The clinical impact of intra- and extracellular miRNAs in ovarian cancer

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Abstract
Ovarian cancer is the most lethal gynecological cancer due to lack of early screening methods and acquired drug resistance. MicroRNAs (miRNAs) are effective post-transcriptional regulators that are transferred by extracellular vesicles, such as exosomes. Numerous studies have revealed that miRNAs are differentially expressed in epithelial ovarian cancer and act either as oncogenes or tumor suppressor genes. Cancer cells secrete exosomes containing miRNAs, which exert various effects on the components of the tumor microenvironment, including cancer-associated fibroblasts, macrophages, and adipocytes. Conversely, cancer cells also receive exosomes from these cells. As a result of cell-to-cell communication, epithelial ovarian cancer acquires a more aggressive phenotype and resistance to multiple drugs. In addition, some circulating miRNAs are protected from RNase degradation in the peripheral blood and can be potential non-invasive biomarkers. In particular, the combination of several circulating miRNAs enhances the accuracy of cancer screening. Likewise, comprehensive analyses revealed specific miRNA signatures in non-epithelial ovarian tumors and several miRNAs contributing to alterations of carcinogenic pathways. Overall, miRNAs play a crucial role in ovarian cancer progression. In this review, we discuss the emerging roles of intra- and extracellular miRNAs in ovarian cancers. In the near future, miRNAs will be practical biomarkers and computational deep learning will help in the clinical application of miRNAs. Moreover, miRNAs are potential therapeutic targets and agents, and there are ongoing clinical trials of miRNA replacement therapy. Therefore, accelerating research on miRNA might improve the prognosis of patients with ovarian cancer.

Keywords
epithelial ovarian cancer, miRNAs, non-epithelial ovarian tumor, non-invasive biomarkers, treatment resistance

Abbreviations: CAF, cancer-associated fibroblast; EMT, epithelial-mesenchymal transition; EOC, epithelial ovarian carcinoma; MET, mesenchymal-epithelial transition; miR-200f, miR-200 family; miRNA, microRNA; PARPi, PARP inhibitors; TAM, tumor-associated macrophages.
INTRODUCTION

Ovarian cancer is the most lethal gynecological cancer, and approximately 300,000 new cases and 18,000 deaths were reported worldwide in 2018. Ovarian tumors show a variety of histological representations and it is now evident that EOC consists of different histological subtypes with distinct molecular biology, tissues of origin, and clinical presentation. Treatment strategies for EOC have dramatically changed with the approval of drugs such as bevacizumab and olaparib. However, clinical challenges such as accurate screening and treatment for drug-resistant EOC remain persistent. Moreover, for non-epithelial malignant tumors, elucidation of the pathology and development of effective therapeutic methods are highly demanded.

Research relating to microRNAs (miRNAs) has been growing exponentially since it was discovered in *C. elegans* in 1993. There are 2,654 annotated mature miRNAs in the human genome; miRNAs modulate the expression of their target genes by interacting with partial complementary 3′-untranslated regions of the genes. Moreover, miRNAs can be encapsulated and delivered to targets by extracellular vesicles such as exosomes, which are small vesicles composed of a lipid bilayer, and mediate cell-to-cell communication in the local and distant microenvironment. Through exosomal transfer, the delivered miRNAs and mRNAs may demonstrate oncogenic functions in recipient cells; thus miRNAs play pivotal roles in cancer progression.

In this review, we discuss the recent findings on miRNA in ovarian cancers, including non-epithelial tumors, and the future prospects of miRNAs for clinical applications.

RECONSIDERING THE IMPACT OF miRNAs IN EOC PROGRESSION

One of the most well-known miRNAs is the miR-200 family (miR-200f), which is composed of the miR-200ba/429 and miR-200c/141...
clusters.\textsuperscript{6} The expression of miR-200f is stimulated by oxidative stress and is upregulated in EOC tissues compared with normal tissues (Figure 1A and Table 1).\textsuperscript{12,13} Importantly, miR-200f is also associated with EMT, and it is demonstrated as a double-negative feedback loop between miR-200f and its target ZEB1/2.\textsuperscript{14} This study reported that high levels of miR-200f inhibit ZEB1 in the epithelial state but, conversely, high levels of ZEB1 inhibit miR-200f in the mesenchymal state. In general, cancer cells gradually acquire more-mesenchymal characteristics, which confer more aggressive invasion-metastatic potential.\textsuperscript{15} However, it cannot be concluded that low expression of miR-200f is associated with more malignant behavior. According to a meta-analysis, high expression of miR-200f was associated with poor survival of patients with EOC.\textsuperscript{16} Moreover, miR-141 and miR-200a promote proliferation, whereas miR-200b inhibits it in EOC cell lines.\textsuperscript{30} Therefore, the functions of miR-200f can be different depending on the miRNA subtype and cell type.

Other miRNAs also contribute to EOC progression, and miR-181a is shown to promote EMT via suppression of SMAD7.\textsuperscript{18} Chr3q26.2 amplification, a frequently observed DNA copy-number alteration, leads to an increased expression of miR-551b-3p, and subsequently upregulates its target gene, STAT3.\textsuperscript{19} STAT3 then activates Wnt/β-catenin signaling through upregulation of miR-92a, which confer stemness properties.\textsuperscript{20} In contrast, miR-193a-3p acts as a tumor suppressor by modulating the MAPK/ERK signaling pathway, and miR-506 inhibits proliferation by targeting CDK4 and CDK6.\textsuperscript{21,22} Reflecting histological subtypes, several miRNAs in the chrXq27.3 cluster are highly expressed in clear cell carcinoma compared with high-grade serous carcinoma.\textsuperscript{23} Downregulation of the chrXq27.3 cluster is associated with an early relapse in patients with advanced EOC.\textsuperscript{24}

In addition, exosomal miRNAs modulate the tumor microenvironment (Figure 1B). Decrease in transfer of miR-124 from EOC cells can result in the transition of quiescent fibroblasts into CAFs.\textsuperscript{25} In CAFs, miR-200f-regulated CXCL12\textsuperscript{z} is shown to promote immunosupression.\textsuperscript{26} Moreover, EOC-derived miR-21-3p, -125b-5p, and -181d-5p remodel macrophages to TAM under hypoxic conditions.\textsuperscript{27} TAM-derived exosomes then confer a Treg/Th17 cell imbalance by transferring miR-21-5p and -29a-3p.\textsuperscript{28} Additionally, malignant ascites has also been reported to contain many cancer-derived exosome.\textsuperscript{29} Upon receiving exosomal miR-99a-5p, peritoneal mesothelial cells enhance the expression of FN1 and vitronectin (VTN) and make cancer invasion easy.\textsuperscript{30}

Therefore, miRNAs have been shown to play different important functions in physiology of cancer progression.

\section*{3 \ THE SIGNIFICANCE OF EXTRACELLULAR miRNAs AS NON-INVASIVE BIOMARKERS IN EOC}

Clinically, measurement of serum CA-125 levels has been widely performed to diagnose EOC, although it is not enough to screen EOC precisely.\textsuperscript{31} Despite the presence of RNases in the plasma, miRNAs exist stably in peripheral blood samples partly because they are encapsulated by exosomes.\textsuperscript{32} Therefore, the application of circulating miRNAs as non-invasive biomarkers has been investigated in various cancers.\textsuperscript{33}

Circulating cancer-derived exosomes increase as EOC progresses, and the miRNA profile of these exosomes is similar to that of their corresponding cancers.\textsuperscript{34} Thus, miR-200f can be a diagnostic marker, as the level of miR-200f is elevated in the peripheral blood of patients with EOC compared with healthy controls (Table 2).\textsuperscript{17,34-36} The levels of miR-200a and miR-200c are associated with disease progression, and the level of miR-200a is shown to be elevated in mucinous and serous carcinomas than in other EOC subtypes.\textsuperscript{26} In addition, exosomal miR-21, -100, and -320 are upregulated, whereas miR-16, -93, -126, and -223 are downregulated in the plasma of patients with EOC compared with healthy controls.\textsuperscript{17} However, the accuracy of a single miRNA as a diagnostic marker is not sufficient due to histological diversity and individual differences.

To improve the accuracy of screening, combination models using several miRNAs have been proposed (Figure 1C). According to a large-scale study performed using 4046 serum samples, a diagnostic model based on 10 miRNAs can distinguish patients with EOC and healthy controls with a high efficiency and accuracy (sensitivity: 99%; specificity: 100%).\textsuperscript{37} The 10 miRNAs obtained in the study are miR-320a, -665, -1275, -3184-5p, -3185, -3195, -4459, 4640-5p, -6076, and -6717-5p. Regarding histological differences, a combination of plasma miR-21, -191, and -1975 can distinguish patients with endometriosis-associated carcinoma and serous carcinoma, whereas miR-21, -362-5p, and -1274a can distinguish patients with endometriosis and endometriosis-associated carcinoma.\textsuperscript{38} Therefore, an appropriate combination of circulating miRNAs will be a potential diagnostic marker in the near future. However, there are several challenges in this research field. Firstly, circulating miRNAs can be influenced not only by the existence of specific cancer but also by presence of many other conditions, such as benign diseases, medication, stress, and any other individual factors. Secondly, depending on the experimental methods, such as next-generation sequencing or microarray, optimal combination, and cut-off value can be varied. Therefore, to efficiently screen EOC patients, further optimization is required.

\section*{4 \ ROLES OF miRNAs IN REGULATING THERAPY RESISTANCE IN EOC}

\subsection*{4.1 \ Conventional chemotherapy}

Over several decades, taxane- and platinum-based chemotherapy has been the standard treatment for EOC.\textsuperscript{2} Downregulation of miR-200f confers paclitaxel resistance, whereas taxane-resistant cell lines with EMT phenotype show decreased expression of miR-200f (Figure 2A).\textsuperscript{12,39} Thus, EMT phenotype is associated with taxane resistance. Inversely, upregulation of miR-200f and miR-591
| miRNA   | Direct target gene | Function                                              | Category | Ref |
|---------|--------------------|-------------------------------------------------------|----------|-----|
| miR-9   | BRCA1              | Sensitization to cisplatin                            | Anti-    | 42  |
| miR-21-3p | SOCS4, SOCS5      | Remodeling of macrophages to TAMs                    | Pro-     | 27  |
| miR-21-5p | STAT3             | Treg/Th17 imbalance                                   | Pro-     | 28  |
| miR-21   | APAF1              | Enhancement of resistance to paclitaxel               | Pro-     | 49  |
| miR-21   | unknown            | Promotion of angiogenesis                             | Pro-     | 55  |
| miR-29a-3p | STAT3            | Treg/Th17 imbalance                                   | Pro-     | 28  |
| miR-92a  | DKK1               | Maintenance of stemness                              | Pro-     | 20  |
| miR-99a-5p | FN1, VTN         | Promotion of cancer cell invasion by remodeling peritoneal mesothelial cells | Pro-     | 30  |
| miR-141  | p38α               | Promotion of cell proliferation/sensitization to paclitaxel | Pro-/anti- | 12  |
| miR-141  | CXCL12β            | Maintenance of immunocompetence                      | Anti-    | 26  |
| miR-141  | unknown            | Sensitization to paclitaxel/enhancement of resistance to carboplatin | Anti-/pro- | 39  |
| miR-141-3p | KEAP1             | Enhancement of resistance to cisplatin               | Pro-     | 41  |
| miR-124  | SPHK1              | Dedifferentiation of CAFs to normal fibroblasts      | Anti-    | 54  |
| miR-125b-5p | SOCS4            | Remodeling of macrophages to TAMs                    | Pro-     | 27  |
| miR-125b | ERBB3              | Inhibition of angiogenesis                           | Anti-    | 51  |
| miR-126-5p | DKK3, AXIN1, BACH1, NFAT5 | Enhancement of resistance to cisplatin               | Pro-     | 46  |
| miR-181a | SMAD7              | Promotion of cellular survival, migration, invasion, and drug resistance | Pro-     | 18  |
| miR-181d-5p | SOCS5            | Remodeling of macrophages to TAMs                    | Pro-     | 27  |
| miR-182  | BRCA1              | Sensitization to radiation and PARP inhibitor         | Anti-    | 60  |
| miR-182  | unknown            | Promotion of cell proliferation, invasion, and metastasis | Anti-    | 61  |
| miR-193a-3p | GRB7              | Suppression of cell proliferation, migration, and invasion | Anti-    | 22  |
| miR-195-5p | PSAT1             | Sensitization to cisplatin and inhibition of angiogenesis | Anti-    | 47  |
| miR-199a | ERBB2              | Inhibition of angiogenesis                           | Anti-    | 51  |
| miR-200a | p38α               | Promotion of cell proliferation/sensitization to paclitaxel | Pro-/anti- | 12  |
| miR-200a | CXCL12β            | Maintenance of immunocompetence                      | Anti-    | 26  |
| miR-200a | Unknown            | Sensitization to paclitaxel/enhancement of resistance to carboplatin | Anti-/pro- | 39  |
| miR-200b | Unknown            | Promotion of cell proliferation                      | Pro-     | 17  |
| miR-200c | NRP1               | Sensitization to PARP inhibitor                       | Anti-    | 63  |
| miR-200 family | ZEB1/2         | Maintenance of epithelial state                      | Anti-    | 14  |
| miR-200 family | IL8, CXCL1      | Inhibition of angiogenesis                           | Anti-    | 56  |
| miR-205  | Unknown            | Promotion of angiogenesis                            | Pro-     | 53  |
| miR-216b | PARP1              | Sensitization to cisplatin                           | Anti-    | 45  |
| miR-223  | PTEN               | Enhancement of resistance to cisplatin               | Pro-     | 50  |
| miR-484  | VAGFB, VEGFR2      | Inhibition of angiogenesis                           | Anti-    | 57  |
| miR-506  | CDK4/6             | Suppression of cell proliferation                    | Anti-    | 21  |
| miR-506  | RAD51              | Sensitization to cisplatin and PARP inhibitor         | Anti-    | 43  |
| miR-509-3p | RAD51, HMGA2      | Sensitization to cisplatin and PARP inhibitor         | Anti-    | 44  |
| miR-551b-3p | STAT3            | Resistance to apoptosis and promotion of survival and proliferation | Pro-     | 19  |
| miR-591  | ZEB1               | Enhancement of resistance to paclitaxel              | Pro-     | 40  |
| miR-622  | KU70, KU80         | Enhancement of resistance to cisplatin and PARP inhibitor | Pro-     | 62  |
| miR-1246 | CAV1               | Enhancement of resistance to paclitaxel              | Pro-     | 48  |

Abbreviations: anti-, anti-oncogenic; CAFs, cancer-associated fibroblasts; pro-, pro-oncogenic; Ref, references; TAMs, tumor-associated macrophages.
enhances paclitaxel sensitivity by targeting ZEB1.12,40 Conversely, the functions of miR-200f in platinum resistance are reversed. Overexpression of miR-141 enhances cisplatin resistance by regulating KEAP1, and miR-141 or miR-200c confer further carboplatin resistance in multidrug-resistant cells (Figure 2B).39,41 Therefore, it can be concluded that the EMT phenotype does not always contribute to drug resistance.

In addition, the expression levels of miR-9, miR-506, and miR-509-3p are associated with a better response to platinum-containing chemotherapy in patients with EOC.42-44 miR-9 mediates the downregulation of BRCA1 and improves cisplatin sensitivity.42 Moreover, miR-506 and miR-509-3p enhance cisplatin sensitivity by targeting RAD51, which is an important gene for DNA repair.43,44 Downregulation of PARP1 by miR-216b also reverses cisplatin resistance in EOC cells.45 Hence, downregulation of these miRNAs targeting DNA repair-related genes is important for cisplatin resistance. Moreover, activation of the Wnt/β-catenin signaling pathway is involved in platinum resistance, and the pathway is shown to be activated by miR-126-5p but suppressed by miR-195-5p.46,47

Furthermore, exosomal transfer of miRNAs may also lead to development of chemoresistance. TAMs receive miR-1246 abundant exosomes derived from paclitaxel-resistant EOC cells, and miR-1246 further promotes paclitaxel resistance.48 In contrast, EOC cells receive miR-21 abundant exosomes from cancer-associated adipocytes and fibroblasts, resulting in paclitaxel resistance.49 Moreover, in hypoxic conditions, miR-223 enriched TAM-derived exosomes promote cisplatin resistance in EOC cells via the PTEN-PI3K/AKT pathway.50

Therefore, various miRNA-related mechanisms are involved in chemoresistance, and combination of different chemotherapies is advisable considering this the aspect of miRNAs.

4.2 | Anti-VEGF therapy

As cancer progresses, the center of cancer tissues become hypoxic, and cancer cells promote angiogenesis. EOC tissues are known to represent high levels of vascular endothelial growth factor A (VEGFA). Therefore, bevacizumab, a monoclonal antibody for all VEGFA isoforms, suppresses angiogenic processes, resulting in prolonged progression-free survival in patients with EOC.3 However, its clinical benefits are usually transient.

Anti-angiogenic miR-125b, -195-5p, and -199a are downregulated in EOC, and these miRNAs target HIF1A and VEGFA (Figure 2C).47,51 Conversely, low expression of angiogenic miR-378 is associated with prolonged survival due to progression-free effects observed due to bevacizumab in EOC patients.52 Moreover, exosomes also contribute to angiogenesis, and EOC-derived miR-141-3p, miR-205, and adipocyte-derived miR-21 promote vascularization of endothelial cells.53,55 Conversely, miR-200f inhibits angiogenesis by modulating the expression of interleukin-8 and CXCL1 in cancer cells and directly affecting endothelial cells.56 In addition, miR-484 modulates tumor vasculature by targeting VEGFB and VEGFR2, and it alters the chemosensitivity of EOC cells in vivo but not in vitro.57

It is suggested that, not only VEGFA, but also activation of alternative angiogenic pathways is associated with bevacizumab tolerance.57,58 Therefore, intervention with these miRNAs and their targets may reverse bevacizumab resistance.

4.3 | Poly ADP ribose polymerase (PARP) inhibitors (PARPi)

PARP1 and PARP2 proteins mediate the repair of single-stranded DNA breaks, whereas BRCA1 and BRCA2 proteins play critical roles in the repair of double-stranded DNA breaks called homologous recombination repair.59 PARPi are the first clinically approved drugs designed to exploit synthetic lethality and show a higher response to patients with germline BRCA1/2 mutations or acquired BRCA1/2 mutations.4,59

Based on the concept of synthetic lethality, miR-182 overexpressing breast cancer cells are hypersensitive to PARPi by targeting BRCA1 (Figure 2D).60 However, miR-182 also has functions related to aggressive behaviors by regulating multiple tumor suppressor genes.61 Therefore, BRCA1 targeting therapy seems ambivalent. Moreover, miR-506 and miR-509-3p enhance the efficacy of PARPi as well as cisplatin by targeting RAD51.43,44 However, despite the presence of BRCA-mutation, resistance to PARPi can occur. High expression of miR-622 is associated with poor survival in EOC with BRCA1 mutation, and miR-622 also induces PARPi resistance by downregulating the Ku complex during the cell cycle in BRCA1-mutant in vitro.62 Conversely, miR-200c reversed PARPi resistance by targeting NRP1 which is overexpressed in PARPi-resistant cells.63 Therefore, miRNAs are also powerful modulators of PARPi response.

In conclusion, because various targets of miRNAs are involved in treatment resistance, miRNAs can act as potential therapeutic targets.

5 | NOVEL FINDINGS of miRNAs IN NON-EPITHELIAL MALIGNANT OVARIAN NEOPLASMS

Germ cell tumors (GCTs) are frequently diagnosed in childhood and adolescent age, and are derived from primordial germ cells, embryonic precursors of egg and sperm (Figure 3A).64 According to miRNA microarrays, miR-371-373 and miR-302 clusters are overexpressed in malignant GCTs compared with nonmalignant tissues, regardless of histological subtype.65,66 In particular, these clusters show even higher overexpression in yolk sac tumors, whereas miR-146b-5p, -155, and -182 are overexpressed in germinomas.66,67 Members of the miR-302 cluster and miR-200f, having high expression in yolk sac tumors, are also associated with the TGF-β/BMP signaling pathway.68 Moreover, differentially expressed mRNAs and proteins are identified in GCTs, and elucidation of their differentiation processes is expected.64
Mature teratoma is a benign GCT. However, secondary malignant transformation can occur in postmenopausal women, and its clinical presentation is far from that of other GCTs. 68 Comprehensive miRNA sequencing revealed that 4 miRNAs (miR-26a-5p, -99a-5p, -151a-3p and -378a-3p) are dysregulated in this malignancy compared with mature teratoma or normal ovary, suggesting that these miRNAs might be involved in its carcinogenesis (Figure 3B). 69

| miRNA | Sample type | Methodology | Number of samples | Clinical observation | Ref |
|-------|-------------|-------------|-------------------|---------------------|-----|
| miR-21 | Blood (exosome) | Microarray | EOC (n = 106) vs healthy (n = 29) | AUC: 0.740, SN: 61%, SP: 82% | 17 |
| miR-100 | Blood (exosome) | Microarray | EOC (n = 106) vs healthy (n = 29) | AUC: 0.710, SN: 62%, SP: 73% | 17 |
| miR-200a | Serum | qRT-PCR | EOC (n = 28) vs healthy (n = 28) | AUC: 0.675, SN: 85.7%, SP: 35.7% | 35 |
| miR-200b | Serum | qRT-PCR | EOC (n = 28) vs healthy (n = 28) | AUC: 0.686, SN: 64%, SP: 86% | 17 |
| miR-200c | Serum | qRT-PCR | EOC (n = 28) vs healthy (n = 28) | AUC: 0.722, SN: 85.7%, SP: 35.7% | 35 |
| miR-320 | Blood (exosome) | Microarray | EOC (n = 106) vs healthy (n = 29) | AUC: 0.658, SN: 56%, SP: 69% | 17 |
| miR-200b and -200c | Serum | qRT-PCR | EOC (n = 28) vs healthy (n = 28) | AUC: 0.784, SN: 78.6%, SP: 46.4% | 35 |
| miR-21, -362-5p, and -1274a | Plasma | qRT-PCR | EAOC (n = 14) vs endometriosis (n = 33) | AUC: 0.92, SN: 57%, SP: 91% | 38 |
| A combination of 10 miRNAs | Serum | Microarray | EOC (n = 333) vs non-EOC (n = 3713) | AUC: 1.00, SN: 99%, SP: 100% | 37 |

Abbreviations: AUC, area under curve; DFS, disease-free survival; EAOC, endometriosis-associated ovarian cancer; EOC, epithelial ovarian cancer; FFPE, formalin-fixed paraffin-embedded; HR, hazard ratio; ISH, in situ hybridization; OS, overall survival; PFS, progression-free survival; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; Ref, reference; SN, sensitivity; SP, specificity; TCGA, The Cancer Genome Atlas.

a miR-320a, -665, -1275, -3184-5p, -3185, -3195, -4459, 4640-5p, -6076, and -6717-5p.
b miR-506, -507 -508-3p, -509-3p, -509-5p, -513a-5p, -513b, and -514.

Sex cord-stromal tumors are frequently associated with DICER1 syndrome, a hereditary cancer predisposition syndrome characterized by the deleterious germline DICER1. 70,71 DICER protein is involved in cleavage of precursor miRNAs by recognizing the 5′-end of RNA, and DICER1-deficient cells results in loss of mature 5p miRNA strands (Figure 3C). 7,8,72,73 Therefore, the major alteration in miRNA expression causes a variety of rare
cancers such as pleuropulmonary blastoma, neuroblastoma, and thyroid carcinoma. GCTs and ovarian embryonal rhabdomyosarcoma are also recognized as DICER1-associated tumors. This syndrome clearly shows the importance of miRNAs in carcinogenesis.

Therefore, research on miRNAs in non-epithelial tumors is currently in progress, and further studies are desired with a view to identify novel therapeutic targets.

6 | FUTURE PERSPECTIVES AND CONCLUSIONS

In EOC, there are no effective screening methods, and treatment for advanced/recurrent disease still remains challenging. Because miRNAs play various roles in cancer progression and treatment resistance, clinical application of miRNAs is highly expected. Firstly, due to the stability of miRNAs in the body fluid, circulating miRNAs can be...
promising non-invasive biomarkers (Figure 4). To improve screening effectiveness, the development of a detection method and target miRNAs is essential to advance this field forward. Recently, deep learning computational frameworks has enabled the utilization of enormous multi-omics data, and it provides more robust prognosis prediction models. Moreover, it aids in accurate target prediction of miRNAs and accelerates miRNA research. Secondly, miRNAs have the potential to be novel therapeutic targets and agents. To perform miRNA replacement therapy, it is essential to develop a suitable delivery system with high specificity for targeting cell and protection them from RNA degradation. In cancer-bearing mouse model, intravenous or peritoneal administration of miRNA or anti-miRNA treatment showed therapeutic effects. In addition, an acceptable toxicity of TargomiRs, which are minicells loaded with miR-16-based mimic miRNA, was shown in a phase 1 study of patients with malignant pleural mesothelioma. Hence, miRNA replacement therapy can be a novel attractive therapy for different malignancies, and it is necessary to study how to carry the specific miRNA depending on the type of cancer.

In conclusion, miRNAs are powerful mediators that alter the tumor microenvironment. The application of miRNAs in the clinical setting is advancing, and research on miRNAs continues to receive much attention.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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