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REVIEW ARTICLE

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Development of miRNA-based therapeutic approaches for cancer patients

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Over the past few decades, siRNA and miRNA have attracted a great deal of attention from researchers and clinicians. These molecules have been extensively studied from the standpoint of developing biopharmaceuticals against various diseases, including heart disease, diabetes and cancers. siRNA suppresses only a single target, whereas each miRNA regulates the expression of multiple target genes. More importantly, because miRNA are also secreted from cancer cells, and their aberrant expression is associated with tumor development and progression, they represent not only therapeutic targets but also promising biomarkers for diagnosis and prognosis. Therefore, miRNA may be more effective tools against cancers, in which multiple signal pathways are dysregulated. In this review, we summarize recent progress in the development of miRNA therapeutics for the treatment of cancer patients, and describe delivery systems for oligonucleotide therapeutics.

KEYWORDS
cancer biology, delivery system, exosomes, miRNA, therapeutics

1 | INTRODUCTION

Several lines of evidence indicate that ncRNA regulate multiple stages of life cycle, including development, differentiation and aging, through the regulation of target gene expression.[1-3] Broadly, ncRNA can be classified into two groups: small ncRNA 18-200 nt in length and lncRNA.[4]

miRNA is a type of small ncRNA. The primary function of miRNA is negative regulation of their target genes at the post-transcriptional level. Specifically, miRNA induce transcript degradation or inhibition of protein translation through sequence-specific binding with the 3′UTR of their target mRNA (Figure 1). miRNA also inhibit the translation or facilitate the cleavage of their target mRNA by binding to their CDS.[5-7] To date, multiple miRNA have been identified in various types of cancers, and these tumor-associated miRNA can be classified into two groups: TS-miRNA and onco-miRNA.[8-11]

Because miRNA are considerably smaller than proteins, they can be introduced to cells by the same techniques used for siRNA.[12-14] Consequently, the emergence of the miRNA field prompted many cancer researchers and clinicians to develop miRNA delivery strategies for the treatment of cancer patients. In this review, we summarize the major findings regarding miRNA function in tumor development, and describe recent advances in the preclinical and clinical development of miRNA delivery systems for cancer therapeutics.

Abbreviations: 3′UTR, 3′-untranslated region; BCR-ABL, breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1; CDS, coding sequences; ce-miRNA, cellular miRNA; CML, chronic myelogenous leukemia; CSC, cancer stem cells; cssDNA, circular ssDNA; EMT, epithelial-to-mesenchymal transition; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; EV, extracellular vesicle; EV, extracellular vesicles; FDA, US Food and Drug Administration; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HCV, hepatitis C virus ; HE, hepatic encephalopathy ; LNA, locked nucleic acid; LSC, leukemia stem cells; nt, nucleotides; onco-miRNA, oncogenic miRNA ; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; SCD1, stearoyl desaturase 1; se-miRNA, secreted miRNA; SPRED1, Sprouty-related EVH1-domain-containing 1; TAM, tumor-associated macrophages ; TGF, transforming growth factor; TKI, tyrosine kinase inhibitors; Treg, regulatory T cells; TS-miRNA, tumor suppressive miRNA; ZO-1, zonula occludens-1.

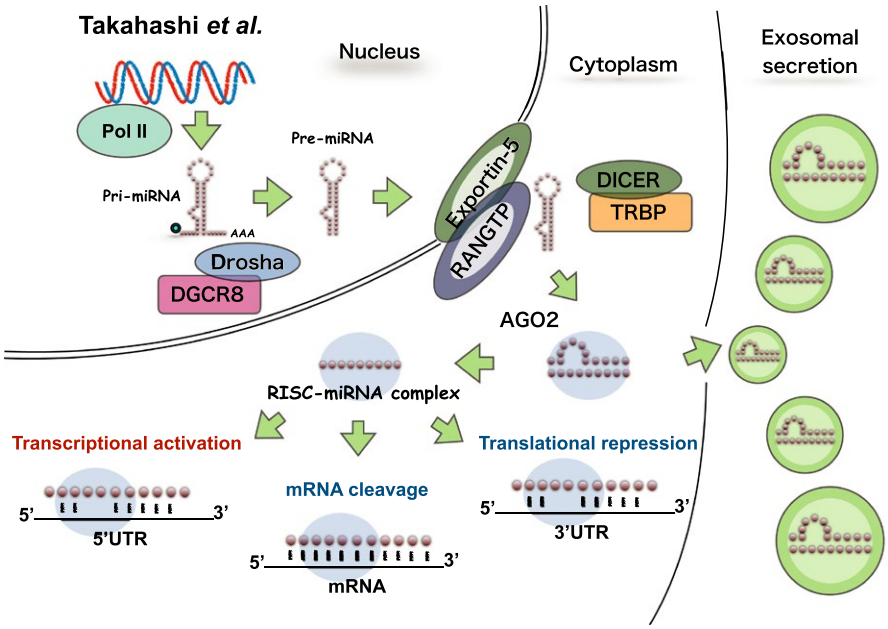

FIGURE 1 Biogenesis of miRNA. Most miRNA are transcribed by RNA polymerase II (RNA pol II) as primary-miRNA (pri-miRNA), and then processed in the nucleus by Drosha-DGCR8 into precursor miRNA (pre-miRNA). The pre-miRNA is exported to the cytoplasm by exportin-5 and further cleaved by a complex containing Dicer and TRBP. The functional strand of mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which contains GW182 and Argonaute protein. As a component of this complex, the mature miRNA regulates gene expression by binding to complementary sequences in the 3′ untranslated region (UTR) or coding regions of its target mRNA, leading to mRNA degradation or translational repression. Alternatively, miRNA can induce translational activation by the 5′UTR of target mRNA. In addition, miRNA can be secreted through the exosomal pathway and regulate gene expression in recipient cells

2 | ROLES OF MIRNA IN CANCER BIOLOGY

The first two miRNA to be reported, lin-4 and let-7, were discovered in the nematode *Caenorhabditis elegans* in 1993 and 2000, respectively.[15-17] Because let-7 is conserved across animal species, from flies to mammals, and plays important roles in the various biological processes, including cancer biology,[18] it has been extensively studied. Subsequently, multiple studies have reported the roles of miRNA in cancer biology, including tumor initiation, drug resistance and metastasis.

miRNA are also important for cell-to-cell communications involved in the inflammatory response, differentiation and tumor progression.[19-21] miRNA are secreted from various types of cells through EV containing membrane proteins such as CD63, CD81 and CD9.[22-24] In the next section, we introduce the functions of TS-miRNA and onco-miRNA in relation to malignant cancer phenotypes such as tumor initiation, EMT, drug resistance and metastasis (Table 1).

3 | TUMORIGENICITY

Over the past decade, CSC have been identified in many common cancer types, ranging from leukemia to solid tumors.[25-33] The main properties of CSC, including tumor-seeding ability and therapy resistance, have been evaluated in immunodeficient mice. Specifically, CSC were isolated by cell sorting for specific markers and then transplanted into immunodeficient mice, where their tumor-seeding abilities were examined.[34]

Yu et al[35] initially identified let-7 as a master regulator of the self-renewal and tumor-seeding ability of breast cancer cells. They observed a high percentage of CSC with the CD44(high)/CD24(-/low) antigen phenotype in tumors isolated from the patients who received chemotherapy. In addition, they showed that CSC exhibited an elevated ability to form mammospheres in vitro and tumors in NOD/SCID mice. Importantly, breast CSC-enriched cells expressed much lower levels of let-7 than parental cells or in vitro differentiated progeny with the CD44(low/-)/CD24(high) antigen phenotype.

Recently, El Helou et al[36] reported that downregulation of miR-600 is significantly associated with poor prognosis in breast cancer patients. miR-600 inhibits WNT signaling, which plays an important role in CSC renewal by directly suppressing SCD1, an enzyme required for the production of active, lipid-modified WNT proteins. Thus, miR-600 inhibits CSC renewal, resulting in suppression of tumor-seeding ability. By contrast, miR-31 promotes the tumor-seeding ability of breast cancer cells through activating WNT signaling.[37]

Exosomal miRNA is also important for regulation of CSC properties. Zhang et al[38] showed that in CML, miR-126 supports the quiescence, self-renewal and engraftment capacity of LSC. Using a mouse model, they demonstrated that endothelial cells in the bone marrow support the CSC properties of LSC by supplying exosomal miR-126 to those cells. They also found that a feedback loop between miR-126 and its target SPRED1 inhibits miRNA maturation.

TABLE 1 Classification of miRNA according to their functions

Phenotype	Cancer	miRNA	Target	References
Tumorigenicity	Breast	Let-7	H-RAS and HMGA2	35
	Breast	miR-600	SCD1	36
	CML	Ex-miR-126	SPRED1	38
Drug resistance	Breast	miR-27b	ENPP1	9
	Liver	miR-221	Caspase-3	39
	CML	miR-377	BCL-XL	40
EMT	Breast	miR-200 family and miR-205	ZEB1 and ZEB2	50
		miR-22	TET family	51
Metastasis	Breast	miR-29b	VEGFA, ANGPTL4, PDGF, LOX and MMP9	61
	Colon	135b	FIH-1	62
	Breast	Ex-miR-105	ZO-1	64
	Breast	Ex-miR-122	PKM-2	65

CML, chronic myelogenous leukemia; EMT, epithelial-to-mesenchymal transition; Ex-miRNA, exosomal miRNA.
Each miRNA is classified according to its role in cancer biology.

Because SPRED1 is a substrate of BCR-ABL, TKI targeting BCR-ABL inhibited the phosphorylation of SPRED1, resulting in an increase in miR-126. On the basis of these findings, they proposed that the combination of TKI and miRNA inhibitors may prevent recurrence in CML patients.

4 | DRUG RESISTANCE

Resistance to chemotherapy is a major obstacle for cancer therapy, and is frequently observed in patients with cancer recurrence. The mechanisms underlying drug resistance can be classified into two main groups: (a) upregulation of drug-transporters and anti-apoptotic genes; and (b) activation of survival pathways. Several studies demonstrate that miRNA are also involved in regulating these drug-resistance pathways.

In breast cancer, miR-27b regulates resistance to docetaxel, an anti-microtubule agent, through its direct target ENPP1.[9] ENPP1 promotes the cell surface localization of ABCG2, which mediates the efflux of several types of small compounds, resulting in acquisition of docetaxel resistance. Therefore, docetaxel in combination with a miR-27b mimic effectively inhibits tumor growth in an animal model of breast cancer.

In HCC, miR-221 induces sorafenib resistance through direct inhibition of caspase 3.[39] More importantly, the expression level of circulating miR-221 in serum is significantly associated with sorafenib resistance in HCC patients. In B-cell lymphoma, miR-377 promotes resistance to ABT199 (venetoclax) by directly targeting B-cell lymphoma-extra large 37.[40] Because venetoclax is a promising small compound for treatment of multiple myeloma and chronic lymphocytic leukemia, it is likely that combination treatment with venetoclax and a miR-377 inhibitor could improve patient outcomes.

In ovarian cancer, elevated expression of nc886, a non-coding RNA induced by TGF-β treatment is associated with a poor prognosis.[41] nc886 inhibits miRNA maturation through the physical interaction with Dicer, resulting in acquisition of invasive ability and drug resistance.

Several groups also succeeded in the selective inhibition of miRNA processing by targeting the Drosha processing site of miRNA.[42,43] Childs-Disney et al[43] reported that neomycin B and its derivative 5′-azido neomycin B bind the Drosha processing site in the miR-525 precursor. Neomycin B is an FDA-approved drug and used for the prevention of HE and bacterial infections caused by cirrhosis. Because up-regulation of miR-525 is observed in approximately 60% of liver cancer tissues and induces the migration and invasion of liver cancer cells by directly targeting ZNF395,[44] neomycin B or its derivative may be a promising drug for the treatment of liver cancer patients. Velagapudi et al[42] also succeeded in the synthesis of a small compound that binds to the Drosha processing site in the miR-96 precursor. miR-96 is reported to be an oncogenic miRNA in breast cancer cells.[45] Consistent with this, a small compound directed against miR-96 efficiently inhibits the growth of breast cancer cells in vitro and in vivo.

5 | EPITHELIAL-TO-MESENCHYMAL TRANSITION

The EMT, a well-studied process in which epithelial cells trans-differentiate into motile mesenchymal cells, is integral for stem cell behavior and development, and it is also observed in the context of malignant tumor behaviors such as drug resistance and metastasis.[46,47] Two recent studies showed that EMT is not necessarily required for metastasis in mouse tumor models,[48,49] arguing that EMT is mainly associated with resistance to chemotherapy. Although the role of EMT in metastasis is not fully elucidated, these studies

suggest that EMT inhibition may improve resistance to conventional chemotherapy.

Gregory et al[50] reported that the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 inhibit the EMT phenotype by directly targeting ZEB1 and ZEB2, which are transcription repressors of E-cadherin. Subsequently, miR-22 was also shown to influence the EMT phenotype through epigenetic regulation of the miR-200 family.[51]

Transforming growth factor-β signaling is important for EMT induction, and several miRNA have been identified as novel regulators of TGF-β-mediated EMT induction. For example, Subramanyam et al[52] reported that miR-302 and -372 promote the reprogramming of human fibroblasts into induced pluripotent stem cells through direct suppression of TGF-β receptor type II and Ras homolog family member, both of which are associated with the EMT phenotype.[53]

In colon cancer, EMT and liver metastasis are induced by exosomal secretion of the miR-200 family, which suppresses the EMT phenotype described above.[50,54] Shelton et al[54] reported that the intracellular levels of miR-200 family members are post-transcriptionally regulated by PKCζ which is reported to act as a tumor suppressor.[55] PKCζ and its substrate ADAR2 play important roles in the accumulation of intracellular miR-200 family members in cancer cells. When PKCζ is downregulated, miRNA of this family are secreted through the exosomal pathway, resulting in EMT induction and metastasis.

6 | METASTASIS

In 2008, Tavazoie et al[56] first reported miR-126 and miR-335 as suppressors of metastasis in breast cancer. Subsequently, multiple miRNA were identified as metastasis-associated miRNA.[57-60] For example, in breast cancer, Chou et al[61] reported that GATA3 suppresses tumor metastasis by upregulating miR-29b. GATA3 is a transcription factor involved in maintenance of the luminal epithelial cell phenotype, and its expression is downregulated or lost in cancer patients. miR-29b directly suppresses expression of pro-metastatic regulators such as VEGFA, ANGPTL4, PDGF, LOX and MMP9. Therefore, GATA3 functions as a tumor suppressor through miR-29b-mediated suppression of pro-metastatic niche. In colon cancer, Valeri et al[62] identified miR-135b as an oncogenic miRNA in colon cancer. Aberrant expression of miR-135b is associated with mutation or inactivation of APC. In addition, miR-135b directly targets factor inhibiting HIF1α, which functions as a tumor suppressor by repressing the HIF1α pathway.[63] Therefore, elevated expression of miR-135b promotes colon cancer metastasis by activating the HIF1α pathway.

In addition to the intracellular miRNA, secreted miRNA are also involved in the regulation of tumor environment and metastasis.[64-67] Zhou et al reported that miR-105 is expressed and secreted specifically by metastatic breast cancer cells, but not in less invasive cells. Exosomal miR-105 destroys the tight junctions by directly targeting ZO-1, resulting in tumor invasiveness and elevated vascular permeability in distant organs.[64] Interestingly, secretion of exosomal miR-105 is induced by the oncoprotein MYC.[68] Moreover, exosomal miR-105 from breast cancer cells activates MYC signaling in cancer-associated fibroblasts by directly targeting MAX-interacting protein 1, a repressor of the c-MYC promoter,[69] resulting in c-MYC-mediated upregulation of glucose and glutamine metabolism and increased growth of neighboring cancer cells.

Fong et al[65] reported that miR-122 also promotes breast cancer metastasis by activating glucose metabolism in pre-metastatic sites. In contrast to the intracellular level of miR-122, which does not significantly differ between non-metastatic and metastatic cancer cells, the amount of exosomal miR-122 is associated with the metastatic capacity of breast cancer cells. Exosomal miR-122 promotes glucose metabolism by directly targeting pyruvate kinase isozymes M2 in pre-metastatic sites, resulting in formation of a pre-metastatic niche.

7 | THERAPEUTIC APPROACHES BASED ON MIRNA REGULATION

As described above, miRNA play important roles in tumor biology and have low toxicity, as exhibited by their endogenous expression in human tissues. Thus, investigations are in progress to develop onco-miRNA antagonizing or TS-miRNA replacement systems (Figure 2). The motivation for attempting to modulate miRNA expression in disease tissues is based on the concept that TS-miRNA are more highly expressed or functional in normal tissues than in tumor tissues, whereas onco-miRNA are upregulated and activated mainly in tumor tissues. Using LNA-modified oligonucleotides, a class of high-affinity bicyclic RNA analogs, miRNA can be easily detected in these tissues, and their functions can be inhibited in in vitro and in vivo studies.[70-72] In addition to LNA-mediated suppression of miRNA, TS-miRNA replacement has been attempted using miRNA mimics, viral vectors expressing miRNA, and small compounds that regulate the endogenous expression of miRNA.[14,73,74]

Because miR-199a is the third most highly expressed miRNA in normal liver and its downregulation correlates with poor prognosis,[75] Callegari et al[76] investigated the tumor suppressive effects of miR-199a using a transgenic mouse model of HCC. For their in vivo delivery experiments, they used lipid nanoparticles that were composed of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-sn-glycerol, methoxypolyethylene glycol, and linoleic acid at a ratio of 50:48:2, and demonstrated that miR-199a exerted anti-tumor activity against mouse HCC.

Expression of miR-21 is elevated in human glioma cells, and LNA against miR-21 can efficiently inhibit tumor growth in a xenograft-model of human glioblastoma.[77,78] MRG-106, an inhibitor of miR-155, has been studied in phase I clinical trials (http://www.miragen.com). To date, MRG-106 has mainly been used to treat blood cancer such as T-cell lymphoma, diffuse large B-cell lymphoma and chronic lymphocytic leukemia. In addition to cancer, LNA-mediated-miRNA inhibition is also effective against other diseases. For example, miR-122 is a liver-specific miRNA that stimulates translation of HCV by

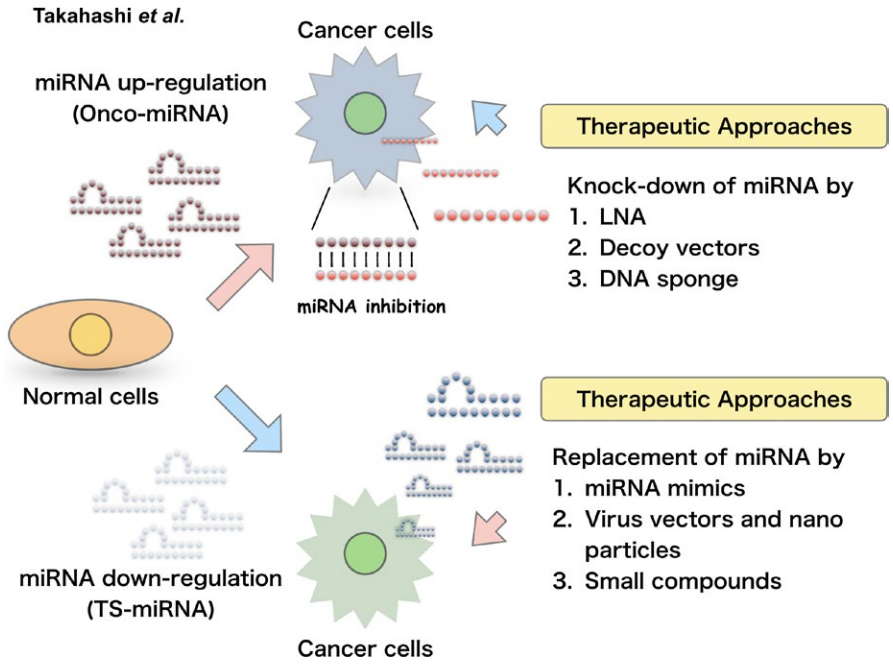

FIGURE 2 Therapeutic approaches that target miRNA. Therapeutic approaches targeting miRNA expression are based on the observation that tumor suppressive miRNA (TS-miRNA) are lost or downregulated in tumor tissues, whereas onco-miRNA are upregulated and activated. onco-miRNA can be inhibited by introduction of locked nucleic acid LNA), decoy vectors or sponge vectors. For replacement of TS-miRNA, miRNA mimics are introduced by viral vectors or nanoparticles, or upregulated by small compounds

interacting with the 5′UTR of the HCV genome.[79-83] This interaction also induces "sponge effects" that repress host genes targeted by miR-122, yielding a fertile environment for long-term replication of HCV.[84] Several studies show that LNA against miR-122 represents a promising approach for HCV treatment in primates.[13,85] Moreover, a clinical study revealed that LNA against miR-122 (miravirsen, SPC3649) achieved prolonged reduction in the levels of viral RNA in HCV patients.[86]

In addition to LNA treatment, miRNA sponges are also being used to inhibit onco-miRNA. miRNA sponges are artificial transcripts that contain multiple tandem high-affinity binding sites for one or more miRNA of interest.[87] Meng et al[88] developed an miRNA sponge using cssDNA. In several types of cancer, miR-9 commonly inhibits its expression of tumor-suppressor genes such as KLF17, CDH1 and LASS2. A cssDNA sponge containing four miR-9 binding sites can efficiently block miR-9 function, restoring endogenous expression of KLF17, CDH1 and LASS2.

Several studies indicate that miRNA modulation is also a promising approach for cancer immunotherapy.[89,90] TAM and regulatory T cells are key components of the tumor microenvironment in various types of cancers.[91,92] TAM develop from monocytes and are categorized into two main functional groups: (a) tumor suppressive type (M1 phenotype); and (b) immunosuppressive type (M2 phenotype). Baer et al[89] reported that let-7 is important for acquisition of the M2 phenotype and promotes tumor-infiltrating cytotoxic T lymphocytes. On the other hand, they also demonstrated that conditional deletion of the DICER in macrophages induces the acquisition of the M1 phenotype. Xu et al[90] reported that miR-424 regulates the PD-L1/PD-1 and CD80/CTLA-4 pathways in drug-resistant ovarian cancer. miR-424 expression was reported to be inversely correlated with PD-L1 and CD80 expression in ovarian cancer patients through its direct targeting of the 3′UTR of PD-L1 and CD80. Therefore, these studies suggest that the inhibition of let-7 or introduction of miR-424 could represent an effective approach for improving cancer immunotherapy.

8 | DELIVERY SYSTEM OF MIRNA

Modification of miRNA with LNA protects the nucleotides from degradation by serum ribonucleases, thereby extending the half-life of oligonucleotides in serum. Accordingly, multiple studies, including clinical trials, have demonstrated that LNA-modified miRNA inhibitors are functional even in vivo (see previous section). To further develop miRNA replacement therapy or LNA-mediated miRNA inhibition, a great deal of work has focused on improving the efficacy and accuracy of miRNA delivery systems. Two main strategies, intra-tumor or systemic delivery, have been considered for miRNA delivery. The advantage of local delivery is that it can selectively deliver miRNA into target tissues without non-specifically introducing miRNA into non-tumor tissues. On the other hand, local delivery is not suitable for metastatic cancer, which is often observed in late stages of disease. To address this need, significant efforts have been made to develop systemic miRNA delivery strategies, in particular by improving targeting delivery systems and transduction efficiency.

To improve the transduction efficacy of oligonucleotides, Hossain et al[93] developed the carbonate apatite-based delivery system. Using this system, several groups succeeded in the efficient transduction of TS-miRNA into colon cancer cells.[94,95] Hiraki et al[94] identified miR-4689 as a TS-miRNA whose expression is downregulated by the K-RAS mutant ($KRAS^{G12V}$). The expression level of miR-4689 is also down-regulated in KRAS mutant colon cancer patients. Their study revealed that miR-4869 inhibits tumor cell proliferation by directly targeting KRAS and AKT. Inoue et al[95] also reported that miR-29b-1-5p is a TS-miRNA and a passenger strand of miR-29b-3p. Compared with

miR-29b-3p, miR-29b-1-5p was significantly downregulated in K-RAS mutant colon cancer patients. In animal experiments, miR-29b-1-5p selectively inhibited the proliferation of K-RAS mutant colon cancer cells.

For selective delivery of miRNA, Orellana et al[96] developed a selective miRNA delivery system without vehicle. Specifically, they directly conjugated a folate ligand, vitamin B9, to the strong TS-miRNA miR-34a.[12] Folate receptor is more highly expressed in epithelial cancers (breast, lung, ovary and colon) and various hematological malignancies than on normal tissues.[97] To stabilize the folate-conjugated miRNA, they also modified the passive strand of miR-34a using 2′-O-methyl RNA bases. This ligand-conjugated miRNA selectively inhibited tumor growth in vitro and in vivo without the use of vehicle. More importantly, Orellana et al confirmed the tumor-suppressive effects of this modified miRNA using an immunocompetent mouse model that mimics aggressive human non-small-cell lung carcinoma.

Several studies demonstrate that exosomes are useful carriers for in vivo siRNA and miRNA delivery.[98,99] Kamerkar et al[98] reported that CD47-positive exosomes derived from normal fibroblast-like mesenchymal cells are more effective than liposomes for in vivo delivery of siRNA against KRAS. CD47 inhibits the phagocytosis of exosomes by monocytes and macrophages. Consequently, CD47-positive exosomes are retained in the blood longer than liposomes, resulting in improved oligonucleotide transduction efficacy. In addition, Pi et al[99] reported that folate ligand- or aptamer-conjugated exosomes are effective for the targeted delivery of oligonucleotides in xenograft models of prostate, breast and colorectal cancer.

9 | CONCLUDING REMARKS

Here, we described recent studies of the functional roles of miRNA in cancer biology and discussed the development of clinical applications based on miRNA mimics or inhibitors. Multiple experiments in animal models demonstrate that single miRNA could suppress multiple oncogenic pathways, indicating that miRNA-based therapeutics represent a generally effective strategy. Therefore, miRNA-based therapeutics could be used to support the conventional therapeutics such as surgical intervention, chemotherapy and radiotherapy, thereby improving treatment for cancer patients.

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CONFLICTS OF INTEREST

Authors declare no conflict of interest for this article.

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