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MicroRNAs and Cancer: Short RNAs Go a Long Way

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MicroRNAs (miRNAs) may be important regulators of gene expression. By modulating oncogenic and tumor suppressor pathways they could, in principle, contribute to tumorigenesis. Consistent with this hypothesis, recurrent genetic and epigenetic alterations of individual miRNAs are found in some tumors. Functional studies are now elucidating the mechanism of action of putative oncogenic and tumor suppressor miRNAs.

Sixteen years ago in back-to-back papers in *Cell*, Ambros, Ruvkun, and their colleagues reported that a small RNA encoded by the *lin-4* locus controls the developmental timing of the nematode *Caenorhabditis elegans* by modulating the expression of the protein-coding gene *lin-14* (Lee et al., 1993; Wightman et al., 1993). At the time, few would have imagined that this discovery marked the birth of a new and far-reaching field of research. Indeed, it took several more years to appreciate that small RNAs like *lin-4* (now termed microRNAs or miRNAs) were not just an interesting peculiarity of the nematode but were an abundant (and pervasive) feature of all Bilateria, including *Homo sapiens* (reviewed in Bartel, 2004).

More recently, biochemical and genetic studies have begun to reveal the physiological functions of individual miRNAs. We now know that miRNAs act by modulating the expression of target genes through sequence complementarity between the so-called “seed” sequence of the miRNA and the “seed-match” present in the target messenger RNA (mRNA). Such binding inhibits the translation and reduces the stability of the mRNA, leading to decreased expression of the target protein. MicroRNAs control a wide array of biological processes, including differentiation, proliferation, and apoptosis. As the deregulation of these very same processes is a hallmark of cancer, there

has been speculation that mutations affecting miRNAs or their functional interactions with oncogenes and tumor suppressor genes might also contribute to tumorigenesis. Here, we summarize recent findings that now strongly support an important role for these tiny RNAs in controlling cell transformation and tumor progression.

A Plethora of Possible Oncogenic Mechanisms

Because miRNAs act by repressing gene expression through direct base-pairing interactions with their target mRNAs, there are several possible mechanisms through which miRNAs could affect tumorigenesis. Overexpression, amplification, or loss of epigenetic silencing of a gene encoding an miRNA that targets one or more tumor suppressor genes could inhibit the activity of an anti-oncogenic pathway. By contrast, the physical deletion or epigenetic silencing of an miRNA that normally represses expression of one or more oncogenes might lead to increased protein expression and a gain of oncogenic potency. More subtle mutations affecting the sequence of the mature miRNA could reduce or eliminate binding to key targets or even drastically change its specificity, thereby altering the balance of critical growth regulatory proteins. Seed-match sequences of target mRNAs could also be the sites of mutation, rendering them free from the repression of a given miRNA or subject to the effects of another (Table 1).

Although only some of these potential mechanisms have been documented in human cancers, over the past 6 years a veritable flood of reports have linked miRNAs to tumor development in one fashion or another. These range from the identification of genomic and gene expression alterations affecting miRNA genes in human cancers to studies in genetically engineered mouse models of the disease. Taken together, the available data provide a compelling case that alterations in miRNA-mRNA regulation can promote tumor development.

As is true for protein-coding genes associated with cancer, the most convincing evidence linking miRNAs to tumorigenesis comes from genetic alterations in cancer cells. Beginning with the work of Carlo Croce and colleagues in 2002, who showed that a pair of neighboring miRNAs are frequently deleted in human chronic lymphocytic leukemia (CLL), there are now additional examples in which miRNA genes are either lost or amplified in tumors (reviewed in Calin and Croce, 2006). Moreover, miRNA expression profiling studies comparing cancer tissue to normal tissue have revealed provocative patterns of miRNA expression (such as the recurrent overexpression or downregulation of individual miRNAs), some of which have been linked to changes in the methylation status of miRNA genes (reviewed in Saito and Jones, 2006). Functional studies performed in cancer cell lines or mouse models of the disease have provided fur-

Table 1. MicroRNAs and Cancer

Mutation/Epigenetic Change	Predicted Functional Consequence	Examples
Deletion of miRNA	Derepression of oncogene	<i>miR-15a-16-1</i>
Epigenetic silencing of miRNA locus	Derepression of oncogene	<i>miR-29, miR-203</i>
Point mutation affecting an miRNA or an miRNA precursor	Reduced affinity for oncogene	N.E.
	Increased affinity for tumor suppressor gene	N.E.
	Reduced processing efficiency	<i>miR-15a~16-1*</i>
	Increased processing efficiency	N.E.
Genomic amplification or translocation of miRNA locus	Increased repression of tumor suppressor gene	<i>miR-17~92, miR-21</i>
Loss of epigenetic silencing of miRNA locus	Increased repression of target tumor suppressor gene	N.E.
Point mutation in oncogene	Decreased or lost affinity for miRNA	N.E.
Point mutation in tumor suppressor gene	Gain or increased affinity for miRNA	N.E.
Rearrangement of 3'UTR (translocation, deletion)	Loss of miRNA-mediated repression	<i>HMGA-2</i>
	Gain of miRNA-mediated repression	N.E.

Shown are potentially oncogenic genetic and epigenetic changes involving miRNAs or their targets. The table includes changes affecting the miRNA gene directly as well as genetic lesions in protein-coding oncogenes and tumor suppressor genes that result in reduced or increased affinity for one or more miRNAs. (N.E., no known examples; *, the mutation is a single base change in *pri-miR-15a~16-1*, immediately downstream of the *pre-miR-16-1* sequence, and the sequence of the mature miRNA is not affected.)

ther support for a direct role of a subset of these miRNAs in tumorigenesis. Similar to the miRNA field as a whole, the discovery of the physiologically relevant targets of these cancer-associated miRNAs is still lagging, but here too, there has been recent progress with interesting candidate targets emerging.

MicroRNAs as Oncogenes

MicroRNAs that are amplified or overexpressed in cancer could act as oncogenes, and a number of putative oncogenic miRNAs have been proposed (for a recent review see Medina and Slack, 2008). An interesting case is represented by *miR-155*, which is upregulated in several hematopoietic malignancies and tumors of the breast, lung, and pancreas (reviewed in Kluiver et al., 2006). The gene encoding the primary transcript for *miR-155* had been identified well before the discovery of miRNAs, as a common proviral DNA insertion site in lymphomas induced by the avian leukosis virus. The absence of an obvious open reading frame remained a puzzling feature of the *BIC* oncogene (as it was initially named) even after it was shown that it could cooperate with *Myc* in inducing hematopoietic tumors. Although the observation that the *BIC* RNA can form extensive secondary structures (including a 145 base pair stem loop that we now know is the precursor to *miR-155*) suggested that the RNA itself could be the oncogenic factor (Tam et al., 1997), its

mechanism of action remained unclear until the identification of *miR-155*. The study of genetically engineered mice with gain- and loss-of-function alleles of *miR-155* has provided valuable insights into its physiological and oncogenic properties. Ectopic expression of *miR-155* in the bone marrow of mice has been reported to induce either polyclonal pre-B cell proliferation followed by full-blown B cell leukemia (Costinean et al., 2006) or myeloproliferation (O'Connell et al., 2008), depending on the system used to drive expression of the transgene.

Although *miR-155* is dispensable for normal B and T cell development, *miR-155*-deficient mice have defective B and T cells (Rodriguez et al., 2007; Thai et al., 2007; Vigorito et al., 2007). In particular, these mice display a reduced germinal center B cell reaction and defective IgG production in response to immunization with either T cell-dependent or -independent antigens (IgM production is normal). These results suggest an important role for *miR-155* in the generation of isotype-switched, high-affinity antibodies. Among the several targets of *miR-155* that may mediate its function is the gene encoding activation-induced cytidine deaminase (AID), which allows immunoglobulin diversification by promoting somatic hypermutation and class-switch recombination in B cells. Two groups have recently demonstrated that mutation of the single *miR-155* binding site in the 3' untranslated region (3'UTR) of the *AID* gene partially

phenocopies deletion of *miR-155* itself (Dorsett et al., 2008; Teng et al., 2008). In both cases, the result is increased levels of AID in germinal center B cells and impaired affinity maturation in response to antigen stimulation, but the phenotypic overlap is only partial. Although class-switch recombination is reduced in mice lacking *miR-155* (Thai et al., 2007; Vigorito et al., 2007), it is increased in mice carrying mutant AID (Dorsett et al., 2008; Teng et al., 2008). Thus, although these experiments elegantly demonstrate how loss of miRNA-mediated regulation of a single target gene can have profound physiological effects, they also serve as a reminder that the phenotypic consequences of loss of an miRNA are likely due to the simultaneous deregulation of many target genes.

Another notable member of the family of oncogenic miRNAs is the *miR-17~92* cluster (reviewed in Mendell, 2008). This cluster, which consists of six miRNAs that are processed from a single primary transcript, was initially linked to cancer based on the observation that it maps to a chromosomal region that is frequently amplified in a subset of human B cell lymphomas (Ota et al., 2004) and overexpressed in a variety of other human cancers. In an important *in vivo* test of the oncogenic potential of *miR-17~92*, He et al. (2005) demonstrated that a truncated version of the cluster (lacking the most distal miRNA, *miR-92*) could cooperate with c-Myc and greatly accelerate tum-

origenesis in a mouse model of B cell lymphoma. Although *miR-17~92* deregulation does not appear to be sufficient to initiate tumorigenesis per se, transgenic mice overexpressing this cluster in lymphocyte progenitor cells develop a lymphoproliferative disorder affecting both B and T cells that eventually results in autoimmunity (Xiao et al., 2008). In contrast, mice carrying a homozygous deletion of the *miR-17~92* locus exhibit premature death of B cells at the pro-B/pre-B stage resulting in lymphopenia (Ventura et al., 2008).

Although the full spectrum of genes regulated by the *miR-17~92* cluster is still unknown, one candidate, the pro-apoptotic gene *Bim*, could be a likely mediator of the B cell phenotype in *miR-17~92* null mice and in *miR-17~92* transgenic mice (Ventura et al., 2008; Xiao et al., 2008). *Bim* is a critical regulator of B cell survival and a potent tumor suppressor gene in the *Eμ-Myc* model of B cell lymphoma (Egle et al., 2004). Its 3'UTR contains multiple binding sites for miRNAs encoded by *miR-17~92*. Consistent with *Bim* being a direct target of *miR-17~92*, its expression is increased in *miR-17~92* null pre-B cells and reduced in B cells from mice overexpressing *miR-17~92*. It is therefore likely that *Bim* suppression by *miR-17~92* contributes to both the tumor-promoting activity of *miR-17~92* overexpression and its physiological function in regulating normal B cell development.

The analysis of mice lacking *miR-17~92* is also shedding light on additional functions of this cluster (Ventura et al., 2008). Mice lacking *miR-17~92* are much smaller than their wild-type littermates, have severely hypoplastic lungs, have an incompletely closed interventricular septum, and die within a few minutes after birth. The lung hypoplasia is of particular interest, as *miR-17~92* overexpression, and occasionally amplification of the locus, has been reported in human lung cancers (Hayashita et al., 2005). The mechanism underlying the lung hypoplasia is currently unclear, but reduced cell proliferation may play a role. Consistent with this hypothesis, forced expression of *miR-17~92* under the control of a lung-specific promoter leads to increased proliferation and blocks differentiation of the lung epithelium in vivo (Lu et al., 2007).

MicroRNAs as Tumor Suppressors

Several miRNAs have been implicated as tumor suppressors based on their physical deletion or reduced expression in human cancer. Beyond these associations, functional studies of a subset of these miRNAs indicate that their overexpression can limit cancer cell growth or induce apoptosis in cell culture or upon transplantation in suitable host animals. This increasingly long list includes at least a dozen miRNAs and miRNA clusters (Medina and Slack, 2008), but we will discuss only a few representative examples here.

The *miR-15a~16-1* cluster of miRNAs has recently emerged as an excellent candidate for the long sought-after tumor suppressor gene on 13q14. This chromosomal region is deleted in the majority of CLLs and in a subset of mantle cell lymphomas and prostate cancers (Calin et al., 2002). There is strong circumstantial evidence that *miR-15a~miR-16-1* is a bona fide tumor suppressor. *miR-15a~16-1* is located in the minimally deleted region in CLL (Calin et al., 2002), and a germline point mutation (a single base change) immediately downstream of the *pre-miR-16-1* sequence has been observed in a few CLL patients (Calin et al., 2005). This mutation has been linked to reduced expression of *miR-16-1*, possibly due to less efficient processing of the precursor RNA, but large-scale studies are needed to determine whether this is indeed a cancer-predisposing mutation. Interestingly, in New Zealand Black mice, a strain that shows a strong predisposition to developing a B cell lymphoproliferative disease reminiscent of human CLL, a similar base change in *pre-miR-16-1* is associated with the development of this lymphoproliferative disease (Raveche et al., 2007).

The tumor suppressor activity of *miR-15a~16-1* is not limited to B cells. More than 50% of human prostate cancers carry a deletion of 13q14. Accordingly, a recent study has shown that inhibition of *miR-15a* and *miR-16* activity leads to hyperplasia of the prostate in mice and promotes survival, proliferation, and invasion of primary prostate cells in vitro (Bonci et al., 2008). In the same study, the therapeutic potential of reconstituting expression of this cluster was illustrated by the significant regres-

sion of prostate tumor xenografts upon intra-tumoral delivery of *miR-15a* and *miR-16-1*. Although the identity of the critical targets of these two miRNAs is still unknown, the list of oncogenes that are directly regulated by *miR-15a* and *miR-16-1* include BCL2, cyclin D1, and WNT3A.

Among the most actively studied of the putative tumor suppressor miRNAs are the members of the *let-7* family (reviewed in Roush and Slack, 2008). The human genome contains a dozen *let-7* family members, organized in eight different loci (http://microrna.sanger.ac.uk/sequences/mirna_summary.pl?fam=MIPF0000002). The first member of the *let-7* family was discovered in *C. elegans*, where it induces cell-cycle exit and terminal differentiation of a particular cell type at the transition from larval to adult life. Consistent with a role in inhibiting tumor development in humans, reduced expression of multiple members of the *let-7* family is frequently observed in lung cancers, where they correlate with poor prognosis (Yanaihara et al., 2006). In addition, various *let-7* genes are located at chromosomal sites deleted in a variety of human cancers. *Let-7* genes can also be directly repressed by the c-Myc oncoprotein (Chang et al., 2008) and their precursor RNAs subjected to inhibition of further processing by lin-28 (Newman et al., 2008; Viswanathan et al., 2008). Functionally, *let-7* represses members of the Ras family of oncogenes (Johnson et al., 2005) as well as the oncogene *HMG2* (Lee and Dutta, 2007; Mayr et al., 2007) and even c-Myc itself (Sampson et al., 2007). In the best example of an oncogenic mutation affecting an miRNA-binding site, translocations involving the *HMG2* oncogene remove functional *let-7* seed-match sequences, causing overexpression of the oncoprotein (Lee and Dutta, 2007; Mayr et al., 2007). Finally, overexpression of *let-7* miRNAs can suppress tumor development in mouse models of breast and lung cancer (Kumar et al., 2008; Yu et al., 2007). Mouse knockout studies have not been reported for any *let-7* family members, and, given the potential for functional overlap within this family, it may be some time before it is clear whether loss of *let-7* in the mouse can promote tumorigenesis.

Recent studies have explored the regulation of miRNAs by tumor suppressor genes. These studies have focused on the miRNAs regulated by p53, a tumor suppressor gene that is frequently inactivated in human cancers (reviewed in He et al., 2007 and references therein). This approach has led to the identification of the *miR-34* family as an important mediator of p53 activity. This family consists of three highly related miRNAs expressed from two separate loci: *miR-34a* from chromosome 1p36 and the *miR-34b/miR-34c* cluster from chromosome 11q23. The transcription of both loci appears to be directly regulated by p53 through binding to conserved sites in the respective promoters. Similar to p53 itself, the expression of *miR-34* can induce cell-cycle arrest or apoptosis. Reduced expression of *miR-34b/miR-34c* has been reported in breast and non-small cell lung cancer cell lines. Furthermore, *miR-34a* is located on 1p36, a region of frequent hemizygous deletion in human neuroblastomas and a variety of other cancers. Interestingly, this region includes another candidate tumor suppressor gene, *CDH5*, that acts by inducing p53 expression via p19Arf. Thus, a deletion of 1p36 can impair the p53 pathway simultaneously upstream and downstream of p53.

MicroRNAs as Modulators of Tumor Progression and Metastasis

In addition to their role in promoting the development of primary tumors, miRNAs have also been implicated in affecting tumor progression, including the lethal metastatic phase of the disease. Several cell biological processes, including those controlling adhesion, migration, and invasion, are involved in allowing primary tumor cells to leave their original locations and to move to another site in the body. Not surprisingly, miRNAs help to regulate these processes, and alterations in miRNA function can influence metastatic potential (reviewed in Ma and Weinberg, 2008 and referenced therein).

Among several putative prometastatic miRNAs, *miR-10b* and *miR-373* are of particular interest. The *miR-10b* miRNA is a direct transcriptional target of Twist1, a known inducer of the epithelial-to-mesenchymal transition (EMT) and metastatic progression. Ectopic

expression of *miR-10b* in nonmetastatic breast cancer cell lines promotes cellular invasiveness and the metastatic spread of transplanted tumors, at least in part as a consequence of the direct repression of the homeobox protein HOXD10. The *miR-373* miRNA was identified in a functional screen for miRNAs that could promote cell migration in vitro (Huang et al., 2008), and its prometastatic potential has been validated in tumor transplantation experiments using breast cancer cells. Of note, *miR-373* has been identified as a potential oncogene (together with *miR-372*) in testicular germ cell tumors (Voorhoeve et al., 2006). However, it has been proposed that the prometastatic and oncogenic properties of this miRNA are due to the regulation of different genes (encoding CD44 and LATS2, respectively).

Studies of breast cancers have also revealed a series of miRNAs that are both underexpressed in advanced cancers and capable of inhibiting cell migration and metastatic spread. Members of the *miR-200* family of miRNAs target the ZEB transcription factors, known inducers of the EMT, and thus reduce cellular migration and invasiveness (Gregory et al., 2008; Park et al., 2008). Based on their differential expression in non-metastatic versus metastatic breast cancer cell lines, *miR-126*, *miR-206*, and *miR-335* were also proposed to be inhibitors of tumor progression (Tavazoie et al., 2008). Indeed, overexpression of these miRNAs can inhibit metastasis in a cell transplantation model, and reduced expression of *miR-126* and *miR-335* correlates with poor metastasis-free survival of breast cancer patients (Tavazoie et al., 2008).

Global Deregulation of MicroRNAs in Cancer

We have focused on the role of specific miRNAs in tumorigenesis, an already extensive and rapidly expanding list. However, recent work has also revealed intriguing changes in the global state of miRNA expression in cancer. Specifically, miRNA expression profiling experiments have demonstrated that most (although not all) miRNAs are underexpressed in tumor tissues compared to normal tissues (Lu et al., 2005). Although it is possible that this phenomenon

reflects the less differentiated states of the tumor cells or their higher proliferation rates, one alternative explanation is that reduced miRNA levels are selected during tumorigenesis because this itself provides some proliferative or survival advantage. These two possibilities are not necessarily mutually exclusive and indeed there is experimental evidence for both. For example, a significant increase in miRNA levels is observed upon induction of differentiation of the cancer cell line HL60 (Lu et al., 2005), consistent with the ability of miRNAs to reinforce transcriptional programs and to help maintain the differentiated state. On the other hand, work with experimental models of lung cancer has shown that genetic or RNAi-based inhibition of miRNA biogenesis can promote tumor formation and progression (Kumar et al., 2007). Finally, widespread transcriptional silencing of miRNAs by c-Myc (Chang et al., 2008) has been reported, suggesting a potential mechanism for the observed global downregulation of miRNAs in transformed cells.

Independent of the functional consequences of miRNA expression patterns in cancer, miRNA profiles have value as diagnostic and prognostic markers of disease. For example, it is sometimes impossible to determine the tissue of origin of a metastatic tumor in patients with unknown primary tumors. Because many miRNAs display exquisite tissue specificity, miRNA profiling of these lesions might prove useful. The initial findings are encouraging, as it appears that miRNA-based classification is more efficient at identifying the tissue of origin of poorly differentiated cancers than is mRNA profiling (Lu et al., 2005; Rosenfeld et al., 2008). MicroRNA profiling of human cancer might guide the choice of the best treatment strategy by providing prognostic information. Indeed, in the two most common forms of non-small cell lung cancers (adenocarcinomas and squamous cell carcinomas), high expression of *miR-155* and low expression of *let-7* correlate with poor prognosis (Yanaihara et al., 2006). Similarly, in colon cancers, elevated expression of *miR-21* is associated with poor survival (Schetter et al., 2008), whereas in chronic lymphocytic leukemias an miRNA “signature” composed of 13 miRNAs is associ-

ated with disease progression (Calin et al., 2005). The results of these and other related reports are promising, but larger-scale studies will be required to validate the usefulness of miRNA profiling in a clinical setting.

A View to the Future

Despite remarkable recent progress, the field of miRNAs and cancer is still in its infancy and many important questions remain to be addressed. Much of the evidence for the existence of oncogenic and tumor suppressor miRNAs would be best characterized as “guilt by association.” With the exception of *miR-155*, which, as discussed above, can induce the formation of B cell leukemia when ectopically expressed in mice, to date none of the putative oncogenic miRNAs have been shown to be sufficient to initiate neoplastic transformation on their own. Likewise, whereas many miRNAs may act as tumor suppressors based on their recurrent deletion or silencing in human cancers or on their growth suppressive properties in cell-based experiments, none has been subjected to germline loss-of-function analysis in mouse models. This absence of evidence should not be construed as evidence of absence, as it likely reflects the youth of the field as well as the complexity of gene targeting experiments involving multiple, functionally related genes. Similarly, although the identification and validation of miRNA targets proceeds at an increasing pace, we still know very little about the cellular circuits controlled by miRNAs in general and by cancer-associated miRNAs in particular. One should resist the temptation to expect that one or a few target mRNAs can fully explain the biological properties of a particular miRNA or, even less so, of an miRNA cluster. More likely, their effects will be found to be the net result of the complex modulation of multiple targets belonging to multiple pathways.

A clearer picture of the role of miRNAs in human cancer will likely emerge as the efforts to resequence the cancer genome reveal the true frequency of mutations in miRNAs and in their target sequences in protein-coding genes (although the latter will require specific analysis of the 3'UTR regions). At the same time, more sophisticated *in vivo* models will

likely help determine the oncogenic and tumor suppressor potential of individual miRNAs and miRNA families. Also, improved experimental and computational methods to identify miRNA targets will provide a more comprehensive understanding of their mechanism of action and of the pathways that they modulate. It is difficult to overestimate the potential impact of these findings. As increasingly effective pharmacological means to modulate miRNA activities are currently being developed (Elmen et al., 2008), identifying miRNAs that are essential for tumor maintenance or for metastasis might provide exciting new therapeutic opportunities. What began 16 years ago as a peculiar discovery in the simple worm has already had a major impact on our understanding of gene regulation. Although not yet a hallmark of cancer, alterations in miRNA function and regulation have rapidly emerged as important players in cancer pathogenesis. Before long, they might influence how the disease is treated as well.

REFERENCES

- Bartel, D.P. (2004). *Cell* 116, 281–297.
- Bonci, D., Coppola, V., Musumeci, M., Addario, A., Giuffrida, R., Memeo, L., D'Urso, L., Pagliuca, A., Biffoni, M., Labbaye, C., et al. (2008). *Nat. Med.* 14, 1271–1277.
- Calin, G.A., and Croce, C.M. (2006). *Oncogene* 25, 6202–6210.
- Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. (2002). *Proc. Natl. Acad. Sci. USA* 99, 15524–15529.
- Calin, G.A., Ferracin, M., Cimmino, A., Di Leva, G., Shimizu, M., Wojcik, S.E., Iorio, M.V., Visone, R., Sever, N.I., Fabbri, M., et al. (2005). *N. Engl. J. Med.* 353, 1793–1801.
- Chang, T.C., Yu, D., Lee, Y.S., Wentzel, E.A., Arking, D.E., West, K.M., Dang, C.V., Thomas-Tikhonenko, A., and Mendell, J.T. (2008). *Nat. Genet.* 40, 43–50.
- Costinean, S., Zanesi, N., Pekarsky, Y., Tili, E., Volinia, S., Heerema, N., and Croce, C.M. (2006). *Proc. Natl. Acad. Sci. USA* 103, 7024–7029.
- Dorsett, Y., McBride, K.M., Jankovic, M., Gzumyan, A., Thai, T.H., Robbiani, D.F., Di Virgilio, M., San-Martin, B.R., Heidkamp, G., Schwickert, T.A., et al. (2008). *Immunity* 28, 630–638.
- Egle, A., Harris, A.W., Bouillet, P., and Cory, S. (2004). *Proc. Natl. Acad. Sci. USA* 101, 6164–6169.
- Elmen, J., Lindow, M., Schutz, S., Lawrence, M., Petri, A., Obad, S., Lindholm, M., Hedtjarn, M., Hansen, H.F., Berger, U., et al. (2008). *Nature* 452, 896–899.
- Gregory, P.A., Bert, A.G., Paterson, E.L., Barry, S.C., Tsykin, A., Farshid, G., Vadas, M.A., Khew-Goodall, Y., and Goodall, G.J. (2008). *Nat. Cell Biol.* 10, 593–601.
- Hayashita, Y., Osada, H., Tatematsu, Y., Yamada, H., Yanagisawa, K., Tomida, S., Yatabe, Y., Kawahara, K., Sekido, Y., and Takahashi, T. (2005). *Cancer Res.* 65, 9628–9632.
- He, L., Thomson, J.M., Hemann, M.T., Hernandez-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S.W., Hannon, G.J., et al. (2005). *Nature* 435, 828–833.
- He, L., He, X., Lowe, S.W., and Hannon, G.J. (2007). *Nat. Rev. Cancer* 7, 819–822.
- Huang, Q., Gumireddy, K., Schrier, M., le Sage, C., Nagel, R., Nair, S., Egan, D.A., Li, A., Huang, G., Klein-Szanto, A.J., et al. (2008). *Nat. Cell Biol.* 10, 202–210.
- Johnson, S.M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K.L., Brown, D., and Slack, F.J. (2005). *Cell* 120, 635–647.
- Kluiver, J., Kroesen, B.J., Poppema, S., and van den Berg, A. (2006). *Leukemia* 20, 1931–1936.
- Kumar, M.S., Erkeland, S.J., Pester, R.E., Chen, C.Y., Ebert, M.S., Sharp, P.A., and Jacks, T. (2008). *Proc. Natl. Acad. Sci. USA* 105, 3903–3908.
- Kumar, M.S., Lu, J., Mercer, K.L., Golub, T.R., and Jacks, T. (2007). *Nat. Genet.* 39, 673–677.
- Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). *Cell* 75, 843–854.
- Lee, Y.S., and Dutta, A. (2007). *Genes Dev.* 21, 1025–1030.
- Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E.A., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., et al. (2005). *Nature* 435, 834–838.
- Lu, Y., Thomson, J.M., Wong, H.Y., Hammond, S.M., and Hogan, B.L. (2007). *Dev. Biol.* 310, 442–453.
- Ma, L., and Weinberg, R.A. (2008). *Trends Genet.* 24, 448–456.
- Mayr, C., Hemann, M.T., and Bartel, D.P. (2007). *Science* 315, 1576–1579.
- Medina, P.P., and Slack, F.J. (2008). *Cell Cycle* 7, 2485–2492.
- Mendell, J.T. (2008). *Cell* 133, 217–222.
- Newman, M.A., Thomson, J.M., and Hammond, S.M. (2008). *RNA* 14, 1539–1549.
- O'Connell, R.M., Rao, D.S., Chaudhuri, A.A., Boldin, M.P., Taganov, K.D., Nicoll, J., Paquette, R.L., and Baltimore, D. (2008). *J. Exp. Med.* 205, 585–594.
- Ota, A., Tagawa, H., Karnan, S., Tsuzuki, S., Karpas, A., Kira, S., Yoshida, Y., and Seto, M. (2004). *Cancer Res.* 64, 3087–3095.

- Park, S.M., Gaur, A.B., Lengyel, E., and Peter, M.E. (2008). *Genes Dev.* 22, 894–907.
- Raveche, E.S., Salerno, E., Scaglione, B.J., Manohar, V., Abbasi, F., Lin, Y.C., Fredrickson, T., Landgraf, P., Ramachandra, S., Huppi, K., et al. (2007). *Blood* 109, 5079–5086.
- Rodriguez, A., Vigorito, E., Clare, S., Warren, M.V., Couttet, P., Soond, D.R., van Dongen, S., Grocock, R.J., Das, P.P., Miska, E.A., et al. (2007). *Science* 316, 608–611.
- Rosenfeld, N., Aharonov, R., Meiri, E., Rosenwald, S., Spector, Y., Zepeniuk, M., Benjamin, H., Shabes, N., Tabak, S., Levy, A., et al. (2008). *Nat. Biotechnol.* 26, 462–469.
- Roush, S., and Slack, F.J. (2008). *Trends Cell Biol.* 18, 505–516.
- Saito, Y., and Jones, P.A. (2006). *Cell Cycle* 5, 2220–2222.
- Sampson, V.B., Rong, N.H., Han, J., Yang, Q., Aris, V., Soteropoulos, P., Petrelli, N.J., Dunn, S.P., and Krueger, L.J. (2007). *Cancer Res.* 67, 9762–9770.
- Schetter, A.J., Leung, S.Y., Sohn, J.J., Zanetti, K.A., Bowman, E.D., Yanaihara, N., Yuen, S.T., Chan, T.L., Kwong, D.L., Au, G.K., et al. (2008). *JAMA* 299, 425–436.
- Tam, W., Ben-Yehuda, D., and Hayward, W.S. (1997). *Mol. Cell. Biol.* 17, 1490–1502.
- Tavazoie, S.F., Alarcon, C., Oskarsson, T., Padua, D., Wang, Q., Bos, P.D., Gerald, W.L., and Massague, J. (2008). *Nature* 451, 147–152.
- Teng, G., Hakimpour, P., Landgraf, P., Rice, A., Tuschl, T., Casellas, R., and Papavasiliou, F.N. (2008). *Immunity* 28, 621–629.
- Thai, T.H., Calado, D.P., Casola, S., Ansel, K.M., Xiao, C., Xue, Y., Murphy, A., Frenthewey, D., Valenzuela, D., Kutok, J.L., et al. (2007). *Science* 316, 604–608.
- Ventura, A., Young, A.G., Winslow, M.M., Lintault, L., Meissner, A., Erkeland, S.J., Newman, J., Bronson, R.T., Crowley, D., Stone, J.R., et al. (2008). *Cell* 132, 875–886.
- Vigorito, E., Perks, K.L., Abreu-Goodger, C., Bunting, S., Xiang, Z., Kohlhaas, S., Das, P.P., Miska, E.A., Rodriguez, A., Bradley, A., et al. (2007). *Immunity* 27, 847–859.
- Viswanathan, S.R., Daley, G.Q., and Gregory, R.I. (2008). *Science* 320, 97–100.
- Voorhoeve, P.M., le Sage, C., Schrier, M., Gillis, A.J., Stoop, H., Nagel, R., Liu, Y.P., van Duijse, J., Drost, J., Griekspoor, A., et al. (2006). *Cell* 124, 1169–1181.
- Wightman, B., Ha, I., and Ruvkun, G. (1993). *Cell* 75, 855–862.
- Xiao, C.C., Srinivasan, L., Calado, D.P., Patterson, H.C., Zhang, B.C., Wang, J., Henderson, J.M., Kutok, J.L., and Rajewsky, K. (2008). *Nat. Immunol.* 9, 405–414.
- Yanaihara, N., Caplen, N., Bowman, E., Seike, M., Kumamoto, K., Yi, M., Stephens, R.M., Okamoto, A., Yokota, J., Tanaka, T., et al. (2006). *Cancer Cell* 9, 189–198.
- Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., Lieberman, J., et al. (2007). *Cell* 131, 1109–1123.