Review Article

MicroRNA: A NEW TOOL FOR CANCER THERAPY

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ABSTRACT

MicroRNAs (miRNAs) are a class of small, endogenous RNAs of 21–25 nucleotides (nts) in length. By targeting specific mRNAs for degradation or translation repression they play an important regulatory role in animals and plants. We present a comprehensive and timely review of the role of miRNAs in cancer. The existing knowledge about miRNAs synthesis, mechanisms for regulation of the genome, practical implications of miRNAs as biomarkers, novel drug targets and therapeutic tools for diagnosis, prognosis, and treatments of human cancers are discussed in this review. The recent findings in miRNA studies is summarized in this review, may add new dimensions to small RNA biology and miRNA therapeutics.

Keywords: McroiRNA, miRNA therapeutics, cancer, biomarker.

1. INTRODUCTION

Modulating the expression level of specific proteins based on sequence complementarities with their target mRNA molecules is contributed by miRNAs, a small, endogenous noncoding RNA molecules. Most of miRNA species identified thus far are encoded in portions of the genome that had been previously thought to be noncoding regions. Diverse processes including cell proliferation, cell death, fat metabolism, neuronal patterning, hematopoietic differentiation, immunity, and control of leaf and flower development are known functions of miRNA[1]. MicroRNAs have recently emerged as an exciting new class of disease biomarker with further potential as the next generation of cancer therapeutics. Since their discovery in 1993, these small, endogenous, non-coding RNAs have been shown to play important regulatory roles in governing gene expression and cellular processes, whilst aberrant expression of miRNAs has been observed in a diversity of pathological events. Importantly, they have been critically implicated in the pathogenesis of most human cancers. Although elucidating their mechanisms of action is still in its infancy, the discovery of miRNAs has uncovered an entirely new and exciting repertoire of molecular factors upstream of gene expression, with great potential for new developments in current diagnostic and therapeutic strategies in the management of cancer patients.

2. MicroRNA SYNTHESIS IN ANIMALS

MicroRNAs are defined as 21–25 nucleotide single-stranded RNAs (ssRNAs), which are produced from hairpin shaped precursors[2]. In animals, genes for miRNAs are transcribed to a primary miRNA (pri-miRNA). The pri-miRNA is processed within the nucleus to a precursor miRNA (pre-miRNA) by Drosha, a class 2 RNase III enzyme. Next, the transport of pre-miRNAs to the cytoplasm is mediated by exportin-5. In the cytoplasm, they are further processed to become mature miRNAs by

Dicer an RNase III type protein and loaded onto the Argonaute (ago) protein to produce the effector RNA-induced silencing complex (RISC). A recent study reported 154C. elegans, 152 Drosophila melanogaster, 337 Daniorerios (zebrafish), 475 Gallus gallus (chicken), 695 human, and 187 Arabidopsis thaliana miRNAs[3]. It is worth noting that the miRNA database "miRBase" reports an indeed larger number of human miRNA than the reported figures. miRNAs have even been reported in simple multicellular organisms[4].

3. Nuclear processing

All animal miRNAs are first processed in the nucleus. The pre-microRNA produced by Pol II is cleaved at the stem of the hairpin structure, which releases an approximately 60–70 nt hairpin structure, known as the precursor miRNA (pre-miRNA)[5,6]. This processing step is performed by Drosha, which requires the DiGeorge syndrome critical region in gene 8 (DGCR8) in humans and Pasha in D. melanogaster or C. elegans as a cofactor[7–11]. Drosha, in conjunction with either DGCR8 or Pasha, forms a large complex known as the microprocessor complex[7,9].

a. Transportation by exportin-5

Pre-miRNAs are transported into the cytoplasm for further processing to become mature miRNAs. The transport of the pre-miRNA occurs through nuclear pore complexes, which are large proteinaceous channels embedded in the nuclear membrane[12]. The transport of the pre-miRNA is mediated by the RanGTP-dependent nuclear transport receptor exportin-5[13–15].

b. Cytoplasmic processing and Argonaute loading

The pre-miRNA is released in the cytoplasm by means of exportin-5(EXP5) and is subsequently processed by an endonuclease cytoplasmic RNase III enzyme Dicer to create a mature miRNA[16–19]. Dicer is a highly specific enzyme that measures about 22 nt from the preexisting terminus of the pre-miRNA and cleaves the miRNA strand.

Dicer is a highly conserved protein that exists in almost all eukaryotic organisms. Some organisms have multiple types of Dicers; for example, D. melanogaster contains Dicer-1 and Dicer-2, each having different roles. Dicer-1 is required for miRNA maturation, whereas Dicer-2 is required for the maturation of siRNA[20].

3. MicroRNA SYNTHESIS IN PLANTS

Genetic studies showed that Dicer like-1 (DCL-1) is solely responsible for plant miRNA processing. The HASTY (HST) homologue of exportin-5 mediates the export of miRNAs from the nucleus to the cytoplasm.

3.1. Genes and their transcription in plants

Recently, advanced genetics, direct cloning and sequencing, and bioinformatics and computational prediction methods have revealed many new miRNAs and their functions in Arabidopsis and other plant species[21]. A recent study reported 959 miRNAs genes from 10 plant species including mosses, dicots, and monocots[21].

3.2. Dicer processing and methylation

Plant miRNA processing is entirely dependent on Dicer-like proteins. Various studies in A. thaliana and other plants have revealed that DCL1 is important for miRNA processing[22]. DCL1 is a nuclear protein which indicates that mature miRNAs in plants might be synthesized in the nucleus[23]. Plant miRNA methylation occurs after Dicer processing, which distinguishes it from animal miRNAs. Hua Enhancer (HEN1), a methyltransferase, may be responsible for methylation and has a general role in miRNA processing in plants[21].

3.3. Argonaute loading and transportation

The resulting methylated miRNA/miRNA* duplex is loaded onto the Ago protein to generate RISC. The Ago family proteins are composed of three distinctive domains: the PAZ, MID, and PIWI domains[24]. The Ago protein PAZ domains bind to RNA and PIWI domains in a folded structure similar to RNase H[25].

4. MECHANISM

MiRNAs guide miRISC to specifically recognize messenger RNA (mRNA) and downregulate gene expression by one of the two posttranscriptional mechanisms: (i) translational repression and (ii) mRNA cleavage (Fig.1). Initially, it was proposed that lin-4 RNA represses translation of C. elegans lin-14 mRNA. Current studies suggest that if miRISC contains a heterologous RNA recognition factor, then it facilitates miRISC to recognize and specifically represses mRNA in spite of lacking miRNA binding sites[26].

4.1. Translation repression

The exact mechanism for the repression of target mRNA translation by miRISC is still unknown. Whether repression occurs at the translational initiation or posttranslational level still needs to be determined. However, the current model suggests that the eIF4F complex is involved in translational initiation. The mRNA molecule becomes circular as a result of this process, and the translation efficiency is thereby improved. In some viral mRNAs, the translation initiation process is facilitated without any initiation factors through internal ribosome sites (IRES), which require only a subset of the initiation factors[27].

4.2. mRNA degradation

Recent studies have suggested that not only the Agocatalyzed mRNA degradation process is responsible for the mRNA degradation, but other mechanisms such as deadenylation, decapping, and exonucleolytic digestion of mRNA are also involved[28–30]. The exact process of target selection has yet to be determined. However, it has been shown that the number, type, and position of mismatches in the miRNA/ mRNA duplex play a critical role in the selection of the degradation or translational repression mechanisms[31].

5. MicroRNAs AND THEIR BIOLOGICAL FUNCTIONS

Recent studies have suggested that a number of miRNAs are able to activate the expression of certain target genes in a sequence-specific manner instead of silencing them. For instance, miR-373 induces expression of E-cadherin and cold-shock domain-containing protein C2 (CSDC2) genes with complementary sequences in their promoters[32]. This novel phenomenon, although largely remaining uncharacterized, is termed "RNA activation" (RNAa). While thus far the exact mechanisms of RNAa remain to be elucidated, the process may require the Ago2 protein and could be associated with histone changes linked to gene activation[33]. It is estimated that over 30% of protein-coding genes in human genome are regulated by miRNAs, suggesting that most of individual miRNAs target multiple protein-coding genes[34]. Therefore, it is convincible that miRNAs play important roles in a wide variety of biological processes. Indeed, accumulated evidence has demonstrated modulation effects of miRNA on development, cell proliferation, differentiation, apoptosis, adhesion, migration and invasion, as well as other biological processes. Thus, expression of this important class of molecules is usually correlated with an array of pathological conditions, among which cancer may represent one of the most relevant diseases related to aberrant expression and/or functions of miRNAs.
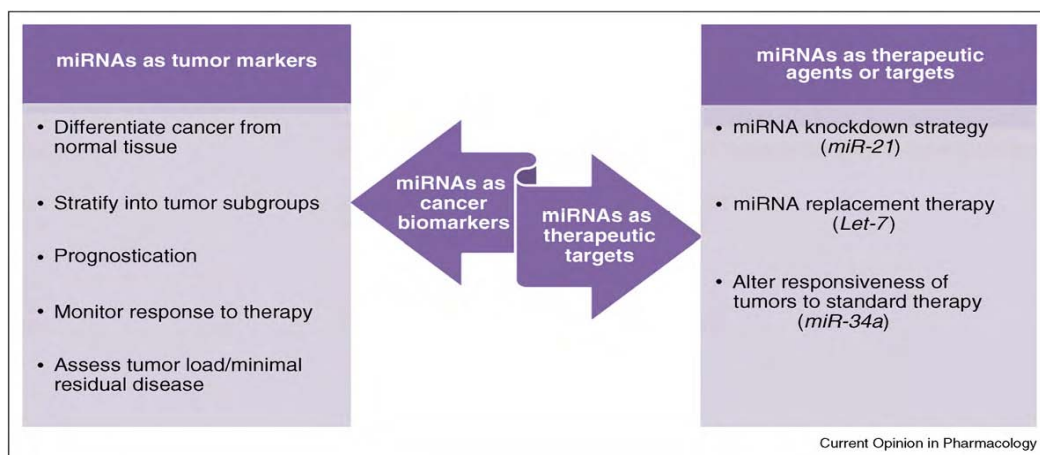
6. MicroRNAs AS BIOMARKERS FOR CANCER

The emergence of miRNAs as modulators of gene expression identifies them as obvious novel candidate diagnostic and prognostic indicators, and potential therapeutic targets. In addition to their tissue specificity, miRNAs hold other unique characteristics that herald them as ideal tumor markers including their stability, ease of detection and association with established clinicopathological prognostic parameters.Potential applications of miRNAs as biomarkers, therapeutic agents and targets for cancer is shown in fig. 2.
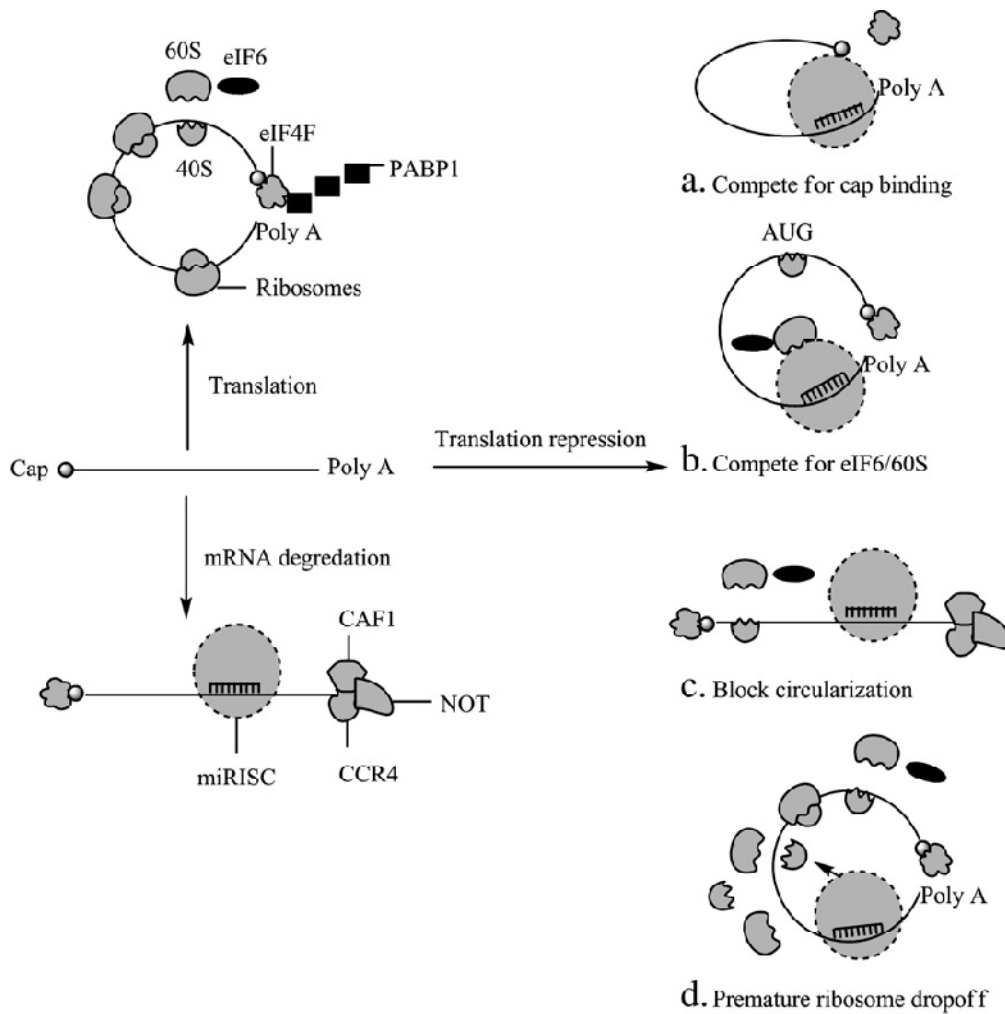
Figure 1: Possible mechanisms for miRNA gene regulation.

Figure 2: Potential applications of miRNAs as biomarkers, therapeutic agents and targets for cancer.

6.1 Breast cancer

Among the leading miRNAs differentially expressed; miR 10b, miR-125b and mR-145 were downregulated whilst miR-21 and miR-155 were consistently over-expressed in breast tumor tissues. miRNAs with prognostic value for breast cancer include miR-10b, miR-21, miR-145, miR-9-3 and let-7; levels of these miRNAs correlate with tumor grade, degree of vascular invasion, lymph node metastases, or metastatic potential[35]. Analogous to the derivation of intrinsic subtypes from gene expression profiles, and the estimation of risk of disease recurrence from multi-gene assays such as Oncotype DX, it is predicted that tumor or circulating miRNA signatures could serve as novel biomarkers and prognostic indicators, and will provide strong rationale for individualized treatment for breast cancer.

6.2 Lung cancer

A number of miRNAs are known to be intimately involved in lung cancer initiation, progression and prognosis. Lung is one of the tissues with the most abundant expression of the let-7 family of miRNAs in its normal non-cancerous state[36]. Tumors have repeatedly been shown to under-express most of the transcripts of the let-7 family; consistent with its known tumor suppressor role. Let-7 regulates several oncogenic pathways, including the RAS pathway where it represses activity of the KRAS oncogene, mutations of which are commonly implicated in adenocarcinoma of the lung.

6.3 Colorectal cancer

The role of miRNAs as biomarkers for colorectal cancer (CRC) is equally as promising and have identified miRNAs which can distinguish cancerous from normal colon tissue; in particular miR-21 was observed to be over-expressed in 87% of colon cancers[37]. miRNAs with tumor suppressor properties which are under-expressed in CRC specimens, and thus potentially function as tumor suppressors, include miR- 31, miR-34a, miR-96, miR-143, miR-145, and let-7a[37,38]. MiR-34a is a well described tumor suppressor miRNA which regulates the p53 pathway and when over expressed induces apoptosis and acute senescence. Conversely reduction of miR-34 expression and function attenuates p53-mediated cell death and is therefore implicated in tumorigenesis, including initiation of CRC[39,40]. It is postulated therefore that loss of mir- 34a expression in colorectal biopsy specimens may be an early biomarker of CRC.

6.4 Prostate cancer

The rationale for miRNAs as biomarkers for prostate cancer is less well defined than for other common cancers given that their investigation in prostate cancer specimens is still in its infancy. Nonetheless emerging data suggest the miRNA expression is clearly dysregulated in prostate tumors and of the 6 profiling studies in this field to date, decreased expression of miR-23b, miR-34a, miR- 100, miR-145 and miR-205 is consistently reported in cancerous tissue compared to normal prostate. In 5 of these 6 miRNA profiling studies, miR-221 and miR-222 are also aberrantly expressed in tumor tissues[41]. The role of miR-141 in initiation and progression of the disease also merits investigation. Nevertheless the growing body of evidence in this field supports miRNAs as promising biomarkers for prostate cancer.

7. MicroRNAs as novel drug targets for cancer treatment

MicroRNAs as novel drug targets or therapeutic tools to develop novel strategies for the treatment of human cancers is suggested by the distinct functions of miRNAs in tumor initiation, progression, and metastasis in human cancers. Plausible approaches could be through either downregulating"oncogenic" miRNAs or upregulating "tumor suppressor" miRNAs. Treatments with LNAantimirs against miR-16, miR-21, and the miR-17-92 cluster were tested in cancer cell lines derived from

glioblastoma, colon cancers, breast cancers, and lung cancers[42]. Administration of LNA-antimir to mice effectively blocks liver-specific miR-122 expression in vivo[43]. Additionally, miR-127 is constitutively expressed in normal cells but epigenetically silenced in cancer cells. Treatment of cancer cells with chromatin-modifying drugs such as 5-Aza-CdR and PBA that inhibits DNA methylation significantly elevated the expression of miR-127 and inhibited cell proliferation, implicating an application of epigenetic approaches for cancer treatments[44].

8. FUTURE PROSPECTIVE AND CONCLUSION

Although the miRNA synthesis pathway in animals and plants has been well researched over the past decade, many questions have yet to be answered. The transition of miRNA applications from bench to bedside, as cancer biomarkers and as therapeutic agents, necessitates addressing several challenges. As biomarkers, various issues regarding miRNA measurement and quantification, particularly in the circulation, need refining. Firstly we need to gain a better understanding of the exact mechanisms by which miRNAs are released into the circulation and if freely circulating miRNA molecules have any functional role in addition to reflecting the presence and pathological features of disease. Functional validation of all miRNAs reported to bedys regulated in cancer, and the identification of their target genes and pathways is also important. With regard to therapeutics whilst progress in this field is rapid and laudable, many obstacles must be overcome for miRNA-based therapies to become a reality in management of common cancers. A significant amount of functional work remains to be done to fully elucidate the mechanisms by which miRNAs contribute to tumorigenesis, and establish a better understanding of the complexity of gene expression regulation by miRNAs. Pharmacological difficulties include developing safe, effective, site-specific delivery mechanisms for miRNA directed therapies. If the current momentum in miRNA translational research can be maintained, this will bring an exciting new dimension to the field of diagnostics and therapeutics for cancer and has the potential to transform current practice to the ideal of individualized care for cancer patients in the near future.

REFERENCES

1. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5: 2004; 522–531.

2. Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T. A uniform system for microRNA annotation. RNA-Publ. RNA Soc. 9: 2003; 277–279.

3. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat. Rev. Mol. Biol. 10: 2009; 126–139.

4. Grimso A, Srivastava M, Fahey B, Woodcroft BJ, Chiang RH, King N, Degnan BM, Rokhsar DS, Bartel DP. Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. Nature. 455: 2008; 1193–1197.

5. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. EMBO J. 21: 2002; 4663–4670.

6. Zeng Y, Cullen BR. Sequence requirements for micro RNA processing and function in human cells. RNA-Publ. RNA Soc. 9: 2003; 112–123.

7. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha–DGCR8 complex in primary microRNA processing. Genes Dev. 18: 2004; 3016–3027.

8. Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature. 432: 2004; 231–235.

9. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. Nature. 32: 2004; 235–240.

10. Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and its D. melanogaster homolog are required for miRNA biogenesis. Curr. Biol. 14: 2004; 2162–2167.

11. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature. 425: 2003; 415–419.

12. Nakielny S, Dreyfuss G. Transport of proteins and RNAs in and out of the nucleus. Cell. 99: 1999; 677–690.

13. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 303: 2004; 95–98.

14. Yi R, Qin Y, Macara LG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 17: 2003; 3011–3016.

15. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA-Publ. RNA Soc. 10: 2004; 185–191.

16. Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell. 106: 2001; 23–34.

17. Hutvagner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNAinterference enzyme Dicer in the maturation of the let 7 small temporal RNA. Science. 293: 2001; 834–838.

18. Ketting RF, Fischer SEJ, Bernstein E, Sijen E, Hannon GJ, Plasterk RHA. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. 15: 2001; 2654–2659.

19. Knight SW, Bass BL. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in Caenorhabditiselegans. Science. 293: 2001; 2269–2271.

20. Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, R.W. Carthew. Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell. 117: 2004; 69–81.

21. Chen X. MicroRNA metabolism in plants. Curr. Top. Microbiol. Immunol. 20: 2008; 117–136.

22. Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. MicroRNAs in plants. Genes Dev. 16: 2002; 1616–1626.

23. Papp I, Mette MF, Aufsatz W, Daxinger L, Schauer SE, Ray A, Winden JVD, Matzke M, Matzke AJ. Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors. Plant Physiol. 132: 2003; 1382–1390.

24. Wahid F, Khan T, Hwang KH, Kim YY. Piwi-interacting RNAs (piRNAs) in animals: the story so far. Afr. J. Biotechnol. 8: 2009; 4002–4006.

25. Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. Science. 305: 2004; 1434–1437.

26. Telfer A, Poethig RS. HASTY: a gene that regulates the timing of shoot maturation in Arabidopsis thaliana. Development. 125: 1998; 1889–1898.

27. Richard WC, Erik JS. Origins and mechanisms of miRNAs and siRNAs. Cell. 136: 2009; 642–655.

28. Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. Genes Dev. 20: 2006; 1885–1898.

29. Giraldez AJ , Mishima Y, Rihel J, Grocock RJ, Dongen SV, Inoue K, Enright AJ, Schier AF. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science. 312: 2006; 75–79.

30. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. Proc. Natl Acad. Sci. USA. 103: 2006; 4034–4039.

31. Aleman ML ,Doench J, Sharp PA. Comparison of siRNA induced off-target RNA and protein effects, RNA-Publ. RNA Soc. 13: 2007; 385–395.

32. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. Micro- RNA-373 induces expression of genes with complementary promoter sequences. ProcNatlAcadSci USA. 105: 2008; 1608–13.

33. Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S et al. Small dsRNAs induce transcriptional activation in human cells. ProcNatlAcadSci USA. 103: 2006; 17337–42.

34. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 120: 2005; 15–20.

35. Iorio MV, Casalini P, Tagliabue E, Menard S, Croce CM. MicroRNA profiling as a tool to understand prognosis, therapy response and resistance in breast cancer. Eur J Cancer. 44: 2008; 2753-2759.

36. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P et al. Conservation of the sequence and temporal expression of let- 7 heterochronic regulatory RNA. Nature. 408: 2000; 86-89.

37. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA. 228: 2008; 425-436.

38. Akao Y, Nakagawa Y, Naoe T. MicroRNA-143 and -145 in colon cancer. DNA Cell Biol. 26: 2007; 311-320.

39. Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, Orntoft TF, Andersen CL, Dobbelstein M. p53-Responsive micrornas 192 and 215 are capable of inducing cell .cycle arrest. Cancer Res. 68: 2008; 10094-10104.

40. He X, He L, Hannon GJ. The guardian's little helper: microRNAs in the p53 tumor suppressor network. Cancer Res. 67: 2007; 11099-11101.

41. Sun T, Wang Q, Balk S, Brown M, Lee GS, Kantoff P. The role of microRNA-221 and microRNA-222 in androgenindependent prostate cancer cell lines. Cancer Res. 69: 2009; 3356-3363.

42. Stenvang J, Silahtaroglu AN, Lindow M, Elmen J, Kauppinen S. The utility of LNA in microRNA-based cancer diagnostics and therapeutics. Semin Cancer Biol. 18: 2008; 89–102.

43. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S et al. LNA-mediated microRNA silencing in non-human primates. Nature. 452: 2008; 896–899.

44. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell. 9: 2006; 435–43.

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