# MicroRNAs and cancer: Key paradigms in molecular therapy (Review)

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**Abstract.** MicroRNAs (miRNAs) are a type of small non-coding RNA molecule that performs an important role in post-transcriptional gene regulation. Since miRNAs were first identified in 1993, a number of studies have demonstrated that they act as tumor suppressors or oncogenes in human cancer, including colorectal, lung, brain, breast and liver cancer, and leukemia. Large high-throughput studies have previously revealed that miRNA profiling is critical for the diagnosis and prognosis of patients with cancer, while certain miRNAs possess the potential to be used as diagnostic and prognostic biomarkers or therapeutic targets in cancer. The present study reviews the studies and examines the roles of miRNAs in cancer diagnosis, prognosis and treatment, and discusses the potential therapeutic modality of exploiting miRNAs.

**Contents**



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## 1. Introduction

MicroRNAs (miRNAs/miRs) were identified in 1993 (1-3). They are a type of small non-coding RNA, between 19-24 nucleotides in length, which perform a critical role in the regulation of gene expression at the post-transcriptional level. miRNAs act by degrading their RNA targets or by repressing the translation of mRNAs (4). In the previous two decades, numerous studies have indicated the important role of miRNAs in the regulation of crucial cellular processes, including proliferation, differentiation, migration, apoptosis, metabolism and the stress response (5). miRNAs have been demonstrated to act as key regulators in the pathogenesis of diseases (6-11), particularly in cancer.

miRNAs provide a novel insight into the study of cancer. Previously, >50% of miRNA genes were revealed to be located in cancer-associated genomic regions and to form central nodal points in cancer development pathways (5), suggesting that miRNAs may perform an important role in the pathogenesis of human cancer. The hypothesis that the dysregulation of miRNAs may perform a fundamental role in the onset, progression and dissemination of numerous types of cancer was primarily confirmed in chronic lymphocytic leukemia (CLL) by Calin *et al* (12), who demonstrated that miR-15a and miR-16-1 were downregulated or deleted in the majority of patients with CLL.

Uncovering the complex role of miRNAs in cancers presents a challenge. Previous studies revealed that miRNAs regulate a number of molecular pathways of cancer by targeting oncogenes and tumor suppressors in tumorigenesis, cancer maintenance and progression (13), involving biological pathways of cancer-stem-cell biology (14), angiogenesis (15), the epithelial-mesenchymal transition, metastasis (16) and drug resistance (17).

miRNAs are widespread and have been estimated to regulate >50% of the human genome (18,19). Results from previous studies revealed that changing the expression of a particular cancer-associated miRNA may alter the expression of a potential oncogenic or anti-oncogenic protein (20), demonstrating that miRNAs may be used as therapeutic targets and tools in cancer treatment.

## 2. The mechanism of miRNAs in cancer

miRNAs overexpressed in cancers were considered to be oncogenes, termed 'oncomirs', which may promote tumor

development by negatively regulating genes, generally those controlling cell differentiation or apoptosis and/or tumor suppressor genes. A certain number of oncomirs exist in the tumor genome, but only a few of them have been well characterized, including miR-21 (21) and the cluster miR-17-92 (22,23).

miR-21 is overexpressed in breast, colorectal, lung and pancreatic cancer, glioblastoma, neuroblastoma, leukemia and lymphoma. miR-21 affects proliferation, apoptosis, migration, invasion and maintenance of cancer cells *in vitro,* and is associated with survival of cancer patients *in vivo* by targeting a tumor-suppressor (21). The miR-17-92 cluster located at chromosome 13q31 is a polycistronic transcript consisting of miRNAs 17, 18a, 19a, 20a, 19b-1 and 92a-1, It is significantly overexpressed in lung cancer and lymphoma (22,23). V-myc avian myelocytomatosis viral oncogene homolog (c-Myc) activates and regulates the miR-17-92 cluster to modulate E2F1 expression and inhibit c-Myc-induced apoptosis through tumor protein p53 pathway (24). Additionally, miR-17-92 inhibits the tumor suppressor genes phosphatase and tensin homolog (25) and RB2 (26) by activating the protein kinase B signaling pathway to promote cancer-cell survival (Fig. 1). Additionally, oncogenic miR-372 and miR-373 promote cell proliferation and tumor development by targeting the tumor suppressor gene large tumor suppressor kinase 2 (27) and neutralizing inhibition of p53-mediated cyclin-dependent kinase in human testicular germ cell tumors.

The expression of tumor suppressor genes is decreased in cancer cells. Tumor suppressor miRNAs negatively inhibit oncogenes and/or genes that control cell differentiation or apoptosis and thus prevent tumor development. miRNAs let-7 and miR-34 family are known to be tumor suppressor genes.

The expression of let-7 is reduced in a number of types of cancer, and is correlated with poor survival (28). The overexpression of let-7 has been demonstrated to inhibit growth of lung cancer cells *in vitro* (29). Results from previous studies have revealed that the reduced expression of let-7 increases the protein expression of the pro-oncogene RAS in lung tumors (29-31) (Fig. 1). A loss of expression of miR-34a is associated with metastasis and recurrence in prostate cancer, while restoration of miR-34 expression is associated with clonogenic cell growth and invasion, apoptosis and cellular activation of chemotherapy and radiation in pancreatic cancer. Another study demonstrated that the miR-34 family may regulate the expression and mutation of p53, while miR-34b and miR-34c target MYC (32-35). A lack of expression of miR-34 family members attenuated p53-dependent and p38-mitogen-activated protein kinase-dependent responses to DNA damage, and led to oncogenesis.

## 3. Cancer stem cells

microRNAs have been demonstrated to perform critical roles in controlling the fate of cancer stem cells (CSCs) (36,37). Numerous genes essential for pluripotency and stem cell function, including Octamer-binding transcription factor 4, *NANOG,* SRY-Homeobox 2 *(SOX2), NOTCH* and B-cell lymphoma 2, are targets of miRNAs, such as miR-296, miR-134, miR-470 and the miR-34 family.

The let-7 family, miR-200 family, and miR-30 are all believed to be important for the regulation of breast cancer stem cells. The let-7 family is downregulated in breast-cancer stem cells. Let-7 family members are associated with tumor formation and metastasis of breast cancer in immunocompromised mice by regulating breast CSCs (38). Let-7 results in the loss of self-renewal (RAS silencing) and enhancement of multi-lineage differentiation (high-mobility group AT-hook 2 (HMGA2) silencing) in CSCs by targeting the 3' untranslated region (UTR) of RAS and HMGA2 genes (39). The miR-200 family, which comprises miR-200a, miR-200b, miR-200c, miR-141 and miR-429, together with miR-145 and miR-146 is highly downregulated in breast CSCs (40), which undergo epithelial-mesenchymal transition (EMT) in response to transforming growth factor β signaling (41). In addition, the stem cell genes *SOX2*, Krüppel like factor 4, polycomb complex protein BMI-1, polycomb protein Suz12, Zinc finger E-box binding homeobox 1 (*ZEB1*), and *ZEB2* are all targets of miR-200 family members (42,43). A low expression of miR-30 inhibits self-renewal of breast cancer stem cells, while antagonism of miR-30 by antisense oligonucleotides enhances self-renewal, tumor regeneration and metastasis in differentiated breast cancer cells (44) (Fig. 2).

## 4. Angiogenesis

Angiogenesis is essential for tumor growth and metastasis (45,46). Previous studies have demonstrated that miRNAs are able to regulate angiogenesis and tumor cell survival (47-51). The miR-17-92 cluster is significantly upregulated in Myc-induced tumors and overexpressed in Ras cells, where it enhances tumor vessel growth in a paracrine manner (47), exhibiting potent tumor angiogenesis-promoting activity. In ovarian cancer, miR-378 enhances tumor angiogenesis, tumor cell survival and growth by targeting ALCAM and EHD1 (48). The overexpression of let-7f and miR-27b exerts pro-angiogenic effects, as shown by the blockade of angiogenesis with 20-O-methyl oligonucleotide inhibitors *in vitro* (49). miR-221 and miR-222 inhibit angiogenesis by targeting at least two important regulators of pro-angiogenic endothelial cell function in tumors (50). Repression of the miR-15-16 cluster was found to be associated with advanced tumor stage and poor prognosis in patients with colorectal carcinoma, and is shown to promote tumor angiogenesis and metastasis by the loss of restriction of its target gene, fibroblast growth factor-2 (FGF2) (51).

## 5. EMT and metastasis

Activation of EMT increases the rates of migration and invasion in tumor cells, while activation of the reverse mesenchymal-to-epithelial transition is required for metastasis outgrowth. Expression of epithelial-cadherin (E-cadherin) by the *Cadherin* 1 gene is essential for retaining an epithelial cell type (52). EMT transcription factors that serve as E-cadherin repressors- such as zinc finger protein (SNAI)1/SNAI2, basic helix-loop-helix proteins including E47, E2-2, Twist-related protein (TWIST)1/TWIST2, and ZEB1/ZEB2, activate cancer cells by triggering EMT (53). The miR-200 family, miR-27 and

miR-205 inhibit ZEB1 and ZEB2 (54-56). In breast cancer, the expression of miR-200 is positively correlated with concentrations of E-cadherin. In kidney-derived cells, the restoration of miR-200 expression is sufficient to reverse the transition (mesenchymal-to-epithelial). In pancreatic epithelial cells, the expression of miR-30 family members is inversely correlated with the mesenchymal phenotype (57). In mesenchymal-like ovarian cancer cell lines, an overexpression of miR-429 reverses EMT (58).

## 6. Clinical applications of miRNAs

*miRNAs as diagnostic indicators*. Numerous tumor-profiling studies have been conducted over the previous 5 years. Several miRNA expression signatures have been identified, which may be used to differentiate between malignant and benign conditions in several organs by screening resected tumors and biopsy samples (59). In leukemia, a 4-miRNA signature was able to differentially diagnose acute lymphoblastic leukemia from acute myeloid leukemia with a sensitivity and specificity of up to 100% (60). In breast cancer, a 97-gene expression profile has been demonstrated to be an improved method for the classification of breast cancer histological grade compared with lymph-node status and tumor size (61). In pancreatic ductal adenocarcinomas, a signature of 7 differentially expressed miRNAs may provide a more accurate diagnosis compared with conventional cytology (62).

*miRNAs as prognostic indicators*. miRNA expression patterns have been identified to predict the outcome and prognosis of cancer in several studies. In breast cancer, 31 miRNAs were demonstrated to be significantly associated with clinical factors, while the overexpression of 17 miRNAs was associated with estrogen-receptor-positive stage I or II breast cancer, with good clinical outcome (63). The overexpression of miR-210 is associated with an increased risk of recurrence and a reduced chance of relapse-free survival (64). miR-155 overexpression exhibits an association with poor post-operative survival in lung cancer and B cell lymphomas (65,66). miR-183 family, miR-183, miR-182 and miR-96 expression has been revealed to correlate with the progression of non-small-cell lung cancer (67). miR-200c expression has been associated with overall survival subsequent to surgery in colorectal cancer (68). According to prognosis, 13 miRNAs were identified with variable expression in CLL.

*miRNAs and cancer treatment*. MicroRNAs possess the capacity to target between tens and hundreds of genes simultaneously. They perform a key role in tumorigenesis as important modulators in cellular pathways by regulating target gene expression through translation repression or mRNA degradation. Thus, miRNAs are attractive candidates for prognostic biomarkers and therapeutic targets in cancer. The identification of miRNAs and their targets is essential for cancer development and metastasis, and therefore may provide exciting therapeutic opportunities. In the present review, potential target genes and a possible mechanism of tumorigenic miRNAs are summarized (69-92) (Table I).

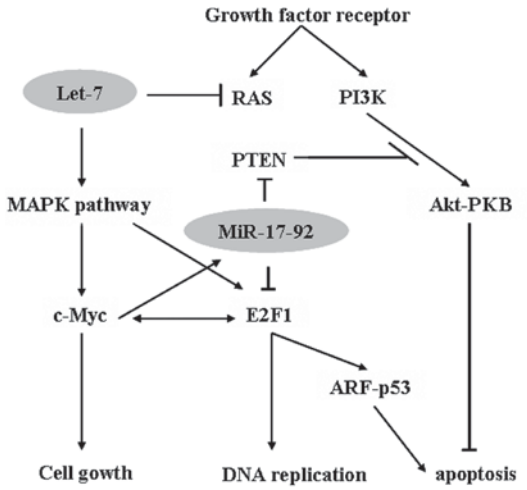


Figure 1. Interaction of miRNAs as oncogenic and tumor suppressor. let-7 suppresses translation of the Ras GTPase genes. The downregulation of let-7 promotes the cell cycle through the Ras-MAPK pathway. miR-17-92 may prohibit oncogene-induced apoptosis. PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide-3 kinase; PKB, protein kinase B; MAPK, mitogen-activated protein kinase; ARF, alternative reading frame protein of p16INK4a locus. miRNA/miR, microRNA; p53, tumor protein 53; E2F1, transcription factor E2F1; Akt, RAC-α serine/threonine-protein kinase.

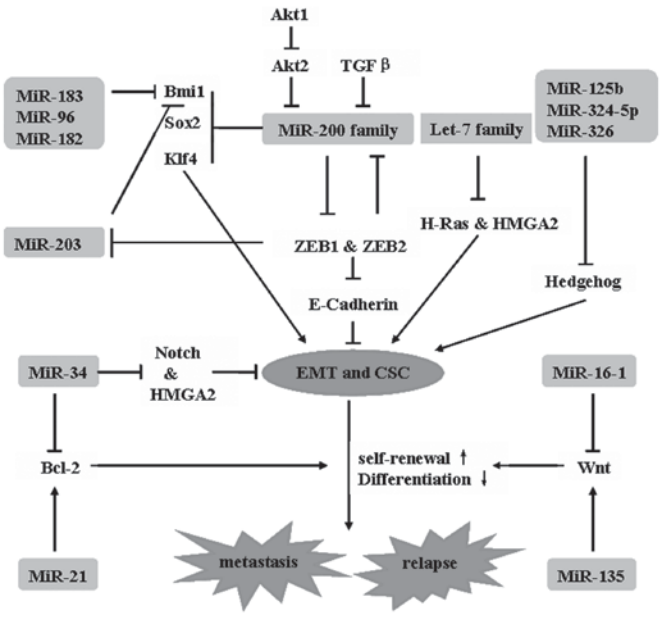


Figure 2. miRNAs associated to breast cancer stem cells and their potential mechanisms. These miRNAs regulate target genes that are involved in the processes of stem cells. The abnormal expression of these potential 'stem cell miRNAs' in cancer indicates that deregulated stem cell genes lead to an increase in the level of self-renewal and a reduction in the intracellular levels of apoptosis in cancer stem cells. This leads to the progression of the cancer. CSC, cancer stem cells; EMT, epithelial-mesenchymal transition; HMGA2, high-mobility group AT-hook 2; Akt1, RAC-α serine/threonine-protein kinase; Akt2; RAC-β serine/threonine-protein kinase; TGF-β, transforming growth factor β; miRNA/miR, microRNA; Klf4, Krüppel like factor 4; BMI-1, polycomb complex protein BMI-1; ZEB1/2, Zinc finger E-box binding homeobox 1/2; H-Ras, transforming protein p21; Bcl2, B-cell lymphoma 2; E-cadherin, epithelial cadherin.

There are several acknowledged approaches to miRNA targeting: Anti-miRNA oligonucleotides (AMOs) are single-stranded molecules that form direct complementarity

Table I. Target genes of candidate microRNAs and the possible mechanisms.

| Cancer | MicroRNAs | Deregulation | Targets | Molecular mechanisms | (Refs.) |
|---|---|---|---|---|---|
| Breast cancer | let-7 | Downregulated | E2F2, c-Myc, KRAS | Reduces the levels of c-Myc and E2F2 proteins<br>Inhibits cell proliferation, KRAS expression and mitogen-activated protein kinase activation | (38) |
| | miR-27a | Upregulated | FOXO1 | Antisense miR-27a inhibits cell cycle traverse and induces cell death | (69) |
| | miR-31 | Downregulated | RhoA | Inhibits local invasion, extravasation or initial survival at a distant site and metastatic colonization | (70) |
| | miR-96 | Upregulated | FOXO1 | Antisense miR-96 inhibits cell cycle traverse and induces cell death | (69) |
| | miR-98 | Downregulated | E2F2, c-Myc | Reduces the levels of E2F2 and c-Myc proteins | (71) |
| | miR-182 | Upregulated | FOXO1, FOXO3 | Antisense miR-182 inhibits cell cycle traverse and induces cell death<br>Stimulates migration and metastatic potential | (69) |
| | miR-205 | Downregulated | HER3 | Inhibits the activation of downstream mediator Akt and increases the responsiveness to tyrosine kinase inhibitors | (72) |
| | miR-9-3 | Downregulated | p53 | Plays a role in the p53-related apoptotic pathway | (73) |
| | miR-375 | Upregulated | SHOX2 | Epithelial-to-mesenchymal transition inducer in breast cancer cells | (74) |
| Gastric cancer | miR-25 | Upregulated | p57 | Suppresses the Cip/Kip family members of cyclin-dependent kinase inhibitors through the 3' untranslated region | (75) |
| | miR-106b | Upregulated | p21, p73 | Suppresses the Cip/Kip family members of cyclin-dependent kinase inhibitors<br>Regulates cell survival by mediating the post-transcriptional downregulation of an ubiquitin ligase, leading to the induction of a proapoptotic regulator in malignant cells | (76) |
| | miR-93, miR-221 | Upregulated | p21, p27, p57 | Suppresses the Cip/Kip family members of cyclin-dependent kinase inhibitors | (77,78) |
| | miR-512 | Downregulated | Mcl-1 | Induces apoptosis of cancer cells | (79) |
| | miR-10b | Upregulated | RhoC | Regulates mRNA expressions of RhoC and urokinase-type plasminogen activator receptor via HOXD10 | (80) |
| Glioma | miR-221 | Downregulated | PTEN/AKT | Mediates epithelial-mesenchymal transition-related gene expressions via regulation of PTEN/Akt signaling | (81,82) |
| | miR-324-5 | Downregulated | GLI1 | Inhibits proliferation | (83) |
| Hepatocellular carcinoma | miR-195 | Downregulated | cyclin D1, E2F3 | Suppresses colony formation and blocks G1-S transition | (84) |
| | miR-16 | Downregulated | Bcl-2 | Regulates apoptosis | (85) |
| | miR-18a | Upregulated | ERα, ESR1 | Represses ERα translation by binding to its mRNA at the 3' untranslated region | (86) |
| | miR-26a | Downregulated | cyclin D2, cyclin E2 | Inhibits cancer cell proliferation, induces tumor-specific apoptosis and protects disease progression without toxicity | (87) |
| | miR-101 | Downregulated | Mcl-1 | Promotes apoptosis and suppresses tumorigenicity | (88) |
| | miR-145 | Downregulated | EGFR, IGF-1R | Inhibits cancer cell growth in epidermal growth factor receptor mutant cancer | (89,90) |

Table I. Continued.

| Cancer | MicroRNAs | Deregulation | Targets | Molecular mechanisms | (Refs.) |
|---|---|---|---|---|---|
| Prostate cancer | miR-331 | Downregulated | HER2/neu | Blocks PI3K/Akt signaling and androgen receptor signaling pathways critical to the development and progression of cancer. | (91) |
| | miR-200, miR-200b | Downregulated | ZEB1, ZEB2 | miR-200 reverses epithelial-to-mesenchymal transition in gemcitabine-resistant cancer cells. miR-200b regulates platelet-derived growth factor-D mediated EMT. | (92) |

E2F2, transcription factor E2F2; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; FOXO1/O3, forkhead box protein O1/O3; RhoA, Ras homolog gene family, member A; HER3, receptor tyrosine-protein kinase erbB-3; p53, tumor protein 53; SHOX2, short stature homeobox 2; p57, cyclin-dependent kinase inhibitor 1C; p21, cyclin-dependent kinase inhibitor 1; p73, tumor protein 73; p27, cyclin-dependent kinase inhibitor 1B; RhoC, Ras homolog gene family, member C; PTEN, phosphatase and tensin homolog; AKT, protein kinase B; GLI1, zinc finger protein GLI1; Bcl-2, B-cell lymphoma 2; ERα/ESR1, estrogen receptor α; Mcl-1, induced myeloid leukemia cell differentiation protein Mcl-1; EGFR, epithelial growth factor receptor; IGF-1R, insulin-like growth factor 1 receptor; HER2/neu, receptor tyrosine-protein kinase erbB-2; ZEB1/2, Zinc finger E-box binding homeobox.

and thus inhibit specific miRNAs. Previous studies have widely used AMOs to target mRNAs and evaluate gene function *in vitro* and *in vivo* (93,94). The chemical modification of the AMOs may improve the hybridization affinity of the target RNA *in vitro* (95), make it resistant to nuclease degradation and activate RNase or other proteins (96). For *in vivo* delivery, altering the protein binding properties of AMOs is necessary to delay plasma clearance and promote uptake into tissues (97,98). AntagomiRs are single-stranded molecules that form complementarity to miRNAs; however, in order to maintain stability while minimizing degradation, they may also be modified with a cholesterol conjugated 20-O-methyl (99,100). Locked nucleic acids (LNAs) have a methylene bridge to functionally lock ribose conformation, which consequently leads to increased binding affinity and stability (101). miRNA sponges function by using multiple complementary 3'UTR mRNA sites for a specific miRNA (102). These sponges competitively bind to miRNA, thus interfering with the normal targeting of a single miRNA by targeting it with antisense oligonucleotides. In addition, the development of stable sponges may assist in recapitulating the effects of downregulation of aberrantly expressed miRNAs (103-105) and nanoparticles, the formulations of which may be used primarily for *in vitro* delivery of miRNAs (106,107).

A small number of studies at present have used this technology for miRNA delivery (108). The results of previous studies demonstrated that by using liposome polycation-hyaluronic acid particles as a carrier for miRNA modified with a tumor targeting monoclonal antibody, a golgin candidate 4 single-chain variable fragment, they were able to target lung metastases in a murine model of metastatic melanoma (109,110).

## 7. Conclusion

In conclusion, miRNAs have changed our understanding of gene expression and set a precedent for the development of novel diagnostic methods and treatments for cancer. To translate these data into clinical application, large cohort studies are required to examine the prognostic and diagnostic value of miRNA panels. In the long term, it is important to identify additional potential targets of miRNA, and to develop safe and specific methods to deliver miRNA-based treatments in order to make the modulation of miRNAs a critical technique for cancer treatment and management.

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