Targeting oncomiRNAs and mimicking tumor suppressor miRNAs: New trends in the development of miRNA therapeutic strategies in oncology (Review)

ROBERTO GAMbari¹, ELEONORA BROGNARA¹, DEMETRIOS A. SPANDIDOS² and ENRICA FABBRI¹

¹Department of Life Sciences and Biotechnology and Biotechnology Center, Ferrara University, Ferrara, Italy; ²Laboratory of Clinical Virology, University of Crete School of Medicine, Heraklion, Crete, Greece

Received March 9, 2016; Accepted April 29, 2016

DOI: 10.3892/ijo.2016.3503

Abstract. MicroRNA (miRNA or miR) therapeutics in cancer are based on targeting or mimicking miRNAs involved in cancer onset, progression, angiogenesis, epithelial-mesenchymal transition and metastasis. Several studies conclusively have demonstrated that miRNAs are deeply involved in tumor onset and progression, either behaving as tumor-promoting miRNAs (oncomiRNAs and metastamiRNAs) or as tumor suppressor miRNAs. This review focuses on the most promising examples potentially leading to the development of anticancer, miRNA-based therapeutic protocols. The inhibition of miRNA activity can be readily achieved by the use of miRNA inhibitors and oligomers, including RNA, DNA and DNA analogues (miRNA antisense therapy), small molecule inhibitors, miRNA sponges or through miRNA masking. On the contrary, the enhancement of miRNA function (miRNA replacement therapy) can be achieved by the use of modified miRNA mimetics, such as plasmid or lentiviral vectors carrying miRNA sequences. Combination strategies have been recently developed based on the observation that i) the combined administration of different antagomiR molecules induces greater antitumor effects and ii) some anti-miR molecules can sensitize drug-resistant tumor cell lines to therapeutic drugs. In this review, we discuss two additional issues: i) the combination of miRNA replacement therapy with drug administration and ii) the combination of antagomiR and miRNA replacement therapy. One of the solid results emerging from different independent studies is that miRNA replacement therapy can enhance the antitumor effects of the antitumor drugs. The second important conclusion of the reviewed studies is that the combination of anti-miRNA and miRNA replacement strategies may lead to excellent results, in terms of antitumor effects.

Contents

1. Introduction
2. Tumor suppressor miRNAs
3. OncomiRNAs and metastamiRNAs
4. Mimicking tumor suppressor miRNAs in miRNA replacement therapy
5. Targeting oncomiRNAs
6. MicroRNAs and epithelial-mesenchymal transition
7. MicroRNAs and neoangiogenesis
8. Selected examples of miRNA therapeutics: mimicking miR-124
9. Selected examples of miRNA therapeutics: mimicking miR-93
10. Selected examples of anti-miRNA therapeutics: targeting miR-221/222
11. Combined treatments: targeting multiple miRNAs
12. Combined treatments: co-administration of antitumor drugs and miRNA therapeutic agents
13. Combining miRNA replacement strategies with anti-miRNAs and siRNA molecules
14. Conclusion

Introduction

MicroRNAs (miRNAs or miRs) are a family of small (19-25 nucleotides in length) non-coding RNAs that have a key role in the regulation of gene expression through the inhibition or the reduction of protein synthesis following miRNA complementary sequence base pairing (1-4). A single or multiple miRNAs can be targeted at the 3' untranslated region (3'UTR),
coding oncoproteins (see the scheme depicted in Fig. 1A). miRNAs exhibiting tumor suppressor properties usually target mRNAs coding for tumor suppressor proteins, whereas miRNAs able to promote cancer target mRNAs and metastamiRNAs) or as tumor suppressor miRNAs (25,26). In general, miRNAs able to promote cancer target mRNAs coding for tumor suppressor proteins, whereas miRNAs exhibiting tumor suppressor properties usually target mRNAs coding oncproteins (see the scheme depicted in Fig. 1A). This has a very important implication in diagnosis and/or prognosis, including the recent discovery that the pattern of circulating cell-free miRNAs in serum allows us to perform molecular analyses on these non-invasive liquid biopsies with deep diagnostic and prognostic implications. This research field has confirmed that cancer-specific miRNAs are present in extracellular body fluids, and may play a very important role in the crosstalk between cancer cells and surrounding normal cells (27-32).

Interestingly, the evidence of the presence of miRNAs in serum, plasma and saliva supports their potential as an additional set of biomarkers for cancer. The extracellular miRNAs are protected by exosome-like structures, small intraluminal vesicles shed from a variety of cells (including cancer cells), with a biogenesis connected with endosomal sorting complex required for transport machinery in multivesicular bodies (29). For instance, miR-141 and miR-221-222 are predicted biomarkers in liquid biopsies from patients with colon cancer (33,34).

On the other hand, tumor-associated miRNAs are suitable targets for intervention therapeutics, as previously reported (35-44) and summarized in Fig. 1B. The inhibition of miRNA activity can be readily achieved by the use of miRNA inhibitors and oligomers, including RNA, DNA and DNA analogues (miRNA antisense therapy) (45-47), small molecule inhibitors, locked nucleic acids (LNAs) (48-53), peptide nucleic acids (PNAs) (54-57), morpholinos (58-60), miRNA sponges (61-67), mowers (68) or through miRNA masking that inhibits miRNA function by masking the miRNA binding site of a target mRNA using a modified single-stranded RNA complementary to the target sequence (69-75). On the contrary, the enhancement of miRNA function (miRNA replacement therapy) can be achieved by the use of modified miRNA mimetics, either synthetic, or produced by plasmid or lentiviral vectors carrying miRNA sequences (76-81).

Alterations in miRNA expression have been demonstrated to be associated with different human pathologies, and guided alterations of specific miRNAs have been suggested as novel approaches for the development of innovative therapeutic protocols (23,24). Studies have conclusively demonstrated that miRNAs are deeply involved in tumor onset and progression, either behaving as tumor-promoting miRNAs (oncomiRNAs and metastamiRNAs) or as tumor suppressor miRNAs (25,26). In general, miRNAs able to promote cancer target mRNAs coding for tumor suppressor proteins, whereas miRNAs exhibiting tumor suppressor properties usually target mRNAs coding oncproteins (see the scheme depicted in Fig. 1A). This has a very important implication in diagnosis and/or prognosis, including the recent discovery that the pattern of circulating cell-free miRNAs in serum allows us to perform molecular analyses on these non-invasive liquid biopsies with deep diagnostic and prognostic implications. This research field has confirmed that cancer-specific miRNAs are present in extracellular body fluids, and may play a very important role in the crosstalk between cancer cells and surrounding normal cells (27-32).

Interestingly, the evidence of the presence of miRNAs in serum, plasma and saliva supports their potential as an additional set of biomarkers for cancer. The extracellular miRNAs are protected by exosome-like structures, small intraluminal vesicles shed from a variety of cells (including cancer cells), with a biogenesis connected with endosomal sorting complex required for transport machinery in multivesicular bodies (29). For instance, miR-141 and miR-221-222 are predicted biomarkers in liquid biopsies from patients with colon cancer (33,34).

On the other hand, tumor-associated miRNAs are suitable targets for intervention therapeutics, as previously reported (35-44) and summarized in Fig. 1B. The inhibition of miRNA activity can be readily achieved by the use of miRNA inhibitors and oligomers, including RNA, DNA and DNA analogues (miRNA antisense therapy) (45-47), small molecule inhibitors, locked nucleic acids (LNAs) (48-53), peptide nucleic acids (PNAs) (54-57), morpholinos (58-60), miRNA sponges (61-67), mowers (68) or through miRNA masking that inhibits miRNA function by masking the miRNA binding site of a target mRNA using a modified single-stranded RNA complementary to the target sequence (69-75). On the contrary, the enhancement of miRNA function (miRNA replacement therapy) can be achieved by the use of modified miRNA mimetics, either synthetic, or produced by plasmid or lentiviral vectors carrying miRNA sequences (76-81).

Several miRNAs exhibit onco-suppressor properties by targeting mRNAs coding oncproteins (82-105). Therefore, these onco-suppressor miRNAs have been found to be often downregulated in tumors. For instance, Fernandez et al (106) recently described the intriguing tumor suppressor activity of miR-340, showing the miR-340-mediated inhibition of multiple negative regulators of p27, a protein involved in apoptosis and cell cycle progression. These interactions with oncprotein-coding mRNA targets determine the inhibition of cell cycle progression, the induction of apoptosis and growth inhibition. The miR-340-mediated downregulation of three post-transcriptional regulators [Pumilio RNA-binding family member (PUM)]1, PUM2 and S-phase kinase-associated protein 2 (SKP2)] correlates with the upregulation of p27. PUM1 and PUM2 inhibit p27 at the translational level, by rendering the p27 transcript available to interact with two oncomiRs (miR-221 and miR-222), while the oncprotein SKP2 inhibits the CDK inhibitor at the post-translational level by triggering the proteasomal degradation of p27, showing that miR-340 affected not only the synthesis but also the decay
of p27. Moreover their data confirm the recent identification of transcripts encoding several pro-invasive proteins such as c-Met, implicated in breast cancer cell migration, RhoA and Rock1, implicated in the control of the migration and invasion of osteosarcoma cells, and E-cadherin mRNA, involved in the miR-340-induced loss of intercellular adhesion (106 and refs within).

Recently, miR-18a was demonstrated to play a protective role in colorectal carcinoma (CRC) by inhibiting the proliferation, invasion and migration of CRC cells by directly targeting the TBP-like 1 (TBPL1) gene. The onco-suppressor activity of miR-18a in CRC tissues and cell lines was supported by the finding that the content of this mRNA is markedly lower in tumor cells with respect to normal control tissues and cells (107). In addition Xishan et al (108) found that miR-320a acts as a novel tumor suppressor gene in chronic myelogenous leukemia (CML) and can decrease the migratory, invasive, proliferative and apoptotic behavior of CML cells, as well as epithelial-mesenchymal transition (EMT), by attenuating the expression of the BCR/ABL oncogene. Furthermore Zhao et al (109) demonstrated that miR-449a functions as a tumor suppressor in neuroblastoma by inducing cell differentiation and cell cycle arrest. Finally, Kalinowski et al (110) and Gu et al (111) demonstrated the significant role of miR-7 in cancer which functions by directly targeting and inhibiting key oncogenic signaling molecules involved in cell cycle progression, proliferation, invasion and metastasis. A partial list of onco-suppressor miRNAs is presented in Table I.

3. OncomiRNAs and metastamiRNAs

miRNAs can act as oncogenes and have been demonstrated to play a causal role in the onset and progression of human cancer (oncomiRNAs) (224-233). Recent findings have nevertheless identified a subclass of miRNAs whose expression is highly associated with the acquisition of metastatic phenotypes and are referred to as miRs endowed with either metastasis-promoting or tumor suppressor inhibitory activities (213,234,235).

Recent data have revealed that miR-25 may act as an onco-miRNA in osteosarcoma, negatively regulating the protein expression of the cell cycle inhibitor, p27. In agreement with this hypothesis restoring the p27 level in miR-25-overexpressing cells was shown to reverse the enhancing effect of miR-25 on Saos-2 and U2OS cell proliferation (236). In addition a recent study published by Siu et al (237), describes miR-96 as a potential target of therapeutics for metastatic prostate cancer, demonstrating the enhanced effects in cellular growth and invasiveness of miR-96 in cell lines (AC1, AC3 and SC1) derived from prostate-specific, Pten/Tp53 double knockout mice and confirmed in tissue samples from prostate cancer patients. miR-96 acts as an oncomiR and metastamiR through TGF-β/mTOR signaling, promoting bone metastasis and contributing to a reduced survival rate in prostate cancer (237). Furthermore Xia et al (238) demonstrated that the overexpression of miR-1908 significantly decreased the expression of PTEN in glioblastoma cells, one of the most frequently mutated tumor suppressors in human cancer, resulting in an increase in proliferation, migration and invasion. Finally Sachdeva et al (239), found that miR-182 targets multiple genes in lung metastasis and regulates intravasation, thus increasing the number of circulating tumor cells (CTCs). Only the simultaneous restoration of miR-182 target genes decreased the number of metastases in vivo, demonstrating that a single miRNA can regulate the metastasis of primary tumors in vivo by the coordinated regulation of multiple genes. Selected examples of oncomiRNAs and metastamiRNAs are presented in Tables II and III. All these miRNAs act by inhibiting tumor suppressor pathways.

4. Mimicking tumor suppressor miRNAs in miRNA replacement therapy

Using the development of anticancer therapies as a representative field of investigation, the therapeutic strategy based on miRNA replacement is targeted to pathological cells which downregulate onco-suppressor miRNAs playing a role in controlling the expression of miRNAs encoding key oncoproteins. The downregulation of these oncogene-targeting miRNAs is clearly the key step for oncogene upregulation leading to tumor onset and progression. Table IV presents selected examples of miRNA replacement therapy in cancer research and treatment (90-92,94-97,99).

As a first representative example, Fig. 2A presents the major results obtained by Wu et al (97), who reported that the in vivo restoration of miR-29b may represent an option for lung cancer treatment. To demonstrate the efficacy of this strategy, they developed a cationic lipoplexes (LPs)-based carrier that efficiently delivered miR-29b both in vitro and in vivo. LPs containing miR-29b (LP-miR-29b) efficiently delivered miR-29b to A549 cells and reduced the expression of the key target, CDK6. In a xenograft murine model, in which LPS efficiently accumulated at tumor sites, the systemic delivery of LP-miR-29b increased miR-29b expression in tumors, down-regulated CDK6 mRNA expression in tumors and, as shown in the upper panels of Fig. 2A, significantly inhibited tumor growth.

A second example of miRNA replacement therapy has been published by Glover et al (304), who reported that miR-7-5p (miR-7) reduces cell proliferation in vitro and induces G1 cell cycle arrest. The systemic miR-7 administration with delivery vesicles reduced adrenocortical carcinoma (ACC) xenograft growth originating from both ACC cell lines and primary ACC cells. As far as the potential mechanisms of action, miR-7 was demonstrated to target Raf-1 proto-oncogene serine/threonine kinase (RAF1). Additionally, miR-7 therapy in vivo led to the inhibition of cyclin dependent kinase 1 (CDK1) (304). Two other methods have also been used to successfully deliver miR-7 in vivo to treat cancer. In a study by Babae et al (305), a miR-7 mimic was systemically delivered using clinically viable, biodegradable, targeted polyamide nanoparticles. This strategy led to the successful inhibition of tumor growth and vascularisation in a glioblastoma xenograft model system. In an earlier study, Wang et al (306) was able to inhibit glioma xenograft growth and metastasis using a plasmid based miR-7 vector systemically delivered by encapsulation in a cationic liposome formulation.

Moreover, Cortez et al (307) revealed a novel function of miR-200c, a member of the miR-200 family, in regulating intracellular reactive oxygen species signaling. They used a lung cancer xenograft model to demonstrate the therapeutic
| MicroRNA | Disease | Biological effects | Target mRNA/pathway | Authors/(Refs.) |
|----------|---------|---------------------|---------------------|----------------|
| miR-1   | Head and neck squamous cell carcinoma (HNSCC), prostate cancer | Inhibition of cell proliferation, invasion, migration and promotion of apoptosis and cell cycle arrest; affected cellular organization of F-actin and impaired tumor cell invasion and filopodia formation | TGLN2, FN1, LASP1, XPO6, TWIST1, EGFR | Nohata *et al* (112); Hudson *et al* (113); Chang *et al* (114) |
| miR-7   | Breast, ovarian cancer | Suppression of cell invasion and metastasis; inhibition of the ability of breast CSCs to metastasize to the brain; inhibition of tumor metastasis and reversed EMT in EOC cell lines | SETDB1, KLF4, EGFR through AKT/ERK1/2 pathway | Zhang *et al* (115); Okuda *et al* (116); Zhou *et al* (117) |
| miR-let-7 | Breast, lung, colon, ovarian cancer | Inhibition of invasion and bone metastasis; reduction of tumor growth, negative regulation of cell cycle-related oncopgenes | RAS, MYC, HMGA2, Snail | Lee and Dutta (83); Sampson *et al* (86); Trang *et al* (92); Dangi-Garimella *et al* (118); Takamizawa *et al* (119); Shi *et al* (120); Johnson *et al* (121) |
| miR-9   | Gastric cancer | Suppression of invasion metastasis | Cyclin D1, Ets1 | Zheng *et al* (122) |
| miR-15a; miR-16-1 | Chronic lymphocytic leukemia (CLL), multiple myeloma, mantle cell lymphoma, prostate cancers, gastric adenocarcinoma | Induction of apoptosis; decreased tumorigenity, evading growth suppressors, resisting cell death | Bcl-2, cyclin D1, WNT3A | Aqeilan *et al* (123); Calin *et al* (124); Pekarsky *et al* (125); Bonci *et al* (126); Kang *et al* (127) |
| miR-16 | Glioblastoma | Repression of endothelial function and angiogenesis | Bmi-1 | Chen *et al* (128) |
| miR-18a | Colorectal cancer | Decrease of cell migration, altered cell morphology, G1/S phase cell cycle arrest, increased apoptosis | CDC42 | Humphreys *et al* (129) |
| miR-25 | Prostate cancer | Inhibition of extravasation *in vivo* | αv, α6 integrins | Zoni *et al* (130) |
| miR-27a | Acute leukemia | Inhibition of cell growth due at least in part, to increased cellular apoptosis | Bax and Bad | Scheibnet *et al* (94) |
| miR-29c | Nasopharyngeal carcinoma | Inhibition of invasion and metastasis | Collagens, Laminin γ1 | Sengupta *et al* (131) |
| miR-29s (miR-29a, miR-29b1, miR-29b2, miR-29c) | Lung cancer, cervical carcinogenesis, cholangiocarcinoma, hepatocellular carcinoma (HCC), mantle cell lymphoma (MCL), melanoma and acute myeloid leukemia (AML) B and T cells | Decrease in cell proliferation and an increase in cell senescence and apoptosis; decreased AML cell growth and impairment of colony formation, longer survival of treated mice; improvement of anti-leukemic activity of decitabine | CDK6, Ppm1d, osteonectin, Mcl-1, KIT, SP1, Bcl-2, DNMT3A, DNMT3B, DNMTs, Tel-1, extracellular matrix genes, FLT3, Cdc42, p85α | Ugalde *et al* (132); Garzon *et al* (133); Garzon *et al* (134); Huang *et al* (98); Kapinas *et al* (135); Mott *et al* (136); Fabbrini *et al* (137); Xiong *et al* (138); Filkowski *et al* (139); Wang *et al* (140); Hu *et al* (141) |
| miR-30b | Laryngeal carcinoma | Antitumor and pro-apoptotic effect *in vivo* and *in vitro* | p53 via MDM2 | Li and Wang (142) |
| miR-31 | Breast cancer, lung adenocarcinoma (stem cells) | Inhibition of multiple steps of metastasis, including invasion, anoikis and colonization | MET-PI3K-Akt, WAVE3 | Hou *et al* (143); Valastyan *et al* (144); Sossey-Alaoui *et al* (145) |
| MicroRNA | Disease                                                                 | Biological effects                                                                                          | Target mRNA/pathway                                                                                     | Authors/(Refs.)                                                                                   |
|----------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| miR-33a  | Chronic myelogenous leukemia (CML), colon carcinoma                    | Decelerated cell proliferation; reduced tumor cell proliferation                                          | Pim-1                                                                                                     | Thomas et al (95); Ibrahim et al (91)                                                             |
| miR-33b  | Breast cancer lung metastasis, osteosarcoma                            | Inhibition of stemness, migration, invasion and metastasis                                                 | HMGA2, SALL4, Twist1, c-MYC                                                                          | Lin et al (146); Xu et al (147)                                                                    |
| miR-34a  | Breast, lung, colon, kidney, prostate, bladder, pancreatic, bone and lung cancer, and melanoma | Blocking of tumor growth; inhibition of cell migration, invasion and metastasis of cancer cells; suppression of prostate CSCs and metastasis; decrease in the production of the chemokine CCL22; disturbance of the bone metastatic niche | Bcl-2, cyclin D1, cyclin E2, CDK4, CDK6, c-MYC, MET, N-MYC, SIRT1, Fra-1, CD44, CCL44, Tgif2 | He et al (148); Bommer et al (149); Fujita et al (150); Leucci et al (151); Saito et al (152); Wei et al (153); Yamakuchi et al (154); Lodygin et al (155); Wiggins et al (90); Yang et al (156); Yang et al (157); Liu et al (158); Krzeszinski et al (159) |
| miR-34b  | Breast, ovarian, endometrial cancer                                    | Tumor suppressor in estrogen-dependent cell growth                                                        | Cyclin D1 and JAG1 in ER+/wild-type p53                                                               | Lee et al (102); Wang et al (160)                                                                   |
| miR-34c  | Breast, ovarian cancer, lung metastasis                                | Inhibition of cell migration; invasion and lung metastasis                                                | Fra-1                                                                                                     | Yang et al (156); Yu et al (161)                                                                   |
| miR-101-3p | Salivary gland adenoid cystic carcinoma                               | Suppression of cell proliferation, invasion and enhanced chemotherapeutic sensitivity                    | Pim-1                                                                                                     | Liu et al (162)                                                                                   |
| miR-122a | Liver tumor and disease                                                | Reduced disease manifestation and tumor incidence                                                         | Klf6                                                                                                      | Tsai et al (163)                                                                                  |
| miR-124  | Intrahepatic, bladder, colorectal and lung cancer, osteosarcoma, neuroblastoma, glioma | Modulation of the intercellular adhesion of leading cells; inhibition of EMT in vitro and suppression of intrahepatic and pulmonary metastasis in vivo; suppression of motility and angiogenesis in bladder cancer cells, of migration and invasion of U-2OS and Saos-2 cells | Integrin β1, ROCK2, EZH2, UHRF1, ROR2, MYO10, DNM13B, PTB/PKM1, PKM2 cascade | Taniguchi et al (164); Huang et al (165); Kato et al (166); Zheng et al (167); Wang et al (168); Zhang et al (169); Sun et al (170); Sun et al (171); Chen et al (172); Zhang et al (173) |
| miR-125a | Cervical cancer                                                       | Suppression of tumor growth, invasion, metastasis                                                        | ARID3B, STAT3                                                                                             | Cowden Dahl et al (174); Fan et al (175)                                                          |
| miR-126  | Non-small cell lung cancer cells, breast, thyroid, liver, colorectal cancer, osteosarcoma | Tumor suppressor genes involved in the control of cell proliferation and cell death, cell migration and blood vessel formation; inhibition of cell proliferation, invasion, migration and tumorigenesis; suppression of tumor metastasis and angiogenesis in hepatocellular carcinoma | EGFL7, SLC7A5, ADAM9, IGFBP2, PTPN1C, MERTK, SDF-1α                                                | Sun et al (176); Xiong et al (177); Wang et al (178); Wen et al (179); Jiang et al (180); Du et al (181); Zhang et al (182); Png et al (183) |
| miR-128  | Glioblastoma, hepatocellular carcinoma, acute lymphoblastic leukemia | Inhibition of angiogenesis and proliferation, inhibition of tumor cell progression                        | WEE1, p70S6K1, Msi1, E2F3a, Bmi-1, EGFR, PDGFRα, PIK3R1                                                | Shi et al (184); Wuchty et al (185); Zhang et al (186); Huang et al (187)                         |
Table I. Continued.

| MicroRNA | Disease | Biological effects | Target mRNA/pathway | Authors/(Refs.) |
|----------|---------|--------------------|---------------------|-----------------|
| miR-133a; miR-133b | Esophageal squamous cell carcinoma | Inhibition of cell proliferation and cell invasion | FSCN1 | Kano et al (188) |
| | | | | |
| miR-135a | Prostate cancer | Inhibition of cell invasion and migration | ROCK1, ROCK2 | Kroiss et al (189) |
| miR-137 | Colorectal cancer | Reduction of invasiveness | FMNL2 | Liang et al (190) |
| miR-143 | Non-small cell lung cancer | Suppression of cell proliferation; inhibition of cell migration and invasion; induction of apoptosis | Lm1k1 | Xia et al (191) |
| miR-145 | Esophageal squamous cell carcinoma, colon carcinoma, gastric cancer, neuroblastoma | Inhibition of cell proliferation and cell invasion; reduced tumor proliferation and increased apoptosis; attenuation of gastric cancer cell migratory and invasive abilities in vitro and suppression of the metastatic cascade in vivo; inhibition of the invasion and metastasis of neuroblastoma cells | FSCN1, c-MYC, ERK5, N-cadherin, HIF-2α | Kano et al (188); Ibrahim et al (91); Gao et al (192); Zhang et al (193) |
| miR-146a/b | Prostate, breast cancer | Inhibition of cell invasion and migration | IRAK1, TRAF6, ROCK1 | Bhaumik et al (194); Lin et al (195) |
| miR-148a | Liver, lung cancer | Inhibition of hepatoma cell migration in vitro and pulmonary metastatic colonization in vivo | MET/Snail signaling | Zhang et al (196) |
| miR-148b | Breast cancer | Inhibition of multiple steps of tumor progression via the regulation of invasion, resistance to anoikis, extravasation, lung metastasis, colonization and chemotherapeutic response | ITGA5, ROCK1, PIK3CA/p110α, NRAS, CSF1 | Cimino et al (197) |
| miR-149 | Breast, lung cancer | Inhibition of basal-like breast cancer cell migration and invasion in vitro; impairment of lung colonization in vivo | Rap1a, Rap1b | Bischoff et al (198) |
| miR-181b | Chronic lymphocytic leukemia | Inhibition of disease progression | Mcl-1, Bcl-2 | Visone et al (199) |
| miR-182 | Glioblastoma | Inhibition of cell growth and cell differentiation | Bcl-2L12, c-MET, HIF2A | Kouri et al (200) |
| miR-193b | Breast cancer, pancreatic ductal adenocarcinoma | Alteration of ERα signaling, such as steroid synthesis and downregulation of the ERα receptor; negative regulation of long non-coding oncogenic RNA | AKR1C2, AKR1C1, YWHAZ (14-3-3 family protein), RNA MIR31HG | Leivonen et al (201); Yang et al (202) |
| miR-198 | Hepatocellular carcinoma | Inhibition of migration and invasion | HGF/c-MET | Tan et al (203) |
| miR-204 | Neuroblastoma, glioma | Stimulation of increased sensitivity to cisplatin treatment and promotion of cell survival; alteration of glioma progression, invasion and migration | TrkB | Bao et al (204); Xia et al (205) |
| miR-205 | Human prostate cancer | Reduction of cell migration/invasion through downregulation of protein kinase C epsilon | CHN1, ErbB3, E2F1, E2F5, ZEB2, PRKCE | Gandellini et al (206) |
potential of the systemic delivery of miR-200c to enhance radiosensitivity in lung cancer. The results obtained suggest that the antitumor effects of miR-200c result partially from its regulation of the oxidative stress response; they further suggested that miR-200c, in combination with radiation, may represent an effective therapeutic strategy in the future.

Recently, Wu et al. (308) reported that the expression of miR-708-5p suppressed lung cancer invasion and metastasis in vitro and in vivo. In particular, it induces apoptosis and suppresses cell migration by inhibiting the cytoplasmic localization of p21, and also weakens the stem cell-like properties of lung cancer cells. In their study, they present the systemic delivery of the PEI/miR-708-5p complexes for miRNA replacement therapy in a mouse model of lung cancer, demonstrating an efficient antitumor activity with no side-effects.

Table I. Continued.

| MicroRNA    | Disease                                           | Biological effects                                                                 | Target mRNA/ pathway | Authors/(Refs.) |
|-------------|---------------------------------------------------|------------------------------------------------------------------------------------|----------------------|-----------------|
| miR-206     | Breast cancer                                     | Inhibition of cell invasion and migration                                          | MET                  | Chen et al (207) |
| miR-214     | Colorectal cancer, liver metastasis               | Suppression of cell migration in vitro; inhibition of liver metastasis of colorectal cancer cells in vivo | FGFR1                | Chen et al (208) |
| miR-218     | Gastric cancer                                    | Suppression of tumor metastases                                                  | ROBO1               | Tie et al (209)  |
| miR-296-5p  | Prostate cancer                                   | Reduction of growth invasion and progression                                       | HMGA1               | Wei et al (210)  |
| miR-302     | Breast cancer                                     | Sensitization of radioresistant breast cancer cells to ionizing radiation         | AKT1, RAD52         | Liang et al (99) |
| miR-302b    | Hepatocellular carcinoma                          | Suppression of cell proliferation                                                | EGFR                 | Wang et al (211) |
| miR-335     | Breast cancer                                     | Inhibition of cell invasion, migration and metastasis                             | SOX4, PTPRN2, MERTK, TNC | Tavazoie et al (212); Hurst et al (213) |
| miR-383     | Medulloblastoma                                    | Control of cell growth                                                            | PRDX3                | Li et al (214)   |
| miR-449     | Gastric cancer, non-small cell lung cancer         | Inhibition of cell proliferation, inhibition of migration and invasion            | GMNN, MET, CCNE2, SIRT1 | Bou Kheir et al (215) |
| miR-493     | Colon, lung cancer                                 | Inhibition of the settlement of metastasized colon cancer cells in the liver; promotion of the death of colon cancer cells; suppression of tumor growth, invasion and metastasis in lungs | IGFR, E2F1, MKK7 | Okamoto et al (217); Gu et al (218); Sakai et al (219) |
| miR-504     | Hypopharyngeal squamous cell carcinoma             | Inhibition of cancer cells proliferation                                          | CDK6                 | Kikkawa et al (220) |
| miR-520c/373 | Breast cancer                                     | Inhibition of cell invasion in vitro and the cell intravasation in vivo           | RELA, TGFRB2        | Keklikoglou et al (221) |
| miR-545     | Pancreatic ductal adenocarcinoma, lung cancer cells | Inhibition of cell growth and proliferation                                        | RIG-1, CDK4         | Song et al (222); Bowen et al (223) |
| miR-596     | Oral squamous cell carcinoma (OSCC)                | Growth inhibition                                                                  | LGALS3BP            | Endo et al (96)  |

5. Targeting oncomiRNAs

The effects of therapeutic molecules against miRNAs have been the object of very recent studies, in part summarized in Table V (309-316). Of course, the endpoint of the treatment of target cells with molecules against selected miRNAs is the alteration of miRNA-regulated genes. As a first example, Wagenaar et al (317) developed potent and specific single-stranded oligonucleotide inhibitors of miR-21 and used them to verify dependency on miR-21 in a panel of liver cancer cell lines. Treatment with anti-miR-21, but not with a mismatch control anti-miRNA, resulted in the significant derepression of direct targets of miR-21 and led to the loss of viability in the majority of HCC cell lines tested. The robust induction of caspase activity, apoptosis and necrosis was noted in the
### Table II. miRNAs exhibiting oncogenic functions.

| MicroRNA | Disease | Biological effects | Target mRNA/pathway | Authors/(Refs.) |
|----------|---------|---------------------|----------------------|-----------------|
| miR-10b | Human esophageal cancer cells, gastric carcinoma | Promotion of migration and invasion | KLF4 | Tian et al (240); Wang et al (241) |
| miR-21 | Breast, colon, pancreatic, lung, prostate, liver and stomach cancer, chronic lymphocytic leukemia; acute myeloid leukaemia, glioblastoma, neuroblastoma | Stimulation of cellular proliferation; action on mitochondrial apoptosis; tumor-suppressive pathways, resisting cell death | PTEN, TPM1, PDCD4, p63, RECK, p53, TGF-β | Chan et al (242); Zhu et al (230); Frankel et al (231); Volinia et al (233) |
| miR-23b | Renal cancer cells | Downregulation of POX (tumor suppressor), increase in HIF signaling | POX | Liu et al (243) |
| miR-27a | Prostate cancer | Increase in the expression of AR target genes and prostate cancer cell growth | PHB | Fletcher et al (244) |
| miR-100 | Myeloid leukemia, glioma | Promotion of cell differentiation, survival and apoptosis | RBSP3, ATM | Ng et al (245); Zheng et al (246) |
| miR-125b | B-cell leukemia | Induction of cell differentiation and transformation | MAP3K11, ARID3B | Knackmuss et al (247) |
| miR-132 | Pancreatic adenocarcinoma (PDAC) | Stimulation of cell proliferation via the β2 adrenergic pathway | Rb1 | Park et al 2011 (248) |
| miR-155 | Lymphoma, leukemia, breast, colon, lung, pancreatic, thyroid brain cancer, diffuse large B-cell lymphoma (DLBCL) | Causes the constitutive activation of signal transducer and activator of transcription 3, sustaining proliferative signaling, resistance of cell death, activation invasion, migration and metastasis | SOCS1, RhoA, FOXO3a, VHL | Kong et al (249); Jiang et al (250); Czyzyk-Krzeska et al (251); Wang et al (252); Ling et al (253); Musilova et al (254) |
| miR-17 | Neuroblastoma | Marked increase of in vitro and in vivo tumorigenesis | p21, BIM | Fontana et al (255) |
| miR-182 | Melanoma | Promotion of melanoma metastases | MITF, FOXO3 | Segura et al (256) |
| miR-214 | Ovarian cancer | Stimulation of cell survival and cisplatin resistance | PTEN | Yang et al (257) |
| miR-221 | Atypical teratoid/rhabdoid tumors (ATRT), osteosarcoma, glioma, breast cancer, follicular thyroid carcinoma (FTC), digestive system carcinoma | Decrease of cell cycle inhibitor p27^{kip1}, tumor development and progression by regulating proliferative signaling pathways, altering telomere and telomerase activity, avoiding cell death from tumor suppressors, autophagy and apoptosis, monitoring angiogenesis, supporting epithelial-mesenchymal transition, and even controlling cell-specific function within the microenvironment | p27^{kip1}, PTEN, KIT, TRPS1, PUMA, PTPt, FOXO3, PIK3R1, TIMP3, TIMP2, DDI4, MDM2, Erα, SOCS3, OCS1, HDAC6, ANGPTL2, BBC3, BMF, RECK, PDLIM2, RelA, p57^{kip2} | Zhang et al (258); Garofalo et al (259); Quintavalle et al (260); Chen et al (261); Matsuzaki et al (262) |
| miR-296 | Brain tumors | Promotion of angiogenesis | HGS | Wurdinger et al (263) |
| miR-301 | Breast cancer | Promotion of growth, proliferation, invasion and metastases | FOXF2, BBC3, PTEN | Shi et al (264) |
| miR-372 | Testicular tumors | Promotion of tumorigenesis in cooperation with RAS | LATS2 | Voorhoeve et al (265) |
| miR-373 | Gastric cancer | Promotion of carcinogenesis | JAK2, PDK1 | Xu et al (266) |
Table II. Continued.

| MicroRNA  | Disease                        | Biological effects                                                                 | Target mRNA/ pathway | Authors/(Refs.)            |
|-----------|--------------------------------|------------------------------------------------------------------------------------|----------------------|---------------------------|
| miR-378   | Breast carcinoma               | Enhancement of cell survival; reduction of caspase-3 activity; promotion of growth and angiogenesis | Sufu, Fus-1          | Lee et al (267)           |
| miR-519a  | Hepatocellular carcinoma, breast cancer | Promotion of tumor growth, proliferation; inhibition of apoptosis; tamoxifen resistance | PTEN/PI3K, AKT/FOXF2  | Tu et al (268); Shao et al (269); Ward et al (270) |
| miR-675   | Colorectal cancer              | Overexpression of H19 (oncofetal non-coding RNA) in cancer tissues                  | RB                   | Tsang et al (271)         |
| miR-1908  | Glioblastoma                   | Promotion of anchorage independent growth in vitro, increasing of tumor forming potential in vivo | PTEN                | Xia et al (238)           |

Figure 2. (A) miRNA replacement therapy: partial list of tumor suppressor miRNAs (in the blue box) and selected examples of the in vivo restoration of miR-29b (97) and of miR-30b (142), leading to the inhibition of tumor cell growth. (B) Targeting oncomiRNAs and metastamiRNAs with antagomiRNAs: partial list of onco/metastamiRNAs and a selected example of the antitumor effects of antagomiR-17-5p (255).
### Table III. miRNAs promoting metastasis.

| MicroRNA  | Disease                          | Biological effects                                                                 | Target mRNA/pathway                                                                 | Authors/(Refs.)                      |
|-----------|----------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------|
| miR-9     | Breast, colon cancer             | Promotion of breast cancer cell motility and invasiveness; enhancement of squamous cell carcinoma CSC expansion and metastasis | CDH1, LIFR, α-catenin                                                              | Ma et al (272); Chen et al (273); White et al (274) |
| miR-10b   | Breast cancer, glioblastoma      | Promotion of EMT, migration, invasion and metastasis                                | TP53, PAX6, NOTCH1, HOXD10                                                         | Ma et al (275); Lin et al (276)       |
| miR-15b   | Pancreatic cancer                | Promotion of EMT                                                                     | SMURF2                                                                              | Zhang et al (277)                     |
| miR-19a/b | Gastric cancer                   | Facilitation of cell migration, invasion and metastasis                              | MXD1                                                                                | Wu et al (278)                        |
| miR-20a   | Cervical, gallbladder cancer      | Facilitation of cancer cell proliferation and metastasis \* \* \* in vitro and increased tumor growth in vivo; induction of EMT | ATG7, TIMP2, Smad7                                                                | Chang et al (279); Zhao et al (280)  |
| miR-21    | Breast, lung, brain, cervical and colorectal cancer, melanoma | Drive to epithelial collective cell migration, invasion, cell metastasis and apoptosis; enhancement of colorectal cell intravasion | TPM1, PDCD4, Maspin (SERPINB5), PTEN, PI3K, Sprouty, p53, cyclin D1, FOXO1, FBXO11, TIPE2, MSH2, hTERT, HIF1α, TIMP3, APAF1 | Zhu et al (230); Dean et al (281); Peacock et al (282); Xu et al (283); Asangani et al (284); Hurst et al (213); Melnik et al (285) |
| miR-96    | Prostate cancer                  | Bone metastasis, enhanced effects on cellular growth and invasiveness                | TGF-β/mTOR signaling                                                               | Siu et al (237)                       |
| miR-105   | Breast cancer                    | Destruction of the integrity of vascular endothelial barriers to promote metastasis  | ZO-1                                                                                | Zhou et al (286)                      |
| miR-122   | Breast cancer                    | Promotion of metastic colonization                                                    | PKM2                                                                                | Fong et al (287)                      |
| miR-135b  | Lung cancer                      | Promotion of cell migration, invasion and metastasis                                 | LATS2, TrCP, NDR2, LZTST1                                                           | Lin et al (288)                       |
| miR-181a  | Breast cancer                    | Promotion of breast cancer metastasis                                                | Bim/TGF-β                                                                           | Taylor et al (289)                    |
| miR-182   | Gallbladder, sarcoma, lung cancer| Promotion of metastasis, circulating tumor cells (CTC); regulation of invasion       | CADM1, RSU1, MTSS1, PA11, TIMP1                                                    | Qiu et al (290); Sachdeva et al (239) |
| miR-183   | Oesophageal carcinoma            | Promotion of proliferation and invasion                                              | PDCD4                                                                               | Ren et al (291)                       |
| miR-200s  | Breast, ovarian cancer           | Activation of invasion and metastasis (but in other cases inhibition)                | ZEB1, ZEB2, SIP1, Sec23a                                                           | Korpal et al (292); Korpal et al (293); Park et al (294); Gregory et al (295) |
| miR-214   | Lung adenocarcinoma, melanoma    | Promotion of migration, invasion and resistance to anoikis of melanoma cells \* \* \* in vitro and the extravasation and lung metastasis formation \* \* \* in vivo; promotion of EMT and metastasis | TFAP2C, Sufu                                                                      | Penna et al (296); Penna et al (297); Long et al (298) |
| miR-296-3p| Prostate cancer                  | Promotion of metastasis                                                              | ICAM1                                                                               | Liu et al (299)                       |
| miR-296-5p| Prostate cancer                  | Promotion of growth and invasion, metastatic progression, and persistence of cancer-initiating cells | Numbl (Klf4 signaling)                                                             | Vaira et al (300)                     |
anti-miR-21-treated HCC cells. Furthermore, the ablation of miR-21 activity resulted in the inhibition of HCC cell migration and in the suppression of clonogenic growth (317).

In another study, using PNAs as anti-miRNA molecules, Fabani et al (318) targeted miR-155, demonstrating the deregulation of mRNA Bat5, Sfp1 and Jarid2. In our laboratory, Brognara et al analyzed the effects of PNAs targeting miR-221 on breast cancer cells (319). In order to maximize uptake in target cells, a polyarginine-peptide (R8) was conjugated, generating an anti-miR-221 PNA displaying very high affinity for RNA and efficient uptake within target cells without the need for transfection reagents. Targeting miR-221 with this PNA molecule resulted in i) a specific decrease in the hybridization levels of miR-221 measured by RT-qPCR, ii) the upregulation of

| MicroRNA   | Disease                                  | Biological effects                                                                 | Target mRNA/pathway | Authors/(Refs.) |
|------------|------------------------------------------|------------------------------------------------------------------------------------|---------------------|-----------------|
| miR-362-5p | Hepatocellular carcinoma                  | Promotion of cell proliferation, migration, invasion \textit{in vitro}; and tumor growth and metastasis \textit{in vivo} | CYLD                | Ni et al (301)  |
| miR-373    | Breast cancer                             | Drives EMT and metastasis                                                          | TXNIP               | Chen et al (302) |
| miR-520c   | Fibrosarcoma, benign prostatic hyperplasia, glioblastoma | Promotion of migration and metastasis                                              | MT1-MMP             | Lu et al (303)  |

Table III. Continued.

| Target mRNA | MicroRNA Disease | Biological effects | Target mRNA/pathway | Authors/(Refs.) |
|-------------|------------------|--------------------|---------------------|-----------------|
| miR-362-5p  | Hepatocellular carcinoma | Promotion of cell proliferation, migration, invasion \textit{in vitro}; and tumor growth and metastasis \textit{in vivo} | CYLD                | Ni et al (301)  |
| miR-373    | Breast cancer                             | Drives EMT and metastasis                                                          | TXNIP               | Chen et al (302) |
| miR-520c   | Fibrosarcoma, benign prostatic hyperplasia, glioblastoma | Promotion of migration and metastasis                                              | MT1-MMP             | Lu et al (303)  |

Table IV. miRNA replacement therapy of cancer: selected examples.

| Tumor type            | miRNA target | Modulated mRNA | Effects following miR treatment | Authors/(Refs.) |
|-----------------------|--------------|----------------|-------------------------------|-----------------|
| Lung cancer           | miR-34a      | Repression of c-Met, Bcl-2; partial repression of CDK4 | Block of tumor growth            | Wiggins et al (90) |
| Colon carcinoma       | miR-33a      | Pim-1          | Reduced tumor proliferation    | Ibrahim et al (91) |
| Colon carcinoma       | miR-145      | c-Myc and ERK5  | Reduced tumor proliferation and increased apoptosis | Ibrahim et al (91) |
| Lung cancer           | miR-let7     | Negative regulation of the cell cycle oncogenes \textit{RAS, MYC and HMGA2} | Reduction of tumor growth         | Trang et al (92) |
| Acute leukemia        | miR-27a      | Bax and Bad    | Inhibition of cell growth due, at least in part, to increased cellular apoptosis | Scheibner et al (94) |
| CML cells             | miR-33a      | Pim-1          | Decelerated cell proliferation | Thomas et al (95) |
| Oral squamous cell carcinoma (OSCC) | miR-596 | LGALS3BP       | Growth inhibition              | Endo et al (96)  |
| Non-small cell lung adenocarcinomas, A549 cells | miR-29b | CDK6, DNMT3B, MCL-1 | Inhibition of tumorigenicity \textit{in vivo} | Wu et al (97) |
| Acute myeloid leukemia | miR-29b     | Downregulation of DNMTs, CDK6, SP1, KIT and FLT3 | Decreased AML cell growth and impairment of colony formation; longer survival of treated mice; improvement of antileukemic activity of decitabine | Huang et al (98) |
| Laryngeal carcinoma   | miR-30b      | p53 via MDM2   | Antitumor and pro-apoptotic effect \textit{in vivo and in vitro} | Li and Wang (142) |
| Breast cancer         | miR-302      | AKT1 and RAD52 | Sensitized radioresistant breast cancer cells to ionizing radiation | Liang et al (99) |
p27Kip1 mRNA and protein expression, measured by RT-qPCR and western blot analysis, respectively. As regards the in vitro effects of anti-miRNA therapy, Yan et al (320) addressed the potential effects of PNA-anti-miR-21 in vivo on the growth of breast cancer cells. In their experiments, MCF-7 cells treated with PNA-anti-miR-21 or PNA-control were subcutaneously injected into female nude mice and detectable tumor masses were observed in few mice in the MCF/PNA-anti-miR-21 group, while much larger tumors were detected in all mice in the MCF/PNA-control group. Both tumor weight and number showed that MCF/PNA-control cells formed larger tumors more rapidly than MCF/PNA-anti-miR-21 cells in nude mice.

As a final example, Cheng et al (57) demonstrated that the PNA anti-miRs with a peptide with a low pH-induced transmembrane structure (pHLIP) target the tumor microenvironment, transport anti-miRs across plasma membranes under acidic conditions, such as those found in solid tumors and effectively inhibit the miR-155 oncomiR in a mouse model of lymphoma.

### 6. MicroRNAs and epithelial-mesenchymal transition

EMT is a powerful process in tumor invasion, metastasis and tumorigenesis, and describes the molecular reprogramming and phenotypic changes that are characterized by a transition from polarized immotile epithelial cells to motile mesenchymal cells (Fig. 3). This process is characterized by the loss of polarity and cell-cell contacts by the differentiated epithelial cells, with deep alterations occurring at the level of tight junctions and desmosomes. The breach of the basement membrane is a following step, leading to the invasion of blood and/or lymphatic vessels by these mesenchymal differentiated cancer cells, which at the end of the process, causes migration, often accompanied by drug resistance (Fig. 3). It is now well-known that several miRNAs are important regulators of EMT. Some of these are miR-7, miR-17/20, miR-22, miR-30, miR-200 and its family members. Most of these miRNAs potentiate EMT, while some well-characterized miRNAs play a suppressive role in EMT. For instance, the metastasis suppressor role of the miR-200 members is strongly associated with the inhibition of EMT. This is well described in the published review by Zhang and Ma (321), and in the studies by Zaravinos et al (322) and Kiesslich et al (323), showing the most recent advances regarding the influence of miRNAs in EMT and the regulatory effects they exert on major signaling pathways in various types of cancer (Fig. 3). In Caski cervical cancer cells, the oncomiR-155 acts as a tumor suppressor and suppresses EGF-induced EMT, decreasing migration/invasion capacities, inhibiting cell proliferation and enhancing the chemosensitivity to DDP in humans (324). Chang et al (279) demonstrated that the overexpression of miR-20a in gallbladder carcinoma cells induced EMT and promoted metastasis via the direct inhibition of Smad7, correlating this miRNA with local invasion, distant metastasis and a poor prognosis in patients with gallbladder carcinoma.

In the ovarian surface epithelium, EMT is considered the key regulator of the post-ovulatory repair process and it can be triggered by a range of environmental stimuli. The aberrant expression of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) in ovarian cancer, and its involvement in the initiation and progression of ovarian cancer have been well demonstrated. The miR-200 family members seem to be strongly associated with EMT and to have a
metastasis suppressor role. miRNA signatures can accurately distinguish ovarian cancer from the normal ovary and can be used as diagnostic tools to predict the clinical response to chemotherapy. Recent evidence suggests a growing list of novel miRNAs (miR-187, miR-34a, miR-506, miRNA-138, miR-30c, miR-30d, miR-30e-3p, miR-370 and miR-106a, among others) that are also implicated in ovarian cancer-associated EMT, either enhancing or suppressing it. MicroRNA-based gene therapy provides a prospective antitumor approach for integrated cancer therapy (325).

As regards the molecular targets of EMT-regulating miRNAs, several are known and validated. Among these, transcription factors play a very important role. For instance, Gao et al (326) identified SOX2 as a key player in EMT, by examining the effects of its overexpression. They demonstrated that SOX2-overexpressing Eca-109 cells exhibited an enhanced cell migration/invasion capacity. Moreover, these cells exhibited characteristics of EMT, that is, a significantly suppressed expression of the epithelial cell marker with a concomitant enhancement in the expression of mesenchymal markers. An increased expression of Slug in SOX2-overexpressing cells suggested the involvement of this transcription factor in SOX2-regulated metastasis. Finally, the expression levels of STAT3/HIF-1α were found to be upregulated in SOX2-expressing cells, and the blockade of these transcription factors resulted in the inhibition of Slug expression at both the protein and mRNA level.

Of interest, is also the finding that miR-221/222, which are involved in EMT as positive regulators, can be transcriptionally controlled by Slug. This was demonstrated by Lambertini et al (327), who showed that Slug silencing significantly decreased the level of miR-221, strongly suggesting that miR-221 is a Slug target gene. This was further confirmed by the characterization of a specific region of the miR-221 promoter that is transcriptionally active and is bound by the transcription factor Slug in vivo.

On the other hand, various miRNAs have been reported to directly target EMT-promoting transcription factors. For instance Qiu et al (328) found that miR-139-5p functions as a suppressor of EMT in HCC and metastasis by targeting ZEB1 and ZEB2, and that it may be a therapeutic target for metastatic HCC. In conclusion, miRNAs targeting and miRNA mimicking strategies are both expected to be suitable for the control of EMT.

7. MicroRNAs and neoangiogenesis

A very important step in tumor dissemination and metastasis is neoangiogenesis. This is a very complex process in which several proteins and protein networks participate, for instance...
interleukin (IL)-8, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietins and matrix metalloproteinases (MMPs). As far as the expression of the IL-8 gene is concerned, the increase in IL-8 gene expression from the healthy brain to low-grade glioma (LGG) can be explained by alterations in the regulatory networks associated with IL-8 gene transcription. Among these, the nuclear factor-kB (NF-kB) network should be proposed, as i) NF-kB is one of the major transcription factors involved in IL-8 gene regulation (329); ii) NF-kB is a marker of glioma onset and progression (330-333); iii) miR-16 inhibits glioma cell growth through the suppression of the NF-kB signaling pathway (334). In addition to transcription factors, miRNAs can directly modulate pro-angiogenic factors. For instance, the increased IL-8 gene expression in high-grade glioma (HGG; with respect to LGG) may be associated with decrease of its inhibitory miRNA, miR-93, at least in a subset of HGG patients. The decrease in miR-93 expression in these HGG patients, in addition to IL-8, may lead to the post-transcriptional upregulation of VEGF, monocyte chemoattractant protein-1 (MCP-1) and platelet-derived growth factor (PDGF)-bb, well recognized markers of the late tumor stages of gliomas (335-337). However, it should be mentioned that HGG samples are highly heterogeneous with respect to miR-93 levels, suggesting the involvement of multiple regulatory pathways in controlling the level of IL-8 gene expression.

8. Selected examples of miRNA therapeutics: mimicking miR-124

One of the better described examples of tumor suppressor miRNAs is miR-124. This miRNA has been found to play a significant role in several types of cancer (168-173,338). Specifically, miR-124 expression is reportedly downregulated in the cells and tissues of esophageal cancer (339), breast cancer (340), renal cell carcinoma (341) and CRC (172). Accordingly, the ectopic expression of miR-124 by target tumor cells inhibits tumor-related parameters in experimental model systems mimicking prostate cancer, medulloblastoma, hepatocellular carcinoma, gastric cancer, glioma, osteosarcoma and CRC.

For instance, Taniguchi et al (164) recently demonstrated that the ectopic expression of miR-124 induced apoptosis and autophagy in colon cancer cells. In addition, miR-124 was demonstrated to target poly(pyrimidinyl)act-binding protein 1 (PTB1), which is a splice of pyruvate kinase muscles 1 and 2 (PKM1 and PKM2), and to induce the switching of PKM isoform expression from PKM2 to PKM1 (164). In addition to this study, Lu et al (342) demonstrated that miR-124a expression was downregulated in human glioma tissues, and that its expression level negatively correlated with the pathological grade of the glioma. The restoration of miR-124a inhibited glioma cell proliferation and invasion in vitro.

Furthermore, they found that miR-124a directly targeted and suppressed IQ motif containing GTPase activating protein 1 (IQGAP1), a well-known regulator of actin dynamics and cell motility (342). Taken together all these data clearly demonstrate that miR-124a is an important tumor suppressor miRNA which is downregulated in cancer cells; accordingly antitumor effects can be achieved following the administration of miR-124, pre-miR-124 or a variety of miR-124 mimics to cancer cells.

Finally, the translational relevance of the role of miR-124 in antitumor drug sensitivity is suggested by the finding that the increased miR-124 expression correlates with an improved breast cancer prognosis, specifically in patients receiving chemotherapy. This finding suggests that miR-124 may potentially be used as a therapeutic agent to improve the efficacy of chemotherapy, including that based on DNA-damaging agents via ATM interactor (ATMIN)- and poly(ADP-ribose) polymerase 1 (PARP1)-mediated mechanisms (343).

9. Selected examples of miRNA therapeutics: mimicking miR-93

A second example of possible miRNA replacement therapy is based on the inhibition of IL-8 and VEGF by the transfection of tumor target cells with pre-miR-93. This was performed in human glioma cell lines (U251 and T98G), as well as on the SK-N-AS neuroblastoma cell line.

The first conclusion of this research activity is that the miRNA, miR-93, is involved in the control of the expression of the IL-8 gene in the glioma U251 and in the neuroblastoma SK-N-AS cell lines (344,345). The effects of these treatments were analyzed by RT-qPCR (looking at the IL-8 mRNA content) or by Bio-plex analysis (looking at IL-8 protein secretion). In addition, Fabbri et al (344) found that the transfection of target cells with pre-miR-93 led to the downregulation of VEGF (see the results depicted in Fig. 4A), suggesting that, as shown in Fig. 4B, miR-93 has effects on the growth of gliomas [by interfering with growth factors, including PDGF, fibroblast growth factor (FGF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF)], as well as on neoangiogenesis.

10. Selected examples of anti-miRNA therapeutics: targeting miR-221/222

Gliomas, as other tumors, express miR-221 at high levels, promoting malignant progression through activation of the Akt pathway and the inhibition of p27Kip1 (346-349). In addition miR-221 mediates the downregulation of other genes, such as PUMA (258), intercellular adhesion molecule 1 (ICAM-1) (350), TIMP metallopeptidase inhibitor 3 (TIMP3) (351) and phosphatase and tensin homolog (PTEN) (352), and may thus be associated with cancer onset and progression (353). Therefore, miR-221 appears to be a specific target for the treatment of gliomas (354,355). Zhang et al (354) reported that the co-suppression of the miR-221/222 cluster suppressed human glioma cell growth by affecting p27Kip1 expression in vitro and in vivo. In our own laboratory, we have also examined the effects of a PNA against miR-221 and showed that it is able to induce a sharp decrease in miR-221 biological activity. The employed PNA carried an Arg(8) peptide to facilitate PNA uptake by target cells. Two studies were published on this specific issue. In the first study by Brognara et al (319), we demonstrated that targeting miR-221 induced a sharp increase in the expression of the miR-221 target p27Kip1 mRNA in a breast cancer cell line (319). In a more recent study of ours, Brognara et al (56)
demonstrated that the PNA against miR-221 can be internalized by glioma cells when linked to a Arg(8) tail (R8), leading to the inhibition of miR-221 functions, associated with the increased expression of p27kip1 in U251 and T98G cells. In addition, the expression of another miR-221 target gene, TIMP3, was upregulated following treatment of the T98G cells with R8-PNA-a221. These data support the concept that targeting miR-221 with antagomiR molecules may provide novel options for developing protocols for the treatment of gliomas. This is supported by the finding that the treatment of all the glioma cell lines with R8-PNA-a221 induced the activation of the early apoptotic pathway (56).

11. Combined treatments: targeting multiple miRNAs

Several tumors express upregulated levels of several miRNAs, suggesting that a possible limit to anti-mRNA therapeutics may be the requirement of the co-targeting of several miRNAs to obtain the programmed biological effects. Moreover, an important anti-miRNA strategy may be associated with the obvious need for the co-targeting of different miRNAs belonging to the same miRNA family.

miRNA-replacement therapy. Yang et al (356) found that the co-transfection of miR-137/197 resulted in a reduction in myeloid cell leukemia 1 (MCL-1) protein expression, as well as in the alteration of the expression of apoptosis-related genes, the induction of apoptosis, and in the inhibition of the viability, colony-forming ability and migration ability of multiple myeloma cells. MCL-1 was further validated as a direct target of miR-137/197. Conversely, the overexpression of MCL-1 partially reversed the effects of miR-137/197. Importantly, the in vivo lentiviral-mediated or intratumor delivery of miR-137/197 induced the regression of tumors in murine xenograft models of multiple myeloma (356).

Anti-miRNA therapy. The co-treatment of target cells with antagomiR molecules selective for different miRNAs has been recently described. For instance, Lee et al (357) investigated the role of miRNAs targeting runt related transcription factor 3 (RUNX3) in early tumorigenesis. Under hypoxic conditions, miR-130a and miR-495 are upregulated and target RUNX3 by binding to its 3'-UTR in gastric cancer cells. Using matrigel plug assay, they found that antagomiRs specific for miR-130a and miR-495 significantly reduced angiogenesis in vivo and hypothesized that the co-targeting of miR-130a and miR-495 may prove to be a potential therapeutic strategy with which to recover RUNX3 expression under hypoxic conditions and in early tumorigenesis (357).

In a recent study, Brognara et al (358) treated glioma cell lines with a combined administration of antagomiR-PNAs targeting miR-221 and miR-222. In fact, the same site recognized by miR-221 in the 3'UTR of target mRNAs can be also identified by miR-222, as suggested by predicted molecular interactions using PUMA 3'UTR as a model system. Therefore, the targeting of miR-221 with antagomiRs may not be sufficient to achieve the complete suppression of miR-221 biological activity due to the presence of miR-222 in target cells. Since miR-221 and miR-222 belong to the same transcriptional unit and are, as expected, co-expressed in tumor cell lines (U251, U373 and T98G), Zhang et al (354) determined whether the co-administration of antagomiRs recognizing miR-221 and miR-222 would lead to a more efficient inhibitory activity on miR-221/222 dependent functions. The results obtained

Figure 4. (A) Transfection of U251 glioma cells with pre-miR-93 leads to the downregulation of interleukin-8 (IL-8) (upper panel) and vascular endothelial growth factor (VEGF; lower panel) protein expression. (B) Scheme outlining the effects of pre-miR-93 on neoangiogenesis and tumor growth in gliomas. Modified from Fabbri et al (344).
demonstrated that the co-suppression of miR-221/222 directly resulted in the upregulation of p27Kip1 in the tested cells and in the inhibition of cell growth by reducing a G1 to S shift in the cell cycle. Consistently, the knockdown of miR-221/222 through antisense 2′-OME-oligonucleotides increased p27Kip1 expression in mice with U251 glioma subcutaneous tumors and markedly reduced tumor growth in vivo through the upregulation of p27Kip1 (354).

In our own laboratory, we have approached the same issue using PNAs. We have previously reported that a PNA targeting miR-221 can be internalized by glioma cells and exert biological effects on miR-221-dependent functions when it is linked to an octaarginine tail (R8) (56). The major results of the more recent study by Brognara et al (358) are the following: i) R8-conjugated PNAs against miR-221 (R8-PNA-a221) and miR-222 (R8-PNA-a222) exhibit selective biological activity on miR-221 and miR-222; ii) when R8-PNA-a221 and R8-PNA-a222 are singularly administered to glioma cells, the specific inhibition of hybridization to miR-221 and miR-222 is obtained following RT-qPCR analysis; iii) both R8-PNA-a221 and R8-PNA-a222 induce the apoptosis of U251, U373 and T98G glioma cells. Finally, the co-administration of R8-PNA-a221 and R8-PNA-a222 was associated with the most prominent effects of this treatment in inducing apoptosis (see the representative experimental results shown in Fig. 5) (358).

12. Combined treatments: co-administration of antitumor drugs and miRNA therapeutic agents

One of the most interesting results obtained to date using miRNA therapeutics is the formal demonstration that, when used in combination with antitumor drugs, satisfactory therapeutic effects may be achieved (359). This has been demonstrated using both miRNA mimicking approaches, as well as anti-miRNA molecules.

miRNA replacement therapy. Gao et al (360), demonstrated that clear-cell renal cell carcinoma is a tumor type which is highly resistant to treatment and that the miR-200 family was involved in the process of mesenchymal-epithelial transition (MET) during renal development. In their study, evidence was provided to indicate that miR-200c sensitizes ccRCC cells to sorafenib or imatinib to inhibit cell proliferation. The combined application of chemotherapeutic drugs and
miR-200c may enhance the efficacy of therapy by promoting both apoptosis and autophagy (360). Another study demonstrating the enhanced effects of the combination of miRNA replacement therapy with antitumor drugs was published by Huang et al (98) with a novel transferrin-conjugated nanoparticle delivery system for synthetic miR-29b (TF-NP-miR-29b), designed for intervention in the treatment of acute myeloid leukemia (AML). The antileukemic activity of TF-NP-miR-29b was evaluated by measuring cell proliferation and colony-forming ability in vitro, as well as in vivo using a leukemia mouse model system. TF-NP-miR-29b treatment significantly downregulated miR-29b targets, such as DNA methyltransferases (DNMTs), CDK6, specificity protein 1 (SP1), KIT and Fms-related tyrosine kinase 3 (FLT3), decreased AML cell growth and impaired colony formation. Mice engrafted with AML cells and then treated with TF-NP-miR-29b had a significantly longer survival compared with the mice treated with TF-NP-scramble or free miR-29b. Furthermore, priming AML cells with TF-NP-miR-29b before treatment with decitabine resulted in a marked decrease in cell viability in vitro and enhanced the antileukemic activity compared to treatment with decitabine alone in vivo, suggesting that miRNA replacement therapy based on the delivery of miR-29b can be proposed for AML therapy also in combination with antitumor drugs.

Moreover, the study by Pogribny et al (361) reported that miR-7 expression directly targeted and significantly inhibited multidrug resistance-associated protein 1 (MRP1), which enhanced sensitivity to cisplatin in cisplatin-resistant breast cancer. Furthermore, an in vitro study by Suto et al (362) demonstrated that miR-7 overexpression enhanced sensitivity to cetuximab and suppressed cell proliferation after treatment with cetuximab in HCT-116 and SW480 cetuximab-resistant CRC cells. Additionally, miR-7 was found to enhance the sensitivity of non-small cell lung cancer (NSCLC) to paclitaxel (PTX) by promoting PTX-induced apoptosis (363). Another recent study demonstrated that the restoration of miR-143 and miR-145 expression in mutant KRAS (HCT116 and SW480) and wild-type KRAS (SW48) colon cancer cells re-sensitized the colon cancer cells to cetuximab by promoting cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC) to induce cell death (364).

In our own laboratory, we further analyzed the possible co-administration of temozolomide (TMZ) and the tumor suppressor pre-miR-124. This was investigated in one neuroblastoma and two glioma cell lines. For miRNA replacement, we employed transfection with pre-miR-124, since miR-124 is a powerful tumor suppressor pro-apoptotic miRNA. In order to demonstrate the activity of the combined treatment, the antiproliferative and pro-apoptotic effects were analyzed. This set of data confirm that miRNA therapeutics can be successfully combined with chemical treatments to obtain greater effects with low doses of reagents. In conclusion, our data showed that, in addition to the combinations between antitumor drugs and antagoniR-based protocols, interesting results can be obtained by the combination of drugs with miRNA replacement agents (Fabbri et al, unpublished data).

**Anti-miRNA therapy.** As regards the use of anti-miRNA molecules, Costa et al (365) developed an efficient delivery system for anti-miR-21 oligonucleotides, showing preferential accumulation within brain tumors and efficient miR-21 silencing, which resulted in increased mRNA and protein levels of the miR-21 target RhoB. Decreased tumor cell proliferation and tumor size, as well as enhanced apoptosis and, to a lesser extent, the improvement of animal survival, were observed in glioblastoma tumor-bearing mice upon the systemic delivery of targeted nanoparticle-formulated anti-miR-21 oligonucleotides and exposure to the tyrosine kinase inhibitor, sunitinib (365). Although further studies are warranted to demonstrate a therapeutic benefit in the clinical context, these findings suggest that miRNA modulation by targeted nanoparticles combined with anti-angiogenic chemotherapy may hold promise as an attractive therapeutic approach. Other studies have reported that the downregulation of miR-21 can induce cell apoptosis and reverse drug resistance in cancer treatments; a synergistic antiproliferative and pro-apoptotic activity was obtained using combined treatment, based on anti-miR-21 molecules and temozolomide (366) or doxorubicin (367) in human glioma cell lines. In our own laboratory, we determined whether the treatment of T98G cells with R8-PNA-a221 or R8-PNA-a222 reverses the resistance of the cells to apoptosis induced by TMZ and found that when R8-PNA-a221 and R8-PNA-a222 are co-administered, the reversion of TMZ resistance was much more efficient as opposed to single treatments (358).

A recent study reported the co-delivery of antagoniR-10b and PTX by a liposomal delivery and showed that it efficiently inhibited tumor growth and reduced the incidence of lung metastasis. In fact, antagoniR-10b impeded the migration of 4 T1 cells in vitro, silencing miR-10b and upregulating Hoxd10 both in vitro and in vivo, while PTX elicited potent tumor cell inhibitory effects (368). The same antitumor efficacy and delivery to the tumor site may be achieved by the dual loading of miR-218 mimic (bio-drug) and temozolomide (chemo-drug) using a new delivery nanogel system approach (369).

**13. Combining miRNA replacement strategies with anti-miRNAs and siRNA molecules**

Xue et al (370) verified the biological activity of novel lung-targeting nanoparticles capable of delivering miRNA mimics and siRNAs to lung adenocarcinoma cells in vitro and to tumors in a genetically engineered mouse model of lung cancer based on the activation of oncogenic Kirsten rat sarcoma viral oncogene homolog (Kras) and the loss of p53 function. The therapeutic delivery of miR-34a, a p53-regulated tumor suppressor miRNA, restored the miR-34a levels in lung tumors, specifically downregulated miR-34a target genes, and attenuated tumor growth. The delivery of siRNAs targeting Kras reduced Kras gene expression and MAPK signaling, increased apoptosis and inhibited tumor growth. The combination of miR-34a and siRNA targeting Kras improved the therapeutic responses as compared to those observed with either small RNA alone, leading to tumor regression. Furthermore, nanoparticle-mediated small RNA delivery plus conventional, cisplatin-based chemotherapy prolonged survival in this model compared to chemotherapy alone. These findings demonstrate that RNA combination therapy is possible in a model of lung cancer and provide preclinical support for the use of small RNA therapies in patients who have cancer (370). A second
example is that published by Nishimura et al (371) who first demonstrated that the siRNA-mediated silencing of EphA2, an ovarian cancer oncogene, resulted in the reduction of tumor growth. Second, they presented evidence that the additional inhibition of EphA2 by an miRNA further ‘boosts’ its antitumor effects. They identified miR-520d-3p as a tumor suppressor upstream of EphA2. The restoration of miR-520d-3p prominently decreased EphA2 protein levels, and suppressed tumor growth and migration/invasion both in vitro and in vivo. The dual inhibition of EphA2 in vivo using nanoliposomes loaded with miR-520d-3p and EphA2 siRNA exhibited synergistic antitumor efficiency and greater therapeutic efficacy than either monotherapy alone. These data emphasize the feasibility of combined miRNA-siRNA therapy, and will have broad implications for innovative gene silencing therapies for cancer and other diseases.

A further example in this very exciting field of investigation was reported by Hu et al (372), studying Bcl-2, a prominent member of the Bcl-2 family of proteins that regulate the induction of apoptosis. They investigated the effect of Bcl-2 siRNAs combined with miR-15a oligonucleotides on the growth of Raji cells. Following transfection of these combined reagents, the protein and mRNA levels of Bcl-2 were markedly decreased. The growth of the cells was significantly inhibited compared with the cells transfected with Bcl-2 siRNA or miR-15a alone and the apoptotic rate significantly increased. These results suggest that the combination of Bcl-2 siRNA and miR-15a oligonucleotides increases the apoptosis of Raji cells, and strongly support the concept that the combination of Bcl-2 siRNA and miR-15a may be a useful approach in the treatment of lymphoma.

Finally, an example of possible combined treatment is shown in Fig. 6, which indicates that the co-treatment of U251 cells with PNs targeting miR-221 or miR-222 in the presence of pre-miR-124 transfection leads to a much higher level of apoptosis as opposed to singularly administered reagents (Fabbri et al, unpublished data).

14. Conclusion

MicroRNA therapeutics in cancer are based on targeting or mimicking miRNAs involved in cancer onset, progression, angiogenesis, EMT and metastasis. This strategy has been proposed several years ago and is based on the well-recognized fact that miRNAs play a key role in the post-transcriptional control of gene expression by the sequence-selective targeting of mRNAs and are key players in several biological functions and pathological processes, including cancer. In this respect, several studies have conclusively demonstrated that miRNAs are deeply involved in tumor onset and progression, either behaving as tumor-promoting miRNAs (oncomiRNAs and metastamiRNAs) or as tumor suppressor miRNAs. In general, miRNAs able to promote cancer target mRNAs coding for tumor suppressor proteins, whereas miRNAs exhibiting tumor suppressor properties usually target mRNAs coding oncoproteins. This has a very important implication in diagnosis and/or prognosis, including the recent discovery that the pattern of circulating cell-free miRNAs in serum allows us to perform molecular analyses on these non-invasive liquid biopsies. This research field has confirmed that cancer-specific miRNAs are present in extracellular body fluids, and may play a very important role in the crosstalk between cancer cells and surrounding normal cells. Interestingly, the evidence of the presence of miRNAs in serum, plasma and saliva supports their potential as an additional set of biomarkers for cancer.

This review has focused on the most promising examples potentially leading to the development of anticancer, miRNA-based therapeutic protocols. The inhibition of miRNA activity can be readily achieved by the use of miRNA inhibitors and oligomers, including RNA, DNA, DNA analogues (miRNA antisense therapy), small molecule inhibitors, miRNA sponges or through miRNA masking. On the contrary, the enhancement of miRNA function (miRNA replacement therapy) can be achieved by the use of modified miRNA mimetics and
Acknowledgements

This study was funded by CIB, by COFIN-2009 and by AIRC (IG 13575: peptide nucleic acids targeting oncomiR and tumor-suppressor miRNAs: cancer diagnosis and therapy). EB is supported by an Umberto Veronesi fellowship. We would like to thank the Horizon-2020 ULTRAPLACAD (ULTRA-sensitive PLAsmonic devices for early Cancer Diagnosis) n.633937 project for supporting the research on circulating miRNAs as diagnostic tools.

References

1. Mazière P and Enright AJ: Prediction of microRNA targets. Drug Discov Today 12: 452-458, 2007.
2. Witkos TM, Koscińska E and Krzyżosiak WJ: Practical aspects of microRNA target prediction. Curr Med Mol 11: 93-109, 2011.
3. Ghelani HS, Rachchh MA and Gokani RH: MicroRNAs as newer therapeutic targets: A big hope from a tiny player. J Pharmacol Pharmacother 3: 217-227, 2012.
4. Krol J, Loedige I and Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11: 597-610, 2010.
5. Sun K and Lat EC: Adult-specific functions of animal microRNAs. Nat Rev Genet 14: 535-548, 2013.
6. Chekulaeva M and Filipowicz W: Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. Curr Opin Cell Biol 21: 452-460, 2009.
7. Guo H, Ingolia NT, Weissman JS and Bartel DP: Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466: 835-840, 2010.
8. Cammuerss S, Strazzieri M, De Rijk P and Del Favero J: Genetic variants in microRNA genes: Impact on microRNA expression, function, and disease. Front Genet 6: 186, 2015.
9. Friedländer MR, Lizzano E, Houben AJS, Bezdan D, Báñez-Coronel M, Kudla G, Mateu-Huertas E, Kagerbauer B, Gonzalez J, Chen KC, et al: Evidence for the biogenesis of more than 1,000 novel human microRNAs. Genome Biol 15: R57, 2014.
10. Cheng WC, Chung IF, Tsai CF, Huang TS, Chen CY, Wang SC, Chang TY, Sun HJ, Chao JY, Cheng CC, et al: Y5M00v2: a small RNA sequencing (smRNA-seq) database for human cancer miRNAs. Nucleic Acids Res 43: D862-D867, 2015.
11. Londin E, Loher P, Telonis AG, Quann K, Clark P, Jing Y, Hatzimichael E, Kirino Y, Honda S, Lally M, et al: Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci USA 112: E106-E111, 2015.
12. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A and Enright AJ: miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34: D140-D144, 2006.
13. Kozomara A and Griffiths-Jones S: miRBase: Annotating high confidence microRNA sequences with deep sequencing data. Nucleic Acids Res 42: D68-D73, 2014.
14. Taccioli C, Fabbi E, Visone R, Volinia S, Calin GA, Fong LY, Gambari R, Bottoni A, Acunzo M, Hagan J, et al: UCbase and miRfunc: A database of ultraconserved sequences and microRNA function. Nucleic Acids Res 37: D141-D148, 2009.
15. Witwer KW: Data submission and quality in microarray-based microRNA profiling. Clin Chem 59: 392-400, 2013.
16. Xie B, Ding Q, Han H and Wu D: miCancer: A microRNA-cancer association database constructed by text mining on literature. Bioinformatics 29: 638-644, 2013.
17. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS and Johnson JM: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433: 769-773, 2005.
18. Peter ME: Targeting of miRNAs by multiple miRNAs: The next step. Oncogene 29: 2161-2164, 2010.
19. Bianchi N, Finotti A, Ferracin M, Lamproti I, Zuccato C, Breveregleri G, Brognara E, Fabbi E, Borgatti M, Negrini M, et al: Increase of microRNA-210, decrease of raptor gene expression and activation of mammalian target of rapamycin regulated proteins following thymarchya treatment of human erythroid cells. PLOS One 10: e0121567, 2015.
20. Subramanian S and Steer CJ: MicroRNAs as gatekeepers of apoptosis. J Cell Physiol 223: 289-298, 2010.
21. Wang Y and Blelloch R: Cell cycle regulation by MicroRNAs in embryonic stem cells. Cancer Res 69: 4093-4096, 2009.
22. Fabbi E, Borgatti M, Montagner G, Bianchi N, Finotti A, Lamproti I, Bezzzeri V, Decheccchi MC, Cabrini G and Gambari R: Expression of microRNA-93 and Inte-Dtin-B during Pseudomonas aeruginosa-mediated induction of proinflammatory responses. Am J Respir Cell Mol Biol 50: 1144-1155, 2014.
23. Faruq O and Vecchione A: microRNA: A diagnostic perspective. Front Med Lausanne 2: 51, 2015.
24. Shalaby T, Fiaschetti G, Baumgartner M and Grotzer MA: Significance and therapeutic value of miRNAs in embryonal neural tumors. Molecules 19: 5821-5862, 2014.
25. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Bica E, Bullrich F, Negrini M, et al: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 101: 2999-3004, 2004.
26. Palermo EI, de Campos SG, Campos M, de Souza NC, Guerreiro ID, Carvalho AL and Marques MM: Mechanisms and role of microRNA deregulation in cancer onset and progression. Genet Mol Biol 34: 363-370, 2011.
27. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ and Wang K: The microRNA spectrum in 12 body fluids. Clin Chem 56: 1733-1741, 2010.
28. Fyafyad-Kazan H, Bitar D, Hamed E, Fieschi M, Lewalle P, Fayad-Kazan M, Badran R, Hamade E, Daheer A, Hussein N, ElDrrani R, et al: Circulating miR-150 and miR-34A in plasma are novel potential biomarkers for acute myeloid leukemia. J Transl Med 11: 31, 2013.
29. Neviani P and Fabebb M: Exosomal microRNAs in the tumor microenvironment. Front. Med. Lausanne 2: 47, 2015.
30. Köberle V, Kronenberg B, Pleil T, Trojan J, Imelmann E, Peveling-Oberleben J, Weiler MW, Elhendawy M, Zeuzem S, Pipher A, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 49: 3442-3449, 2013.
31. He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
32. Westphal M and Lamszus K: Circulating biomarkers for gliomas. Nat Rev Neurol 11: 556-566, 2015.
33. Yau TO, Wu CW, Dong Y, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
34. Köberle V, Kronenberg B, Pleil T, Trojan J, Imelmann E, Peveling-Oberleben J, Weiler MW, Elhendawy M, Zeuzem S, Pipher A, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 49: 3442-3449, 2013.
35. He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
36. Orellana EA and Kasinski AL: MicroRNAs in cancer: A therapeutic strategy against vulvar cancer. Adv Drug Deliv Rev 81: 161-168, 2015.
37. Kontji J, Gibescu JH, Hettinga C, Adema A, Richter M, Halsaen N, Sleuzak-Prochazka I, Ding Y, Kroesen BJ and van der Berg A: Rapid generation of microRNA sponges for microRNA inhibition. PLoS One 7: e29275a, 2012.
38. Li Y, Han Y, Zhang H, Nie L, Jiang Z, Fu P, Gui Y and Cai Z: Synthetic microRNA-mowers targeting miR-183-96-182 cluster or miR-210 inhibit growth and migration and induce apoptosis in bladder cancer cells. PLoS One 7: e52280, 2012.
39. Conti C, Hasfield KD, Barbato S, Carrella S, Pizzio M, Bhat RS, Carabino A, Karali M, Porter LF, Urquhart J, et al: MiR-210 is required for the differentiation of murine embryonic stem cells. Cell 141: 618-631, 2010.
40. Brown BD and Naldini L: Exploiting and antagonizing microRNA interference. Nucleic Acids Res 37: e24, 2009.
41. van Rooij E and Kauppinen S: The utility of LNA in microRNA-based cancer therapeutics: A new class of drugs with potential therapeutic application. Mol Cell Ther 2: 7, 2014.
42. He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
43. Westphal M and Lamszus K: Circulating biomarkers for gliomas. Nat Rev Neurol 11: 556-566, 2015.
44. Yau TO, Wu CW, Dong Y, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
45. Köberle V, Kronenberg B, Pleil T, Trojan J, Imelmann E, Peveling-Oberleben J, Weiler MW, Elhendawy M, Zeuzem S, Pipher A, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 49: 3442-3449, 2013.
46. He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
47. Westphal M and Lamszus K: Circulating biomarkers for gliomas. Nat Rev Neurol 11: 556-566, 2015.
48. Yau TO, Wu CW, Dong Y, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
49. Köberle V, Kronenberg B, Pleil T, Trojan J, Imelmann E, Peveling-Oberleben J, Weiler MW, Elhendawy M, Zeuzem S, Pipher A, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 49: 3442-3449, 2013.
50. He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
51. Westphal M and Lamszus K: Circulating biomarkers for gliomas. Nat Rev Neurol 11: 556-566, 2015.
52. Yau TO, Wu CW, Dong Y, Huang M, Xu C, Kim TK, Etheridge A, Luo Y, Ding Y and Wang K: Current state of circulating microRNAs as cancer biomarkers. Clin Chem 61: 1138-1155, 2015.
53. Köberle V, Kronenberg B, Pleil T, Trojan J, Imelmann E, Peveling-Oberleben J, Weiler MW, Elhendawy M, Zeuzem S, Pipher A, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 49: 3442-3449, 2013.
75. Murakami K and Miyagishi M: Tiny masking locked nucleic acids effectively bind to mRNA and inhibit binding of microRNAs in relation to thermodynamic stability. Biomed Rep 2: 509-514, 2014.

76. Shin KJ, Wall EA, Zavzavadjian JR, Santat LA, Liu J, Hwang JJ, Rebres R, Roach T, Seaman W, Simon MI, et al: A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression. Proc Natl Acad Sci USA 103: 13759-13764, 2006.

77. Askou AJ, Kostic C, Arsenjevic Y, Hollensen AK, Bäck T, Jensen TG, Mikkelsen JG and Croydon TD: Multijigen lentiviral vectors for combined and tissue-specific expression of miRNA- and protein-based antiangiogenic factors. Mol Ther Methods Clin Dev 2: 14064, 2015.

78. Wimbanks CA, Beyar C, Haig A, Qian H, Sepulveda PV and Gregoriev P: miR-206 represses hypertrophy of myogenic cells but not muscle fibers via inhibition of HDAC4. PLoS One 8: e73589, 2013.

79. Montgomery RL, Yu G, Latimer PA, Stack C, Robinson J, Wiggins JF, Ruffino L, elnar K, Omotola M, Patrawala L, Peng Y, Laser J, Shi G, Mittal K, Melamed J, Lee P and Wei JJ: Müller DW and Bosserhoff AK: Integrin beta 3 expression is modulated in a cell-biological mode of migration and invasion. Mol Neurobiol 47: 131-144, 2013.

80. Bader AG: miR-34 - a microRNA replacement therapy is headed for the clinic. Front Genet 3: 120, 2012.

81. Kwekkeboom RF, Lei Z, Doevendans PA, Musters RJ and Rebres R, Roach T, Seaman W, Simon MI: A single Chimeric cancerstem cells to imatinib treatment. Cancer Lett 360: 245-256, 2015.

82. Lee YJ, Lee YJ, Ho CC, Hong QS, Yu SL, Tzeng CR, Yang PC and Chen HW: miRNA-34b as a tumor suppressor in estrogen-dependent growth of breast cancer cells. Breast Cancer Res 13: R116, 2011.

83. Huang Y, Pe B, Yang Y, Shi J and Zhao H: MicroRNA-181 functions as a tumor suppressor in non-small cell lung cancer (NSCLC) by targeting Bel-2. Tumour Biol 36: 3381-3387, 2015.

84. Su R, Lin HS, Zhang XH, Yin XL, Ning HM, Liu B, Zhao PF, Gong JN, Shen C, Song L, et al: MiR-181 family. Regulators of miR-204 acrolein and miR-206/133b clusters: Dysregulation and functional cardiovascular diseases: Opportunities and challenges. Clin Sci (Lond) 127: 351-365, 2014.

85. Sherr CJ: Principles of tumor suppression. Cell 116: 235-246, 2004.

86. Lee YS and Dutta A: The tumor suppressor microRNA let-7 regulates the HMG2 oncoprotein, Genes Dev 21: 1025-1030, 2007.

87. Mayr C, Hemmann MT and Bartel DP: Disrupting the pairing effect of miRNA- and protein-based antiangiogenic factors. Mol Ther 19: 1116-1122, 2011.

88. Dinulescu DM, Lengyel E and Peter ME: Let-7 prevents early oncogenic transformation. Science 315: 1576-1579, 2007.

89. Brown D and Bader AG: Development of a lung cancer therapy: MicroRNA mimicry replacement therapy. Cancer Res 70: 7027-7030, 2010.

90. Koeberle R, Aigner A and Hartmann R: MicroRNA replacement therapy sensitizes breast cancer cells to ionizing radiation. Pharm Rev 30: 1008-1016, 2013.

91. Müller HG, Rasmussen AP, Andersen HH, Johnsen KB, Henriksen M and Duroux M: A systematic review of microRNA in glioblastoma multiforme: Micro-modulators in the multigenic model of migration and invasion. Mol Neurobiol 47: 131-144, 2013.

92. Hershkovitz-Rokah O, Modai S, Pasmanik-Chor M, Toren A, Shamron N, Raanani P, Shilipberg O and Granot R: Restoration of miR-424 suppresses BCR-ABL activity and sensitizes CML cells to imatinib treatment. Cancer Lett 360: 245-256, 2015.

93. Scheibner A, Teaboldt B, Hauer MC, Chen X, Cherukuri S, Buechner J, Tømte E, Haug BH, Henriksen JR, Løkke C, Flægstad T and Einvik C: Tumour-suppressor microRNAs let-7 and miR-204 are modulated in a cell-biological mode of migration and invasion. Mol Neurobiol 47: 131-144, 2013.

94. Bader AG, Boer J, Matzke K, Mayr C, Arsenjevic Y, Hollensen AK, Bäck T, Jensen TG, Mikkelsen JG and Croydon TD: Multijigen lentiviral vectors for combined and tissue-specific expression of miRNA- and protein-based antiangiogenic factors. Mol Ther Methods Clin Dev 2: 14064, 2015.

95. Liu YN: EGF receptor pomotes prostate cancer bone metastasis by cationic lipoplexes for lung cancer. Mol Ther Nucleic Acids 2: 3240-3250, 2015.

96. Chang YS, Chen WY, Yin JJ, Sheppard-Tillman H, Huang J and Liu YN: EGF receptor promotes prostate cancer bone metastasis by downregulating miR-1 and activating TWIST1. Cancer Res 75: 3077-3086, 2015.

97. Zhang H, Cai K, Wang J, Wang X, Cheng K, Shi F, Jiang L, Zhang Y and Dou J: MiR-7: inhibited directly by lincRNA HOTAIR, directly inhibits SETDB1 and reverses the EMT of breast cancer stem cells by downregulating the STAT3 pathway. Stem Cells 32: 2858-2868, 2014a.

98. Okuda H, Xing F, Pandey PR, Sharma S, Watabe M, Pai SK, Mo YY, Iizumi-Gairani M, Hirota S, Liu Y, et al: MiR-7 suppresses brain metastasis of breast cancer stem-like cells by modulating KLK4. Cancer Res 73: 1434-1444, 2013.
117. Zhou X, Hu Y, Dai L, Wang Y, Zhou J, Wang W, Di W and Qiu L: MicroRNA-7 inhibits tumor metastasis and reverses epithelial-mesenchymal transition through AKT/ERK1/2 inactivation. Cell Cycle 22: 291-303, 2012.

114. Ferrari P, Gualtieri M, Martinelli C, Bonafè M, Pande S and Talu C: miR-29 suppresses the proliferation, invasion and metastasis of gastric cancer cells through targeting cyclin D1 and Ets1. PLoS One 8: e55719, 2013.

113. Teixeira F, van’t Veer L, Beerenkamp J, van der Flier A, Feron V, Geurts van Kessel C, van der Graaf J, van der Saag P, de Weger R and Jonkers J: Role of miR-34a/b/c in breast cancer. Cell Cycle 14: 3895-3908, 2015.

112. Wang H, Li Z, Li X, Wang H, Shi X, Li Q, Zhou Z, Wu S, Xiao J, Li Q, Gao Y and Liu J: MicroRNA-29a/b/b regulates GAS5 expression and cell proliferation in breast cancer. Oncogene 34: 4321-4330, 2015.

111. Zhang L, Li Q, Zhang Y, Liu Y, Mao J and Wang D: miR-29 expression in breast cancer cells inhibits cell proliferation and metastasis in vitro. Oncogene 33: 5750-5758, 2014.

110. Wang J, Wang W, Qiu Z, Zhai Y, Zhao S, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b suppressed cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

109. Zhang L, Wang Y, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b inhibits cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

108. Wang J, Wang W, Qiu Z, Zhai Y, Zhao S, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b suppressed cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

107. Wang X, Wang Y, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b inhibited cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

106. Wang J, Wang W, Qiu Z, Zhai Y, Zhao S, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b suppressed cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

105. Wang J, Wang W, Qiu Z, Zhai Y, Zhao S, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b inhibited cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.

104. Wang J, Wang W, Qiu Z, Zhai Y, Zhao S, Wang J, Li X, Wang Y, Zhang T and Li J: miR-29a/b suppressed cell proliferation and invasion in breast cancer cells. Journal of Experimental & Therapeutic Oncology 12: 187-195, 2015.
158. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S, et al: The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by targeting CD44. Nat Med 17: 211-215, 2011.

159. Krzeszinski JY, Wei W, Huyhn H, Jin Z, Wang X, Chang TC, Xie XJ, He L, Mangala LS, Lopez-Berestein G, et al: miR-34a blocks osteosarcoma and bone metastasis by inhibiting osteoclastogenesis and Tgif2. Nature 512: 431-435, 2014.

160. Wang LG, Ni Y, Su BH, Mu XR, Shen HC and Du J: MicroRNA-34b functions as a tumor suppressor and acts as a nodal point in the feedback loop with Met. Int J Oncol 42: 957-962, 2013.

161. Yu Z, Kim J, He L, Creighton CJ, Gunaratne PH, Hawkins SM and Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Yang Y, Zhang A, Maric D, Anolik R, Zenklusen JC, Zhang A, Maric D, Anolik R, Zenklusen JC, Zambetti GP, Gilmour DS: Prediction of associations between microRNAs and gene expression in glioma biology. PLoS One 6: e14681, 2011.

162. Zhang Y, Chao T, Li R, Liu W, Chen Y, Yan X, Gong Y, Yin B, Liu W, Wang Q, et al: MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3. J Mol Med Berl 87: 43-51, 2009.

163. Huang CY, Huang XP, Zhu JY, Chen ZG, Li XJ, Zhaoh X, Huang S, He JB, Lian F, Zhao WN, et al: miR-128-3p suppresses hepatic colorectal carcinoma proliferation by regulating Pdk1 and is correlated with the prognosis of HCC patients. Oncol Rep 33: 2889-2898, 2015.

164. Wang Q, Wang X, Wang Y, Wang XF, et al: miR-128 inhibits tumor growth and angiogenesis by targeting p506K1. PLoS One 7: e32709, 2012.

165. Wuchty S, Arjona D, Li A, Kostiarov Y, Walling J, Ahn S, Zhu C, Maric D, Anolik R, Zenklusen JC, Zhang A, Maric D, Anolik R, Zenklusen JC, Zambetti GP, Gilmour DS: Prediction of associations between microRNAs and gene expression in glioma biology. PLoS One 6: e14681, 2011.

166. Zhang Y, Chao T, Li R, Liu W, Chen Y, Yan X, Gong Y, Yin B, Liu W, Wang Q, et al: MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3. J Mol Med Berl 87: 43-51, 2009.

167. Huang CY, Huang XP, Zhu JY, Chen ZG, Li XJ, Zhaoh X, Huang S, He JB, Lian F, Zhao WN, et al: miR-128-3p suppresses hepatic colorectal carcinoma proliferation by regulating Pdk1 and is correlated with the prognosis of HCC patients. Oncol Rep 33: 2889-2898, 2015.
199. Visone R, Veronese A, Rassenti LZ, Balatti V, Pearl DK, Acunzo M, Volinia S, Taccioli C, Kipps TJ and Croce CM: miR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. Blood 118: 3072-3079, 2011.

200. Koike F, Hurley J, Danieli WA, Hu Y, Hao L, Peng CY, Merkel TJ, Queisser MA, Ritter C, et al: miR-182 integrates apoptosis, growth, and differentiation programs in glioblastoma. Genes Dev 29: 732-745, 2015.

201. Leivenon SK, Rokka A, Oslings P, Kohonen P, Corhals GL, Karlson O, and Perälä M: Identification of miR-193b targets in breast cancer cells and systems biological analysis of their functional impact. Mol Cell Proteomics 10: M110.003522, 2011.

202. Yang H, Liu P, Zhang J, Peng X, Lu Z, Yu S, Meng Y, Tong WM and Chen J: Long non coding RNA miR-1314H exhibits oncogenic properties of pancreatic ductal adenocarcinoma and is negatively regulated by miR-193b. Oncogene; Nov 9, 2015 (Epub ahead of print).

203. Tan S, Li R, Ding K, Lobic PE and Zhu T: miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/MET pathway. FEBS Lett 585: 2229-2234, 2011.

204. Bao W, Wang HH, Tian FJ, He XY, Qiu MT, Wang JY, Zhang HJ, Wang LH and Wan X: A TrkB-STAT3-miR-204-5p regulatory circuitry controls proliferation and invasion of endometrial carcinoma cells. Mol Cancer 12: 155, 2013.

205. Xia Z, Liu F, Zhang J and Liu L: Decreased expression of miR-99a-5p contributes to glioma progression and promotes glioma cell growth, migration and invasion. PLoS One 10: e0132399, 2015.

206. Gandellini P, Polini M, Longoni N, Pennati M, Binda M, Colecchia M, Salvioni R, Supino R, Moretti R, Limonta P, et al: miR-205 exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase C epsilon. Cancer Res 69: 2287-2295, 2009.

207. Chen QY, Jiao DM, Yan L, Wu QY, Hu HZ, Song J, Yan J, Wu LJ, Xu LQ and Shi JG: Comprehensive gene and microRNA expression profiling reveals miR-206 inhibits MET in lung cancer metastasis. Mol Biosyst 11: 2290-2302, 2015.

208. Chen DL, Wang ZQ, Zeng ZL, Wu WJ, Zhang DS, Luo W, Qiu MZ, Wang DS, Ren C, et al: Identification of microRNA-214 as a negative regulator of colorectal cancer cell liver metastasis by way of regulation of fibroblast growth factor receptor 1 expression. Hepatology 60: 598-609, 2014.

209. Tie J, Pan Y, Zhao L, Wu K, Liu J, Sun S, Guo X, Wang B, Gang Y, Zhang Y, et al: MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo receptor. PLoS Genet 6: e1000870, 2010.

210. Wei JJ, Wu X, Peng Y, Shi G, Basturk O, Yang X, Daniels G, Osman I, Ouyang J, Hernando E, et al: Regulation of the HMG1A expression by microRNA-296 affects prostate cancer growth and invasion. Clin Cancer Res 17: 1297-1305, 2011.

211. Wang L, Yao J, Shi X, Hu L, Li Z, Song T and Huang C: MicroRNA-203 regulates suppression of EGF receptor signaling in human hepatocellular carcinoma SMCC-7721 cells. BMC Cancer 13: 448, 2013.

212. Tavazoei SF, Alarcón C, Oskarsson T, Hsing C, Zhang JD, Limonta P, et al: miR-214 inhibits pancreatic cancer cell growth, migration and invasion of hepatocellular carcinoma cells. Cancer Sci 105: 425-430, 2014.

213. Visone R, Veronese A, Rassenti LZ, Balatti V, Pearl DK, Acunzo M, Volinia S, Taccioli C, Kipps TJ and Croce CM: miR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. Blood 118: 3072-3079, 2011.

214. Li KK, Pang JC, Lau KM, Zhou L, Mao Y, Wang Y, Poon WS and Fan H: HGF-MIR-383 is downregulated in medulloblastoma and targets peroxiredoxin 3 (PRDX3). Brain Pathol 23: 413-425, 2013.

215. Bou Kheir T, Futoma-kazmierczak E, Jacobsen A, Krogh A, Hurst DR, Edmonds MD and Welch DR: Metastamir: The Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Wang L, Yao J, Shi X, Hu L, Meng Y, Tong WM and Chen J: Long non coding RNA miR-1314H exhibits oncogenic properties of pancreatic ductal adenocarcinoma and is negatively regulated by miR-193b. Oncogene; Nov 9, 2015 (Epub ahead of print).

216. Okamoto K, Ishiguro T, Midorikawa Y, Ohata H, Izumiya M, Luo W, Huang B, Li Z, Li H, Sun L, Zhang Q, Qiu X and Wang E: MicroRNA-449a is downregulated in non-small cell lung cancer and inhibits migration and invasion by targeting c-Met. PLoS One 8: e64759, 2013.

217. Okamoto K, Ishiguro T, Midorikawa Y, Ohata H, Izumiya M, Tsuchiya N, Sato A, Sakai H and Nakagama H: miR-493 induction during carcinogenesis blocks metastatic settlement of colon cancer cells in liver. EMBO J 31: 1752-1763, 2012.

218. Gu Y, Cheng Y, Song Y, Zhang Z, Deng M, Wang C, Zheng G and Z: MicroRNA-493 suppresses tumor growth, invasion and metastasis of lung cancer by regulating E2F1. PLoS One 9: e102602, 2014.
Fletcher CE, Dart DA, Sita-Lumsden A, Cheng H, Rennie PS, Ng WL, Yan D, Zhang X, Mo YY and Wang Y: Over-expression of miR-221/222 in gastric carcinoma. Hum Pathol 44: 1278-1285, 2013.

Chan JA, Krichevsky AM and Kosik KS: MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65: 6029-6033, 2005.

Liu W, Zabirnyk O, Wang H, Shiao YH, Nickerson ML, Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D and Wang J and Wu J: Role of miR-155 in breast cancer. Front Biosci 17: 2350-2355, 2012.

Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, Forloni M, Boldrini R, Donfrancesco A, Federici V, Giacomini P, Alvarez-Diaz S, Zakrzewski J, Blochin E, Rose A, Bogunovic D, Khalil S, Anderson LM, Perantoni AO and Phang JM: miR-23b targets proline oxidase, a novel tumor suppressor protein in renal cancer. Oncogene 29: 4914-4924, 2010.

Fletcher D, Dart DA, Sita-Lumsden A, Cheng H, Rennie PS and Bevan CL: Androgen-regulated processing of the oncomir miR-27a, which targets Prohibitin in prostate cancer. Hum Mol Genet 21: 3112-3127, 2012.

Ng WL, Yan D, Zhang X, Mo YY and Wang Y: Over-expression of miR-100 is responsible for the low-expression of ATM in the human glioma cell line M059J. DNA Repair (Amst) 9: 1170-1175, 2010.

Zheng YS, Zhang H, Zhang XJ, Feng DD, Luo XQ, Zeng CW, Lin KY, Zhou H, Qu LH, Zhang P, et al: MiR-100 regulates cell differentiation and survival by targeting RBPJ, a phosphatase-like tumor suppressor in acute myeloid leukemia. Oncogene 31: 80-92, 2012.

Knackmuss U, Lindner SE, Aneichyk T, Krotkamp B, Knust Z, Liu W, Zabirnyk O, Wang H, Shiao YH, Nickerson ML, Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D and Wang J and Wu J: Role of miR-155 in breast cancer. Front Biosci 17: 2350-2355, 2012.

Park JK, Henry JC, Jiang J, Esau C, Gusev Y, Lerner MR, Postier RG, Brackett DJ and Schmittgen TD: miR-132 and miR-212 are increased in pancreatic cancer tissues and target the retinoblastoma tumor suppressor. Biochem Biophys Res Commun 406: 518-523, 2011.

Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D and Cheng QJ: MicroRNA-155 regulates cellular survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. J Biol Chem 285: 17869-17879, 2010.

Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, Liu MF and Wang ED: MicroRNA-155 functions as an oncomir in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 70: 3119-3127, 2010.

Czyzyk-Krzeska MF and Zhang X: MiR-155 is at the heart of oncogenic pathways. Oncogene 33: 677-678, 2014.

Wang J and Wu J: Role of miR-155 in breast cancer. Front Biosci (Landmark Ed) 17: 2350-2355, 2012.

Ling N, Gu J, Lei Z, Li M, Zhao J, Zhang HT and Li X: microRNA-155 regulates cell proliferation and invasion by targeting FOXO3a in glioma. Oncol Rep 30: 2111-2118, 2013.

Jiang S, Zhang HW, Li MH, He XH, Li Y, Gu H, Liu MF and Wang ED: MicroRNA-155 functions as an oncomir in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 70: 3119-3127, 2010.

Fontana L, Fiori ME, Albini S, Cifaldi L, Giovinazzi S, Tu K, Liu Z, Yao B, Han S and Yang W: MicroRNA-519a promotes tumor growth by targeting PTEN/P13K/AKT signaling in hepatocellular carcinoma. Int J Oncol 48: 965-974, 2016.

Shao J, Cao J, Liu Y, Mei H, Zhang Y and Xu W: MicroRNA-519a promotes proliferation and inhibits apoptosis of hepatocellular carcinoma cells by targeting FOXF2. FEBS Open Bio 5: 893-899, 2015.

Ward A, Shukla K, Balwierz A, Soons Z, König R, Sahin O and Wiemann S: MicroRNA-519a is a novel oncomir conferring tamoxifen resistance by targeting a network of tumour-suppressor genes in ER+ breast cancer. J Pathol 235: 368-379, 2014.

Tsang WP, Ng Ek, Ng SS, Jin H, Yu J, Sung JJ and Kwok TT: Aberrant miR-182 expression promotes melanoma metastasis by functionally interacting with DOK2. Cancer Res 76: 6163-6172, 2016.

Chang Y, Liu C, Yang J, Liu G, Feng F, Tang J, Hu L, Li L, Tufarelli C and Lund JN: Inflammation and MiR-21 pathways mediate proliferation and invasion of gastric cancer cells by targeting DUSP4. J Transl Med 14: 238, 2016.

Zhao JH: miR-221/222: Promising biomarkers for breast cancer. Tumour Biol 34: 1361-1370, 2013.

Möllby R and Sunaert H, Tsang J, Waldron L, Pintilie M, Hui AB, Sykes J, P’ng C, Miller N, et al: MicroRNA-301 mediates proliferation and invasion in human breast cancer. Cancer Res 71: 2926-2937, 2011.

Vorhoeve PM, Le Sage C, Schriemer M, Gillis AJ, Stoop H, Nagel R, Liu YP, van Duijse J, Drost J, Griekspoor T, et al: A genome-wide screen implicates miR-372 and miR-373 as oncogenes in testicular germ cell tumors. Adv Exp Med Biol 670: 79-89, 2011.

Wang J and Wu J: Role of miR-155 in breast cancer. Front Biosci (Landmark Ed) 17: 2350-2355, 2012.

Lee DY, Deng Z, Wang Z and Yang BB: MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. Proc Natl Acad Sci USA 104: 20350-20355, 2007.

Tu K, Liu Z, Yao B, Han S and Yang W: MicroRNA-519a promotes tumor growth by targeting PTEN/P13K/AKT signaling in hepatocellular carcinoma. Int J Oncol 48: 965-974, 2016.

Shao J, Cao J, Liu Y, Mei H, Zhang Y and Xu W: MicroRNA-519a promotes proliferation and inhibits apoptosis of hepatocellular carcinoma cells by targeting FOXF2. FEBS Open Bio 5: 893-899, 2015.

Ward A, Shukla K, Balwierz A, Soons Z, König R, Sahin O and Wiemann S: MicroRNA-519a is a novel oncomir conferring tamoxifen resistance by targeting a network of tumour-suppressor genes in ER+ breast cancer. J Pathol 235: 368-379, 2014.

Tsang WP, Ng Ek, Ng SS, Jin H, Yu J, Sung JJ and Kwok TT: Oncofetal H19-derived miR-675 regulates tumor suppressor RB1 in human colorectal cancer. Carcinogenesis 31: 350-358, 2010.

Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, Teruya-Feldstein J, Reinhardt F, Onder T, Valastyan S, et al: miR-9, a MYC/MYCН-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 12: 247-256, 2010.

Chen D, Sun Y, Wei Y, Zhang P, Rezaeean AH, Teruya-Feldstein J, Gupta S, Liang H, Lin HK, Hung MC, et al: LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. Nat Med 18: 1511-1517, 2012.

White RA, Neiman JM, Reddi A, Han G, Birlea S, Mitra D, Dionne L, Fernandez P, Murao K, Bian L, et al: Epithelial stem cell mutations inhibit tumor suppressor interactions to promote squamous cell carcinoma metastasis. J Clin Invest 123: 4390-4404, 2013.

Ma L, Teruya-Feldstein J and Weinberg RA: Tumour invasion and metastasis initiated by microRNA-212222 overexpression in human glioblastoma increases invasiveness by targeting the protein phosphate PTPγ. Oncogene 31: 858-868, 2012.

Chen WX, Hu Q, Qiu MT, Zhong SL, Xu JJ, Tang JH and Zhao JH: miR-221/222: Promising biomarkers for breast cancer. Tumour Biol 34: 1361-1370, 2013.
283. Xu J, Zhang W, Lv Q and Zhu D: Overexpression of miR-21 promotes the proliferation and migration of cervical cancer cells via the inhibition of PTEN. Oncol Rep 33: 3108-3116, 2015.

284. Avidan A, Asadpour S, Gharabaghi A, Amin R, Khatami AR, Albrecht JH, Colburn NH, Post S and Allgayer H: MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor PdcD4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27: 2128-2136, 2008.

285. Melnik BC: MiR-21: An environmental driver of malignant melanoma. J Invest Dermatol 132: 2022, 2015.

286. Zhou W, Chang LW, Yang CL, Lin JC, Chen CC, Pan SH, Wu CT, Chen HY, Yang SC, Hong TM, et al: MicroRNA-135b promotes lung cancer metastasis by regulating multiple targets in the Hippo pathway and LzT51. Nat Commun 4: 1877, 2013.

287. Taylor MA, Soesma-Allaoui K, Thompson CL, Danielpou D and Schiemann WP: TGFB1 upregulates miR-18a expression to promote breast cancer metastasis. J Clin Invest 123: 150-163, 2013.

288. Qiu Y, Luo X, Kan T, Zhang Y, Yu W, Wei Y, Shen N, Bi Y and Jiang X: TGFB1 upregulates miR-182 expression to promote gallbladder cancer metastasis by targeting CADM1. Mol Biol Rep 40: 593-601, 2013.

289. Ren LH, Chen WX, Li S, He XY, Zhang ZM, Li M, Cao RS, Hao B, Zhang HJ, Qiu HQ, et al: MicroRNA-183 promotes proliferation and invasion in oesophageal squamous cell carcinoma by targeting cell cycle G1 arrest. Br J Cancer 111: 2003-2013, 2014.

290. Korpal M, Lee ES, Hu G and Kang Y: The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 283: 14910-14914, 2008.

291. Korpal M, Ell BJ, Buffa FM, Ibrahim T, Blanco MA, Celia-Terrassa T, Mercatali L, Khan Z, Goodarzi H, Hua Y, et al: Direct targeting of Sec23a by miR-200s influences cancer cell chemo-resistance and promotes metastatic colonization. Nat Med 17: 1009-1021, 2011.

292. Park SM, Gaur AB, Lengyel E and Peter ME: The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 22: 894-907, 2008.

293. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Colburn JH, Maldonado DA, Satpathy JK, Colburn NH, Post S and Allgayer H: MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor PdcD4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27: 2128-2136, 2008.

294. Melnik BC: MiR-21: An environmental driver of malignant melanoma. J Invest Dermatol 132: 2022, 2015.

295. Zhou W, Fong MY, Zhou W, Liu L, Tsykin A, Colburn JH, Colburn NH, Post S and Allgayer H: MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor PdcD4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27: 2128-2136, 2008.

296. Melnik BC: MiR-21: An environmental driver of malignant melanoma. J Invest Dermatol 132: 2022, 2015.

297. Zhou W, Chang LW, Yang CL, Lin JC, Chen CC, Pan SH, Wu CT, Chen HY, Yang SC, Hong TM, et al: MicroRNA-135b promotes lung cancer metastasis by regulating multiple targets in the Hippo pathway and LzT51. Nat Commun 4: 1877, 2013.

298. Taylor MA, Soesma-Allaoui K, Thompson CL, Danielpou D and Schiemann WP: TGFB1 upregulates miR-18a expression to promote breast cancer metastasis. J Clin Invest 123: 150-163, 2013.

299. Qiu Y, Luo X, Kan T, Zhang Y, Yu W, Wei Y, Shen N, Bi Y and Jiang X: TGFB1 upregulates miR-182 expression to promote gallbladder cancer metastasis by targeting CADM1. Mol Biol Rep 40: 593-601, 2013.

300. Ren LH, Chen WX, Li S, He XY, Zhang ZM, Li M, Cao RS, Hao B, Zhang HJ, Qiu HQ, et al: MicroRNA-183 promotes proliferation and invasion in oesophageal squamous cell carcinoma by targeting cell cycle G1 arrest. Br J Cancer 111: 2003-2013, 2014.
320. Yan LX, Wu QN, Zhang Y, Li YY, Liao DZ, Hou JH, Fu J, Zeng MS, Yun JP, Wu QL, et al. Knockdown of miR-21 in human breast cancer cell lines inhibits proliferation, in vitro migration and in vivo tumor growth. Breast Cancer Res 13: R2, 2011.

321. Zhang J and Ma L: MicroRNA control of epithelial-mesenchymal transition and metastasis. Cancer Metastasis Rev 31: 653-662, 2012.

322. Zaravinos A, Radioucic J, Lambrou GI, Volanis D, Delakas D, Stathopoulos EN and Spandidos DA: Expression of miRNAs involved in angiogenesis, tumor cell proliferation, tumor suppressor inhibition, epithelial-mesenchymal transition and activation of metastasis in bladder cancer. J Urol 188: 615-623, 2012.

323. Kesslich T, Pichler M and Neureiter D: Epigenetic control of epithelial-mesenchymal transition in human cancer. Mol Clin Oncol 1: 3-11, 2013.

324. Lei C, Wang Y, Huang Y, Yu H, Huang Y, Wu L and Huang L: Up-regulated miR155 reverses the epithelial-mesenchymal transition induced by EGF and increases chemosensitivity to cisplatin in human Caki cervical cancer cells. PLoS One 7: e52310, 2012.

325. Koutsaki M, Spandidos DA and Zaravinos A: Involvement of nuclear factor-kappa B in miR-124-mediated suppression of PTEN, p27(kip1), p57(kip2), and PUMA. Am J Cancer Res 3: 299-312, 2013.

326. Ueda R, Kohanbash G, Sasaki K, Fujita M, Zax X, Kastenhuber ER, McDonald HA, Potier MM, Lotze MT, et al. Dicer-regulated microRNAs 222 and 223 promote resistance of cancer cells to cytotoxic T-lymphocytes by down-regulation of ICAM-1. Proc Natl Acad Sci USA 106: 10746-10751, 2009.

327. Zhang J, Han L, Ge Y, Zhou X, Zhang A, Zhang C, Zhong Y, You Y, Pu P and Kang C: miR-221/222 promote malignant progression of glioma through activation of the Akt pathway. Int J Oncol 36: 913-920, 2010.

328. Zhang C, Jiang T, Wang J, Cheng J, Pu P and Kang C: MiR-221/222 promote the growth of malignant glioma cells by regulating its target genes, molecular targets of CNS tumors. Dr Miklos Garami (Ed). ISBN: 978-953-307-736-9. InTech, pp 461-482, 2012.

329. Sarkar D, Dubayho B, Ali S, Goncalves P, Kolppera SL, Sethi S, Philip PA and Li Y: Down-regulation of miR-221 inhibits proliferation of pancreatic cancer cells through up-regulation of PTEN, p27(kip1), p57kip2, and PUMA. Am J Cancer Res 3: 465-477, 2013.

330. Zhang C, Kang C, You Y, Pu P, Yang W, Zhao P, Wang G, Zhang A, Jia Z, Han L, et al. Co-suppression of miR-221/222 cluster suppresses human glioma cell growth by targeting p27kip1 in vitro and in vivo. Int J Oncol 34: 1653-1660, 2009.

331. Zhang R, Peng B, Xin T, Guo H, Xing Y, Xu S, Feng B, Liu B and Pang Q: Plasma miR-221/222 family as novel diagnostic and prognostic biomarkers for glioma. Mol Neurobiol 53: 1452-1460, 2016.

332. Yang Y, Li F, Saha MN, Abdi J, Qiu L and Chang H: miR-137 promotes glioma cell proliferation and tumor angiogenesis by targeting BCL2. Oncotarget 6: 33269-33278, 2015.

333. Lee SH, Jung YD, Choi YS and Lee YM: Targeting of RUNX3 by miR-130a and miR-495 cooperatively increases cell proliferation and tumor angiogenesis in gastric cancer cells. Oncotarget 6: 33269-33278, 2015.
360. Gao C, Peng FH and Peng LK: MiR-200c sensitizes clear-cell renal cell carcinoma cells to sorafenib and imatinib by targeting heme oxygenase-1. Neoplasma 61: 680-689, 2014.

361. Pogribny IP, Filkowski JN, Tryndyak VP, Golubov A, Shpyleva SI and Kovalchuk O: Alterations of microRNAs and their targets are associated with acquired resistance of MCF-7 breast cancer cells to cisplatin. Int J Cancer 127: 1785-1794, 2010.

362. Suto T, Yokobori T, Yajima R, Morita H, Fujii T, Yamaguchi S, Altan B, Tsutsumi S, Assao T and Kuwano H: MicroRNA-7 expression in colorectal cancer is associated with poor prognosis and regulates cetuximab sensitivity via EGFR regulation. Carcinogenesis 36: 338-345, 2015.

363. Liu R, Liu X, Zheng Y, Gu J, Xiong S, Jiang P, Jiang X, Huang E, Yang Y, Ge D, et al: MicroRNA-7 sensitizes non-small cell lung cancer cells to paclitaxel. Oncol Lett 8: 2193-2200, 2014.

364. Gomes SE, Simões AE, Pereira DM, Castro RE, Rodrigues CM and Borralho PM: miR-143 or miR-145 overexpression increases cetuximab-mediated antibody-dependent cellular cytotoxicity in human colon cancer cells. Oncotarget: Jan 25, 2016 (Epub ahead of print).

365. Costa PM, Cardoso AL, Nóbrega C, Pereira de Almeida LF, Bruce JN, Canoll P and Pedroso de Lima MC: MicroRNA-21 silencing enhances the cytotoxic effect of the antiangiogenic drug sunitinib in glioblastoma. Hum Mol Genet 22: 904-918, 2013.

366. Qian X, Ren Y, Shi Z, Long L, Pu P, Sheng J, Yuan X and Kang C: Sequence-dependent synergistic inhibition of human glioma cell lines by combined temozolomide and miR-21 inhibitor gene therapy. Mol Pharm 9: 2636-2645, 2012.

367. Zhang S, Han L, Wei J, Shi Z, Pu P, Zhang J, Yuan X and Kang C: Combination treatment with doxorubicin and microRNA-21 inhibitor synergistically augments anticancer activity through upregulation of tumor suppressing genes. Int J Oncol 46: 1589-1600, 2015.

368. Zhang Q, Ran R, Zhang L, Liu Y, Mei L, Zhang Z, Gao H and He Q: Simultaneous delivery of therapeutic antagonoms with paclitaxel for the management of metastatic tumors by a pH-responsive anti-microbial peptide-mediated liposomal delivery system. J Control Release 197: 208-218, 2015.

369. Fan L, Yang Q, Tan J, Qiao Y, Wang Q, He J, Wu H and Zhang Y: Dual loading miR-218 mimics and Temozolomide using AuCOOH-FA-CS drug delivery system: Promising targeted anti-tumor drug delivery system with sequential release functions. J Exp Clin Cancer Res 34: 106, 2015.

370. Xue W, Dahlman JE, Tammela T, Khan OF, Sood S, Dave A, Cai W, Chirino LM, Yang GR, Bronson R, et al: Small RNA combination therapy for lung cancer. Proc Natl Acad Sci USA 111: E3553-E3561, 2014.

371. Nishimura M, Jung EJ, Shah MY, Lu C, Spizzo R, Shimizu M, Han HD, Ivan C, Rossi S, Zhang X, et al: Therapeutic synergy between microRNA and siRNA in ovarian cancer treatment. Cancer Discov 3: 1302-1315, 2013.

372. Hu X, Li W, Liu G, Wu H, Gao Y, Chen S, He D and Zhang Y: The effect of Bcl-2 siRNA combined with miR-15a oligonucleotides on the growth of Raji cells. Med Oncol 30: 430, 2013.