

Special Focus Review

Micromanipulating cancer

microRNA-based therapeutics?

Fabio Petrocca and Judy Lieberman*

Immune Disease Institute; Program in Cellular and Molecular Medicine; Children's Hospital Boston and Department of Pediatrics; Harvard Medical School; Boston, MA USA

Key words: cancer, microRNA, RNAi, delivery, therapeutics, oncomir

Because microRNAs regulate cancer cell differentiation, proliferation, survival and metastasis, manipulating microRNA function, either by mimicking or inhibiting miRNAs implicated in cancer, could provide a powerful therapeutic strategy to interfere with key pathways for cancer progression. This review will explore some of the opportunities and obstacles to harnessing microRNA biology for cancer therapy.

Introduction

The discovery of the first microRNA (miRNA), *lin-4*, a small non-coding stem loop RNA controlling larval development in *C. elegans*, by Lee and Ambros¹ in 1993 opened up a new role for small RNAs in regulating gene expression. The field exploded after Fire and Mello described dsRNA-induced gene silencing in worms in 1998,² and Elbashir and Tuschl then demonstrated in 2001 that the same process is conserved in mammalian cells and can be harnessed using synthetic small RNA duplexes (siRNAs) to silence expression of genes with high specificity.³ Since then siRNAs have been used as a powerful research tool for manipulating gene expression to understand the function of individual genes or to perform unbiased genetic screens in mammalian systems. Researchers in academia and industry are also actively investigating how to harness this technology to manipulate gene expression to treat a diverse array of diseases.

miRNAs are the most abundant class of small non-coding dsRNAs in somatic eukaryotic cells. Recent estimates suggest that as many as 90% of human protein-coding genes might be regulated by endogenous small RNAs.⁴ miRNAs block the translation and/or accelerate the degradation of mRNAs that contain partially complementary sequences most often, but not exclusively, in their 3'UTRs.⁵⁻⁷ The rules that govern miRNA binding and gene silencing are not completely understood, and identification of miRNA-regulated target genes is still a challenge. The dominant

model is that each miRNA subtly downregulates the expression of hundreds of genes, serving as a rheostat to fine tune cellular pathways. However, the expression of some genes can be dramatically inhibited, and these may be key target genes for changing the developmental or functional direction of a cell. For other genes, such as those for which haploinsufficiency has profound functional consequences, modest gene silencing can cause significant physiological effects. By acting on multiple genes in the same pathway, miRNAs can orchestrate complex combinatorial programs of gene expression and thereby act as master regulators of cell differentiation, survival, proliferation and response to environmental cues.

Most of the preclinical and proof-of-concept clinical investigations of adapting RNAi for therapeutics so far have sought to introduce synthetic siRNAs into tissues to knock-down expression of a single disease-causing gene. The current state of the art is that chemical modifications of the siRNA seem able to control off-target gene silencing caused by recognition of partially complementary sequences (a miRNA-type effect) as well as nonspecific immunostimulation secondary to siRNA recognition by immune sensors (such as TLRs, RIG-I) of pathogenic dsRNA.⁸ The major obstacle to siRNA-based therapeutics is intracellular delivery, since siRNAs need to get into the cytoplasm to engage the RNAi machinery, and they do not cross mammalian cell membranes on their own. (It has been estimated that hundreds of small biotech and larger pharmaceutical companies are currently working on this problem, a sign of how significant the problem is and of the potential opportunities that would be opened up by solving it.) This obstacle is less formidable for silencing gene expression in situations where siRNAs can be administered locally or in the liver where siRNA-containing particles or certain types of oligonucleotides can be efficiently delivered. The mature miRNA is processed into a dsRNA that resembles an siRNA, except that base pairing is imperfect and the duplex typically contains small bulges.⁹ Because of their chemical similarity, many of the therapeutic issues that confront siRNA-based therapeutics also apply to manipulating (either inhibiting or enhancing) the endogenous miRNA pathway.

miRNA-based therapeutics could potentially provide an opportunity to impact broad cellular programs to cause cancer cell differentiation, induce cell cycle arrest and apoptosis, inhibit metastasis or sensitize cells to radio/chemotherapy agents.

*Correspondence to: Judy Lieberman; Immune Disease Institute; Program in Cellular and Molecular Medicine; Children's Hospital Boston and Department of Pediatrics; Harvard Medical School; Boston, MA USA; Email: lieberman@idi.harvard.edu

Submitted: 01/20/09; Revised: 04/24/09; Accepted: 05/14/09

Previously published online as an RNA Biology E-publication:
<http://www.landesbioscience.com/journals/rnabiology/article/9013>

Although a cancer cell might readily mutate to evade any particular siRNA drug, it probably would be less likely to be able to evade a miRNA-based therapeutic that regulates the expression of hundreds of genes. This review will explore some of the opportunities and obstacles to harnessing miRNA biology for cancer therapy.

miRNAs and Cancer: Rounding Up the Usual Suspects

The first link between miRNAs and cancer was identified by Croce and colleagues when the miR-15a-16-1 cluster was implicated as a putative tumor suppressor gene mapping to chr 13q14, a small genomic region frequently deleted or translocated in chronic lymphocytic leukemia (CLL).¹⁰ Subsequent analysis of diverse human tumors showed that miRNAs were frequently associated with sites of chromosomal instability or amplification¹¹ and defined a subset of cancer-related miRNAs recurrently involved in a variety of cancers.^{12,13} Amongst these, the miR-17-92, miR-106b-25 and miR-221-222 clusters, miR-155, let-7, miR-34a, miR-200 and others have been shown to play important roles in a variety of oncogenic processes. Other miRNAs are expressed only in tumors of a limited

tissue type and may be the most accurate way of defining not only the cell of origin, but also the state of differentiation of a tumor.¹⁴ Moreover, miRNA signatures specify tumor subtypes¹⁵ and can help predict prognosis and response to therapy.¹⁶

Cancer-associated miRNAs (termed oncomirs) can act as either oncogenes or tumor suppressors (Fig. 1). So far only a small number of genes regulated by oncomirs have been described. It is likely that many additional important oncomir-regulated targets/pathways as well as additional oncomirs will continue to be described in this fast-moving field. For reasons of space, only a few of the salient miRNAs first implicated in cancer are discussed below; the reader is referred to more comprehensive recent reviews for further information.^{17,18}

A causal relationship between dysregulated miRNA expression and cancer became evident when miR-17-92 was shown to accelerate the onset of Myc-induced high-grade leukemias in mice reconstituted with hematopoietic stem cells overexpressing Myc and miR-17-92 transgenes.¹⁹ The leukemogenic mechanism likely involved miR-25/92 suppression of the proapoptotic bcl-2 family member Bim, since Bim-mediated apoptosis is an important safeguard against Myc-induced cellular proliferation.²⁰

microRNA	Change in Expression	Types of cancer	Validated targets
miR-21	Up	Breast, Colon, Lung, Pancreas, Prostate, Stomach, Biliary, Brain, Liver, Multiple Myeloma.	CDK6, PDCD4, CDKN1A, FAS, IL6R, SOCS5, APAF1, NFIB, TPM1
miR-17/20/93/106	Up	B-cell lymphoma/leukemia, Lung, Stomach, Colon, Pancreas, Prostate, Thyroid, Neuroendocrine, Neuroblastoma	E2F1, CDKN1A, NCOA3, RUNX1, VEGFA
miR-25/92	Up	B-cell lymphoma/leukemia, Lung, Stomach, Colon, Pancreas, Prostate, Thyroid, Neuroendocrine, Neuroblastoma	BCL2L11, CDKN1C
miR-221/222	Up	Thyroid, Pancreas, Stomach, Prostate, Melanoma	CDKN1B, CDKN1C, KIT
miR-34	Down	Liver, Prostate, Breast, Lung, Neuroblastoma	NOTCH1, BCL2, E2F3, MYCN, DLL1, VEGFA, CCND1, CDK6, SIRT1
miR-15/16	Down	Chronic lymphocytic leukemia, Prostate	BCL2, PDCD4, MCL1, RAB21, ACTR1A, JUN, PRIM1
miR-155	Up	B-cell lymphoma/leukemia, Lung, Breast, Nasopharyngeal carcinoma	BACH1, AGTR1, AID, LDOC1, MATR3, TM6SF1, AGTR1, SHIP1
let-7	Down	Lung, Breast	LIN28, hRAS, kRAS, HMGA2, TRIM71, NF2
miR-141/200	Up Down	Ovarian, Prostate Kidney	ZEB1, ZEB2
miR-372/373	Up	Breast, Testicular germ cell	LATS2, CD44, CD24, MYBL1, VEGFA
miR-335	Down	Breast	SOX4, MERTK

Figure 1. Well-documented tumor-associated miRNAs. miRNAs, frequently deregulated in human primary tumors, modulate the expression of some oncogenes and tumor suppressor genes. The list of experimentally validated target genes and references for them can be found at: <http://mirecords.umn.edu/miRecords/>.

Construction of the first miRNA transgenic mouse, which overexpresses miR-155, a miRNA frequently upregulated in various leukemias, in B cells, proved that a single miRNA was sufficient to trigger high-grade pre-B cell leukemia/lymphoma.²¹ Since then, additional in vivo studies have shown that miR-17-92 plays a crucial role in protecting pre-B cells from apoptosis in mice genetically deficient in miR-17-92 or Dicer,^{22,23} whereas overexpressing the same miRNAs in B-cells of transgenic mice expands the B-cell compartment and leads to autoimmunity.²⁴ Bim was identified as an important miRNA target since the Dicer-knockout phenotype can be partially rescued on a Bim-null genetic background.

To initiate and maintain a tumor, cancer cells must acquire the ability to proliferate autonomously, evade apoptosis and self-renew. To expand, progress and metastasize, solid tumors also need to be able to stimulate angiogenesis, invade normal tissue boundaries (basement membranes, extracellular matrix, nearby blood vessels) and become motile. Cancer-related miRNAs have been implicated in each of these steps. For example, the CDK inhibitors, p21, p27 and p57, the gatekeepers of the G₁/S and G₂/M cell cycle checkpoints, are silenced by miR-17/93/106,^{25,26} miR-221/222,^{27,28} and miR-25/92,²⁹ respectively. These miRNAs, upregulated in

most human tumors, may coordinate a program to prime cells for uncontrolled proliferation. Similarly, BCL2 and Bim, whose reciprocal balance regulates survival and apoptosis, are regulated by miR-15a-16,³⁰ and miR-25/92,²³⁻²⁵ respectively. Downregulation of miR-15a-16 and/or upregulation of miR-25/92 shifts the balance in favor of BCL2, thus inhibiting apoptosis. In fact, miR-15a-16 expression is downregulated in most cases of CLL and prostate cancer, whereas miR-25/92 are upregulated in most human cancers. miR-34a, which is transactivated by p53,³¹ one of the main tumor suppressor genes frequently inactivated in human tumors, acts as a tumor suppressor miRNA by inducing cell cycle arrest and apoptosis in breast and lung cancer cells. This miRNA inhibits the expression of cyclin D1 and the cyclin-dependent kinase CDK6, which drive cell cycle progression from G₁ to S phase, as well as SIRT1, a histone deacetylase that suppresses p53 transcriptional activity and the expression of its targets p21 and PUMA.^{32,33}

miRNAs also play a major role in regulating cancer cell self-renewal. A self-renewing pool of immature cancer cells within a tumor, termed tumor-initiating cells or cancer stem cells (CSC), are thought to serve as a self-renewing reserve to generate new cancer cells. These cells are also relatively resistant to chemotherapy and radiotherapy and likely play a major role in tumor recurrence after therapy.³⁴ The let-7 family of miRNAs, which is not expressed in CSC (or other types of normal stem cells), acts as a master regulator of self-renewal in breast CSC.^{35,36} Processing of the let-7 primary transcripts to the mature miRNA is inhibited at several steps by lin28, which is only expressed in stem cells.³⁷ These miRNAs inhibit the expression of the RAS, HMGA2 and MYC oncogenes.³⁸⁻⁴¹ let-7 may also enhance sensitivity to chemotherapy drugs (unpublished data).

In addition to enhancing tumor cell growth, the miR-17-92 cluster also supports tumor angiogenesis by inhibiting the expression of thrombospondin-1, a tumor suppressor that prevents the growth of new blood vessels.⁴² Invasion of adjacent tissue and metastasis by epithelial cancer cells is another key step in tumor progression and is thought to involve a process termed epithelial-mesenchymal transition (EMT). In this context, epithelial cancer cells at the periphery of the tumor engage in a complex cross-talk with stromal cells leading to a global re-programming of gene expression with loss of epithelial traits (such as E-cadherin expression) and acquisition of mesenchymal properties, including invasion and motility. The miR-141/200 family is involved in the control of EMT by regulating the transcriptional repressors ZEB1 and ZEB2 that induce EMT by repressing E-cadherin transcription.⁴³⁻⁴⁵ As a consequence, cells become motile by removing E-cadherin-mediated cell-cell adhesive interactions and liberating E-cadherin-bound β -catenin to translocate to the nucleus to activate genes that induce and maintain the mesenchymal phenotype. Moreover, the transcription factor Twist, a master regulator of EMT, activates miR-10b that in turn silences HOXD10, resulting in increased expression of the prometastatic gene RHOC,⁴⁶ whereas miR-335 suppresses the transcription factor SOX4 and inhibits metastasis.⁴⁷

Manipulating miRNAs for Cancer Prevention or Therapy

Because oncomirs control major programs of tumorigenesis, manipulating miRNAs by inhibiting oncogenic miRNAs or mimicking tumor suppressor miRNAs in cancer cells is an attractive approach to cancer therapy. The evidence supporting this idea comes from experiments performed *in vitro* or with *ex vivo* transduced tumor cells in xenotransplanted immunodeficient mice or miRNA-expressing transgenic mice. However, only a few examples of tumor inhibition from *in vivo* administration of miRNA mimics or antisense oligonucleotides (ASOs) have been reported. Locked nucleic acid (LNA) inhibitors of miR-21 inhibited glioma growth by inducing apoptosis when injected intracranially, especially when coadministered with neural precursor cells expressing a secretable variant of the cytotoxic agent tumor necrosis factor-related apoptosis inducing ligand (S-TRAIL).⁴⁸ Cholesterol-conjugated ASOs (antagomirs⁴⁹) targeting miR-221 and miR-222, which suppress p27 expression, inhibited the outgrowth of a prostate cancer cell line in nude mice when injected intratumorally.⁵⁰ In another model, antagomirs were used to suppress tumor angiogenesis in tumor xenografts by antagonizing miR-296.⁵¹

Other studies have shown that mimicking tumor suppressor miRNAs could be of therapeutic benefit. In particular, lentiviral transduction of glioma cells with miR-128 inhibited tumor outgrowth in xenotransplanted mice by suppressing expression of the stem-cell promoting epigenetic modifier Bmi-1,⁵² whereas administration of let-7 into the lungs of mice bearing an activated K-RAS transgene by intranasal adenoviral infection inhibited the outgrowth of lung adenocarcinomas that develop spontaneously in these mice.⁵³ Moreover, *ex vivo* and *in vivo* lentiviral transduction of let-7 in non small cell lung cancer cells inhibited tumor outgrowth.⁵⁴

Although these studies provide encouraging support for the potential of miRNA-based cancer therapy, none of these examples comes close to demonstrating an effective antitumor response in a scenario that accurately mimics late stage cancer, when tumor cells are disseminated. As for siRNA-based therapy, the biggest obstacle to miRNA-based therapy is intracellular delivery. Local delivery of small nucleic acids is easier to achieve with present methods than systemic delivery, which would be required for metastatic disease (Fig. 2). However, even local delivery to some tissues, such as the lung, without using viral vectors, is still far from certain. Some of the early reports that suggested therapeutic delivery of siRNAs in the lung may actually have been measuring nonspecific immunostimulatory effects of siRNAs on TLR receptors. However, some of the mucosal surfaces of the body may readily take up small RNAs into the cytoplasm for efficient gene silencing. The good news is that small RNAs can penetrate deep into some tissues, potentially providing a way to transduce not only localized epithelial cancers (which might be better treated by surgery), but also locally invasive tumors. One attractive opportunity worth considering is prevention of malignant transformation of genetically or environmentally caused dysplastic conditions that are localized to the mucosae. Examples of this include head and neck dysplastic lesions secondary to alcohol and tobacco use

or the cancers that arise from them, which typically spread locally; dysplasias in the genital or oral cavity caused by oncogenic human papilloma virus serotypes; EBV-related nasopharyngeal carcinoma; or familial colonic adenoma syndromes. For herpesvirus-mediated cancers, viral miRNAs, such as the viral homologue of miR-155, could also be antagonized. Some skin cells (such as the hair follicles) may also be good targets, and other skin cells may be transduced if the keratinized outer layer is gently disrupted. Local injection of small RNAs, particularly if formulated with targeting molecules or particles, at some sites, such as the eye or central nervous system, may also be effective, but this needs to be more carefully studied to distinguish on and off target effects and consider efficiency of delivery and silencing in specific cell types within a tissue.

Another site where delivery is more tractable is the liver since unconjugated locked nucleic acid (LNA) oligonucleotides have been shown to inhibit miR-122 in the liver of primates to lower blood cholesterol.⁵⁵ This approach might be useful for treating primary hepatocellular carcinoma or metastases after surgical removal of primary tumors that metastasize almost exclusively to the liver (i.e., colon cancer).

The greatest need for new therapies is for systemic treatment of disseminated disease. The problem of intracellular delivery may be more tractable for antagonizing miRNAs than for mimicking them since miRNAs can be inhibited by using single-stranded antisense constructs, which more readily get into cells than double-stranded RNAs. A decade of research developing ASO therapeutics has improved delivery of single-stranded molecules and is being applied to developing miRNA inhibitors. In addition to liver cells, antagomirs also target cardiac tissue when administered systemically in mice: after a month of anti-miR-133 infusion via a subcutaneous minipump, animals developed a marked increase in key hypertrophic parameters, such as diastolic left ventricular posterior wall and diastolic interventricular septum thickness, left ventricular mass index and the ratio of left ventricle weight to body weight.⁵⁶

However, delivery of antagomirs is still very inefficient and the doses required for effective miRNA inhibition suggest that the cost will be prohibitive. The miR-122 LNA ASO appears to be more efficient and doses of 3–10 mg/kg (vs. 40–80 mg/kg for antagomirs⁴⁹) target the liver well enough to lower cholesterol in non-human primates.⁵⁵ Although delivery may be easier to accomplish to inhibit a miRNA, the biological effect may be greater by mimicking a miRNA that is not expressed than by incompletely

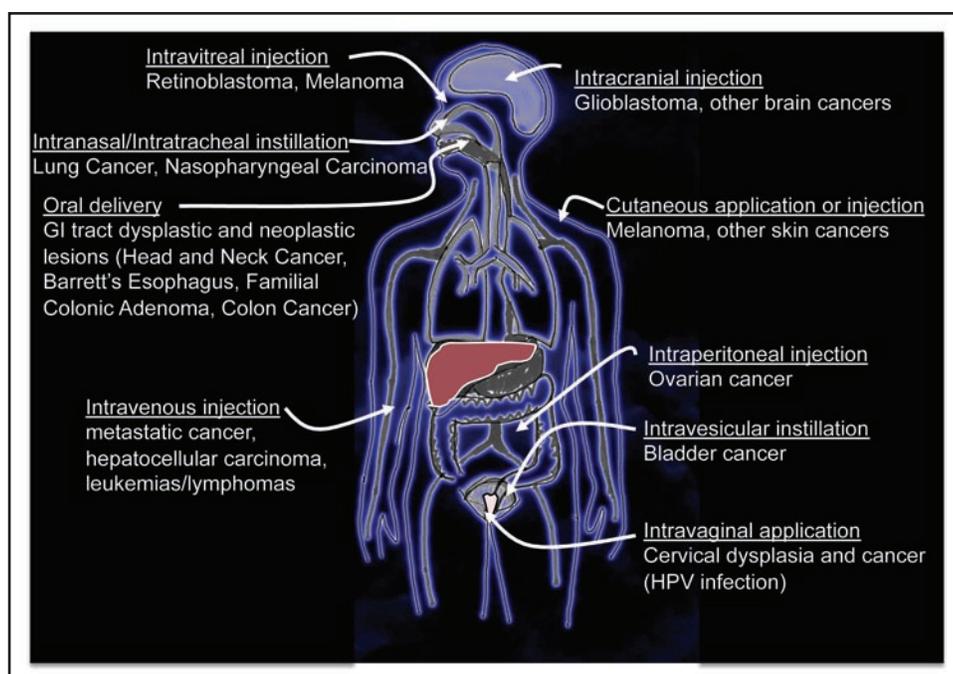


Figure 2. Strategies for delivery of small RNAs or antisense oligonucleotides to prevent or treat cancer. Specific, efficient and non-toxic RNAi delivery strategies, currently under investigation, will support translational efforts to develop RNAi-based therapies to prevent and treat a wide array of premalignant and malignant human conditions. Some of the approaches most likely to work to target different tumors are indicated. Currently available technology has been well documented to deliver small RNAs to the liver (shown in brown) after intravenous injection of liposomes or antisense oligonucleotides or to the genital tract (pink) after intravaginal delivery of lipoplexed or cholesterol-conjugated RNAs. Local injection at other sites may also be effective, but efficient delivery has not always been rigorously demonstrated. In some cases, off-target effects may have contributed to beneficial effects on disease in animal models.

inhibiting a miRNA that is. It is premature to predict which of the delivery methods that have been described so far or are in development, including different types of liposomes, polymer complexes, nanoparticles, targeting peptides, RNAs conjugated to targeting small molecules or RNA aptamers, reviewed in,^{8,57} will provide practical solutions to deliver miRNAs efficiently to metastatic tumors. Although PEGylated liposomes are already used in the oncology clinic to deliver doxorubicin and amphotericin B, the cationic lipids needed to engineer small RNA-containing liposomes may need to be further developed to reduce toxicity. More than one delivery approach may work, and some may be better suited for one type or location of cancer than another. Preferential uptake of RNA-containing particles into cancers via their leaky blood vessels compared to the relatively impermeant vessels of normal tissues may facilitate small RNA delivery to tumors and lead to reduced toxicity. We favor further pursuing cell-specific targeted delivery since it will not only reduce the dose, but also reduce toxicity to normal cells.^{58,59}

Efforts to develop miRNA-based therapy for cancer are still in their infancy. In addition to delivery, many questions remain. Because some miRNAs may accomplish their function primarily by suppressing the expression of only one or a few key target genes, it is not clear whether manipulating miRNA function will be more effective than silencing one or a few oncogenes or genes required

for cell proliferation or survival. Experiments that directly compare silencing a few key oncogenes with inhibiting or activating an oncomir in animal models will help answer this question. On the other hand, the chemical modifications being used for siRNA therapeutics to enhance stability and minimize off-target effects might not be suitable for miRNA drugs since they may suppress the weak binding of an imperfectly complementary miRNA to its physiological targets.

Small RNA therapeutics, both siRNA and miRNA mimics/inhibitors, open up ways around some of the biggest problems encountered with traditional cancer chemotherapy. For one, they broaden the universe of potential drug targets beyond the list of “druggable” targets. Important cancer-promoting genes (such as MYC and other transcription factors) are not considered viable conventional drug targets, but can be readily inhibited by siRNAs or miRNAs. If the delivery problem can be solved, identifying siRNAs or miRNAs to silence key genes can be done rapidly compared to the time it takes to identify a small molecule lead compound and optimize its activity. Moreover, the multidrug transporters that contribute to drug resistance may not function to efflux small RNAs. There is no reason to believe that CSC that are resistant to conventional chemotherapy will be selectively resistant to RNA therapeutics. In fact introducing tumor suppressor miRNAs, such as let-7, may cause CSC to differentiate into less malignant cells that are more susceptible to conventional therapy. Drug resistance through mutation of target gene sequences is likely to be as much (actually probably more) of a problem for siRNA drugs as for conventional drugs. However, it may be much more difficult for cancer cells to resist the actions of a miRNA on multiple gene targets, which is one of the reasons we think miRNA-based therapy may prove superior for cancer.

The next few years should prove exciting as researchers explore whether the promise of manipulating miRNAs for cancer therapy can work. We are cautiously optimistic, but realize that cancer cells may be able to evade all of our best attempts at control.

References

- Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993; 75:843-54.
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998; 391:806-11.
- Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; 411:494-8.
- Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; 19:92-105.
- Bagga S, Bracht J, Hunter S, Massier K, Holtz J, Eachus R, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell* 2005; 122:553-63.
- Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, et al. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 2006; 312:75-9.
- Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, et al. Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* 2005; 309:1573-6.
- de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. *Nat Rev Drug Discov* 2007; 6:443-53.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism and function. *Cell* 2004; 116:281-97.
- Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and downregulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002; 99:15524-9.
- Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004; 101:2999-3004.
- Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005; 435:834-8.
- Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006; 103:2257-61.
- Shell S, Park SM, Radjabi AR, Schickel R, Kistner EO, Jewell DA, et al. Let-7 expression defines two differentiation stages of cancer. *Proc Natl Acad Sci USA* 2007; 104:11400-5.
- Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. *Cancer Res* 2007; 67:8699-707.
- Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005; 353:1793-801.
- Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs—the micro steering wheel of tumour metastases. *Nat Rev Cancer* 2009; 9:293-302.
- Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. *Cell* 2009; 136:586-91.
- He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. *Nature* 2005; 435:828-33.
- Egle A, Harris AW, Bouillet P, Cory S. Bim is a suppressor of Myc-induced mouse B cell leukemia. *Proc Natl Acad Sci USA* 2004; 101:6164-9.
- Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA* 2006; 103:7024-9.
- Koralov SB, Muljo SA, Galler GR, Krek A, Chakraborty T, Kanellopoulou C, et al. Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. *Cell* 2008; 132:860-74.
- Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* 2008; 132:875-86.
- Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol* 2008; 9:405-14.
- Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, et al. E2F1-regulated microRNAs impair TGFbeta-dependent cell cycle arrest and apoptosis in gastric cancer. *Cancer Cell* 2008; 13:272-86.
- Ivanovska I, Ball AS, Diaz RL, Magnus JF, Kibukawa M, Schelter JM, et al. MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. *Mol Cell Biol* 2008; 28:2167-74.
- Visone R, Russo L, Pallante P, De Martino I, Ferraro A, Leone V, et al. MicroRNAs (miR)-221 and miR-222, both overexpressed in human thyroid papillary carcinomas, regulate p27^{Kip1} protein levels and cell cycle. *Endocr Relat Cancer* 2007; 14:791-8.
- Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafre SA, Farace MG. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27^{Kip1}. *J Biol Chem* 2007; 282:23716-24.
- Kim YK, Yu J, Han TS, Park SY, Namkoong B, Kim DH, et al. Functional links between clustered microRNAs: suppression of cell cycle inhibitors by microRNA clusters in gastric cancer. *Nucleic Acids Res* 2009; 37:1672-81.
- Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA* 2005; 102:13944-9.
- Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell* 2007; 26:745-52.
- Sun F, Fu H, Liu Q, Tie Y, Zhu J, Xing R, et al. Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest. *FEBS Lett* 2008; 582:1564-8.
- Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci USA* 2008; 105:13421-6.
- O'Brien CS, Farnie G, Howell SJ, Clarke RB. Are stem-like cells responsible for resistance to therapy in breast cancer? *Breast Dis* 2008; 29:83-9.
- Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 2007; 131:1109-23.
- Ibarra I, Erlich Y, Muthuswamy SK, Sachidanandam R, Hannon GJ. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. *Genes Dev* 2007; 21:3238-43.
- Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. *Science* 2008; 320:97-100.
- Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS is regulated by the let-7 microRNA family. *Cell* 2005; 120:635-47.
- Akao Y, Nakagawa Y, Naoe T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol Pharm Bull* 2006; 29:903-6.
- Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, et al. MicroRNA let-7a downregulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res* 2007; 67:9762-70.

41. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* 2007; 315:1576-9.
42. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet* 2006; 38:1060-5.
43. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 2008; 22:894-907.
44. Korpai M, Lee ES, Hu G, 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* 2008; 283:14910-4.
45. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; 10:593-601.
46. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007; 449:682-8.
47. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, et al. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008; 451:147-52.
48. Corsten MF, Miranda R, Kasmieh R, Krichevsky AM, Weissleder R, Shah K. MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. *Cancer Res* 2007; 67:8994-9000.
49. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005; 438:685-9.
50. Mercatelli N, Coppola V, Bonci D, Miele F, Costantini A, Guadagnoli M, et al. The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. *PLoS ONE* 2008; 3:4029.
51. Wurdinger T, Tannous BA, Saydam O, Skog J, Grau S, Soutschek J, et al. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. *Cancer Cell* 2008; 14:382-93.
52. Godlewski J, Nowicki MO, Bronisz A, Williams S, Otsuki A, Nuovo G, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res* 2008; 68:9125-30.
53. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, et al. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* 2008; 7:759-64.
54. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, et al. Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc Natl Acad Sci USA* 2008; 105:3903-8.
55. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. *Nature* 2008; 452:896-9.
56. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, et al. MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 2007; 13:613-8.
57. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov* 2009; 8:129-38.
58. Song E, Zhu P, Lee SK, Chowdhury D, Kussman S, Dykxhoorn DM, et al. Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors. *Nature Biotech* 2005; 23:709-17.
59. McNamara JO, 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. *Nature Biotech* 2006; 24:1005-15.