Leading Edge
Essay
Cell

# Lin28: A MicroRNA Regulator with a Macro Role

Srinivas R. Viswanathan[1] and George Q. Daley[1,*]
[1]Stem Cell Transplantation Program, Division of Pediatric Hematology/Oncology, Children's Hospital Boston and Dana Farber Cancer Institute, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and the Howard Hughes Medical Institute, Boston, MA 02115, USA
*Correspondence: george.daley@childrens.harvard.edu
DOI 10.1016/j.cell.2010.02.007

**Lin28, a highly conserved RNA-binding protein, has emerged as a modulator of the processing of the *let-7* microRNA. This role for Lin28 has important implications for our mechanistic understanding of pluripotency, the timing of development, and oncogenesis.**

MicroRNAs (miRNAs) are a class of small RNAs with diverse regulatory roles. The discovery of miRNAs and closely related small-interfering RNAs (siRNAs) is a watershed moment in biology that has changed traditional views of genetic regulation and has placed small RNAs alongside transcription factors as critical regulators of gene expression. But what regulates these regulators? Recent studies indicate that miRNA biogenesis can be posttranscriptionally regulated by *trans*-acting factors. The Lin28/*let-7* partnership is the best characterized example of the relationship between an miRNA and its posttranscriptional regulator and may shed light on the posttranscriptional regulation of other miRNAs. Here, we highlight recent advances in our understanding of the posttranscriptional regulation of the *let-7* miRNA by the conserved RNA-binding protein Lin28 and show how this impacts our understanding of pluripotency, development, and cancer.

Lin28 was first characterized in the nematode *Caenorhabditis elegans* as an important regulator of developmental timing (Ambros and Horvitz, 1984; Moss et al., 1997). Mutations in the *lin-28* gene and in other so-called "heterochronic genes" perturb developmental progression in the worm, such that characteristic events occur either earlier or later than normal. The mammalian homologs of *lin-28*, Lin28 and Lin28b, bind to the terminal loops of the precursors of *let-7* family miRNAs and block their processing into mature miRNAs (Heo et al., 2008; Newman et al., 2008; Rybak et al., 2008; Viswanathan et al., 2008; Piskounova et al., 2008). Interestingly, *let-7* is itself a heterochronic gene in the worm, suggesting that the interaction between Lin28 and *let-7* is important for regulating the timing of development.

## Lin28, Reprogramming, and Pluripotency

Both *lin-28* and *let-7* are highly conserved across evolution (Moss et al., 1997; Pasquinelli et al., 2000), and recent data are beginning to uncover roles for Lin28 in mammalian development. Several groups have reported the reprogramming of adult human fibroblasts to induced pluripotent stem (iPS) cells using the same combination of factors (OCT4, SOX2, KLF4, and c-MYC) originally discovered and used by Shinya Yamanaka to reprogram mouse fibroblasts back to a pluripotent state. However, James Thomson's group created iPS cells from adult human fibroblasts using a slightly different cocktail of factors: OCT4, SOX2, NANOG, and LIN28 (reviewed in Yamanaka, 2008). Together with recent evidence that Lin28 represses the processing of a *let-7* precursor into the mature *let-7* miRNA, this raises the tantalizing possibility that repression of *let-7* is important in establishing the pluripotent state.

In support of this notion, the *let-7* miRNA is present in low amounts or is absent in a variety of different stem and progenitor cell populations. Furthermore, as organisms age, the self-renewal capacity of their neural stem cells declines, apparently due to an increase in *let-7* and a corresponding decrease in the expression of *HMGA2*, a target gene that is silenced by *let-7* (Nishino et al., 2008). Mature miRNAs of the *let-7* family are poorly expressed in undifferentiated embryonic stem cells (ESCs) but their primary *let-7* precursor transcripts are readily detected (Thomson et al., 2006). This suggests that mature *let-7* miRNAs are maintained at low levels posttranscriptionally in ESCs and their target genes are not repressed. Recent evidence suggests that *let-7* opposes the actions of a family of ESC cell-cycle-regulating miRNAs that maintain self-renewal (Melton et al., 2010). In the reprogramming cocktail used by Yu et al. (2007), LIN28 functionally replaces c-MYC, and c-MYC is a known target of *let-7* (Kumar et al., 2007), which supports the notion that LIN28 promotes reprogramming by preventing production of mature *let-7* miRNAs. Several reports also indicate that Lin28 can affect protein levels by regulating mRNA stability (Polesskaya et al., 2007; Xu et al., 2009; Qiu et al., 2009), and that Lin28 directly promotes the translation of *OCT4* mRNA (Qiu et al., 2009) and may modulate cell proliferation by enhancing the translation of various cell-cycle regulators in mouse ESCs (Xu et al., 2009). Therefore, Lin28 may promote reprogramming through both miRNA-dependent and miRNA-independent pathways. In some cases, it is also possible that regulation of mRNA stability may be a secondary consequence of Lin28-mediated modulation of miRNA processing.

## Lin28 and Cancer

In addition to c-MYC, several other oncogenes are known to be targets of *let-7*, including K-Ras and cyclin D1 (reviewed in Roush and Slack, 2008). Therefore, *let-7* may act as a tumor suppressor miRNA, and its loss has been linked to

oncogenesis (Johnson et al., 2005; Takamizawa et al., 2004). Mammalian genomes contain multiple *let-7* family members processed from distinct precursor transcripts. In settings where *let-7* family members are coexpressed and show functional redundancy, genetic events that inactivate individual *let-7* members may not have dramatic phenotypic consequences. However, given that LIN28/LIN28B regulates all *let-7* family miRNAs, could LIN28/LIN28B contribute to oncogenesis by coordinately inactivating *let-7* family miRNAs? We have found that LIN28/LIN28B can promote transformation by repressing *let-7* miRNAs, and that activation of LIN28/LIN28B occurs in many different human tumors with a frequency of ~15% (Viswanathan et al., 2009).

It is indeed intriguing, but perhaps no coincidence, that all currently described reprogramming factors—OCT4, SOX2, KLF4, c-MYC, NANOG, and LIN28—have been linked to oncogenesis. This underscores the notion that the complex genomic reprogramming that accompanies induced pluripotency shares many commonalities with the complex, albeit aberrant, genomic reprogramming that accompanies neoplastic transformation. Strikingly, LIN28 and LIN28B are specifically activated in the subset of tumors that are poorly differentiated and carry the worst prognosis (Viswanathan et al., 2009). Aggressive, high-grade tumors are frequently characterized by impaired differentiation, and pathologists often use the differentiation status of a tumor as a metric for scoring cancer severity. When expressed in the proper genetic and epigenetic context, LIN28 together with OCT4, SOX2, and NANOG can promote the reprogramming of a terminally differentiated cell to a pluripotent ESC-like cell. But when aberrantly expressed, LIN28/LIN28B may contribute to the development of an aggressive, poorly differentiated tumor. Indeed, a recent report suggests that several different types of aggressive poorly differentiated tumors, including basal-like breast cancers and high-grade bladder carcinomas, express an ESC-like gene signature (Ben Porath et al., 2008).

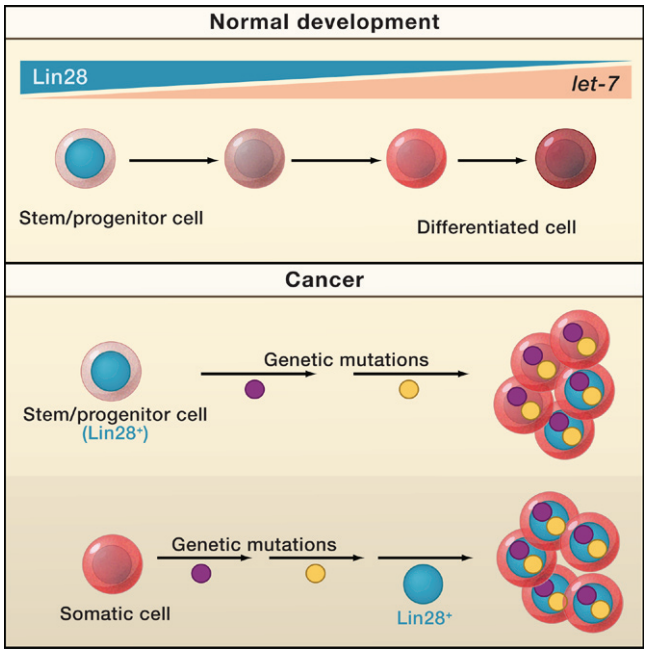


**Figure 1. *Let-7* in Development and Tumorigenesis**

(Top) During normal development, the RNA-binding protein Lin28 is highly expressed in stem and progenitor cells. Lin28 blocks processing of *let-7* microRNA (miRNA) precursor molecules into mature miRNAs , thereby maintaining expression of genes that drive self-renewal and proliferation. As progenitor cells differentiate, Lin28 expression decreases, which allows *let-7* processing and increased production of mature *let-7* miRNAs. *Let-7* miRNAs repress the expression of genes involved in self-renewal resulting in lineage commitment and terminal differentiation.

(Bottom) Tumors that express Lin28 might arise in two ways. A stem/progenitor cell expressing Lin28 could become transformed through acquisition of several genetic mutations. Alternatively, a somatic cell that has been transformed through acquisition of genetic mutations could activate Lin28 expression, through genetic or epigenetic mechanisms, in the later stages of tumorigenesis. Lin28-positive tumors are poorly differentiated and more aggressive than Lin28-negative tumors.

Poorly differentiated tumors might arise either from stem or progenitor cells that acquire additional malignant hits or from somatic cells that dedifferentiate by activating components of the pluripotency machinery (Figure 1). In the case of LIN28B, rare amplification or translocation events might explain activation in some cases (Viswanathan et al., 2009). A more common mechanism, however, might be transcriptional activation by an upstream factor or factors during tumor progression. In support of this notion, c-Myc binds to both the *Lin28* (Dangi-Garimella et al., 2009) and *LIN28B* (Chang et al., 2009) loci and activates expression of these genes. However, c-Myc activation in tumors is far more common than *LIN28/LIN28B* activation—the need for transcriptional coactivators or epigenetic inaccessibility of the *LIN28/LIN28B* loci may explain this. Finally, in some cases, LIN28/LIN28B may be expressed in a rare somatic progenitor cell of origin that becomes transformed (Figure 1). Regardless of the mechanism of activation, which may vary from tumor to tumor, cancers expressing LIN28/LIN28B appear to be dependent on these proteins for growth (Chang et al., 2009; Dangi-Garimella et al., 2009; Viswanathan et al., 2009).

The precise manner by which LIN28 contributes to tumorigenesis presents a compelling avenue for future investigation, and there are several intriguing possibilities. Rare breast tumor-initiating cells express low levels of all *let-7* family miRNAs (Yu et al., 2007). Meanwhile reduced *let-7* levels have been shown to confer radioresistance, whereas increased *let-7* levels confer radiosensitivity (Weidhaas et al., 2007). Knocking down LIN28B expression in a radioresistant lung cancer cell line increased *let-7* levels and decreased expression of *K-RAS*, a *let-7* target, resulting in enhanced radiosensitivity (Jeong et al., 2009). Therefore, tumor-initiating cells expressing LIN28/LIN28B may persist after chemotherapy and may seed relapse in some patients.

Emerging evidence suggests that HMGA2, another target of *let-7*, actively promotes the epithelial-to-mesenchymal transition (EMT), a key event in development and metastasis (Dangi-Garimella et al., 2009; Shell et al., 2007). The observation that LIN28 and LIN28B are specifically activated in advanced stage and high-grade tumors suggests that they play a role later during tumorigenesis. Furthermore, Lin28 and *let-7* have been placed directly within a metastasis sig-

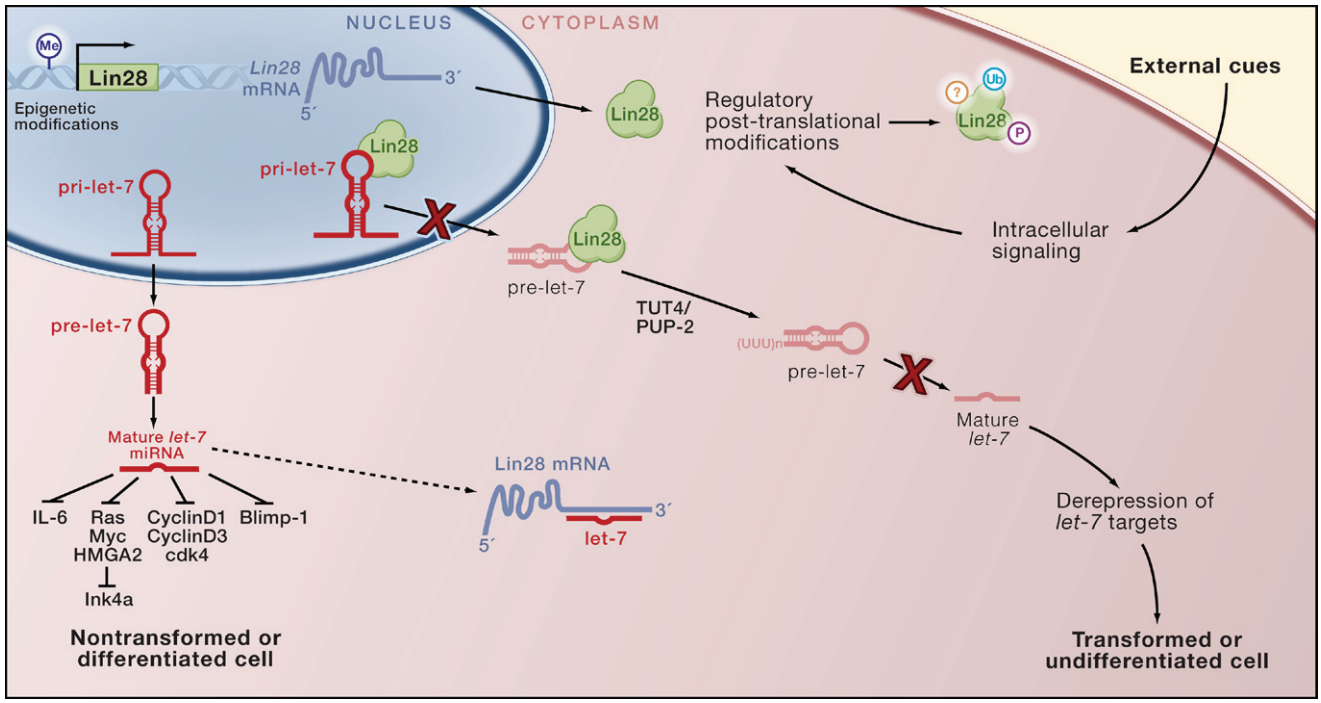


**Figure 2. The Lin28/*let-7* Regulatory Loop**

In pluripotent cells or transformed cells, the *Lin28* locus is epigenetically accessible and poised for transcriptional activation by pluripotency or oncogenic transcription factors. The Lin28 protein may be posttranslationally modified by phosphorylation, ubiquitination, or other modifications, which may affect its subcellular localization and activity. In the presence of Lin28, the pre-let-7 precursor is uridylated by TUT4 and targeted for degradation, thus blocking its processing into the mature *let-7* miRNA. This leads to derepression of *let-7* target genes, including oncogenes such as Ras and c-Myc and cell-cycle genes such as cyclin D1 and cyclin D3. In differentiated cells or nontransformed cells, the *lin28* locus is epigenetically inaccessible or the factors that transcriptionally activate *lin28* are not present. In the absence of Lin28, the pre-let-7 precursor can be efficiently processed into mature *let-7* miRNAs, which block expression of pro-mitogenic factors (e.g., Ras, c-Myc, HMGA2), and factors involved in cell-cycle progression (cyclin D1, cyclin D3, cdk4) and maintenance of the pluripotent or multipotent state (e.g., Blimp-1). Upon initiation of differentiation, *let-7* miRNA targets the *lin28* mRNA for posttranscriptional repression (dotted arrow), further relieving repression of pre-let-7 precursor processing and establishing a positive-feedback loop that helps to promote differentiation.

naling cascade (Dangi-Garimella et al., 2009). Notably, Lin28 shares many parallels with Twist, a factor recently identified as an inducer of EMT and a key mediator of metastasis (Dangi-Garimella et al., 2009; Mani et al., 2008). Both Lin28 and Twist are encoded by developmentally regulated genes, are associated with advanced malignancy and regulatory circuits involving c-Myc, and both strongly cooperate with other oncogenes in assays of cell transformation (Mani et al., 2008; Valsesia-Wittmann et al., 2004). Elucidating whether LIN28 and LIN28B have a direct role in promoting metastasis is a potentially rewarding avenue for future work.

Several studies place Lin28/Lin28b within an important regulatory network involving c-Myc and *let-7* (Chang et al., 2009; Dangi-Garimella et al., 2009). Lin28/Lin28b represses *let-7*, which is itself able to repress Lin28/Lin28b by binding to the 3′UTR of *Lin28/Lin28b* transcripts, thus forming a double-negative-feedback loop. A second feedback loop exists between Lin28 and c-Myc: Lin28/Lin28b derepresses c-Myc by repressing *let-7*, and c-Myc transcriptionally activates both Lin28 and Lin28b (Chang et al., 2009; Dangi-Garimella et al., 2009). Notably, derepression of c-Myc might also contribute to the transcriptional repression of diverse miRNAs and transcriptional activation of certain oncogenic miRNAs (Chang et al., 2008). This Lin28/*let-7*/c-Myc loop may partially explain the widespread deregulation of miRNAs observed in many human malignancies (Lu et al., 2005).

There is also a positive-feedback loop involving NF-κB, Lin28b, *let-7*, and IL-6: NF-κB induces expression of *Lin28b*, leading to repression of *let-7* and expression of the gene encoding IL-6 (a *let-7* target). IL-6 can itself activate NF-κB, completing a positive-feedback loop. In an experimental system, transient induction of the Src tyrosine kinase activates NF-κB and initiates this positive-feedback loop, resulting in neoplastic transformation. This loop operates in cancer cell lines and breast tumor-initiating cells and has been suggested to link the inflammatory response, of which IL-6 is a component, to cancer (Iliopoulos et al., 2009). Both of these loops may function in normal development: double negative-feedback loops have been postulated to play a central role in gene regulatory networks by forming "bistable switches" that reinforce binary cell fate decisions (Martinez et al., 2008).

## Lin28: A Developmental Regulator

Several genome-wide association studies (GWAS) have implicated *LIN28B* as a regulator of human development. Hirschhorn and colleagues identified markers near 12 loci that account for 2% of the population variation in human height; 1 of the 12 loci identified was

*LIN28B*, and several other loci encode *let-7* targets (Lettre et al., 2008). More recently, several independent GWAS have identified common polymorphisms near *LIN28B* as being associated with a woman's age at menarche (reviewed in Hartge, 2009). Together with established data in the worm, the identification of relevant human genetic variations suggests that the Lin28/*let-7* axis may be an important regulator of cell growth and development in evolutionarily distant organisms. Subtle variations in the activity of this pathway may result in height or size differences among individuals of the same species, and more dramatic perturbations of this pathway may drive oncogenesis. Thus, Lin28/Lin28b may be similar to PI3-kinase, mTOR, and components of the Hippo pathway, which contribute to the regulation of organ growth and size and may be deregulated in some cancers.

Several recent reports suggest that Lin28 may cooperate with a terminal uridylyl transferase (TUTase) to regulate blockade of *let-7* production (Figure 2). Lin28 and Lin28b are associated with a TUTase activity that results in the addition of a poly-U tail to the *let-7* precursor, pre-let-7 (Heo et al., 2008). Uridylated pre-let-7 cannot be efficiently processed by the enzyme Dicer and is targeted for degradation. Three groups have now identified the enzyme responsible for this activity in both mammalian cells (TUT4/zcchc11) (Heo et al., 2009; Hagan et al., 2009) and in *C. elegans* (PUP-2) (Lehrbach et al., 2009). Together, these reports outline an evolutionarily ancient pathway in which Lin28 recognizes pre-let-7 via a motif in its terminal loop and recruits a TUTase to mark pre-let-7 for degradation. Lin28/TUT4-mediated uridylation of other pre-miRNAs with similar loop motifs has also been reported (Heo et al., 2009). Given that polymerases are facile targets for pharmacological inhibition by small molecules, TUT4 may prove to be an attractive pharmaceutical target for manipulating the LIN28/*let-7* axis in cancer cells.

The terminal loops of miRNA precursors serve essential regulatory roles (Trabucchi et al., 2009; Michlewski et al., 2008; Guil and Caceres, 2007; Piskounova et al., 2008); for example, hnRNPA1 binds to the terminal loop of pri-*miR-18a* and enhances Drosha-mediated processing of this precursor into the mature miRNA (Guil and Caceres, 2007). The RNA-binding protein KSRP binds to the terminal loop of a subset of miRNAs, including *let-7*, and enhances their processing (Trabucchi et al., 2009). Indeed, it is quite likely that the expression of many miRNAs is controlled at the level of processing, with the terminal loop serving as a point of regulation (Michlewski et al., 2008). The factors that control miRNA biogenesis are likely to confer an additional layer of precision, complexity, and versatility to gene regulation. The therapeutic targeting of such regulatory factors may be a viable strategy to reduce or enhance the expression of specific miRNAs in the setting of human disease.

Recent studies of Lin28 and *let-7* have highlighted the fact that normal development, somatic cell reprogramming, and oncogenesis share common pathways. Going forward, it will be critical to define precise roles for Lin28/Lin28b in each of these settings. This will allow us to understand how the Lin28/*let-7* pathway functions in physiological development, how it is deregulated during tumorigenesis, and how it can be experimentally exploited to enhance the production of pluripotent cells. More broadly, further investigation of the Lin28/*let-7* partnership may lend insight into the mechanisms and consequences of the regulation of miRNA expression by *trans*-acting factors.

## ACKNOWLEDGMENTS

GQD is a member of the Scientific Advisory Board of MPM Capital, iPierian, and Epizyme.

## REFERENCES

Ambros, V., and Horvitz, H.R. (1984). Science *226*, 409–416.

Ben Porath, I., Thomson, M.W., Carey, V.J., Ge, R., Bell, G.W., Regev, A., and Weinberg, R.A. (2008). Nat. Genet. *40*, 499–507.

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.

Chang, T.C., Zeitels, L.R., Hwang, H.W., Chivukula, R.R., Wentzel, E.A., Dews, M., Jung, J., Gao, P., Dang, C.V., Beer, M.A., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 3384–3389.

Dangi-Garimella, S., Yun, J., Eves, E.M., Newman, M., Erkeland, S.J., Hammond, S.M., Minn, A.J., and Rosner, M.R. (2009). EMBO J. *28*, 347–358.

Guil, S., and Caceres, J.F. (2007). Nat. Struct. Mol. Biol. *14*, 591–596.

Hagan, J.P., Piskounova, E., and Gregory, R.I. (2009). Nat. Struct. Mol. Biol. *16*, 1021–1025.

Hartge, P. (2009). Nat. Genet. *41*, 637–638.

Heo, I., Joo, C., Cho, J., Ha, M., Han, J., and Kim, V.N. (2008). Mol. Cell *32*, 276–284.

Heo, I., Joo, C., Kim, Y.K., Ha, M., Yoon, M.J., Cho, J., Yeom, K.H., Han, J., and Kim, V.N. (2009). Cell *138*, 696–708.

Iliopoulos, D., Hirsch, H.A., and Struhl, K. (2009). Cell *139*, 693–706.

Jeong, S.H., Wu, H.G., and Park, W.Y. (2009). Exp. Mol. Med. *41*, 912–918.

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.

Kumar, M.S., Lu, J., Mercer, K.L., Golub, T.R., and Jacks, T. (2007). Nat. Genet. *39*, 673–677.

Lehrbach, N.J., Armisen, J., Lightfoot, H.L., Murfitt, K.J., Bugaut, A., Balasubramanian, S., and Miska, E.A. (2009). Nat. Struct. Mol. Biol. *16*, 1016–1020.

Lettre, G., Jackson, A.U., Gieger, C., Schumacher, F.R., Berndt, S.I., Sanna, S., Eyheramendy, S., Voight, B.F., Butler, J.L., Guiducci, C., Illig, T., et al. (2008). Nat. Genet. *40*, 584–591.

Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., et al. (2005). Nature *435*, 834–838.

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). Cell *133*, 704–715.

Martinez, N.J., Ow, M.C., Barrasa, M.I., Hammell, M., Sequerra, R., Doucette-Stamm, L., Roth, F.P., Ambros, V.R., and Walhout, A.J.M. (2008). Genes Dev. *22*, 2535–2549.

Melton, C., Judson, R.L., and Blelloch, R. (2010). Nature *463*, 621–626.

Michlewski, G., Guil, S., Semple, C.A., and Cßceres, J.F. (2008). Mol. Cell *32*, 383–393.

Moss, E.G., Lee, R.C., and Ambros, V. (1997). Cell *88*, 637–646.

Newman, M.A., Thomson, J.M., and Hammond, S.M. (2008). RNA *14*, 1539–1549.

Nishino, J., Kim, I., Chada, K., and Morrison, S.J. (2008). Cell *135*, 227–239.

Pasquinelli, A.E., Reinhart, B.J., Slack, F., Martindale, M.Q., Kuroda, M.I., Maller, B., Hayward, D.C., Ball, E.E., Degnan, B., Muller, P., et al. (2000). Nature *408*, 86–89.

Piskounova, E., Viswanathan, S.R., Janas, M., LaPierre, R.J., Daley, G.Q., Sliz, P., and Gregory, R.I. (2008). J. Biol. Chem. *283*, 21310–21314.

Polesskaya, A., Cuvellier, S., Naguibneva, I., Duquet, A., Moss, E.G., and Harel-Bellan, A. (2007). Genes Dev. *21*, 1125–1138.

Qiu, C., Ma, Y., Wang, J., Peng, S., and Huang, Y. (2009). Nucleic Acids Res. Published online December 4, 2009. 10.1093/nar/gkp1071.

Roush, S., and Slack, F.J. (2008). Trends Cell Biol. *18*, 505–516.

Rybak, A., Fuchs, H., Smirnova, L., Brandt, C., Pohl, E.E., Nitsch, R., and Wulczyn, F.G. (2008). Nat. Cell Biol. *10*, 987–993.

Shell, S., Park, S.M., Radjabi, A.R., Schickel, R., Kistner, E.O., Jewell, D.A., Feig, C., Lengyel, E., and Peter, M.E. (2007). Proc. Natl. Acad. Sci. USA *104*, 11400–11405.

Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., Harano, T., Yatabe, Y., Nagino, M., Nimura, Y., et al. (2004). Cancer Res. *64*, 3753–3756.

Thomson, J.M., Newman, M., Parker, J.S., Morin-Kensicki, E.M., Wright, T., and Hammond, S.M. (2006). Genes Dev. *20*, 2202–2207.

Trabucchi, M., Briata, P., Garcia-Mayoral, M., Haase, A.D., Filipowicz, W., Ramos, A., Gherzi, R., and Rosenfeld, M.G. (2009). Nature *459*, 1010–1014.

Valsesia-Wittmann, S., Magdeleine, M., Dupasquier, S., Garin, E., Jallas, A.C., Combaret, V., Krause, A., Leissner, P., and Puisieux, A. (2004). Cancer Cell *6*, 625–630.

Viswanathan, S.R., Daley, G.Q., and Gregory, R.I. (2008). Science *320*, 97–100.

Viswanathan, S.R., Powers, J.T., Einhorn, W., Hoshida, Y., Ng, T.L., Toffanin, S., O'Sullivan, M., Lu, J., Phillips, L.A., Lockhart, V.L., et al. (2009). Nat. Genet. *41*, 843–848.

Weidhaas, J.B., Babar, I., Nallur, S.M., Trang, P., Roush, S., Boehm, M., Gillespie, E., and Slack, F.J. (2007). Cancer Res. *67*, 11111–11116.

Xu, B., Zhang, K., and Huang, Y. (2009). RNA *15*, 357–361.

Yamanaka, S. (2008). Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 2079–2087.

Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., Lieberman, J., and Song, E. (2007). Cell *131*, 1109–1123.