MicroRNA in Lung Cancer

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In the last decade, researchers have identified a novel mode of gene regulation in the form of a family of small RNAs. In 1993, investigators first identified in *C. elegans* a small RNA, *lin-4*, which affected developmental timing by forming a duplex with the 3′UTR of another gene, *lin-14*, thus preventing *lin-14* translation.[1] The identification of *lin-4* represented the first small RNA-regulating target mRNA expression. In 2000, investigators identified another small RNA *let-7* that regulated the gene *let-4*; however, unlike *lin-4*, *let-7* was conserved across among several species.[2] Both *lin-4* and *let-7* seemed critical to normal cell differentiation.[3] Since this initial discovery, many small RNAs have been identified as potential regulators of gene expression and have been termed microRNAs (miRNAs, mirs). MiRNAs are a family of small noncoding RNAs (approximately 21–25 nucleotides (nt) long) expressed in many organisms, including animals, plants, and viruses. MiRNAs originate from stem-loop precursors and are candidates for the regulation of both translation and mRNA degradation by base pairing to complementary sites of target mRNA.[4] To date, several hundred miRNAs have been identified in animals, plants and viruses, but the biological function has only been identified in a few miRNAs.[5] The biological functions and mechanisms by which miRNAs are regulated remain poorly understood. A single miRNA may bind to and regulate several target mRNAs, whereas several miRNAs may bind and regulate the same target mRNA.[4] Data suggest that miRNAs seem to be integral to several biological functions, including gene regulation, apoptosis, hematopoetic development, and maintenance of cell differentiation. MiRNAs have been identified in several human neurodegenerative metabolic diseases and malignancies.[4]

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MiRNA PROCESSING

MiRNAs may be located in several genomic locations, such as within introns of protein coding genes or within introns or exons of noncoding RNAs.[6] MiRNAs may exist either separately from other miRNAs, functioning as individual transcription units, or in clusters.[5] As illustrated in Figure 1, within the nucleus, miRNAs are transcribed as long primary transcripts by RNA polymerase II into primary miRNAs (pri-miRNAs), which range from hundreds to thousands of nucleotides in length.[6] While in the nucleus, Drosha, an RNase III, in conjunction with either DiGeorge syndrome (critical region gene 8 [DGCR8] in humans) or Pasha (double-stranded RNA binding domain dsRBD protein in *Drosophila* and *C. elegans*), cleaves both strands of the pri-miRNA to release a 70- to 100-nucleotide stem loop, termed the precursor miRNA (pre-miRNA).[7] Cleavage by Drosha is essential for determining the eventual mature miRNA structure. The pre-miRNA is subsequently exported from the nucleus to the cytoplasm by the Exportin5/RanGTP.[7] Once in the cytoplasm, a second RNase III termed Dicer, in conjunction with a dsRBD, cleaves the pre-miRNA, releasing an approximately 22-nucleotide RNA duplex (mature miRNA and its complement miRNA*).[8] Only one strand of the miRNA/miRNA* duplex is released to enter the protein complex of miRNA-containing ribonucleoprotein particles (miRNPs), and the other strand is degraded.[6] MiRNPs guide miRNAs to the target RNA to regulate protein expression by either translational inhibition or mRNA degradation.[8]

MiRNAs bind to the 3′UTR complementary sequences of target RNA, and the degree of complementary pairing results in mRNA degradation and/or translational repression.[9] Investigators initially identified the first eight bases of miRNA starting from the 5′ end (termed the miRNA seed) as important and sufficient for the miRNA–mRNA interaction.[10] However, investigators suggest that mechanisms for targeting may be much more complicated.[11]

MICRORNA IN HUMAN CANCERS

Dysregulation of miRNAs may result in alteration in biological functions relevant to certain malignancies. Our current understanding of miRNA function in cancer is minimal.[5] Epigenetic alterations including DNA methylation and somatic gene alterations are potential candidates for mechanisms of regulation.[4] The observation that many miRNAs are located within genomic regions relevant to carcinomas suggests that miRNAs function as either oncogenes or tumor suppressors.[5] Researchers first identified a link between miRNA expression and human malignancy with the observa-

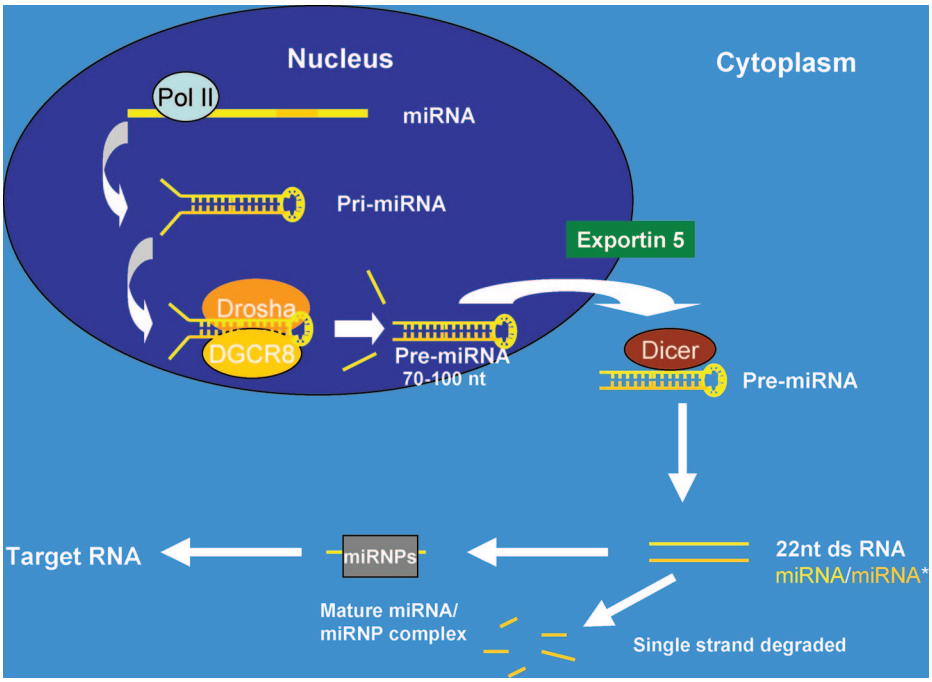

FIGURE 1. MicroRNA processing. Within the nucleus, miRNAs are transcribed as long primary transcripts by RNA polymerase II into primary miRNAs (pri-miRNAs). Drosha, an RNase III, in conjunction with DiGeorge syndrome critical region gene 8 (DGCR8), cleaves both strands of the pri-miRNA to release a 70- to 100-nucleotide stem loop, termed the precursor miRNA (pre-miRNA). The pre-miRNA is exported from the nucleus to the cytoplasm by the Exportin5/RanGTP. Once in the cytoplasm, a second RNase III termed Dicer, in conjunction with a dsRBD, cleaves the pre-MiRNA, releasing an approximately 22-nucleotide RNA duplex (mature miRNA and its complement miRNA*). One strand of the miRNA/miRNA* duplex is released to enter the protein complex of miRNA-containing ribonucleoprotein particles (miRNPs), and the other strand is degraded. MiRNPs guide miRNAs to the target RNA to regulate protein expression by either translational inhibition or mRNA degradation.

tion that there was a downregulation or deletion of miRNAs *mir-15-a* and *mir-16-1* in locus 13q14 in patients with B-cell chronic lymphocytic leukemia (CLL).[12] More than 50% of cases of CLL can be attributed to a deletion at 13q14.[5] Since that discovery, researchers have identified abnormal expression of miRNAs in several types of malignancies, including CLL, colorectal neoplasia, Burkettt's lymphoma, large-cell lymphoma, breast cancer, lung cancer, and hepatocellular and thyroid carcinoma.[13–15] Furthermore, initial expression profiling of miRNAs in patient cohorts with different solid tumors suggests distinct miRNA signature patterns based on tumor type.[3] Investigators have suggested that miRNAs may be used as biomarkers in the diagnosis, prognosis, and treatment of cancers.

MiRNA IN LUNG CANCER

In 2005, Johnson et al.[16] first identified a connection between miRNA expression and genes relevant to lung cancer. The investigators observed an inverse relationship between *let-7* and RAS protein expression in human cancer cell lines. Furthermore, microarray analysis of human cancerous tissue demonstrated decreased *let-7* expression in lung cancer but not in adjacent normal lung tissue, colon cancer, or breast cancer.[17] Researchers also identified a correlation between expression of *let-7* and postoperative survival after curative resection of lung cancers.[18] There have been few studies examining miRNA expression profiling in human lung cancer. Recently, Yanaihara et al.[4] used microarray analysis to identify distinct miRNA profiles between lung cancer and noncancerous tissue. In addition, the investigators identified five distinct miRNAs that predicted prognosis among patients with adenocarcinoma.

MiRNAs are a novel family of small RNAs that are integral to several biological functions and that regulate gene expression in many human diseases, including malignancies. The identification of this new layer of gene regulation and the potential targets may prove important in diagnosis, treatment, and prognosis in lung cancers.

MiRBase (http://www.sanger.ac.uk/Software/Rfam/mirna) provides up-to-date access to sequences, names of novel miRNA genes, and targets.[19,20]

REFERENCES

1. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;75:843–854.
2. Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000; 403:901–906.
3. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005;435:834–838.
4. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006;9: 189–198.

5. Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. *Cancer Res.* 2005;65:3509–3512.
6. Du T, Zamore PD. microPrimer: the biogenesis and function of microRNA. *Development* 2005;132:4645–4652.
7. Kim VN, Nam JW. Genomics of microRNA. *Trends Genet* 2006;22: 165–173.
8. Vatolin S, Navaratne K, Weil RJ. A novel method to detect functional microRNA targets. *J Mol Biol* 2006;358:983–996.
9. Pillai RS. MicroRNA function: multiple mechanisms for a tiny RNA? *RNA* 2005;11:1753–1761.
10. Berezikov E, Plasterk RH. Camels and zebrafish, viruses and cancer: a microRNA update. *Hum Mol Genet* 2005;14:R183–R190.
11. Berezikov E, Cuppen E, Plasterk RH. Approaches to microRNA discovery. *Nat Genet* 2006;38(Suppl):S2–S7.
12. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002;99: 15524–15529.
13. Di Leva G, Calin GA, Croce CM. MicroRNAs: fundamental facts and involvement in human diseases. *Birth Defects Res C Embryo Today* 2006;78:180–189.
14. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 2005;65:7065–7070.
15. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and nontumorous tissues. *Oncogene* 2006;25:2537–2545.
16. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell* 2005;120:635–647.
17. Eder M, Scherr M. MicroRNA and lung cancer. *N Engl J Med* 2005; 352:2446–2448.
18. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004;64:3753–3756.
19. Griffiths-Jones S, Grocock RJ, van Dongen S, et al. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006;34:D140–D144.
20. Griffiths-Jones S. The microRNA Registry. *Nucleic Acids Res* 2004;32: D109–D111.

