the therapeutic effects. When using compound 1 (10 mg kg⁻¹) to treat xenografted tumor-bearing mice, compound 1 induced significant inhibition of tumor growth. However, TRBP knock out tumors showed resistance to treatment by compound 1. Additionally, overexpression of AGO2 and Dicer are also closely related to cancer development. Jeang *et al.* developed a miRNA-dependent tumor cell model.²⁹ Cells were pre-treated with 2 μM compound 4 or 1 μM compound 5 and subcutaneously implanted into nude mice, resulting in notable inhibition of tumor formation.

Therapeutic applications of synthetic oligonucleotides

To down-regulate oncogenic miR-21 to achieve therapeutic effects, Tian *et al.* used PAMAM-NGOs to deliver anti-miR-21

Fig. 8 The therapeutic effect of compound 17 (Targapremir-210) on tumor proliferation *in vivo*. MDA-MB-231 cells stably expressing luciferase were used for tumor plantation. (A) Bioluminescence imaging of mice bearing MDA-MB-231 tumors pre-treated *in vitro* with compound 17 or anti-miR-210 (top) or administered via i.p. injection (bottom). (B) Extracted tumors and (C) quantification of the miR-210, GPD1L, and HIF-1 α levels in tumor tissues from mice injected with compound 17 or anti-miR-210. Adapted from ref. 41 with permission.

into A549 non-small-cell lung cancer cells.⁶⁴ After treatment with 100 nM anti-miR-21, an ~80% reduction in migration and invasion of A549 cells was detected. Similarly, Kang *et al.* used cationic polymers to deliver anti-miR-21 into U87 tumor-bearing mice through intravenous injection.⁷⁹ In comparison to control treatments, anti-miR-21 treatments (1.6 µg) led to an ~50% decrease in tumor growth. In order to treat lung cancers, Sun *et al.* used SLNs to deliver tumor-suppressing miR-34a.⁶² After systemic administration of miR-34a (0.5 mg) into tumor-bearing mice, tumor growth and metastasis were remarkably inhibited. It is worth noting that Sun *et al.* recently developed a “smart” system for the delivery of miR-34a to treat breast cancer.⁸⁰ Cationic polymers were coated with matrix metalloproteinase-2 (MMP-2) peptide substrates, which led to specific accumulation of miR-34a in tumor sites with high expression levels of MMP-2. To demonstrate this, they compared the delivery efficiency of several carriers, including native polymers (CP2K), polymers with the MMP-2 substrate (En-CNP), and polymers with a mutated MMP-2 substrate (En-unCNP). After administration of miR-34a (10 µg) encapsulated with the different carriers into 4T1 tumor-bearing mice, inhibition of the tumor-growth and prolonged survival rates were observed in the En-CNP groups (Fig. 9A and B). Naked miR-34a did not show any effect. En-unCNP showed a similar efficiency to CP2K, demonstrating that the presence of the MMP-2 substrate is important for the targeted delivery of miR-34a. The therapeutic effects were also further confirmed through the immunohistochemical staining of tumor sections (Fig. 9C). Additionally, the pHLP peptide efficiently delivered anti-miR-155 in a mouse model of lymphoma, resulting in significant therapeutic outcomes.⁵⁶ Simultaneous delivery of tumor-suppressing miR-

205 and anti-miRNA toward oncogenic miR-221 by hydrogels into a triple negative breast cancer mouse model also led to remarkable inhibition of tumor growth.⁶⁷

Combined use of small molecule regulators of miRNAs with synthetic oligonucleotides

Since small molecule regulators of miRNAs and synthetic oligonucleotides function *via* two different pathways, their combined use to inhibit the same miRNA may lead to amplified therapeutic effects. For example, Yao *et al.* used mesoporous silica nanoparticles to simultaneously deliver anti-miR-122 (200 nM) and an inhibitor of miR-122 (compound 11, 2 µM) into liver cancer cells.⁸¹ In comparison with the single use of each agent, a significant reduction in miR-122 levels (~70%) and increase in target gene expressions (~8-fold) were achieved by the combinatory inhibition. Similarly, they delivered anti-miR-21 (150 nM) and an inhibitor of miR-21 (compound 7, 4.5 µM) into ovarian cancer HeLa cells, resulting in significant suppression of the miR-21 levels (~90%) and promotion of cellular apoptosis of HeLa cells.⁸² Moreover, Maiti *et al.* recently developed a series of dual-binding small molecules targeting miR-27a, which were synthesized by linking neomycin (9) with a bisbenzimidazole using linkers with different lengths.⁸³ These conjugates showed high binding affinities toward pre-miR-27a (equilibrium association constant $K_a = 1.2\text{--}7.4 \times 10^8 \text{ M}^{-1}$). Upon treatment of MCF-7 cells with these conjugates (5 µM), a significant reduction in the mature miR-27a levels (~65%) and inhibition of cell proliferation were achieved. Similarly, as reported by Duca *et al.*, by modifying with natural and artificial nucleobases, compound 9 was also able to bind to pre-miR-372 and pre-miR-373 and inhibit their production in AGS cells.⁸⁴ Liang *et al.* found that fluorophore-modified neomycin could bind to pre-miR-21.⁸⁵ By modifying neomycin with a Dicer inhibitory unit, *N*-hydroxyimide, they developed a series of bifunctional small molecules. Using artificial HEK293T cells that were transfected with a plasmid to express pre-miR-21, they tested the inhibitory activity of these bifunctional small molecules in a cellular environment. The expression levels of miR-21 decreased in a dose-dependent manner and an ~76% reduction was induced by 5 µM of the small molecules.

Conclusions and perspectives

miRNAs have been identified as key gene regulators that are involved not only in physiological processes but also in pathologies. Regulation of aberrantly expressed miRNAs represents a novel and efficient way to achieve therapeutic outcomes. Molecules that are currently reported to regulate miRNAs mainly include small molecules that have been identified by high-throughput screening and synthetic oligonucleotides that can replace or inhibit miRNAs. Even though small molecule regulators of miRNAs are effective in miRNA regulation, the detailed pathways responsible for their inhibitory or activating effects on endogenous miRNAs are still unclear, greatly hindering their application in therapeutics. To

Fig. 9 Targeted delivery of miR-34a to inhibit tumor growth. (A) Growth curves of 4T1 tumors in mice treated with miR-34a with delivery by different carriers. (B) Survival analysis. (C) Analysis of 4T1 tumor sections by H&E, Ki-67, and TUNEL staining. Adapted from ref. 80 with permission.

address this issue, chemical-biological methods using small molecules with bio-orthogonal tags to reveal their targets in combination with bio-orthogonal chemistry should be employed in the future. Moreover, many miRNAs have been identified to have specific functions in disease development. There are still only a few small molecule regulators that have been found to regulate endogenous miRNAs. To achieve therapeutic treatments by regulating miRNAs, there is a great demand for other potential small molecule regulators. In the case of synthetic oligonucleotides, their poor cellular permeability and easy degradation in serum mean that additional carriers are required to deliver them into cells. The biocompatibility and biodegradability of these carriers are critical for their future applications in clinics. Further development and improvement of current carriers are needed before proceeding to therapeutics. Moreover, since miRNAs can simultaneously regulate multiple genes, the question of how to avoid side effects due to the regulation of other genes by synthetic oligonucleotides still remains to be answered. Nevertheless, it is encouraging that synthetic oligonucleotides have shown promise in pre-clinical trials. The combined use of small molecules and synthetic oligonucleotides to realize amplified effects may further improve the therapeutic efficiency of regulating endogenous miRNAs. With the further identification of small molecule regulators of miRNAs and the development of strategies for the delivery of synthetic oligonucleotides, therapeutic treatments in clinics with miRNAs as endogenous targets will be achieved in the future.

Abbreviations

MicroRNA	miRNA
Antisense miRNA oligonucleotides/miRNA inhibitors	AMOs/ antimiRs
Primary miRNA	pri- miRNA
Precursor miRNA	pre- miRNA
RNA-induced silencing complex	RISC
3'-Untranslated region	3'-UTR
Argonaute 2	AGO2
TAR RNA-binding protein	TRBP
Fluorescence resonance energy transfer	FRET
Green fluorescent protein	GFP
Small-interfering RNA	siRNA
Poly(C)-binding protein 2	PCBP2
Myogenic miRNA	myomiR
Phosphorothioate	PS
Locked nucleic acid	LNA
Peptide nucleic acid	PNA
Phosphorodiamidate morpholino oligonucleotide	PMO
Solid lipid nanoparticle	SLN
Polyethyleneimine	PEI
Polyamidoamine	PAMAM
Nanographene oxide	NGO
Low molecular weight protamine	LMWP

pH (low) insertion peptide	pHLIP
Programmed cell death 4	PDCD4
Mammalian target of rapamycin	mTOR
Glycerol-3-phosphate dehydrogenase 1-like enzyme	GPD1L
Matrix metalloproteinase-2	MMP-2
Library of Pharmacologically Active Compounds	LOPAC
Food and Drug Administration	FDA
Half maximal effective concentration	EC ₅₀
Half maximal inhibitory concentration	IC ₅₀
Myogenic differentiation protein	myoD
RNA helicase A	RHA

Conflicts of interest

The authors declare no competing interest.

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