Enjoy the Silence: The Story of *let-7* MicroRNA and Cancer

Jérôme Torrisani¹, Laurie Parmentier¹, Louis Buscail^{1,2} and Pierre Cordelier^{1,*}

¹INSERM U 858 – I2MR – Equipe 12, 1 Avenue Jean Poulhès BP 84225 31432 Toulouse Cedex 4, France; ²Service de Gastroentérologie, CHU Rangueil, 1 Avenue Jean Poulhès, 31059 Toulouse, France

Abstract: Cancer is a multi-step disease involving dynamic changes in the genome. However, studies on cancer genome so far have focused most heavily on protein-coding genes, and our knowledge on alterations of the functional noncoding sequences in cancer is largely absent. MicroRNAs (miRNAs) are endogenous small noncoding RNAs weighing 20 to 23 nucleotides that negatively regulate gene expression at the posttranscriptional level by base pairing to the 3' untranslated region of target messenger RNAs. Hundreds of miRNAs have been identified in humans and are evolutionarily conserved from plants to animals. These tiny but potent molecules regulate various physiological and pathological pathways such as cell differentiation and cell proliferation. Recently, miRNA alterations have been linked to the initiation and the progression of human cancer. As a consequence, miRNA-expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify miRNA genes that might represent downstream targets of activated oncogenic pathways, or that target protein-coding genes involved in cancer. Of importance, pioneering studies described *let-7* miRNA as a negative regulator of the oncogenic family of Ras guanosine triphosphatases in both *Caenorhabditis elegans* and human tumor cell lines. Later, *let-7* expression deregulation was reported in several cancers, suggesting that *let-7* may act as a tumor suppressor. This review will discuss the late insights in *let-7* function, the relationship between *let-7* and tumorigenesis, and the potential for modulating *let-7* expression for the treatment of cancer.

Received on: March 17, 2007 - Revised on: April 24, 2007 - Accepted on: May 4, 2007

INTRODUCTION

MicroRNAs are endogenous, non-coding small RNAs that repress target genes expression at the post-transcriptional level by base pairing to the 3' untranslated region of multiple target messenger RNAs. They are conserved through species, and form an important class of regulators that participate in multiple physiological and pathological processes including metabolism, proliferation, cell death, differentiation and development, insulin secretion from pancreatic beta cells, viral infection and cancer [1-7]. In this review, we briefly described the regulation of miRNAs biogenesis. We focused on novel insights into *let-7* miRNA regulation and function. The relationship between *let-7* and tumorigenesis, and the potential for modulating *let-7* expression for the treatment of cancer, will also be discussed.

SILENCING, THE PAST...

Late 1993, Lee *et al.* and Wightmann *et al.* first identified *lin-4*, a non-coding gene that encoded a 21-nucleotide transcript containing sequence complementarity to the 3' untranslated region (3'-UTR) of *lin-14* mRNA [8, 9]. *Lin-4* pairing with *lin-14* mRNA UTR resulted in potent inhibition of its translation. Subsequently, a second RNA called *let-7* (for lethal-7) was characterized in *Caenorhabditis elegans* [10]. At first, *let-7* was thought to specifically participate in developmental timing of *Caenorhabditis elegans*. These findings resulted in describing this new class of regulator as

stRNAs for small temporal RNAs. In 2001, hundreds of these RNAs were discovered in worms, flies and human cells and called for the first time microRNAs. [11]. Today, miRNAs are estimated to account for greater than 3% of all human genes that control the expression of thousands of target mRNAs.

MAKING miRNA: GENOMIC ORGANIZATION AND TRANSCRIPTIONAL CONTROL

MicroRNAs' encoding genes can be either organised in clusters as polycistrons, inserted in introns of mRNAs, or in isolated genomic regions. In general, miRNA expression is thought to be driven by polymerase II-promoters [12], due to tissue-specific or developmental-stage-specific transcription. One particular microRNA, *let-7*, was discovered in the early 1990's and it was found that in humans and mice, the *let-7* family consists of 12 genes encoding for nine distinct miRNA (*let-7a* to *let-7i*). For *let-7*, existence of the TRE (Temporal Regulatory Element) implicates polymerase II [13]. However, for other miRNA such as miR-1, transcription is dependent on SRF (serum response factor), MyoD, and Mef2 factors when Myc was demonstrated to regulate a specific miRNA cluster located on chromosome 13 [14]. This transcription produces long primary miRNAs (pri-miRNA) which are usually capped at the 5' end and polyadenylated similar to protein encoding RNA (mRNA). The varying regulation observed for transcription of specific miRNAs indicates that their expression is controlled allowing for their temporal and spatial patterns.

DNA methylation and histone modification also play crucial roles in chromatin remodeling and general regulation of gene expression in mammalian development and human

*Address correspondence to this author at the Inserm U 858 – I2MR – Equipe 12, 1 Avenue Jean Poulhès BP 84225 31432 Toulouse Cedex 4, France; Tel: (33)561322404; Fax: (33)561322403; E-mail: pierre.cordelier@toulouse.inserm.fr

diseases [15]. DNA hypermethylation on CpG dinucleotides associated to hypoacetylated histones results in a chromatin compaction that leads to an inhibition of transcription [16]. Recent studies have implicated both these two epigenetic control systems in miRNA expression. In fact, Saito *et al.* identified 17 out of 313 human miRNAs upregulated by the combined treatment of human cancer cells and normal fibroblasts by the DNA-demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase (HDAC) inhibitor 4-phenylbutyric acid. The miRNA upregulation, in particular miR-127, was associated with a partial DNA demethylation and an elevated level of both acetylated histone H3 and methylated histone H3-lysine 4. Moreover, Lujambio *et al.* [17] observed that miRNA-124a transcription is inhibited following CpG island hypermethylation in human tumors from different cell types. Interestingly, this loss of expression of miRNA-124a correlated with the activation of oncogenic cyclin D kinase 6, and the phosphorylation of the tumor suppressor *retinoblastoma*. Correspondingly, Scott *et al.* reported a rapid alteration of miRNA levels in a breast cancer cells in response to a treatment by the hydroxamic acid histone deacetylase inhibitor LAQ824 [18]. Furthermore, miRNA array analysis revealed a decreased expression of the *let-7* family upon LAQ824 treatment. More recently, Brueckner *et al.* demonstrated that *let-a3* is heavily methylated in normal lung samples when the tumorous *let-7-a3* gene remains unmethylated [19]. In colon cancer-derived cells, epigenetic activation of the *let-7-a3* locus resulted in enhanced miRNA expression and in oncogenic changes in transcription profiles and tumorigenicity. However, the epigenetic control of miRNA expression is under recent scrutiny. For example, a study from Haber's group showed that miRNA expression can not be induced by demethylating agents or HDAC inhibitors in a lung cancer cell line. Although one explanation for such discrepancy might reside in using different cell lines as well as different regimen of HDAC inhibitors [20], so additional work is required to ascertain the role of epigenetics in microRNA expression.

MAKING miRNA: PROCESSING MANAGEMENT

Once synthesized, pre-miRNAs form several stem-loop secondary structures, before cleavage by a complex formed by Drosha (a RNase III endonuclease) and DGCR8/Pasha (protein containing double stranded RNA binding domains) in the nucleus. This first step of maturation is the release of 60 to 70-nucleotides-long precursors that are exported to the cytoplasm by Ran-GTP and the Exportin-5. Once cytoplasmic, the RNase-III endonuclease Dicer digests both strands of the duplex to generate ≈ 20 nucleotides double-stranded RNAs. Following helicase activity, a single-stranded mature miRNA is incorporated into the ribonucleoprotein complex RISC (RNA Induced Silencing Complex). Finally, miRNAs incorporated into RISC regulate mRNA expression by triggering either mRNA cleavage/degradation or by repressing the translation machinery [4]. Interestingly, a recent study reports the identification of the complete primary transcripts for the *let-7* miRNA in *C. elegans* [21]. The authors reveal the existence of two polyadenylated transcripts, which serve as substrates for a trans-splicing reaction that involves a Spliced Leader sequence (SL1). This study effectively demonstrates that cis acting sequences and trans-acting factor are

important steps of *let-7* miRNA biogenesis. It provides another layer of regulation and control for miRNA synthesis.

ROCK, PAPER, SCISSORS?: miRNA FUNCTION

In animals, miRNAs usually control gene expression through complementary elements at the 3' untranslated regions (UTRs) of their target messenger RNAs. The mature miRNA is incorporated into an effector complex, the miRNP for miRNA-containing ribonucleo-protein particles (miRNP). These miRNP are then directed to processing bodies also called GW bodies or P/GW bodies, which are cytoplasmic compartments involved in mRNA metabolism, degradation, and translational control [22, 23]. The role of the miRNA embedded into the miRNP is to ensure target mRNA recognition. Interestingly, most of the investigated animal miRNAs bind to multiple, partially complementary sites in the 3'-UTRs. It is supposed that regulation is achieved by complete complementation which then leads to the destruction of the target mRNAs [24, 25]. However, contrary to these findings, imperfect base-pairing is shown to result in translation repression [2, 8, 9]. Furthermore, other studies have detected both miRNAs and their targets on polysomes strongly suggesting that protein synthesis initiation, elongation or stability might be impaired [26-29].

The mode of action of the miRNA *let-7a* is controversial. While the miRNA *let-7a* is recently demonstrated to sediment with the actively translating polysomal fractions in Hela cells, in Richter *et al.*, it is demonstrated that the nascent capped protein originating from *let-7's* target mRNA may be destroyed immediately, or "lost in translation". Interestingly, cap-independent-translation initiated by internal ribosome entry site (IRES) elements was quite refractory to *let-7* inhibitory action [29, 30]. Nevertheless, Pillai *et al.* provide evidence that, in mammalian cells, *let-7* miRNP inhibits translation at the initiation step [29]. Indeed, the authors suggest that the inhibition may involve interference either with the recruitment of eIF4E (eukaryotic Initiation Factor 4E) to the m7G cap or with the eIF4E-eIF4G interaction. The onset of such findings has brought forth the notion that the inhibition of translation by different miRNAs can follow diverse route.

While present findings show that microRNAs are probable in all species, and are implicated in several biological processes, only a fraction of miRNA targets are functionally validated [31, 32]. Historically, *let-7* was found to bind to the 3' UTR of *lin-41* and *hbl-1* (*lin-57*), to inhibit their translation in nematodes [10, 33-36], and control the developmental transition from the L4 stage into the adult stage [10, 33, 34]. An evolutionarily conserved regulation of the RAS family of growth control proteins by *let-7*, from Nematodes, to human cancer cells has also been demonstrated. Recently, *let-7* implication in cancerogenesis has been extended to the repression of High Mobility Group A2, thus preventing oncogenic transformation in many tumors [37].

MicroRNA SIGNATURE IN CANCER

miRNAs are aberrantly expressed or mutated in both solid and haematopoietic tumors as determined by use of fast and reliable high throughput techniques (microarray plat-

forms or bead-based flow cytometry [38]. As stated previously in this review, many abnormally expressed miRNAs in tumors potentially target transcripts of oncogenes involved in tumorigenesis. Such examples include the targeting of the Ras oncogenes by *let-7* family members, BCL2 anti-apoptotic gene by the *miR-15a/miR-16-1*, E2F1 transcription factor by the *miR-17-92* cluster, or BCL6 anti-apoptotic gene by *miR-127* [39, 40]. Of particular importance is that miRNA expression patterns correlate quite perfectly with clinical and biological characteristics of tumors and metastasis, including tissue type, differentiation, aggression and response to therapy [38]. In addition, miRNAs were found to be more stable but also to show more diversity of expression in human cancers, enabling fewer to be quantified for diagnosis, as compared to mRNAs [38]. How miRNA expression is regulated in cancer remains unclear since no correlation between pri-miRNA and mature miRNA expression in tumors is known, as compared with normal tissue samples [41]. These data strongly suggest that the miRNA alterations that occur in tumors might not be exclusively due to altered pri-miRNA transcription. Reduced expression of *let-7* miRNA has been observed in colon cancers [42] and lung cancers [43]. Moreover, when overexpressed in colon cancer cells, *let-7* miRNA leads to a growth proliferation associated with a reduced level of RAS protein [44].

Recently, preliminary data on the microRNA expression abnormalities in pancreatic endocrine, acinar tumors, and pancreatic adenocarcinoma have been reported [45-47]. This is noteworthy because pancreatic ductal carcinoma is the fifth leading cause of cancer related deaths in Western countries, with an increasing incidence over the last 40 years [48, 49]. Because of the late diagnosis, due to a long and silent clinical phase during tumor development, and the lack of early diagnostic markers, pancreatic cancer prognosis is very poor [50]. So far no effective therapies have been established to alleviate this devastating and often fatal end-stage condition. As such, there is an urgent need for the development of new modalities to improve the diagnosis and subsequent treatment of pancreatic cancer. To date, we have established the pattern of expression of miRNAs that we call potentiating, *miR-211*, *mir-301*, and the inhibitory miRNAs; *miR-21*, *miR-221*, *miR-222*, *miR-16*, *miR-15a*, *miR-17-5-p*, *let-7*. These miRNA expression profiles have been attained through pancreatic ductal carcinoma-derived cell lines, tumors and fine needle aspiration (FNA) from patients suffering from pancreatic cancer. Using Taqman-based RT-PCR, we identified a global down regulation of anti-oncogenic miR in pancreatic ductal carcinoma samples, when oncogenic miRNA were elevated (unpublished data). We further identified a global down-expression of *let-7* miRNA family members in 30 FNA of pancreatic ductal carcinoma tumors. The lack of *let-7* expression in pancreatic ductal carcinoma tumors was confirmed by in situ RT-PCR on tissues micro arrays (unpublished data). Therefore, we are currently generating a better understanding of the molecular mechanisms involved in pancreatic ductal carcinoma oncogenesis which we believe will lead to the development of new molecular markers for either early diagnosis or targeted therapy. Our data has confirmed previously reported *let-7* alteration of expression in pancreatic ductal carcinoma, and describe the

potential clinical and therapeutic interest of studying miRNA equipment in pancreatic ductal carcinoma.

miRNA AS THERAPEUTIC AGENTS: FEUDS OR FRIENDS?

Recent evidence demonstrate that miRNAs can be actors in carcinogenesis, and are consequently referred to as 'oncomirs' [39, 40]. They are shown to actively participate in tumorigenic processes, but also inhibit tumor cell proliferation and metastasis. In addition to the miRNAs themselves, the factors involved in the biogenesis of miRNAs have also been associated with various tumorigenic processes. As a consequence, gene therapies based on miRNAs transfer could be of interest to impair tumor progression. miRNAs such as *let-7*, *miR-15* and *miR-16*, which negatively regulate the RAS oncogenes, and BCL2, respectively, are promising candidates for cancer treatment.

Transient overexpression of miRNAs in cells can be achieved by transfection of double-stranded RNA molecules that mimic dicer's cleavage product. Using this approach, *let-7* mi RNA was recently demonstrated to be a potential growth suppressor in human colon cancer cells [44]. We extended this observation to pancreatic cancer-derived cells (unpublished data). In another example, Hossain A *et al.* used synthetic miRNA *17-5p* to inhibit breast cancer cell proliferation [51]. An alternate method includes introducing pri-miRNA sequences into DNA plasmids, which are shown to be sufficient to yield mature miRNAs [52]. This method was successfully used to express *let-7* in lung cancer cells [43]. This is of particular interest when using inducible or tissue-specific polII promoters to tightly control miRNA production. In addition, miRNA production can be achieved using adenovirus [53] or retrovirus systems [41] to overcome the low transfection efficiency of primary cells or to deliver miRNA *in vivo* with high efficacy. A downfall, however is that many miRNA promote cell proliferation. Oligoribonucleotides complementary to the targeted miRNA have been used to silence miRNA function in cell lines. This approach has been recently used to demonstrate *miR-21* promotion of tumor growth [54], or to characterize the effect of multiple miRNA on Hela cells and A549 cells proliferation [55]. Silencing of miRNAs in mice has recently been achieved by administration of cholesterol-conjugated single-stranded RNAs complementary to miRNAs, termed 'antagomirs' [56], which then efficiently targeted *miR-122*, a highly enriched miRNA in the liver. While these findings have led to an extraordinary amount of information and is suggestive of therapeutic advances, it remains to be determined whether antagomirs can discriminate between members of miRNA families. Synthetic miRNA, such as RNAi, might be prone to off-target, unwanted side effects, such as interferon induction and RNase L and toll-like receptor activation. In addition, one must keep in mind that excess RNAi was fatal to mice. Grimm *et al.* recently reported that overexpressed recombinant pre-miRNAs overwhelmed and saturated exportin-5 [57]. As a consequence, normal cellular pre-miRNAs processing was inhibited, as their cytoplasmic maturation, and function, leading to cell death [57]. Taken together, tightly controlled expression of miRNA is a key factor for successful therapy.

CONCLUSIONS AND PERSPECTIVES

In 2000, the original *C. elegans* miRNAs *let-7* controlling the developmental timing of progenitor cell maturation was discovered. Now, miRNAs such as *let-7* are estimated to comprise 1%–5% of the mammalian genome [4, 5, 58], making them one of the most abundant classes of regulators. miRNA have now been demonstrated to be evolutionary conserved, and to performs regulatory functions in numerous biological processes including developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation, and morphogenesis [1, 2]. miRNAs have been demonstrated to function as oncogenes, or tumor suppressors. Further, evidence for the regulation of these translational repressors in carcinogenesis at the level of pri-miRNA expression and/or of miRNA processing have accumulated and offered potential new therapeutic advances. Taken together, anti-oncogenic miRNA such as *let-7* is an exceptionally promising candidate prognostic and therapeutic advance in the treatment of cancer.

ACKNOWLEDGEMENTS

The authors would like to thank Dina Arvanitis, PhD, for careful reading of the manuscript.

REFERENCES

- [1] Alvarez-Garcia, I., Miska, E. A. MicroRNA functions in animal development and human disease. *Development* **2005**, *132*: 4653-4662.
- [2] Ambros, V. The functions of animal microRNAs. *Nature* **2004**, *431*: 350-355.
- [3] Bagga, S., Pasquinelli, A. E. Identification and analysis of microRNAs. *Genet Eng (N Y)* **2006**, *27*: 1-20.
- [4] Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **2004**, *116*: 281-297.
- [5] Bentwich, I., Avniel, A., Karov, Y., Aharonov, R., Gilad, S., Barad, O., Barzilai, A., Einat, P., Einav, U., Meiri, E., Sharon, E., Spector, Y., Bentwich, Z. Identification of hundreds of conserved and non-conserved human microRNAs. *Nat. Genet.* **2005**, *37*: 766-770.
- [6] Schwab, R., Palatnik, J. F., Riester, M., Schommer, C., Schmid, M., Weigel, D. Specific effects of microRNAs on the plant transcriptome. *Dev. Cell* **2005**, *8*: 517-527.
- [7] Zamore, P. D., Haley, B. Ribo-gnome: the big world of small RNAs. *Science* **2005**, *309*: 1519-1524.
- [8] Lee, R. C., Feinbaum, R. L., Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **1993**, *75*: 843-854.
- [9] Wightman, B., Ha, I., Ruvkun, G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* **1993**, *75*: 855-862.
- [10] Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R., Ruvkun, G. The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* **2000**, *403*: 901-906.
- [11] Lee, R. C., Ambros, V. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* **2001**, *294*: 862-864.
- [12] Kim, V. N. MicroRNA biogenesis: coordinated cropping and dicing. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*: 376-385.
- [13] Johnson, S. M., Lin, S. Y., Slack, F. J. The time of appearance of the *C. elegans* *let-7* microRNA is transcriptionally controlled utilizing a temporal regulatory element in its promoter. *Dev. Biol.* **2003**, *259*: 364-379.
- [14] O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V., Mendell, J. T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **2005**, *435*: 839-843.
- [15] Bird, A. P. The relationship of DNA methylation to cancer. *Cancer Surv.* **1996**, *28*: 87-101.
- [16] Ng, H. H., Zhang, Y., Hendrich, B., Johnson, C. A., Turner, B. M., Erdjument-Bromage, H., Tempst, P., Reinberg, D., Bird, A. MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat. Genet.* **1999**, *23*: 58-61.
- [17] Lujambio, A., Ropero, S., Ballestar, E., Fraga, M. F., Cerrato, C., Setien, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Gitt, A., Spiteri, I., Das, P. P., Caldas, C., Miska, E., Esteller, M. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res.* **2007**, *67*: 1424-1429.
- [18] Scott, G. K., Mattie, M. D., Berger, C. E., Benz, S. C., Benz, C. C. Rapid alteration of microRNA levels by histone deacetylase inhibition. *Cancer Res.* **2006**, *66*: 1277-1281.
- [19] Brueckner, B., Stresemann, C., Kuner, R., Mund, C., Musch, T., Meister, M., Sultmann, H., Lyko, F. The human *let-7a-3* locus contains an epigenetically regulated microRNA gene with oncogenic function. *Cancer Res.* **2007**, *67*: 1419-1423.
- [20] Diederichs, S., Haber, D. A. Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing. *Cancer Res.* **2006**, *66*: 6097-6104.
- [21] Bracht, J., Hunter, S., Eachus, R., Weeks, P., Pasquinelli, A. E. Trans-splicing and polyadenylation of *let-7* microRNA primary transcripts. *RNA* **2004**, *10*: 1586-1594.
- [22] Liu, J., Rivas, F. V., Wohlschlegel, J., Yates, J. R., 3rd, Parker, R., Hannon, G. J. A role for the P-body component GW182 in microRNA function. *Nat Cell Biol* **2005**, *7*: 1261-1266.
- [23] Rehwinkel, J., Behm-Ansmant, I., Gatfield, D., Izaurralde, E. A crucial role for GW182 and the DCP1: DCP2 decapping complex in miRNA-mediated gene silencing. *RNA* **2005**, *11*: 1640-1647.
- [24] Bagga, S., Bracht, J., Hunter, S., Massirer, K., Holtz, J., Eachus, R., Pasquinelli, A. E. Regulation by *let-7* and *lin-4* miRNAs results in target mRNA degradation. *Cell* **2005**, *122*: 553-563.
- [25] Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S., Johnson, J. M. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* **2005**, *433*: 769-773.
- [26] Kim, J., Krichevsky, A., Grad, Y., Hayes, G. D., Kosik, K. S., Church, G. M., Ruvkun, G. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc. Natl. Acad. Sci. USA* **2004**, *101*: 360-365.
- [27] Nelson, P. T., Hatzigeorgiou, A. G., Mourelatos, Z. miRNP: mRNA association in polyribosomes in a human neuronal cell line. *RNA* **2004**, *10*: 387-394.
- [28] Olsen, P. H., Ambros, V. The *lin-4* regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev. Biol.* **1999**, *216*: 671-680.
- [29] Pillai, R. S., Bhattacharyya, S. N., Artus, C. G., Zoller, T., Cougot, N., Basyuk, E., Bertrand, E., Filipowicz, W. Inhibition of translational initiation by *Let-7* MicroRNA in human cells. *Science* **2005**, *309*: 1573-1576.
- [30] De Gregorio, E., Baron, J., Preiss, T., Hentze, M. W. Tethered-function analysis reveals that eIF4E can recruit ribosomes independent of its binding to the cap structure. *RNA* **2001**, *7*: 106-113.
- [31] Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da Piedade, I., Gunsalus, K. C., Stoffel, M., Rajewsky, N. Combinatorial microRNA target predictions. *Nat. Genet.* **2005**, *37*: 495-500.
- [32] Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P., Burge, C. B. Prediction of mammalian microRNA targets. *Cell* **2003**, *115*: 787-798.
- [33] Abrahante, J. E., Daul, A. L., Li, M., Volk, M. L., Tennessen, J. M., Miller, E. A., Rougvie, A. E. The *Caenorhabditis elegans* hunchback-like gene *lin-57/hbl-1* controls developmental time and is regulated by microRNAs. *Dev. Cell* **2003**, *4*: 625-637.
- [34] Lin, S. Y., Johnson, S. M., Abraham, M., Vella, M. C., Pasquinelli, A., Gamberi, C., Gottlieb, E., Slack, F. J. The *C. elegans* hunchback homolog, *hbl-1*, controls temporal patterning and is a probable microRNA target. *Dev. Cell* **2003**, *4*: 639-650.
- [35] Slack, F. J., Basson, M., Liu, Z., Ambros, V., Horvitz, H. R., Ruvkun, G. The *lin-41* RBCC gene acts in the *C. elegans* heterochronic pathway between the *let-7* regulatory RNA and the LIN-29 transcription factor. *Mol. Cell* **2000**, *5*: 659-669.
- [36] Vella, M. C., Choi, E. Y., Lin, S. Y., Reinert, K., Slack, F. J. The *C. elegans* microRNA *let-7* binds to imperfect *let-7* complementary sites from the *lin-41* 3'UTR. *Genes Dev.* **2004**, *18*: 132-137.
- [37] Mayr, C., Hemann, M. T., Bartel, D. P. Disrupting the Pairing Between *let-7* and *Hmga2* Enhances Oncogenic Transformation. *Science* **2007**.

- [38] 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., Downing, J. R., Jacks, T., Horvitz, H. R., Golub, T. R. MicroRNA expression profiles classify human cancers. *Nature* **2005**, *435*: 834-838.
- [39] Esquela-Kerscher, A., Slack, F. J. Oncomirs - microRNAs with a role in cancer. *Nat. Rev. Cancer* **2006**, *6*: 259-269.
- [40] Slack, F. J., Weidhaas, J. B. MicroRNAs as a potential magic bullet in cancer. *Future Oncol.* **2006**, *2*: 73-82.
- [41] He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S. W., Hannon, G. J., Hammond, S. M. A microRNA polycistron as a potential human oncogene. *Nature* **2005**, *435*: 828-833.
- [42] Michael, M. Z., SM, O. C., van Holst Pellekaan, N. G., Young, G. P., James, R. J. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol. Cancer. Res.* **2003**, *1*: 882-891.
- [43] Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., Harano, T., Yatabe, Y., Nagino, M., Nimura, Y., Mitsudomi, T., Takahashi, T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res.* **2004**, *64*: 3753-3756.
- [44] 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*: 903-906.
- [45] Lee, E. J., Gusev, Y., Jiang, J., Nuovo, G. J., Lerner, M. R., Frankel, W. L., Morgan, D. L., Postier, R. G., Brackett, D. J., Schmittgen, T. D. Expression profiling identifies microRNA signature in pancreatic cancer. *Int. J. Cancer* **2007**, *120*: 1046-1054.
- [46] Roldo, C., Missiaglia, E., Hagan, J. P., Falconi, M., Capelli, P., Bersani, S., Calin, G. A., Volinia, S., Liu, C. G., Scarpa, A., Croce, C. M. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. *J. Clin. Oncol.* **2006**, *24*: 4677-4684.
- [47] Szafranska, A. E., Davison, T. S., John, J., Cannon, T., Sipos, B., Maghnouj, A., Labourier, E., Hahn, S. A. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* **2007**.
- [48] Rosewicz, S., Wiedenmann, B. Pancreatic carcinoma. *Lancet* **1997**, *349*: 485-489.
- [49] Safioleas, M. C., Moulakakis, K. G. Pancreatic cancer today. *Hepato-gastroenterology* **2004**, *51*: 862-868.
- [50] Jemal, A., Thomas, A., Murray, T., Thun, M. Cancer statistics, **2002**. *CA Cancer. J. Clin.* **2002**, *52*: 23-47.
- [51] Hossain, A., Kuo, M. T., Saunders, G. F. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol. Cell Biol.* **2006**, *26*: 8191-8201.
- [52] Chen, C. Z., Li, L., Lodish, H. F., Bartel, D. P. MicroRNAs modulate hematopoietic lineage differentiation. *Science* **2004**, *303*: 83-86.
- [53] Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P., Stoffel, M. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* **2004**, *432*: 226-230.
- [54] Meng, F., Henson, R., Lang, M., Wehbe, H., Maheshwari, S., Mendell, J. T., Jiang, J., Schmittgen, T. D., Patel, T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* **2006**, *130*: 2113-2129.
- [55] Cheng, A. M., Byrom, M. W., Shelton, J., Ford, L. P. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res.* **2005**, *33*: 1290-1297.
- [56] Krutzfeldt, J., Rajewsky, N., Braich, R., Rajeev, K. G., Tuschl, T., Manoharan, M., Stoffel, M. Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* **2005**, *438*: 685-689.
- [57] Grimm, D., Streetz, K. L., Jopling, C. L., Storm, T. A., Pandey, K., Davis, C. R., Marion, P., Salazar, F., Kay, M. A. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature* **2006**, *441*: 537-541.
- [58] Berezikov, E., Guryev, V., van de Belt, J., Wienholds, E., Plasterk, R. H., Cuppen, E. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* **2005**, *120*: 21-24.